STUDY PROTOCOL

Dietary Composition and Energy Expenditure during Weight-Loss Maintenance

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Principal Investigators – David S. Ludwig, MD, PhD – Cara B. Ebbeling, PhD

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PROJECT SUMMARY

Many overweight and obese people can lose weight for a few months, but most have difficulty maintaining weight loss over the long term. One explanation for the poor long-term outcome of weight loss diets relates to behavior, in that motivation to adhere to restrictive regimens typically diminishes with time. An alternative explanation is that weight loss elicits biological adaptations – specifically a decline in energy expenditure and an increase in hunger – that promote weight regain. We recently examined this question in a cross-over feeding study with 21 overweight or obese young adults and found that total energy expenditure (TEE) during weight-loss maintenance was 325 kcal/d greater on a low-carbohydrate diet compared to a conventional low-fat (high-carbohydrate) diet (*Ebbeling et al. JAMA 2012*). A moderate-carbohydrate diet elicited intermediate effects on TEE. We also found potentially important dietary effects on insulin resistance, cortisol excretion, and other chronic disease risk factors. The purpose of the proposed study is to follow-up our initial findings using a parallel design, so that each of three test diets can be examined for 20 weeks, substantially extending duration compared to the 4-week periods in our cross-over study. Following 12±2% weight loss on a standard run-in diet, 150 adults (aged 18 to 65 years) will be randomly assigned to one of three weight-loss maintenance diets controlled for protein content (20% of energy) and varying widely in dietary carbohydrate-to-fat ratio: High-carbohydrate (**HI**, 60% of energy from carbohydrate, 20% fat), Moderate- carbohydrate (**MOD**, 40% carbohydrate, 40% fat), Low-carbohydrate (**LO**, 20% carbohydrate, 60% fat). During the weight-loss maintenance phase, energy intake will be adjusted to prevent changes in body weight. The primary outcome will be change in total energy expenditure (indirect calorimetry using stable isotopes) through 20 weeks. Secondary outcomes will include resting energy expenditure (indirect calorimetry using respiratory gas exchange), physical activity (accelerometry), measures of insulin resistance and skeletal muscle work efficiency, components of the metabolic syndrome, and hormonal and metabolic measures that might inform an understanding of physiological mechanisms. In addition, we will test for effect modification by key baseline covariates, including insulin secretion. We also will assess weight change during a 2-week *ad libitum* feeding phase, as an objective measure of dietary effects on hunger. The study will be performed in collaboration with a local university and high school, providing a novel and feasible method for feeding subjects in dining halls and monitoring compliance.

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1. SPECIFIC AIMS

We recently examined the effects of dietary composition on energy metabolism in a cross-over feeding study with 21 overweight or obese young adults and found that total energy expenditure (TEE) during weight-loss maintenance was 325 kcal per day greater with a low-carbohydrate diet compared to a conventional low-fat (high-carbohydrate) diet.[1] A moderate-carbohydrate diet elicited intermediate effects on TEE. We also found potentially important dietary effects on insulin resistance, leptin sensitivity, cortisol excretion, and cardiovascular disease (CVD) risk factors. However, the short duration of each test diet, only 4 weeks, limits conclusions regarding long-term effects. Here we propose a follow-up study using a parallel design, so that each of three test diets can be examined for 20 weeks, and a sample size of N=150 adults, ensuring robust power.

Specific Aim #1: To evaluate the effects of 3 diets varying widely in carbohydrate-to-fat ratio (high-carbohydrate, moderate-carbohydrate, low-carbohydrate) on energy expenditure during weight-loss maintenance.

Hypotheses

1a Total energy expenditure during weight-loss maintenance will differ among test diets through 20 weeks.

1b Resting energy expenditure during weight-loss maintenance will differ among test diets through 20 weeks.

Primary outcome: total energy expenditure (assessed by indirect calorimetry using stable isotopes).
Secondary outcomes: resting energy expenditure (assessed by indirect calorimetry using respiratory gas exchange), physical activity (assessed by accelerometry).
Potential effect modification: including insulin secretion (insulin at 30 minutes after standard 75-g oral glucose load).

Specific Aim #2: To evaluate the effects of 3 diets varying widely in carbohydrate-to-fat ratio on chronic disease risk factors during weight-loss maintenance.

Hypothesis

2 Chronic disease risk factors during weight-loss maintenance will differ among test diets through 20 weeks.

Secondary outcomes: insulin sensitivity and insulin secretion (assessed by frequently-sampled oral glucose tolerance test (OGTT), urine C-peptide, glycemic control (HgA1c, 1,5-anhydroglucitol), lipid profiles (total cholesterol, HDL-cholesterol, LDL-cholesterol, non-HDL cholesterol, triglycerides), lipoprotein particle subfraction distribution, coagulopathy (PAI-1, fibrinogen), inflammatory mediators (hsCRP, IL-6), blood pressure, sleep (assessed by accelerometry).

Specific Aim #3: To evaluate physiological mechanisms potentially relating dietary carbohydrate-to-fat ratio to metabolism and risk for chronic disease – including CVD, type 2 diabetes, and cancer.

Hypothesis

3 Differences among diets in measures of skeletal muscle work efficiency, body composition, insulin sensitivity and secretion, anabolic and catabolic hormones, gut microbiome, and metabolomics profiles will provide additional physiological insights into the effects of dietary composition on health outcomes during weight-loss maintenance.

Secondary outcomes: skeletal muscle work efficiency (assessed by cycle ergometry), body composition (assessed by a multi-component model), insulin sensitivity and secretion, urine C-peptide, thyroid functions (thyroxine [T4], free T4, rT3, TSH), growth hormone action (IGF-1, IGF binding proteins), reproductive hormones (LH, FSH, testosterone [total and free], estradiol), stress hormones (24-hour urinary cortisol and catecholamines), leptin, adiponectin (total, high-molecular weight), ghrelin, gut microbiome (saved stool samples), metabolomics profiles (saved serum samples).

Specific Aim #4: To evaluate the effects of 3 diets varying widely in carbohydrate-to-fat ratio on voluntary food intake and body weight during an *ad libitum* feeding phase.

Hypothesis

4 Body weight will differ among diets during the 2-week *ad libitum* feeding phase (implemented after the test phase of weight-loss maintenance).

Secondary outcome: body weight.

2. RESEARCH STRATEGY

2.A. Overview. This RCT will comprise three phases, as shown in **Figure 1**, using a feeding protocol. The purpose of the run-in phase is to obtain baseline measurements and restrict energy intake to achieve a 12±2% decrease in body weight, and then stabilize body weight. The purpose of the test phase is to compare the metabolic effects of high–, moderate–, and low–carbohydrate diets during weight-loss maintenance (SA#1, SA#2) and the physiological mechanisms underlying these effects (SA#3). The purpose of the *ad libitum* feeding phase is to evaluate the effects of these diets on body weight (SA#4). Food will be prepared in kitchens at FSU and Assabet Valley Regional Technical High School (AV) by chefs hired to work on the study in collaboration with study dietitians and food service personnel. Subjects (n=150) will be FSU students, faculty, and staff who are regularly on campus (FSU or AV), along with members of the greater MetroWest community who are able to come to campus for meals. We anticipate that many of the enrolled students will live in residence halls. We will ask all subjects, regardless of residence status, to consume meals under observation by study staff in the dining hall. Compliance with this request for campus-based participants will be defined as consumption of no fewer than two meals per day (Monday – Friday) in the dining hall to accommodate school, work, and family schedules. Compliance with this request for community-based participants will be defined as consumption of no less than one meal per day (Monday – Friday) in the dining hall to accommodate work and family schedules. Other meals and the evening snack will be packaged for take-out. The primary outcome is TEE through 20 weeks.

