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A randomized study of dietary composition during weight-loss maintenance: Rationale, study design, intervention, and assessment

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ABSTRACT

Background: While many people with overweight or obesity can lose weight temporarily, most have difficulty maintaining weight loss over the long term. Studies of dietary composition typically focus on weight loss, rather than weight-loss maintenance, and rely on nutrition education and dietary counseling, rather than controlled feeding protocols. Variation in initial weight loss and insufficient differentiation among treatments confound interpretation of results and compromise conclusions regarding the weight-independent effects of dietary composition. The aim of the present study was to evaluate three test diets differing in carbohydrate-to-fat ratio during weight-loss maintenance.

Design and dietary interventions: Following weight loss corresponding to 12 ± 2% of baseline body weight on a standard run-in diet, 164 participants aged 18 to 65 years were randomly assigned to one of three test diets for weight-loss maintenance through 20 weeks (test phase). We fed them high-carbohydrate (60% of energy from carbohydrate, 20% fat), moderate-carbohydrate (40% carbohydrate, 40% fat), and low-carbohydrate (20% carbohydrate, 60% fat) diets, controlled for protein content (20% of energy). During a 2-week *ad libitum* feeding phase following the test phase, we assessed the effect of the test diets on body weight.

Outcomes: The primary outcome was total energy expenditure, assessed by doubly-labeled water methodology. Secondary outcomes included resting energy expenditure and physical activity, chronic disease risk factors, and variables to inform an understanding of physiological mechanisms by which dietary carbohydrate-to-fat ratio might influence metabolism. Weight change during the *ad libitum* feeding phase was conceptualized as a proxy measure of hunger.

1. Introduction

In the US, about 50% of adults with overweight or obesity are trying to lose weight, often by following energy-restricted diets [1]. While many experience some initial success, most have difficulty maintaining clinically significant weight loss over the long term [2,3]. A common explanation for weight regain relates to behavior, in that motivation to adhere to a dietary prescription typically diminishes with time. Indeed, behavioral intervention trials indicate a direct association between adherence and weight loss, regardless of dietary treatment [4–6]. An alternative explanation relates to biology, in that weight loss elicits adaptations that promote weight regain, including a decline in energy expenditure and an increase in hunger [7,8]. Whether macronutrient composition influences these adaptations remains a subject of debate [9,10].

For most of the last half century, dietary fat restriction was the primary focus of clinical practice guidelines and public health

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recommendations for weight loss and chronic disease risk management. Alternative strategies that focus on modifying carbohydrate amount and paying attention to carbohydrate source (choosing foods with a low glycemic index) have gained attention over the last two decades [11,12]. Lowering dietary glycemic load [13–15] using these strategies may produce beneficial effects on metabolism and hunger compared to conventional low-fat (high-carbohydrate) diets. Acutely, beneficial effects may include hormonal changes that increase availability of metabolic fuels in the late postprandial period (attenuated insulin levels, for example), and thereby decrease hunger and voluntary food intake [16,17]. Chronically, lowering glycemic load appears to lessen the fall in resting and total energy expenditure (TEE) that predictably occurs during weight loss [18–20], although the mechanisms for this effect remain speculative. While some nutrition experts advocate decreasing carbohydrate to reduce dietary glycemic load [21], others contend that substantial benefit can be achieved with a moderate decrease in carbohydrate intake so long as the carbohydrate source has a low glycemic index [22]. Still others argue that clinical care for patients with obesity and general public health messages should remain focused on lowering energy intake, pointing out that fat is an easily over-consumed and energy-dense macronutrient [23].

The primary aim of this study was to evaluate the effect of three diets varying widely in carbohydrate-to-fat ratio (high-carbohydrate, moderate-carbohydrate, low-carbohydrate) on energy expenditure during weight-loss maintenance, using a controlled feeding protocol. The primary outcome was TEE, assessed by doubly-labeled water methodology. Outcomes for additional specific aims are presented in Section 5 of this protocol paper.

2. Study design and infrastructure

The study was a randomized controlled trial (RCT) comprising run-in, test, and *ad libitum* feeding phases (Fig. 1). The purpose of the run-in phase was to obtain baseline measurements and restrict energy intake to achieve a 12 ± 2% decrease in body weight. Participants who were unable to achieve this level of weight loss were dismissed from the study prior to randomization. The purpose of the test phase was to compare the metabolic effects of high-, moderate-, and low- (HI-, MOD-, and LO-) carbohydrate diets during weight-loss maintenance and explore physiological mechanisms underlying these effects. We assessed study outcomes at baseline (BSL), post-weight loss (PWL, time 0), and midpoint (MID, weeks 8–10) and end (END, weeks 18–20) of the test phase. The purpose of the *ad libitum* feeding phase was to evaluate the effects of the test diets on change in body weight, as a proxy measure of hunger.

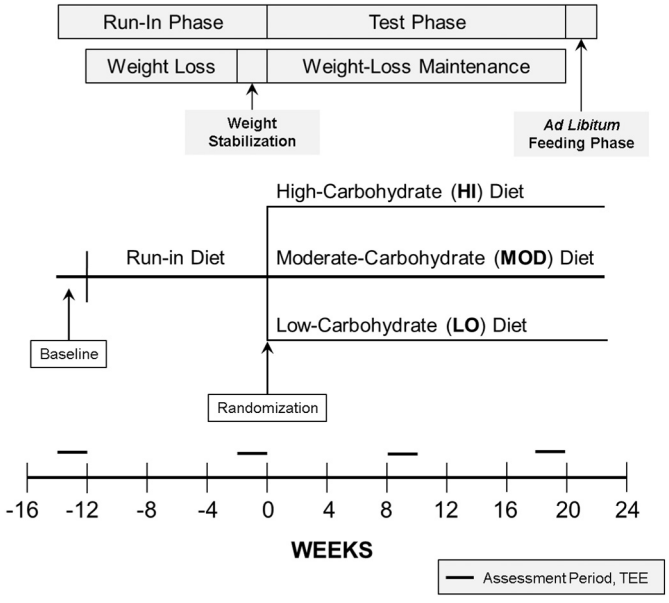

Fig. 1. Study design.