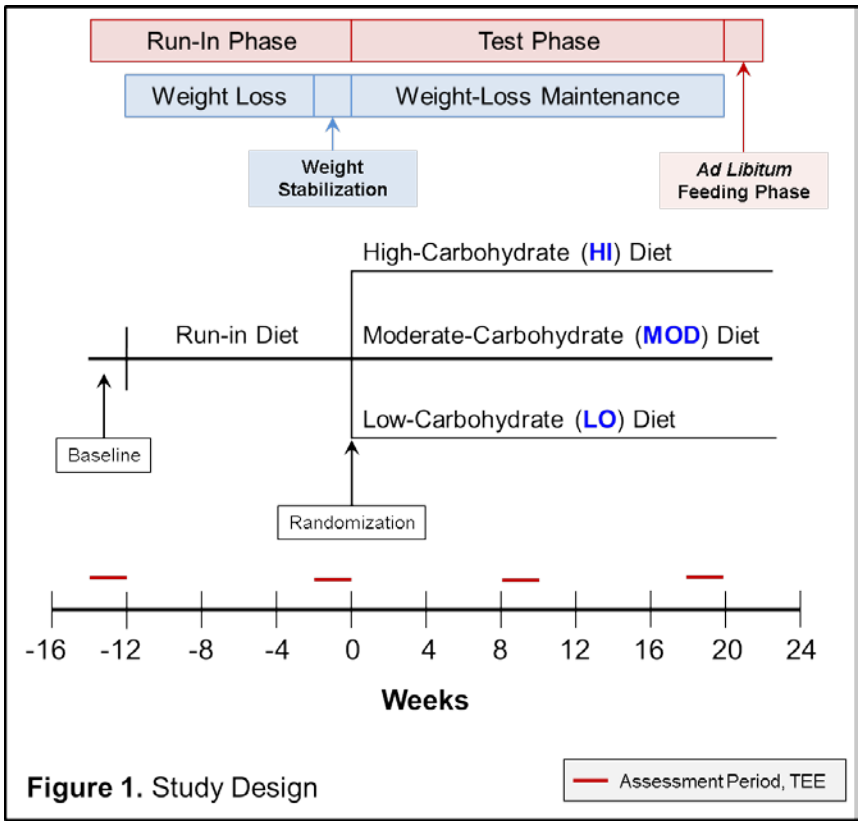

Figure 1. Study Design

2.B. Timeline. The proposed 4-year study consists of an initial start-up period; recruitment, preparation, and implementation periods for each of 3 cohorts; and a data management and analysis period (**Table 1**). During the start-up period, we will develop the diets, establish protocols, prepare a manual of operations, hire and train staff, and begin recruitment. For each cohort, recruitment will occur during the spring semester prior to study participation. We will prepare for the cohort during the summer so that participation can begin in the fall semester and continue through the next spring semester. The data management and analysis period will be devoted to preparation of data sets and programs for statistical analysis.

Table 1. Study timeline.

	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Year 1 *(2013-2014)*												
Study Start-up												
Recruitment, Cohort 1								n=25				
Year 2 *(2014-2015)*												
Preparation, Cohort 1												
Implementation, Cohort 1												
Recruitment, Cohort 2								n=65				
Year 3 *(2015-2016)*												
Preparation, Cohort 2												
Implementation, Cohort 2												
Recruitment, Cohort 3								n=60				
Year 4 *(2016-2017)*												
Preparation, Cohort 3												
Implementation, Cohort 3												
Data Management and Analysis												

We carefully considered the academic schedule when developing the timeline. Subjects will begin participation upon their return to campus for the fall semester. They will complete the 12-week run-in phase at the end of November and the first few weeks of the test phase in December, before winter break. In January, subjects will return to campus for the spring semester. They will complete the 10-week assessments at the end of January and beginning of February. Finally, they will complete the 20-week assessments in April and *ad libitum* feeding period in May, at the end of the academic year. We will provide subjects with packaged food during breaks. In addition, we will give subjects Wi-Fi scales (Withings Inc., Cambridge, MA). Subjects will weigh themselves daily during the study. The scales will be linked to a patient monitoring Website called SetPoint Health (SPH). We will use the SPH Website (https://fs2.sphpro.com) to remotely monitor subject body weight and to track self-reported food intake (amount of provided food consumed). We will contact subjects by telephone, if necessary, to promote compliance.

2.C. Diets. Energy content and macronutrient composition of the run-in and test diets are summarized in **Table 2**. For all diets, total energy intake will be distributed across meals and snacks throughout the day: 22.5% for breakfast, 32.5% for lunch, 32.5% for dinner, and 12.5% for an evening snack. The macronutrient composition of every meal will reflect the composition of each respective diet. We will instruct subjects to consume their meals at regularly scheduled times and not skip meals. Concerning *ad libitum* consumption of non-caloric beverages and artificial sweeteners, we will advise participants to have no more than 3 servings per day of each of the following items: beverages containing artificial sweeteners, caffeinated beverages, packets of artificial sweeteners, and gum and mints containing artificial sweeteners. We will ask subjects not to consume alcoholic beverages (and exclude heavy consumers and binge drinkers from the study, **2.D**). To ensure micronutrient adequacy and minimize the influence of micronutrient differences among test diets, we will give each subject a daily multi-vitamin and mineral supplement throughout the study.

Table 2. Dietary energy and macronutrient composition.

Dietary Variable		Run-In Phase	Test Phase		
		Energy Restriction	High (**HI**) Carbohydrate Diet	Moderate (**MOD**) Carbohydrate Diet	Low (**LO**) Carbohydrate Diet
Energy	(% of weight maintenance needs)	60	100	100	100
Carbohydrate	(% of total energy)	45	60	40	20
Fat	(% of total energy)	30	20	40	60
Protein	(% of total energy)	25	20	20	20
Sodium	(mg)	–	3000	3000	3000
Added Sugar	(% of total carbohydrate)	–	15	15	15
	(% of total energy)	–	9	6	3
Saturated Fat	(% of total fat)	–	35	35	35
	(% of total energy)	–	7	14	21
Fiber	(g)	–	35	30	25

Run-in Diet. The run-in diet will be consistent with recommendations specified by the Institute of Medicine,[2] with protein intake at the upper end of an acceptable range to enhance satiety during weight loss.[3] Consistent with the methodology used in our preliminary study,[1] we will calculate individual energy needs as the arithmetic product of REE, estimated using a regression equation,[4,5] and a physical activity factor of 1.5.[6] To avoid overestimation of energy needs, we will use measured body weight adjusted for ideal body weight (IBW) as the weight variable (([measured weight – IBW] × 0.25) + IBW) in the regression equation. To estimate IBW, we will determine the sex- and height-specific midpoints of weight ranges for medium frames, using Metropolitan Life Insurance Company Height-Weight Tables. Energy intake will be restricted to 60% of calculated needs to promote weight loss, without feeding any subject <1200 kcal/d. The goal is to reduce body weight to a level that is 12±2% below baseline weight. The amount of food provided will be adjusted, if necessary, to achieve the target weight loss over 9 to 10 weeks.

Test Diets. We will compare 3 diets varying in carbohydrate and fat, as specified in **Table 2**, while controlling for protein. We will stabilize body weight at the end of the run-in phase, prior to the start of the test phase. Energy needs for weight stabilization will be estimated based on recent rate of weight loss (energy intake during weight loss [kcal/day] + (rate of weight loss [kg/day] × 7700 kcal/kg]). Although the conversion factor of 7700 kcal/kg is not appropriate for calculations of long-term, energy balance,[7,8] this calculation is useful for estimating energy needs over the short term. Added sugars will contribute no more than 15% of total carbohydrate. Saturated fat will comprise approximately 35% of total fat, with the remainder distributed between mono- and polyunsaturated fat. Targets for sodium, added sugar, fat, and fiber will be achieved based on daily intakes averaged over one week. We will use many of the same foods, in differing amounts, across diets and systematically replace foods when necessary to achieve the specified macronutrient targets. As such, the diets will reflect gradients in amounts of foods rich in carbohydrate and fat. The quantity of non-starchy vegetables will be approximately the same across diets. *High-Carbohydrate Diet.* This diet will contain 60% of total energy from carbohydrate and 20% from fat, based on contemporary public health recommendations[9] that emphasize sources of carbohydrate such as whole grains, vegetables, fruits, legumes, and low-fat dairy products. *Moderate-Carbohydrate Diet.* This diet will contain 40% of total energy from carbohydrate and 40% from fat. These targets will be achieved by decreasing the quantity of grains relative to the high-carbohydrate, decreasing fruits, adding foods containing fat (e.g., nuts, seeds, sauces, spreads, toppings), decreasing amounts of legumes when necessary, and including some higher fat dairy products. *Low-Carbohydrate Diet.* This diet will contain 20% of total energy from carbohydrate and 60% from fat. Relative to the moderate-carbohydrate diet, these targets will be achieved by eliminating all grains, removing some fruits, adding more foods containing fat, further decreasing amounts of legumes, and increasing some higher fat dairy products.

Adjusting Energy Intake to Achieve Weight Stability. During the test phase of the study, we will monitor body weight using the SetPoint Health Website and adjust energy intake to achieve weight stability, defined as weight change of no more than ±2 kg. We will measure weight every day and regress weight (g) on time (days). A slope of ≥15 g per day over 14 days will indicate the need to adjust energy intake to achieve stability. Adjustments will be made, based on deviation from the post-weight loss anchor weight and the slope of the regression line, no more frequently than every 2 weeks.

2.D. Subjects. We will recruit adults who meet the criteria outlined in **Table 3.** These criteria will be assessed during visits with potential subjects and in communication with primary care providers.

Table 3. Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
• Aged 18 to 65 years (FSU students, faculty, staff, community members). • BMI ≥ 25 kg/m^2. • Weight ≤ 350 lbs (159 kg). • Medical clearance from a primary care provider. • Plans to matriculate as a student at FSU or work on campus throughout the academic year of enrollment in the study. • Willingness and ability to come to campus throughout the academic year of enrollment in the study. • Willingness to eat and drink only the foods and beverages on the study menus during participation, with no food allergies or aversions. • Willingness to eat in the dining hall. • Willingness to abstain from consuming alcohol during participation. • Academic and social clearance from the FSU Office of Enrollment and Student Development (student subjects) or Criminal Offender Record Information (CORI) check and Sex Offender Registry Information (SORI) check (community-based subjects).	• Change in body weight exceeding ±10% during prior year. • Recent adherence to a special diet. • Recent adherence to a vigorous physical activity regimen (as indicated by participation in a varsity sport). • Chronic use of any medication or dietary supplement that could affect study outcomes. • Current smoking (1 cigarette in the last week). • Heavy baseline alcohol consumption (> 10 drinks/week) or history of binge drinking (≥ 5 drinks in 1 day, anytime in past 6 months). • Physician diagnosis of a major medical illness or eating disorder. • Abnormal laboratory screening tests (hemoglobin A1c, TSH, hematocrit<30%, BUN, creatinine, ALT>200% of normal upper limit). • Plans for a vacation during the study that would preclude adherence to prescribed diets.
Additional exclusion criteria for females	
• Irregular menstrual cycles. • Any change in birth control medication during the 3 months prior to enrollment. • Pregnancy during the 6 months prior to enrollment. Lactation during the 3 months prior to enrollment.	

Rationale for Criteria. Students (FSU), faculty, staff, and community members aged 18 to 65 who are regularly on campus (FSU and AV), and members of the greater MetroWest community who are willing and able to come to campus will be eligible to participate in the study. We specify a BMI inclusion criterion starting at 25 kg/m^2, which corresponds to the conventional definition of overweight. We will not enroll anybody who weighs more than 350 lbs (159 kg), corresponding to the upper limit for some assessment equipment. Recent and substantial weight change, adherence to a special diet or vigorous physical activity regimen, use of medications or dietary supplements, smoking, or excessive alcohol consumption will be exclusionary due to possible confounding of study outcomes. Abnormal laboratory tests, indicative of unrecognized illness, also will be exclusionary. We will use a cut-point corresponding to 200% of the normal upper limit for ALT given the high prevalence of fatty liver among obese individuals and recognizing that the first line of treatment for fatty liver typically is weight loss.[10] Vacation plans over winter or spring break that involve international travel or that do not permit adequate and safe food storage and food preparation are exclusionary given the high likelihood of noncompliance for over a week. Additional exclusion criteria for females are specified to diminish confounding from unpredictable changes in reproductive hormone cycles.

Recruitment and Screening. We will recruit ethnically and racially diverse subjects by posting flyers, newspaper advertisements, and Internet announcements at FSU and AV during the spring semester. When an individual responds, we will implement a multi-step screening and enrollment process: *1)* telephone conversation, *2)* informational visit, *3)* medical clearance from a primary care provider, *4)* screening visit, and *5)* informed consent visit. During the telephone conversation, we will provide an overview of the study and ask preliminary screening questions. If the individual is provisionally eligible based on the telephone conversation, we will invite him/her to an informational visit during which we will explain the study protocol as outlined in the consent form and further assess provisional eligibility. If the individual remains provisionally eligible, we will request medical clearance in writing from his/her primary care provider (with verbal and written permission from the provisionally eligible subject to make this request). We will request academic and social clearance in writing from the FSU Office of Enrollment and Student Development for all provisionally eligible student

subjects. We will obtain permission to do CORI/SORI background checks for provisionally eligible subjects for community-based participants, in compliance with University and School District policies. Any potential subjects for whom we cannot obtain medical, academic, or social clearance will be ineligible for the study. At the screening visit, we will obtain a blood sample for analysis of hemoglobin A1c, TSH, hematocrit, BUN, creatinine, ALT. Subjects will be recruited in three cohorts at the rate presented in **Table 1**.

Retention. We designed the study to promote retention as follows. *1)* We will bring research operations to the FSU campus where adults spanning a wide age range attend classes or work during the academic year. We also will recruit community-based participants who work or live in metropolitan and residential areas in close proximity to FSU or AV. *2)* The study protocol includes a run-in phase to promote weight loss, prior to randomization. Individuals who lose weight at the expected rate will have demonstrated their capacity to comply with arguably the most rigorous phase of our study. *3)* We will collaborate with professional chefs to develop a cycle menu of palatable meals and snacks corresponding to specifications of the run-in and test diets (**Table 2**). *4)* We carefully considered logistical feasibility when selecting outcomes and determining assessment schedules. *5)* The study provides benefits to subjects, including weight loss.

Compensation. We will offer compensation for time and any inconvenience imposed by study participation and to cover the cost of the meal plan. All enrolled participants will receive a study stipend totaling $3,280. Resident student participants, who are required to purchase a full meal plan according to FSU policies, will also receive up to $3,220 as reimbursement for the cost of the meal plan. Non-resident students, faculty, staff, and community-based participants will receive study meals and snacks valued at up to $3,220. Thus, the maximum total value of compensation is $6,500, dispersed at eight specific time points during the study, as presented in **Table 4**. This plan is not considered coercive given the rigors of study participation, but encourages study completion.

Table 4. Reimbursement and Compensation Schedule.

		Resident FSU Students			Non-Resident FSU Students, Faculty, Staff, Community members	
Week	Phase or Event	Monthly Meal Plan *	Monthly Stipend *	Additional Stipend †	Monthly Stipend *	Additional Stipend †
		Reimbursement	Compensation		Compensation	
-9	Run-In	$400	$100	$200	$100	$200
-4	Run-In	$400	$120		$120	
1	Test	$400	$120	$300	$120	$300
6	Test	$400	$130		$130	
11	Test	$400	$140	$400	$140	$400
16	Test	$400	$150		$150	
21	Ad libitum	$400	$170	$400	$170	$400
	Completion	$420	$550	$500	$550	$500

* Monthly payments for participating in the intervention. Each payment includes reimbursement for the meal plan (resident students only) and a stipend as compensation for time and effort to adhere to the study diets.

† Additional stipend as compensation for time and effort required for measurement of study outcomes at specified time points.

2.E. Randomization. A blocked randomization design will be employed to ensure close balance among the three diet arms at any point in the study. The randomization will be stratified by feeding site (FSU, AV), sex, ethnicity-race (non-Hispanic white, other), age (18–39.9 years and 40.0–65.9 years), and BMI (overweight: 25.0–29.9 kg/m^2, obese: ≥30.0 kg/m^2) to ensure balance at the completion of enrollment within every subcategory, regardless of size. Enrollment logs, one for each stratum, will be prepared with a numerical sequence of identifiers. Diet assignment lists, identical to the enrollment logs except for the addition of a randomly chosen diet, will be prepared under supervision of the study biostatistician, using specialized software developed for that purpose. The diet assignments will be randomly permuted within blocks of 3, 6, and 9, and the blocks themselves will be randomly permuted. Each upcoming assignment will thus be unpredictable, preventing any deliberate or inadvertent bias on the part of those conducting enrollment. The assignment list will be kept in the private custody of a clinical research specialist who, confirming adequate weight loss during the run-in phase, will relay the diet assignment of each subject to intervention staff. All staff involved in assessing study outcomes and conducting analyses of biospecimens will be masked to diet assignments.