Researchers from Boston Children's Hospital and Harvard Medical School partnered with faculty and staff at Framingham State University (FSU), Sodexo, and Assabet Valley Regional Technical High School (AV). The study was known as the Framingham State Food Study, or (FS)2. We decided against a hospital-based feeding study, recognizing challenges associated with recruiting participants willing and able to travel to a research center in the city on a daily basis, limited space in research kitchens, inability to provide freshly prepared meals three times per day, and cost. Rather, we formed a novel collaboration with FSU, located about 20 miles west of Boston, and Sodexo, the food service contractor at FSU. We had access to ample on-site facilities including large and well-equipped commercial kitchens, a dining area where participants ate supervised meals, and on-campus space that was transformed into a research center for conducting assessments. We established a satellite feeding site at AV, located about 10 miles north of Framingham, to expand our catchment area and meet recruitment goals. Collaborating with the school nutrition program at AV, we had access to a commercial kitchen and dining space. We hired a contractor to build kiosks for preparing and serving study foods adjacent to dining areas in both locations (FSU and AV).

All study protocols were approved by the Institutional Review Board at Boston Children's Hospital. We obtained written informed consent prior to baseline assessments. The stipend for full study participation was $3280, and study meals and snacks were valued at $3220, for total compensation of $6500. The study was conducted between August 2014 and May 2017.

The leadership team included two principal investigators, two study directors (one from BCH to oversee all day-to-day operations, another from FSU to serve as a liaison with BCH regarding study infrastructure), an associate study director, a nutrition research manager, a Sodexo director of dining services, a biostatistician, and a data and quality manager. This team stayed in close communication with executive staff at FSU (e.g., vice president of academic affairs and provost, executive vice president) and AV (*e.g.,* superintendent-director, director of business operations). Study staff included employees with varying levels of effort from BCH (study physician, support dietitian, diet technician, statistical programmer, research assistants and study coordinators), FSU (financial coordinator, faculty and staff who volunteered on study work groups), Sodexo (program manager/lead dietitian, staff dietitian, chefs), and AV (food service director, head chef, sous chef). We also hired *per diem* nurses, radiology technologists, and research assistants to help with study visits, meal preparation (*e.g.,* weighing and serving foods), and data entry. Most of the research assistants were students at FSU.

3. Participants

3.1. Eligibility criteria

We enrolled faculty, staff, students, and residents of communities surrounding FSU and AV who met the eligibility criteria listed in Table 1. We specified BMI ≥ 25 kg/m^2, which corresponds to the conventional definition of overweight, as an inclusion criterion but did not enroll anybody who weighed > 350 lbs. (159 kg), to avoid exceeding upper weight limits for some assessment equipment. We excluded anyone who reported recent and substantial weight change or behaviors that could confound study outcomes (adherence to a special diet or vigorous-intensity physical activity regimen, use of medications or dietary supplements, smoking, excessive alcohol consumption). We excluded those who had abnormal results from screening laboratory tests, indicative of unrecognized illness. We did not enroll individuals who had vacation plans which precluded shipment of food (*e.g.,*

Table 1
Eligibility criteria.

Inclusion 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
- 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 participants only)
- Provision of written permission to conduct Criminal Offender Record Information (CORI) and Sex Offender Registry Information (SORI) checks (community-based participants only)

Exclusion criteria
- 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 or intensive training for events such as a marathon or triathlon)
- 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
- 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

international travel). We specified additional exclusion criteria for females to diminish confounding from changes associated with birth control medication, pregnancy, or lactation.

3.2. Recruitment and screening

We contracted with marketing (Wing Press, Inc., Framingham, MA), video production (MediaBoss, Framingham, MA), and advertising (Buyer Advertising, Inc., Newton, MA) professionals to develop and implement a recruitment campaign. We utilized electronic (study website with video overview of the study, answers to frequently asked questions, and sample menus; e-mail messages; Facebook and online newspaper advertisements) and print (brochures, direct mailings, newspaper advertisements) media. We also held face-to-face informational sessions on the FSU campus. For each of three cohorts, recruitment occurred during the spring semester prior to the respective academic year (August–May) of study participation. We enrolled participants in waves for each cohort.

When an individual responded to an advertisement for the study, we implemented 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 provided an overview of the study and asked preliminary screening questions. If the individual was provisionally eligible based on the telephone conversation, we invited him/her to an informational visit during which we explained the study protocol as outlined in the consent form and further assessed provisional eligibility. If the individual remained provisionally eligible, we requested medical clearance in writing from his/her primary care provider (with written permission from the provisionally eligible participant to make this request). We requested academic and social clearance in writing from the FSU Office of Enrollment and Student Development for all provisionally eligible students. As a safety precaution, we obtained permission to do Criminal Offender Record Information (CORI) and Sex Offender Registry Information (SORI) checks for provisionally eligible individuals who were not affiliated with FSU or AV. At the screening visit, we obtained a blood sample for analysis of hemoglobin A1c (HbA1c), thyroid stimulating hormone (TSH), hematocrit, blood urea nitrogen (BUN), creatinine, and alanine aminotransferase (ALT). At the informed consent visit, we reconfirmed all eligibility criteria, obtained written informed consent, and invited participants to "opt in" for certain data collection and ancillary studies (Section 7). Of 1685 individuals who began the screening process, 234 were eligible for the study and provided informed consent prior to baseline data collection, as shown in the flow diagram (Fig. 2).

3.3. Randomization

We randomized 164 participants who successfully completed the run-in phase, using a blocked randomization design to ensure close balance among the three diet arms at every point in the study. The randomization was stratified by sex, ethnicity-race (non-Hispanic white, other), age (18–39.9 years, 40.0–65.9 years), BMI (overweight: 25.0–29.9 kg/m^2, obese: ≥ 30.0 kg/m^2), and feeding site (FSU, AV) to ensure balance at the completion of enrollment within every subcategory, regardless of size. Enrollment logs, one for each stratum, were prepared with a numerical sequence of identifiers. Diet assignment lists, identical to the enrollment logs except with the addition of a randomly chosen diet, were prepared using specialized software. The diet assignments were randomly permuted within blocks of 3, 6, and 9, and the blocks themselves were randomly permuted. Each upcoming assignment was thus unpredictable, preventing any deliberate or inadvertent bias on the part of staff conducting enrollment. The assignment list was maintained in a secure electronic folder accessible only to staff responsible for randomization. All staff members assessing study outcomes and conducting analyses of biospecimens were masked to diet arm assignment.

4. Dietary interventions

Targets for energy content and macronutrient composition of the run-in and test diets are summarized in Table 2. For all diets, energy was distributed 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 and snack reflected the composition of each respective diet. We instructed participants to consume their meals at regularly scheduled times and not to skip meals. We advised participants to have no more than three servings per day of each of the following non-caloric items: beverages containing artificial sweeteners, caffeinated beverages, packets of artificial sweeteners, and gum or mints containing artificial sweeteners. We asked participants not to consume alcoholic beverages. To ensure micronutrient adequacy and minimize the influence of micronutrient differences among test diets, we gave each participant a daily multi-vitamin and mineral supplement (Centrum Adult, Pfizer Consumer Healthcare, Madison, NJ) with breakfast throughout all phases of the study. Energy and nutrient composition of the diets were calculated using Food Processor Nutrition Analysis Software (ESHA Research Inc., Salem, OR).

4.1. Run-in phase

The targeted macronutrient composition of the run-in diet was consistent with recommendations specified by the Institute of Medicine [24], with protein intake at the upper end of an acceptable range to enhance satiety during weight loss [25]. We estimated individual energy needs as the arithmetic product of resting requirements estimated using a regression eq. [26,27] and a physical activity factor of 1.5. To avoid overestimation of energy needs, we used measured body weight

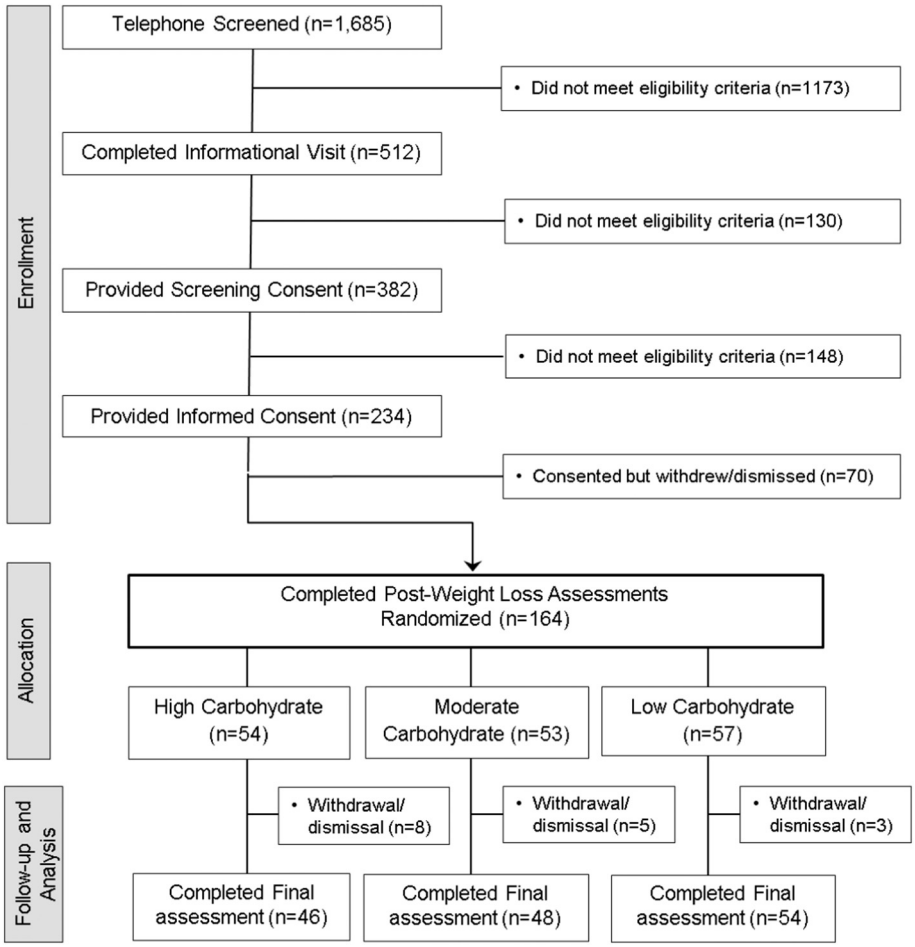

Fig. 2. Flow diagram.