2.F. Assessment of Outcomes. We will assess study outcomes under free-living conditions during visits to on-campus research facilities, as listed in **Table 5** and described below for each Specific Aim.

Table 5. Study outcomes, covariates, and effect modifiers.

	Run-In Phase		Test Phase		*Ad libitum* Feeding Phase
	Baseline	Post-Weight Loss	Midpoint	End	
	-14 to -12 weeks	-2 to 0 weeks	8 to 10 weeks	18 to 20 weeks	21 to 22 weeks
Study Outcomes Corresponding to each SA					
SA#1					
TEE	X	X	X	X	
REE	X	X	X	X	
Physical activity	X	X	X	X	
SA#2					
Insulin sensitivity and secretion (OGTT) *	X	X	X	X	
Urine C-peptide	X	X		X	
Glycemic control (HgA1c, 1,5-anhydroglucitol)	X	X	X	X	
Lipid profiles (TC, HDL-C, LDL-C, non-HDL-C, TG)	X	X	X	X	
Lipoprotein particle subfraction distribution	X	X	X	X	
Coagulopathy (PAI-1, Fibrinogen)	X	X	X	X	
Inflammatory mediators (C-reactive protein, IL-6)	X	X	X	X	
Blood pressure	X	X	X	X	
Sleep	X	X	X	X	
SA#3					
Skeletal muscle work efficiency (cycle ergometry)	X	X		X	
Body composition (multi-component model) *	X	X		X	
Insulin sensitivity and secretion (OGTT) *	X	X	X	X	
Urine C-peptide	X	X		X	
Thyroid functions (T4, Free T4, rT3, TSH)	X	X		X	
Growth hormone action (IGF-1, IGF-BPs)	X	X		X	
Reproductive hormones (LH, FSH, E2, total and free TST)	X	X		X	
Stress hormones (urine cortisol, urine catecholamines)	X	X		X	
Leptin, adiponectin (total, high-molecular weight), ghrelin	X	X	X	X	
Gut microbiome	X	X		X	
Serum metabolomics profile	X	X	X	X	
SA#4					
Body weight					X
Covariates and Effect Modifiers					
Sex	X				
Race	X				
Ethnicity	X				
Body weight, BMI *	X	X	X	X	
Body composition (multi-component model) *	X	X		X	
Insulin sensitivity and secretion (OGTT) *	X	X	X	X	
Obesity-related genes	X				
Palatability of Test Diet				X	

Abbreviations. OGTT, oral glucose tolerance test; HgA1c, Hemoglobin A1c; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; non-HDL-C, non-high-density lipoprotein cholesterol; TG, triglycerides; PAI-1, plasminogen activator inhibitor-1; IL-6, interleukin-6; T4, thyroxine; rT3, Reverse triiodothyronine; TSH, thyroid stimulating hormone; LH, luteinizing hormone; FSH, follicle stimulating hormone; TST, testosterone; E2, estradiol.

* Variables derived from the OGTT and body composition assessment will be assessed as outcomes and as covariates.

Energy Expenditure (SA #1). Macronutrient composition could affect energy expenditure directly because metabolic pathways vary in energetic efficiency or indirectly through hormonal responses that regulate metabolic pathways.[11,12] Dietary carbohydrate, in particular, may control disposition of macronutrients via effects on the rise in blood glucose after eating and secretion of insulin and other hormones.[13] In our preliminary study, we observed a significant inverse trend by dietary glycemic load for TEE and REE. While we did not see any differences in physical activity across diets in our preliminary study, here we hypothesize that dietary composition may influence level of physical activity due to differences in availability of metabolic fuels with a longer intervention, consistent with animal data,[14] or intrinsic skeletal muscle work efficiency as regulated by leptin.[15-18] We will measure TEE under free-living conditions over 21-day periods at baseline, at post-weight loss (week 0), and during intervals ending at weeks 10 and 20 of the test phase. We will assess REE and physical activity at the same time points.

Total energy expenditure (with doubly labeled water, DLW). Following oral administration of the stable isotopes of hydrogen (^{2}H) and oxygen-18 (^{18}O) in the form of $^{2}H_{2}{}^{18}O$, the ^{18}O is eliminated from the body as both carbon dioxide and water, and the ^{2}H is excreted exclusively as water. The difference between the urinary elimination rates of ^{18}O and ^{2}H provides a measure of carbon dioxide production (rCO_2) used to estimate TEE. The dose of DLW will be a mixed cocktail containing 0.086 g of $^{2}H_{2}O$ at 99.98 atom % ^{2}H and 1.38 g of 10% ^{18}O per kg body weight. Urine samples will be collected before each dose and at regular intervals over 14 days after each dose. Isotopic enrichment data will be obtained by gas-isotope-ratio mass spectrometry,[19] converted to atom percent, and used to model rCO_2. TEE will be calculated from rCO_2 using the equation of Ravussin et al,[20] with the food quotient (FQ) as an estimate of respiratory quotient (RQ).[21] *Resting energy expenditure***.** We will measure REE by indirect calorimetry using respiratory gas exchange after a 12-hour overnight fast. The calorimetry system will be calibrated according to the manufacturer's specifications (TrueOne 2400, Parvo Medics, Sandy, UT). Room temperature will be maintained at a constant level, and lighting and noise will be minimized to limit variability in measurements. The subject will be awake and reclining. Oxygen consumption and carbon dioxide production will be measured for 30 minutes, and REE will be calculated by the Weir equation[22] using data averaged over the last 20 minutes. We will assess REE on two separate mornings. If the two measurements are not within 10%, we will obtain a third measurement. We will take the average of the two closest measurements as the best estimate of REE. *Physical activity***.** While measures of TEE and REE can be used in combination to obtain an estimate of physical activity level,[23] this approach does not provide information regarding quality of physical activity. Thus, we will assess physical activity with an accelerometer (ASP-BTLE, Actigraph LLC, Pensacola, FL). The device measures and sums the magnitude of accelerations, and data are expressed as intensity counts per minute. We will ask each subject to wear the accelerometer on the right hip for 7 days per assessment, except when sleeping, bathing, or participating in water activities. Daily physical activity will be quantified as total counts and minutes of moderate- to vigorous-intensity physical activity, consistent with published methodology.[24] We also will ask participants to complete daily physical activity diaries, recording each activity performed during 15-minute time blocks throughout each day the monitor was worn [25]. We will use information from the diaries to confirm the times that the physical activity monitors were worn during waking hours [26].