adjusted for ideal body weight (IBW) as the weight variable (([measured weight – IBW] × 0.25) + IBW) in the regression equation, based on methods used in a previous study [18]. To estimate IBW, we determined the sex- and height-specific midpoints of weight ranges for medium frames, using Metropolitan Life Insurance Company Height-Weight Tables. Energy intake was restricted to 60% of estimated needs to achieve a target weight loss equating to 12 ± 2% of baseline body weight, with a minimum of 1200 kcal/d. We asked participants to weigh themselves daily using Wi-Fi scales (Withings Inc., Cambridge, MA), from which we could retrieve data (as explained below), and the amount of food provided was increased or decreased as necessary to reach the target weight loss over 9 to 10 weeks. Previous studies have indicated metabolic adaptation characterized by declines in resting energy expenditure (REE) and TEE with weight loss corresponding to 10% of baseline weight [7,18,20,28,29]. With 20% weight loss, REE may not decline further [7,28], but TEE may continue to decline at a slower rate due to shifts in non-REE [28]. We selected the 12 ± 2% weight loss target to ensure significant metabolic adaptation without

Table 2
Targets for dietary energy and macronutrient composition.

Dietary variable		Run-in phase	Test phase		
		Energy restriction	High (HI)	Moderate (MOD)	Low (LO)
			Carbohydrate diet	Carbohydrate diet	Carbohydrate diet
Energy[a]	(% of weight maintenance needs)	60	100	100	100
Carbohydrate[b]	(% of total energy)	45	60	40	20
Fat[b]	(% of total energy)	30	20	40	60
Protein[b]	(% of total energy)	25	20	20	20
Sodium[c]	(mg)	–	3000	3000	3000
Added sugar[c]	(% of total carbohydrate)	–	15	15	15
	(% of total energy)	–	9	6	3
Saturated fat[c,d]	(% of total fat)	–	35	35	35
	(% of total energy)	–	7	14	21
Fiber[c]	(g)	–	35	30	25

[a] Distributed throughout the day: 22.5% for breakfast, 32.5% for lunch, 32.5% for dinner, and 12.5% for an evening snack.
[b] Target for each meal.
[c] Target for daily average over each week.
[d] Remainder of total fat distributed between mono- and polyunsaturated fat.

imposing greater burden (on participants and the study) of a larger weight loss target.

4.2. Test phase

The three test diets varied in carbohydrate and fat but were controlled for protein (Table 2). We used many of the same foods, in differing amounts, across diets and systematically replaced foods when necessary to achieve the specified macronutrient targets. As such, the diets reflected gradients in amounts of foods rich in carbohydrate and fat (Table 3). The high-carbohydrate diet contained 60% of energy from carbohydrate and 20% from fat, consistent with contemporary public health recommendations that emphasize sources of carbohydrate such as whole grains, vegetables, fruits, legumes, and low-fat dairy products [30]. The moderate-carbohydrate diet contained 40% of energy from carbohydrate and 40% from fat. Relative to the high-carbohydrate diet, these targets were achieved by decreasing the quantity of grains and 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. The low-carbohydrate diet contained 20% of energy from carbohydrate and 60% from fat. Relative to the moderate-carbohydrate diet, these targets were achieved by eliminating all grains, removing some fruits, adding more foods containing fat, further decreasing amounts of legumes when necessary, and increasing some higher fat dairy products. The quantities of non-starchy vegetables were approximately the same across diets. There was no high-fructose corn syrup in any of the diets.

We stabilized body weight at the end of the run-in phase, prior to the start of the test phase. The energy level for weight stabilization was estimated based on recent rate of weight loss for each participant (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 [31,32], this calculation is useful for estimating energy needs over the short term. During the test phase, we monitored body weight and adjusted energy intake to achieve weight stability, defined as weight change not exceeding ± 2 kg. We obtained weight measurements from Wi-Fi scales every day and regressed weight (g) on time (days). A slope ≥ 15 g per day over 14 days indicated the need to adjust energy intake to achieve stability. Considering deviation from the PWL anchor weight and slope of the regression line, we made adjustments in energy intake when necessary but not more frequently than every 2 weeks.

4.3. Ad Libitum feeding phase

We instructed participants regarding 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. Participants continued to consume test diets with specified proportions of macronutrients, according to the

Table 3
Example menu (per 2000 kcal).

HI carbohydrate menu	MOD carbohydrate menu	LO carbohydrate menu
Breakfast		
Egg white, 115 g	Egg white, 115 g	Egg white, 115 g
Canola oil, 3 g	Canola oil, 6 g	Canola oil, 6 g
	–	Butter (salted), 7 g
	Salt, 0.4 g	Salt, 0.5 g
	Cheddar cheese (shredded), 9 g	Cheddar cheese (shredded), 22 g
Ranchero sauce, 15 g	Ranchero sauce, 20 g	Ranchero sauce, 20 g
Grilled kielbasa, 15 g	Grilled kielbasa, 30 g	Grilled kielbasa, 30 g
Multigrain english muffin, 62 g	Multigrain english muffin, 29 g	–
Strawberry fruit spread, 20 g	Strawberry fruit spread,10 g	–
100% Orange juice, 165 g	100% Orange juice, 138 g	100% Orange juice, 118 g
Lunch		
Vegetarian sloppy joe, 75 g	Vegetarian sloppy joe, 70 g	Vegetarian sloppy joe, 80 g
Grapes, 285 g	Grapes, 167 g	Grapes, 100 g
Parmesan crisps, 26 g	Parmesan crisps, 30 g	Parmesan crisps, 42 g
–	–	Bibb leaf lettuce, 65 g
–	–	Green bell pepper, 45 g
–	–	Olive oil, 6 g
–	–	Parmesan cheese, 11 g
–	Macademia nuts, 20 g	Macademia nuts, 26 g
Whole wheat sourdough bread, 74 g	Whole wheat sourdough bread, 45 g	–
Greek yogurt (vanilla, nonfat), 110 g	Greek yogurt (vanilla, nonfat), 100 g	–
Dinner		
Leaf spinach, 100 g	Leaf spinach, 100 g	Leaf spinach, 100 g
Herbed grilled salmon, 55 g	Herbed grilled salmon, 90 g	Herbed grilled salmon, 80 g
Orange sections, 180 g	Orange sections, 165 g	Orange sections, 95 g
–	Dry roasted peanuts, 8 g	Dry roasted peanuts, 33 g
–	Cheddar cheese, 10 g	Cheddar cheese, 15 g
Long grain and wild rice, 115 g	Long grain and wild rice, 100 g	–
Whole wheat bread, 27 g	Whole wheat bread, 22 g	–
Greek yogurt (vanilla, nonfat), 160 g	–	–
Dried cranberries, 20 g	–	–
Milk, skim, 80 g	Milk, 2%, 120 g	Milk, 3.25%, 180 g
–	–	Salt, 0.3 g
Snack		
Toasted lentil salad, 35 g	Toasted lentil salad, 35 g	Toasted lentil salad, 35 g
–	–	Olive oil, 5 g
Macaroni, 31 g	–	–
Semi-soft cheese, 21 g	Semi-soft cheese, 34 g	Semi-soft cheese, 36 g
Blueberries, 170 g	Blueberries, 145 g	Blueberries, 55 g

respective diet arms to which they were randomly assigned. We provided the same amount of food relative to that provided for weight stabilization at the end of the test phase and instructed participants 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."

4.4. Support and monitoring

We asked participants to eat at least one meal per day, Monday through Friday, in the dining area at FSU or AV. These meals were supervised by dietitians and other support staff (diet technician, FSU upperclassman trained in counseling) who provided encouragement to study participants, answered their questions, and thereby fostered a positive participant experience. Dietitians facilitated monthly group sessions to reinforce instructions, offer support, and address topics of interest (*e.g.*, managing special occasions, nutritional overview of the test diets, self-monitoring and behavior modification for weight management).

A study-specific online portal was developed for tracking and communication (SetPoint Health, Needham, MA). We tracked body weight, meal and snack consumption, dietary compliance, and information obtained from questionnaires. We asked participants to weigh themselves daily in the privacy of their homes or dormitory rooms using calibrated Wi-Fi scales (Withings Inc., Cambridge, MA). Each scale sent the daily weight data to the participant's mobile device (smartphone or tablet) *via* Bluetooth. The weight data were synced to the Withings cloud and then to the portal *via* the Withings Application Program Interface (API). For supervised meals, research assistants entered the weights of leftovers into the portal; for unsupervised (take-out) meals, we asked participants to record the proportion of each provided food or beverage item consumed using a form on the portal that was pre-populated with a daily menu that included all foods and beverages in every meal and snack. We also asked participants to complete questionnaires on the portal regarding consumption of any non-study foods and beverages (including non-caloric beverages and non-nutritive sweeteners), abnormal symptoms, medications (prescription and over-the-counter), and physical activity. We used the portal to remind participants of their appointment times and to convey messages from principal investigators and announcements from study staff.

5. Study outcomes

Table 4 provides an overview of measurements, including the time point at which we assessed each outcome. We assessed outcomes under free-living conditions and during visits to a research center at FSU. Blood samples were collected following a 12-hour overnight fast, except where indicated otherwise. Biospecimens were frozen in a −80°C freezer at FSU and then transferred to the Biobank Core Laboratory at BCH. Biochemical analyses, except where indicted otherwise, were done in the Clinical and Epidemiological Research Laboratory (CERLab) in the Department of Laboratory Medicine at BCH at the end of the study. An overview of laboratory methods used in the CERLab is available from the corresponding author.

5.1. Energy expenditure and physical activity

Specific Aim #1 was to evaluate the effects of the three diets on energy expenditure during weight-loss maintenance. Macronutrient composition could affect energy expenditure directly because metabolic pathways vary in energetic efficiency or indirectly through hormonal responses that regulate metabolic pathways [16,33]. 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 [34]. We measured TEE under free-living conditions during 14-day periods (within 21-day windows). We assessed REE and physical activity within the same assessment windows.

5.1.1. Total energy expenditure

We assessed TEE by doubly-labeled water (DLW) methodology. Following oral administration of water labeled with stable isotopes of hydrogen (^{2}H, deuterium) and oxygen-18 (^{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 urinary disappearance rates of ^{18}O and ^{2}H provides a measure of carbon dioxide production (rCO_2), used to estimate TEE [35].