Chronic Disease Risk Factors (SA#2). The effect of macronutrient composition on conventional CVD risk remains a topic of controversy, confounded in part by diet-induced differences in body weight. The proposed study offers a special opportunity to examine the effects of different macronutrient diets on novel, as well as conventional, CVD risk factors, independent of body weight and taking into account diet-related effects on relative adiposity. We will assess chronic disease risk factors following an overnight fast at baseline, post-weight loss (week 0), and at weeks 10 and 20 of the test phase. *Insulin sensitivity and insulin secretion.* We will conduct an OGTT, using a standard 75-gram dose of dextrose (Trutol™, ThermoFisher Scientific, Waltham, MA). Blood for determination of plasma glucose and serum insulin will be obtained by indwelling venous catheter at −10, −5, 0, 10, 20, 30, 60, 90, and 120 minutes relative to the start time of dextrose consumption. The hand and forearm will be placed in a warming box set at 50-55°C (~120-130°F) to arterialize venous blood samples. Using plasma glucose and serum insulin data, we will calculate indexes of peripheral and hepatic insulin sensitivity as described by Abdul-Ghani et al.[27] Insulin levels during the first 30 minutes following the dose of dextrose will be used to assess insulin secretion. We will measure C-peptide in a 24-hour urine sample as an indicator of daily insulin secretion [28]. We will assess HbA1c in a fasting blood sample as an integrated measure of mean glycemia over approximately three months [29] and 1,5-anhydroglucitol (1,5-AG) as marker of diet-induced glucose excursions [30]. *Lipid profiles, coagulopathy, inflammation, leptin, and adiponectin.* Serum total cholesterol, HDL-cholesterol, and triglycerides will be measured using standardized assays. Serum LDL cholesterol will be measured using direct enzymatic/spectrophotometric methodology. Non-HDL-cholesterol will be calculated as an indicator of atherogenic particle concentration.[31,32] Lipoprotein particle subfraction distribution will be assessed by nuclear magnetic resonance (NMR) spectroscopy (Liposcience Inc., Raleigh, NC) to further evaluate metabolic risk. Plasma PAI-1 and fibrinogen will be measured as indicators of coagulopathy, and levels of high-sensitivity C-reactive protein and IL-6 will be measured as markers of chronic inflammation. *Blood pressure.* Consistent with current guidelines,[33] we will measure blood pressure by auscultation at the right arm using a sphygmomanometer (System 5, American Diagnostic Corporation, Hauppauge, New York), 3 times at each assessment visit. *Sleep.* We will measure sleep using the same accelerometer used to assess physical activity (ASP-BTLE, Actigraph LLC, Pensacola, FL). We will ask each subject to wear the accelerometer on the non-dominant wrist for 7 nights per assessment. We will quantify total sleep time, sleep onset latency, wake after sleep onset, and sleep efficiency. We also will ask subjects to complete a daily sleep diary.

Physiological Mechanisms (SA#3). We will measure skeletal muscle work efficiency and other outcomes at baseline, post-weight loss (week 0), and week 20 of the test phase. *Skeletal muscle work efficiency.* We will measure work efficiency during graded cycle ergometry according to published methods.[15,16] In brief, following a 10-minute warm-up period, subjects will pedal at 60 rpm against graded resistance to generate power corresponding to grades of 10W, 25W, and 50W in 4-minute stages. We will measure oxygen uptake and carbon dioxide production by indirect calorimetry and convert oxygen consumption to energy expenditure based on respiratory exchange ratio. We will define skeletal muscle work efficiency at each grade as power generated per increase in energy expenditure above resting. *Other hormonal axes that affect or respond to energy balance*. We will assess thyroid functions (Thyroxine [T4], free T4, rT3, TSH), growth hormone action (IGF-1, IGF-BP3), reproductive hormones (LH, FSH, testosterone, estradiol), stress hormones (24-hour urinary cortisol, 24-hour urinary catecholamines), leptin, adiponectin (total, high molecular weight), and ghrelin using standard procedures. *Body composition***.** We will assess body composition using a multi-component model with measures of total body volume from air displacement plethysmography (ADP, BodPod, Cosmed USA Inc., Concord, CA), total body water by isotope dilution, and bone mineral content by dual-energy x-ray absorptiometry (DXA, Horizon A, Hologic Inc., Bedford, MA). We will instruct participants to fast for at least 5 hours prior to the DXA and ADP measurements. *Gut microbiome***.** We will save stool samples to assess changes in gut flora previously associated with metabolic changes and obesity in humans (e.g.,relative abundance of *Firmicutes* and *Bacteroidetes* species) following published methods.[34-36] This assessment will be done only for subjects who "opt in" to collect stool samples. *Metabolomics profiles.* We will save serum samples and use liquid chromatography tandem mass spectrometry for metabolomics profiling. Such a platform has proven to be highly informative of pathophysiologic status, providing for example quantitative data regarding disease development before the appearance of conventional disease markers.[37-39]

Weight Change (SA#4). We will allow voluntary food intake during the 2-week *ad libitum* feeding phase at the conclusion of the study, providing a complementary approach to the preceding 20-week test phase of weight-loss maintenance. Subjects will continue to consume test diets with specified proportions of macronutrients, according to random assignment. We will provide the same amount of food relative to that provided for weight stabilization and instruct subjects as follows: "Have your meals according to your usual schedule. Eat as much or as little of each meal as you like until you are satisfied. If you finish your meal and are hungry before the next meal, eat something of your own choosing until you are satisfied. If you do not finish your meal, do not eat anything before the next meal. We ask that you eat until satisfied, but avoid overeating to the point of feeling too full. Do not drink alcohol during the free feeding phase." *Body weight.* Subjects will weigh themselves daily, using Wi-Fi scales linked to the SetPoint Health Website, throughout the *ad libitum* feeding phase so that we can assess weight change.

Covariates and Effect Modifiers. Variables that will be included as covariates or effect modifiers in statistical analyses are listed in Table 6. *Demographic data.* At baseline, we will collect self-report data with regard to sex, race, and ethnicity. *Body weight and composition.* We will measure body weight at the time of each study assessment using an electronic scale and body composition using a multi-component model, as described above at the time points denoted in Table 6. *Insulin sensitivity and secretion.* We will conduct an OGTT, as described above at the time points denoted in Table 6. *Obesity-related genes.* The effects of diet on study outcomes may be modified by obesity-related genes. For participants who "opt in," we will isolate and save buffy coat from blood samples for extracting DNA. Genetic studies may include, but not be limited to, candidate gene analysis and whole genome/exome sequencing. We have particular interest in amylase gene copy number [40]. *Palatability of Test Diet.* We will measure perceived palatability (tastiness) of the test diets using a 10-cm Visual Analog Scale (VAS).

2.G. Data Management. We will follow clinical data management best practices. Essential data management activities will include design and development of case report forms (CRFs); design, development, and maintenance of the data management system; and preparation of datasets.

Case Report Forms. We will develop customized CRFs to capture data required to test our hypotheses, with particular attention to minimizing data entry errors and ensuring efficient, consistent, and unambiguous data abstraction from source documents. The design will include simple introductions for each section, obvious skip patterns, and standard coding and formatting conventions. We will write a standard operating procedure describing how to complete each CRF (question-by-question guide). A clinical research specialist will complete a critical review of all CRFs prior to pre-testing to evaluate the form for programmability. Staff then will pre-test CRFs to evaluate the logic, wording, and general construction. Pre-testing also will include evaluation of overall feasibility of the data collection plan and procedures. The principal investigators and the study biostatistician will approve the final set of CRFs prior to database programming.

Data Management System. We will use the Research Electronic Data Capture (REDCap) software application to collect and manage data. REDCap is designed to comply with HIPAA regulations. The research team will work collaboratively with personnel in the Clinical Research Center at Boston Children's Hospital to build a study-specific data dictionary for use in implementing the REDCap system. The web-based system offers an intuitive interface for data entry, with real time validation rules (automated data type and range checks). Data entered in REDCap will be stored in a password-protected database hosted on a secure, firewall-protected server. Only authorized users will have access to data in the REDCap system. All users will undergo REDCap training by the Harvard Catalyst resource specialist and will follow standard operating procedures for data entry into REDCap. The system is supported by the Clinical Research Information Technology and Research Computing Departments, with nightly back-up of data.

Preparation of Datasets. Data for primary and secondary outcomes, process measures, and covariates will be recorded on CRFs and entered into the REDCap data management system. The study biostatistician will oversee transfer of data to statistical analysis programs.

2.H. Statistical Methods. We will follow the *a priori* analysis plan described below, with a sample size of N=150 adults ensuring robust power.

Analysis Plan. The primary outcome measure of the trial is total energy expenditure (TEE) per kg body weight, measured at four time points: baseline (pre-weight loss), week 0 (post-weight loss, pre-randomization), week 10 (midway through the test phase), and week 20 (end of test phase). The primary null hypothesis is that the time course of TEE between week 0, week 10, and week 20 will be the same for all three diets: high (HI), moderate (MOD), or low (LO) carbohydrate-to-fat ratio. An alternative hypothesis is detailed below.