The dose of DLW was a mixed cocktail containing 0.086 g of ^2H_2O (99.98 atom % ^{2}H) and 1.38 g $H_2{}^{18}O$ (10 atom % ^{18}O) per kg body weight (Sigma Aldrich, St Louis, MO). Each dose was weighed to the nearest 0.01 g into a dosing bottle. Participants consumed the DLW and then 20 mL of filtered water, dispensed into the dosing bottle to capture any DLW adhering to the sides of the bottle and thus ensure complete consumption of the weighed DLW. Spot urine samples were collected on the day before and immediately prior to each dose and then at regular intervals over 14 days after each dose, for a total of nine samples (two pre-dose, seven post-dose), never from the first morning void. Isotopic enrichment of each urine sample was measured in duplicate in the USDA/ARS Children's Nutrition Research Center's Gas-Isotope-Ratio Mass Spectrometry (GIRMS) Laboratory at Baylor College of Medicine (Houston, TX) [36–38]. Enrichment values were converted to atom percent (AP) [39] following normalization to the Vienna-Standard Mean Ocean Water (V-SMOW)/Standard Light Antarctic Precipitation (SLAP) scale according to the International Atomic Energy Agency [40]. The AP values were then normalized for the amount and enrichment of the dose [41]:

$$AP_{normalized}(t) = ((AP(t) - AP(0)/(AP_{dose} - AP_{water})) \times (a\ MW_{H2O}/WA)$$

where t is time after dosing; AP(0) is mean enrichment of two pre-dosing samples; AP_{dose} and AP_{water} are enrichments of dose and water, respectively; a is gram amount of dose diluted for analysis; MW_{H2O} is molecular weight of water (18.02 g/mol); W is gram amount of tap water used to dilute the dose; and A is gram amount of dose administered to the participant.

Diverging monoexponential disappearance curves were fitted simultaneously to the normalized data using nonlinear least-squares regression analysis [42]:

$$AP_{normalized,deuterium}(t) = (1/N_H) \times \exp.(-k_H t)$$

$$AP_{normalized,oxygen-18}(t) = (1/N_O) \times \exp.(-k_O t)$$

where N_H, k_H and N_O, k_O are isotope dilution spaces and fractional disappearance rates for deuterium and ^{18}O, respectively. From these parameters, we derived the ratio of dilution spaces, N_H/N_O, and the production rate of carbon dioxide:

$$rCO_2 = 0.4554 \times (k_O N_O - k_H N_H)$$

using a correction factor of 0.4554 for isotope fractionation [43,44]. TEE was calculated from rCO_2 using the equation of Ravussin et al. [45]:

$$TEE\ (kcal/d) = rCO_2 \times 22.4 \times (1.2321 + 3.815/FQ)$$

with the food quotient (FQ) as an estimate of respiratory quotient. Using the methods of Black et al. [46], we assumed FQ to be 0.85 at baseline and estimated FQ from dietary composition at PWL (0.87) and MID and END (HI, 0.90; MOD, 0.85; LO, 0.79).

For quality control, we obtained a spot urine sample one week prior to dosing at PWL, MID, and END to confirm negligible isotopic decay related to carry over effects of the previous dose. We used the jackknife

Table 4
Study outcomes, covariates, and effect modifiers.

	Run-in phase		Test phase		*Ad libitum*
	BSL	PWL	MID	END	Feeding phase
	− 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 Specific Aim (SA) of the parent study					
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) [a]	X	X	X	X	
Urine C-peptide	X	X		X	
Glycemic control (HbA1c, 1,5-anhydroglucitol)	X	X	X	X	
Lipid profiles (TC, HDL-C, LDL-C, non-HDL-C, TG)	X	X	X	X	
Coagulopathy (PAI-1, Fibrinogen)	X	X	X	X	
Inflammatory mediators (hsCRP, IL-6)	X	X	X	X	
Blood pressure	X	X	X	X	
SA#3					
Skeletal muscle work efficiency (cycle ergometry)	X	X		X	
Body composition (multi-component model)[a]	X	X		X	
Insulin sensitivity and secretion (OGTT)[a]	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-BP3)	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	
Metabolomics profile (saved samples)	X	X	X	X	
Gut microbiome (saved samples)[b]	X	X		X	
SA#4					
Body weight					X
Study outcomes for ancillary studies					
Lipoprotein particle subfraction distribution[c]	X	X		X	
Sleep[c]	X	X	X	X	
Psychological health[b]	X	X		X	
Cognition[b]	X	X		X	
Weight bias[b]	X	X		X	
Postprandial metabolic fuels[b]			10–15 weeks		
Adipocyte biology[b]		X	10–15 weeks		
Brain activity[b]				14–20 weeks	
Covariates and effect modifiers					
Sex	X				
Ethnicity	X				
Race	X				
Age	X				
Body weight, BMI[a]	X	X	X	X	
Body composition (multi-component model)[a]	X	X		X	
Insulin sensitivity and secretion (OGTT)[a]	X	X	X	X	
Obesity-related genes[b]	X				
Palatability of test diet				X	

Abbreviations. TEE, total energy expenditure; REE, resting energy expenditure; OGTT, oral glucose tolerance test; HbA1c, 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; hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; T4, thyroxine; rT3, reverse triiodothyronine; TSH, thyroid stimulating hormone; IGF-1, insulin-like growth factor-1; IGH-BP3, insulin-like growth factor-binding protein 3; LH, luteinizing hormone; FSH, follicle stimulating hormone; E2, estradiol; TST, testosterone; BMI, body mass index.

[a] Assessed as outcomes and covariates.

[b] Assessed only for participants who "opted in".

[c] Related to SA#2 of the parent study.

procedure to detect and correct bias of estimated TEE due to outliers [47]. We specified pool size ratio (N_H/N_O) outside the range of 1.015–1.060 as an initial screen for detection of analytical problems [41].