The analytic framework for addressing both primary and secondary hypotheses will be repeated-measures analysis of variance (ANOVA), with the outcomes of interest as dependent variables and study arm (diet: HI, MOD, LO) as a three-level independent variable. Although covariates are theoretically balanced by the randomization and thus should have little influence, we will adjust the ANOVA for a number of baseline and time-varying covariates in order to reduce residual variance and improve our power to detect diet differences. These include the outcome of interest at baseline (pre-weight loss); change in body weight over the test phase (weeks 0–20); demographic characteristics (sex, race, ethnicity, age); baseline anthropometric measures (body-mass index, percentage lean mass, percentage weight lost pre-randomization); and design variables (study site, cohort, enrollment wave). We will employ an autoregressive covariance structure to account for potentially diminishing within-subject correlation over time. To minimize the influence of extreme values on the fitted model, we will employ an outlier-deletion algorithm equivalent to robust regression with iterative reweighting.[41] A single subject who developed a disqualifying medical condition (hypothyroidism, as documented by 2 elevated TSH values) post-randomization will be excluded from the primary analysis.

To test the primary hypothesis, we will construct appropriate contrasts from parameters of the fitted repeated-measures model (namely, adjusted mean TEE at week 10 and week 20 – adjusted mean TEE atweek 0, HI vs. MOD vs. LO), and test their significance with a 2-df F-test and critical p-value 0.05. If the overall null hypothesis is rejected, pairwise contrasts between study arms will be constructed and compared to zero. The principle of closed testing[42] dictates that in this special situation of three groups, compared pairwise only if the overall null hypothesis is rejected, we may make each pairwise comparison with a critical p-value of 0.05 and still preserve the Type I error rate for the family of four comparisons at 5%.

In secondary analyses, we will test each covariate for effect modification (covariate × diet interaction) and, if significant effects are found, construct separate estimates for the diet effects by covariate stratum. Additional secondary analyses will be conducted with insulin-30 (from baseline OGTT) as a covariate. The use of time-varying covariates will allow us to test hypotheses of mediation by diet-related behavior and other process measures, secondary to the primary hypothesis of intervention efficacy. We will also perform a "per protocol" analysis, excluding any subject whose weight was out of target range at the 20-week time point (defined as weight change of no more than ±2 kg relative to the PWL anchor weight), or who began taking an exclusionary medication.

Secondary outcomes will be assessed similarly to TEE. Measures with skewed distribution will be log-transformed for analysis and re-transformed to natural units for reporting, with changes and differences (Δlog) expressed as ratios (exp(Δlog)) or percentages (100% × (ratio–1)).

In all analyses, except "per protocol," we will follow the intention-to-treat principle, ascribing the randomly assigned diet to each subject regardless of degree of compliance. To test for biased dropout, we will compare baseline characteristics of completers with those of non-completers, using standard procedures (Student t, Wilcoxon rank-sum, Fisher exact test). We will use inverse probability weighting to compensate for missing

data, constructing a logistic model for missingness based on the baseline characteristics that differed between completers and non-completers. SAS software (SAS Institute Inc., Cary, NC) will be used for all computations.

Sample Size, Power, and Detectable Effects. For comparability with pilot data from prior studies, our sample-size calculation is formulated in terms of changes in TEE uncorrected for body weight between week 0 and week 20. The primary null hypothesis is that the mean value of ΔTEE, defined as change in total energy expenditure at week 20 of the test phase compared to week 0 (post-weight loss), will be the same in all three diet arms. To estimate our power to detect deviations from this null hypothesis, we use the power characteristics of a simple one-factor, three-level ANOVA using covariate-adjusted residual variance estimates taken from our pilot data. As a conservative, minimal-impact alternative, pictured in the inset panel of **Figure 2**, we hypothesize that one of the three diets (high carbohydrate-to-fat ratio, or HI) will differ from the other two. The power of the ANOVA test to reject the null hypothesis increases with the magnitude of difference, as detailed in **Figure 2** for three illustrative sample sizes. These curves were derived from the non-central F distribution under a range of alternatives from 0 to 400 kcal/d, taking account of the parallel-group design (as contrasted with our pilot study, a 3-arm crossover design) and assuming a standard deviation of 412 kcal/d for ΔTEE among subjects as observed in the pilot data. Our proposed sample size, 45 completers per diet (allowing for 10% attrition from recruited sample of 50), provides 80% power to detect a difference of 237 kcal/d, as indicated by the point on the middle curve. This is a smaller effect than was discerned in the pilot, where mean ΔTEE under a high-carbohydrate (low-fat) diet differed from the average under moderate- and low-carbohydrate diets by 263 kcal/d. The smaller sample size, 25 per arm, would provide only 50% power for that magnitude of effect, while the larger sample size, 75 per arm, would be much more costly and would reduce the effect detectable with 80% power only to 175 kcal/d. We thus believe our design choice to be optimal in terms of feasibility and statistical power.

Detectable-effect figures for a variety of secondary outcomes are tabulated in **Table 6**. Based on the same design, alternative hypothesis, and analytic strategy, each figure represents 0.575 standard deviations of the outcome, a moderate effect size by conventional benchmarks.

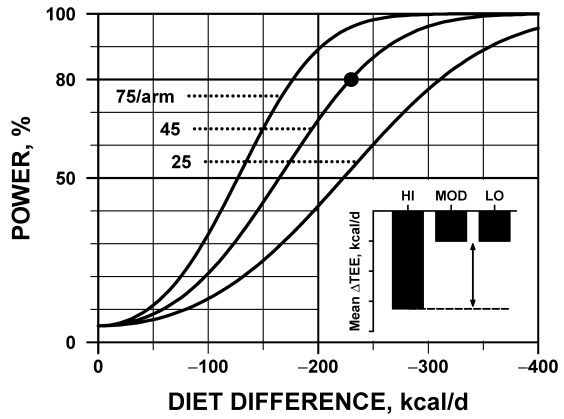

Figure 2. Power of one-way ANOVA to detect deviation of mean ΔTEE under one diet from the other two (inset). Each curve represents a fixed sample size. Dot indicates detectable effect, 237 kcal/d at 80% power, with proposed 45/arm. Curves based on non-central F distribution, 5% Type I error, between-subject standard deviation 412 kcal/d as in preliminary data.

Table 6. Detectable difference for primary and secondary outcomes. Under minimal-impact alternative hypothesis that one diet will differ from the other two in mean 20-week change. Derived from non-central F distribution assuming 45 completers per diet arm, 5% Type I error, and standard deviation as observed in pilot study.[1]

Outcome	Detectable effect
Total energy expenditure (TEE)	237 kcal/d
Resting energy expenditure (REE)	89 kcal/d
Moderate-vigorous physical activity	8 min/d
Leptin*	19%
Urinary cortisol*	31%
C-reactive protein*	68%
Peripheral insulin sensitivity index†	0.57

* Log-transformed for analysis, detectable effect expressed as percentage difference.
† Derived from oral glucose tolerance test: rate of decline of serum glucose between 60 and 120 minutes (mg/dL/hr) divided by time-weighted mean serum insulin (μIU/mL) between baseline and 120 minutes.

3. PROTECTION OF HUMAN SUBJECTS

3.A. Risks To The Subjects

3.A.1. Human Subjects Involvement, Characteristics, and Design. The proposed procedures involving human subjects are described in detail above with regard to study design (**2.A**), inclusion and exclusion criteria (**2.D**), recruitment and retention strategies (**2.D**), randomization (**2.E**), dietary intervention (**2.C**), and sample size (**2.H**). Key personnel have completed training in the protection of human subjects.

We will send de-identified urine samples to the Baylor College of Medicine via Federal Express for gas-isotope-ratio mass spectrometry, as part of the protocol utilizing doubly-labeled water to assess total energy

expenditure (**2.F**). Each sample will contain only a study-specific subject identification (ID) number and information regarding the date and time of sample collection to ensure integrity of the data collection process. Serum, plasma, and other urine samples collected for assessment of chronic disease risk factors and physiological mechanisms will be labeled in the same manner and then triaged to appropriate laboratories or a biorepository.

We will use the Web-based platform of SPH to track subject weight and food intake. SPH will require access to the following Protected Health Information (PHI): study ID number, subject name, telephone number, and email address. Subjects will interact with the SPH Website via computer or through a free smartphone application or "app;" therefore subject IP addresses and other electronic identifiers may be known and stored. A Business Associate Agreement and a Web Site Agreement will be executed between SPH and the Clinical Trials Business Office at BCH. A copy of SPH's HIPAA-compliant IT Data Security Standards will be filed with the Institutional Review Board.