5.1.2. Resting energy expenditure

We measured REE by indirect calorimetry using respiratory gas exchange methodology with a ventilated hood system (TrueOne 2400, Parvo Medics, Sandy, UT) after a 12-hour overnight fast. The instrumentation was calibrated according to the manufacturer's specifications. Room temperature was maintained at a constant level, and lighting and noise were minimized to limit variability in measurements. Participants were reclining and awake during measurements. Oxygen consumption and carbon dioxide production were measured for 30 min, and REE was calculated by the Weir eq. [48] using minute-by-minute data averaged over the last 20 min. We measured REE on two separate mornings at each assessment time point and, if the two measurements were not within 10%, obtained a third measurement on another morning within the assessment window. We used the mean of the two closest measurements as the best estimate of REE.

5.1.3. Physical activity

While measures of TEE and REE can be used in combination to obtain an estimate of physical activity energy expenditure [49], this approach does not provide information regarding quality of physical activity. Thus,

we assessed physical activity using a triaxial accelerometer (wGT3x-BT, ActiGraph LLC, Pensacola, FL). The device measures and sums the magnitude of accelerations in three planes of movement, yielding data expressed as counts per minute. We asked each participant to wear the accelerometer on the right hip for seven days per assessment, except when sleeping, bathing, or participating in water activities. In addition, participants completed daily physical activity diaries, recording each activity performed during 15-minute time blocks throughout each day of monitoring [50]. We used information from the diaries to confirm times when physical activity monitors were worn during waking hours [51]. Daily physical activity was quantified as total counts and minutes of moderate- to vigorous-intensity physical activity using the ActiLife Data Analysis Platform (version 6.13.3, ActiGraph LLC, Pensacola, FL), consistent with published methodology [51,52].

5.2. Chronic disease risk factors

Specific Aim #2 was to evaluate the effects of the three diets on chronic disease risk factors during weight-loss maintenance. We assessed novel, as well as conventional, risk factors following a 12-hour overnight fast.

5.2.1. Insulin sensitivity, insulin secretion, and glycemic control

We conducted an oral glucose tolerance test (OGTT), using a standard 75-gram dose of dextrose (Trutol™, ThermoFisher Scientific, Waltham, MA). Blood for determination of plasma glucose and insulin was obtained by indwelling venous catheter at − 10, − 5, 0, 10, 20, 30, 60, 90, and 120 min relative to the start time of dextrose consumption. The hand and forearm were placed in a warming box set at 65°C (150°F) to arterialize venous blood samples [53]. We collected glucose and insulin data for calculating indexes of peripheral and hepatic insulin sensitivity, as described by Abdul-Ghani et al. [54]. Insulin level at 30 min following the dose of dextrose (insulin-30) was a proxy measure of insulin secretion, consistent with previous studies [55–57]. We measured C-peptide in a 24-hour urine sample as an indicator of daily insulin secretion [58]. We assessed HbA1c in a fasting blood sample as an integrated measure of mean glycemia over approximately three months [59] and 1,5-anhydroglucitol (1,5-AG) as marker of diet-induced glucose excursions [60].

5.2.2. Lipid profiles

We measured serum total cholesterol, HDL-cholesterol, LDL-cholesterol (direct enzymatic spectrophotometric methodology) and triglycerides. Non-HDL-cholesterol was calculated as an indicator of atherogenic particle concentration [61,62]. The CERLab is certified by the Centers for Disease Control and Prevention/National Heart, Lung, and Blood Institute Lipid Standardization Program.

5.2.3. Coagulopathy and inflammation

We measured plasma plasminogen activator inhibitor type 1 (PAI-1) [63] and fibrinogen [64] as indicators of coagulopathy. We measured serum high-sensitivity C-reactive protein (hs-CRP) and interleukin-6 (IL-6) as markers of chronic inflammation [65].

5.2.4. Blood pressure

We measured blood pressure by auscultation at the right arm using a sphygmomanometer (System 5, American Diagnostic Corporation, Hauppauge, New York), according to standard procedures [66]. Measurements were taken three times at each assessment visit, with the last two measurements averaged for data analysis.

5.3. Physiological mechanisms

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

5.3.1. Skeletal muscle work efficiency

We conducted graded cycle ergometry to measure skeletal muscle work efficiency, according to published methods [67,68]. In brief, following a 10-minute warm-up period, participants pedaled at 60 rpm against graded resistance to generate power corresponding to 10 W, 25 W, and 50 W in 4-minute stages. We measured oxygen uptake and carbon dioxide production by indirect calorimetry and converted oxygen consumption to energy expenditure based on respiratory exchange ratio. Skeletal muscle work efficiency at each grade was calculated as power generated per increase in energy expenditure above resting. We instructed participants to fast for 5 h prior to the cycling test.

5.3.2. Body composition

We assessed body composition using a multi-component model [69] to estimate fat mass with measures of total body volume from air displacement plethysmography (ADP; BodPod, Cosmed USA Inc., Concord, CA), bone mineral content and body mass by dual-energy x-ray absorptiometry (DXA; Horizon A, Hologic Inc., Bedford, MA), and total body water (TBW) by isotope dilution. We instructed participants to fast for 5 h prior to the visit for ADP and DXA measurements and not exercise or drink > 8 oz of water within 2 h of the measurements. We used $H_2{}^{18}O$ pool size (N_O), from the DLW protocol described above, as an estimate of TBW (correcting for the 1% overestimation of TBW due to isotope exchange with nonaqueous exchangeable oxygen in the body) [70]. The multi-component equation of Wang (eq. 11, [69]) was used to calculate fat mass from measured total body volume, bone mineral content, body mass, and TBW.