3.A.2. Sources of Materials. Data will be obtained directly from subjects for research purposes only, as described in **2.F**. Daily weight and food intake data will be collected from subjects through the SPH Website or smartphone "app." During the screening process, we will obtain permission from subjects, in writing, to request medical clearance from their primary care providers. We will also obtain permission from subjects, in writing, to register them with SPH, and to obtain their preference for receiving study reminders (email or text message). Laboratory testing will be performed on blood, stool, and urine samples obtained at the time of the study. SPH will be used to collect data on subject weight and food intake. Data will not be obtained from other sources.

All data will be held in strict confidence such that only personnel listed in the protocol on file with the IRB will have access to individually identifiable private information about the subjects. Any publication or report resulting from this work will maintain the anonymity of study subjects. A study identification (ID) assignment log will be the only link between subject ID codes and identifiable information. The log will be kept in a password protected electronic file, accessible only to study staff for the purposes of enrolling and tracking subjects. Tracking information will be stored in a separate database that is not linked to the data management system (**2.G**). All specimens and case report forms will be labeled with study ID codes, without individually identifiable private information.

3.A.3. Potential Risks. The project has been designed to keep risks to the lowest level possible. All subjects will be fully screened prior to enrollment to rule-out the presence of pre-existing or complicating medical conditions. The dietary treatments will satisfy all nutritional requirements, and diets similar to these have been consumed for extended periods (i.e., > 1 year) with safety. Our low-carbohydrate diet, with ~60% of energy from fat, is similar to the Atkins Diet consumed by millions of Americans for months to years, and less restrictive than the classic ketogenic diet, with 80% of energy from fat, recommended for chronic consumption in some clinical settings (e.g., epilepsy management). Some inconvenience may be caused by the frequency of assessment visits and the requirement to consume only study foods. Slight pain and small bruises may be expected from insertion of intravenous lines for blood draws. The subject may feel lightheaded or nauseated with fasting prior to assessment visits and during the oral glucose tolerance test (OGTT). Total radiation exposure from DXA (about 4 millirem per scan) is considered small in comparison to estimated annual background radiation dose for the average person living in the United States (300 millirem). The SPH Web site satisfies HIPAA regulations for data security; therefore the risk of loss of confidentiality is minimized to the fullest-possible extent.

3.B. Adequacy of Protection Against Risks

3.B.1. Recruitment and Informed Consent. Recruitment procedures are described in detail above (**2.D**). A full explanation of the study will be provided to each subject by verbatim reading of the consent form approved by the IRB at BCH. Written informed consent will be obtained from each subject by study personnel, under the supervision of Drs. Ebbeling and Ludwig, who have been appropriately trained in the protection of human subjects. Study personnel will provide as much time as needed for each subject to review protocols and ask questions. The consent process will be performed in a private room at Framingham State University (FSU). Signed consent forms will be filed in a locked cabinet at BCH, and a copy will be given to the subject for future reference.

3.B.2. Protection Against Risk. We will take several steps to protect against possible risk. A physician will be on call 24-hour per day should any problems arise. A member of the BCH research team will be available to assist all subjects with questions or issues around the use and security of the SPH Website. The OGTT and all

blood draws will be performed by a nurse. Adverse events will be reviewed and action will be taken as described below (**3.E**). Abnormal serum electrolytes have rarely occurred on carbohydrate-restricted diets, and we will check serum electrolytes (sodium, potassium, chloride, bicarbonate, calcium, magnesium and phosphate) in any subject assigned to the low-carbohydrate diet who reports potentially-related symptoms (e.g., unexplained muscle aches or fatigue).

3.C. Potential Benefits of the Proposed Research to the Subject and Others

Benefits to the subjects include medical assessment that might identify a treatable pre-existing illness and substantial weight loss resulting from energy-restriction during the run-in phase. The results of DXA scans and blood tests will be sent to the PCP at the end of the study, when all participants have completed all of the study measurements. In addition, the study will demonstrate the effects of different macronutrient diets on metabolism and chronic disease risk factors, a topic of great significance to society in general. These benefits are believed to considerably outweigh the risks and possible inconvenience of participating in this study.

3.D. Importance of the Knowledge to be Gained

The study will provide important information regarding the effects of different macronutrient diets, a topic of great potential significance to society in general. We believe that the benefits (**3.C**) and knowledge to be gained considerably outweigh the risks and inconvenience to study subjects.

3.E. Data and Safety Monitoring (DSM) Plan

The dietary interventions are modeled after popular dietary patterns, and the study does not employ pharmacological agents or notably invasive procedures. Thus, the DSM Plan will focus on enrollment and drop-out rates. However, subjects will be closely monitored by an Endocrinology Fellow and Dr. Ludwig, in close communication with the safety officer (Dr. Agus, see below). Serious adverse events will be promptly reported to the IRB at BCH.

The frequency of data review is summarized in the **Table 7**. The Study Director (Ms. Klein) will be responsible for assembling the data and producing summary reports for data review. Reports will be sent to the PIs (Drs. Ludwig and Ebbeling), biostatistician (Dr. Feldman), and safety officer.

Table 7. Frequency of data review.

Data type	Frequency of review
Subject accrual	Monthly during recruitment
Drop-out rate	Monthly
Data collection	Quarterly
Adverse events	Upon occurrence

Subject Accrual, Drop-out Rate. Subject accrual rate, with careful attention to inclusion/exclusion criteria, will be reviewed monthly during the recruitment phases. This review will also assess targeted/planned enrollment of ethnic-racial groups to ensure diversity. Drop-out rate will be monitored on a monthly basis.

Data collection. A clinical research specialist will review all data collection forms on a monthly basis for completeness, accuracy, and compliance with standardized protocols. A statement reflecting the results of the review will be sent to the IRB as part of the annual continuing review.

Adverse events. All serious, unexpected, and study-related adverse events (hospitalization, serious illness or disability) will be evaluated by the Endocrinology Fellow, under the supervision of Dr. Ludwig, and the safety officer within 24 hours of recognition by study personnel and promptly reported to the IRB at BCH. All non-serious events will be reviewed by the Endocrinology Fellow within 1 week of recognition. If an adverse event occurs, the Endocrinology Fellow, under the direction of Dr. Ludwig, will communicate with the subject's primary care physician to ensure necessary medical or professional intervention. A case report form will be used to monitor adverse events. All events will be reported to the IRB and safety officer as part of the annual continuing review.

Qualifications and Responsibilities of the Safety Officer. The safety officer for this trial will be Michael Agus, MD, an Assistant in Critical Care Medicine, with appointments to the Division of Endocrinology and Medicine Critical Care Program at BCH. Dr. Agus is a practicing physician licensed in the state of Massachusetts, with experience in the fields of critical care medicine, endocrinology, and clinical research. As safety officer, Dr. Agus will be available as needed for consultation regarding serious and non-serious adverse events.

3.F. Trial Registration (ClinicalTrials.gov Requirements)

Drs. Ebbeling and Ludwig will register the proposed study in ClinicalTrials.gov prior to screening or enrolling any subjects.

3.G. Inclusion of Women and Minorities

We will enroll both males and females. The distribution of ethnic-racial groups will reflect the diversity of the community at FSU. We will reach out to students, faculty, and staff using a variety of venues (**2.D**).

4. BIBLIOGRAPHY AND REFERENCES CITED

1. Ebbeling CB, Swain JF, Feldman HA, et al. Effects of dietary composition on energy expenditure during weight-loss maintenance. *JAMA.* 2012;307:2627-2634. PMID: 22735432.
2. Institute of Medicine. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids. Washington (DC): The National Academies Press; 2002.
3. Halton TL, Hu FB. The effects of high protein diets on thermogenesis, satiety and weight loss: a critical review. *J Am Coll Nutr.* 2004;23:373-385. PMID: 15466943.
4. Mifflin MD, St Jeor ST, Hill LA, Scott BJ, Daugherty SA, Koh YO. A new predictive equation for resting energy expenditure in healthy individuals. *Am J Clin Nutr.* 1990;51:241-247. PMID: 2305711.
5. Frankenfield D, Roth-Yousey L, Compher C. Comparison of predictive equations for resting metabolic rate in healthy nonobese and obese adults: a systematic review. *J Am Diet Assoc.* 2005;105:775-789. PMID: 15883556.
6. Pereira MA, FitzerGerald SJ, Gregg EW, et al. A collection of Physical Activity Questionnaires for health-related research. *Med Sci Sports Exerc.* 1997;29:S1-205. PMID: 9243481.
7. Katan MB, Ludwig DS. Extra calories cause weight gain--but how much? *JAMA.* 2010;303:65-66. PMID: 20051571.
8. Hall KD, Heymsfield SB, Kemnitz JW, Klein S, Schoeller DA, Speakman JR. Energy balance and its components: implications for body weight regulation. *Am J Clin Nutr.* 2012;95:989-994. PMID: 22434603.
9. U.S. Department of Agriculture and U.S. Department of Health and Human Services. Dietary Guidelines for Americans, 2010. 7th Edition, Washington, DC: U.S. Government Printing Office, December 2010.
10. York LW, Puthalapattu S, Wu GY. Nonalcoholic fatty liver disease and low-carbohydrate diets. *Annu Rev Nutr.* 2009;29:365-379. PMID: 19575599.
11. Feinman RD, Fine EJ. Thermodynamics and metabolic advantage of weight loss diets. *Metab Syndr Relat Disord.* 2003;1:209-219. PMID: 18370664.
12. Ludwig DS. The glycemic index: physiological mechanisms relating to obesity, diabetes, and cardiovascular disease. *JAMA.* 2002;287:2414-2423. PMID: 11988062.
13. Feinman RD, Fine EJ. Nonequilibrium thermodynamics and energy efficiency in weight loss diets. *Theor Biol Med Model.* 2007;4:27. PMID: 17663761.
14. Scribner KB, Pawlak DB, Aubin CM, Majzoub JA, Ludwig DS. Long-term effects of dietary glycemic index on adiposity, energy metabolism, and physical activity in mice. *Am J Physiol Endocrinol Metab.* 2008;295:E1126-1131. PMID: 18780772.
15. Baldwin KM, Joanisse DR, Haddad F, et al. Effects of weight loss and leptin on skeletal muscle in human subjects. *Am J Physiol Regul Integr Comp Physiol.* 2011;301:R1259-1266. PMID: 21917907.
16. Goldsmith R, Joanisse DR, Gallagher D, et al. Effects of experimental weight perturbation on skeletal muscle work efficiency, fuel utilization, and biochemistry in human subjects. *Am J Physiol Regul Integr Comp Physiol.* 2010;298:R79-88. PMID: 19889869.
17. Rosenbaum M, Goldsmith R, Bloomfield D, et al. Low-dose leptin reverses skeletal muscle, autonomic, and neuroendocrine adaptations to maintenance of reduced weight. *J Clin Invest.* 2005;115:3579-3586. PMID: 16322796.
18. Rosenbaum M, Vandenborne K, Goldsmith R, et al. Effects of experimental weight perturbation on skeletal muscle work efficiency in human subjects. *Am J Physiol Regul Integr Comp Physiol.* 2003;285:R183-192. PMID: 12609816.
19. Wong WW, Lee LS, Klein PD. Deuterium and oxygen-18 measurements on microliter samples of urine, plasma, saliva, and human milk. *Am J Clin Nutr.* 1987;45:905-913. PMID: 3578092.
20. Ravussin E, Harper IT, Rising R, Bogardus C. Energy expenditure by doubly labeled water: validation in lean and obese subjects. *Am J Physiol.* 1991;261:E402-409. PMID: 1909495.
21. Black AE, Prentice AM, Coward WA. Use of food quotients to predict respiratory quotients for the doubly-labelled water method of measuring energy expenditure. *Hum Nutr Clin Nutr.* 1986;40:381-391. PMID: 3771290.
22. Weir JB. New methods for calculating metabolic rate with special reference to protein metabolism. *J Physiol.* 1949;109:1-9. PMID: 15394301.
23. Martin CK, Das SK, Lindblad L, et al. Effect of calorie restriction on the free-living physical activity levels of nonobese humans: results of three randomized trials. *J Appl Physiol.* 2011;110:956-963. PMID: 21292847.

24. Metzger JS, Catellier DJ, Evenson KR, Treuth MS, Rosamond WD, Siega-Riz AM. Patterns of objectively measured physical activity in the United States. *Med Sci Sports Exerc.* 2008;40:630-638. PMID: 18317384.
25. Bouchard C, Tremblay A, Leblanc C, Lortie G, Savard R, Theriault G. A method to assess energy expenditure in children and adults. *Am J Clin Nutr.* 1983;37:461-467. PMID: 6829488.
26. Keadle SK, Shiroma EJ, Freedson PS, Lee IM. Impact of accelerometer data processing decisions on the sample size, wear time and physical activity level of a large cohort study. *BMC Public Health.* 2014;14:1210. PMID: 25421941.
27. Abdul-Ghani MA, Matsuda M, Balas B, DeFronzo RA. Muscle and liver insulin resistance indexes derived from the oral glucose tolerance test. *Diabetes Care.* 2007;30:89-94. PMID: 17192339.
28. Hall KD, Chen KY, Guo J, et al. Energy expenditure and body composition changes after an isocaloric ketogenic diet in overweight and obese men. *Am J Clin Nutr.* 2016;104:324-333. PMID: 27385608.
29. American Diabetes Association. Glycemic Targets. *Diabetes Care.* 2017;40:S48-S56. PMID: 27979893.
30. Juraschek SP, Miller ER, 3rd, Appel LJ, Christenson RH, Sacks FM, Selvin E. Effects of dietary carbohydrate on 1,5-anhydroglucitol in a population without diabetes: results from the OmniCarb trial. *Diabet Med.* 2017. PMID: 28574153.
31. Miller M, Ginsberg HN, Schaefer EJ. Relative atherogenicity and predictive value of non-high-density lipoprotein cholesterol for coronary heart disease. *Am J Cardiol.* 2008;101:1003-1008. PMID: 18359322.
32. Di Angelantonio E, Sarwar N, Perry P, et al. Major lipids, apolipoproteins, and risk of vascular disease. *JAMA.* 2009;302:1993-2000. PMID: 19903920.
33. Pickering TG, Hall JE, Appel LJ, et al. Recommendations for blood pressure measurement in humans and experimental animals: Part 1: blood pressure measurement in humans: a statement for professionals from the Subcommittee of Professional and Public Education of the American Heart Association Council on High Blood Pressure Research. *Hypertension.* 2005;45:142-161. PMID: 15611362.
34. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. *Nature.* 2006;444:1022-1023. PMID: 17183309.
35. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature.* 2006;444:1027-1031. PMID: 17183312.
36. Ley RE, Backhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. Obesity alters gut microbial ecology. *Proc Natl Acad Sci U S A.* 2005;102:11070-11075. PMID: 16033867.
37. Christofk HR, Vander Heiden MG, Harris MH, et al. The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth. *Nature.* 2008;452:230-233. PMID: 18337823.
38. Lewis GD, Wei R, Liu E, et al. Metabolite profiling of blood from individuals undergoing planned myocardial infarction reveals early markers of myocardial injury. *J Clin Invest.* 2008;118:3503-3512. PMID: 18769631.
39. Sabatine MS, Liu E, Morrow DA, et al. Metabolomic identification of novel biomarkers of myocardial ischemia. *Circulation.* 2005;112:3868-3875. PMID: 16344383.
40. Rukh G, Ericson U, Andersson-Assarsson J, Orho-Melander M, Sonestedt E. Dietary starch intake modifies the relation between copy number variation in the salivary amylase gene and BMI. *Am J Clin Nutr.* 2017. PMID: 28539377.
41. Rousseuw PJ, Leroy AM. *Robust Regression and Outlier Detection*. New York, NY: John Wiley & Sons; 1987.
42. Bauer P. Multiple testing in clinical trials. *Stat Med.* 1991;10:871-889; discussion 889-890. PMID: 1831562.