5.3.3. Hormonal axes

We assessed hormonal axes that affect or respond to energy balance. We measured thyroid functions (serum thyroxine [T4], free T4, reverse triiodothyronine [rT3], and thyroid stimulating hormone [TSH]), growth hormone action (serum insulin-like growth factor-1 [IGF-1] and IGF-binding protein-3 [IGF-BP-3]), reproductive hormones (serum testosterone, estradiol, luteinizing hormone, and follicle stimulating hormone), and stress hormones (24-hour urinary cortisol and catecholamines). We also measured hormones that regulate several physiological functions associated with obesity-related chronic disease [71,72], including serum leptin and total and high-molecular weight adiponectin (secreted from adipose tissue) and plasma ghrelin (secreted primarily from the stomach).

5.3.4. Saved samples

We saved aliquots of serum for future analysis of metabolomics profiles, recognizing that profiling can provide important information pertaining to not only physiological mechanisms but also dietary adherence, as indicated in a previous study [73]. In addition, participants were invited to “opt in” to collect stool samples for future analysis of gut microbiome. We obtained stool samples in triplicate using an established protocol [74]. We saved extra aliquots of serum, plasma, and urine in a − 80°C freezer in the biorepository at BCH for possible future analyses.

5.4. Weight change with Ad Libitum feeding

Specific Aim #4 was to evaluate the effects of the three test diets, during *ad libitum* feeding, on weight change. Throughout the *ad libitum* feeding phase, participants continued to weigh themselves daily using Wi-Fi scales.

6. Covariates

Covariates or effect modifiers are listed in Table 4. Methods for assessing body weight, body composition, and insulin sensitivity and secretion are described above. We collected demographic data (sex,

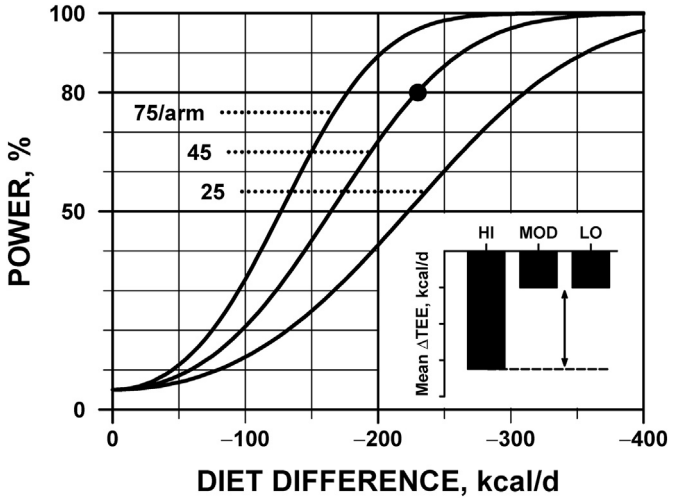

Fig. 3. Statistical power.
Power of one-way ANOVA to detect deviation of mean ΔTEE under one diet from the other two (inset). Each curve represents a fixed sample size. Dot indicates detectable effect, 237 kcal/d at 80% power, with proposed 45/arm. Curves based on non-central F distribution, 5% Type I error, between-subject standard deviation 412 kcal/d as in preliminary data.

ethnicity, race, age) by self-report. We isolated and saved buffy coat from blood samples for extracting DNA from participants who "opted in" for genetic studies. These studies may include, but will not be limited to, candidate gene analysis and whole genome/exome sequencing. We have particular interest in amylase gene copy number [75]. We measured perceived palatability (tastiness) of the test diets using a 10-cm Visual Analog Scale (VAS).

7. Ancillary studies

The parent study, which is the focus of this protocol paper, provided an opportunity for data collection pertaining to several ancillary studies. These studies were led by faculty and trainees from HMS or FSU. Outcomes pertaining to lipoprotein particle subfraction distribution, sleep, psychological health, cognition, and weight bias are listed in the ClinicalTrials.gov registry for the parent study (NCT02068885). Two of the ancillary studies required substantial resources and effort, in terms of additional study visits for hospital-based assessments, and have separate registries (Metabolic Fuels and Adipocyte Biology: NCT02235038; Brain Reward Activity: NCT02300857). The outcomes for which we invited participants to "opt in" are denoted in Table 4.

8. Statistical methods

8.1. Analysis plan

We will follow an *a priori* analysis plan. The primary outcome measure will be TEE per kg body weight, measured at four time points: pre-weight loss (BSL), week 0 (PWL, pre-randomizaiton), week 10 (MID), and week 20 (END). According to the primary null hypothesis, the time course of TEE between week 0, week 10, and week 20 will be the same for all three diets.

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 power to detect diet differences. These include the outcome of interest at BSL (pre-weight loss); change in body weight over the test phase (weeks 0–20); demographic characteristics (sex, ethnicity, race, age); anthropometric measures (BMI and percentage lean mass at BSL, 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 [76]. A single participant who developed a disqualifying medical condition (hypothyroidism, as documented by two 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 at week 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 [77] 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 also will perform a "per protocol" analysis, excluding any participant 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 analyzed similarly to TEE. Measures with skewed distribution will be log-transformed for analysis and retransformed 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 participant 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.

8.2. Sample size, power, and detectable effects

For comparability with pilot data from prior studies, our sample-size calculation was formulated in terms of changes in TEE uncorrected for body weight between week 0 and week 20. As noted above, 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 used the power characteristics of a simple one-factor, three-level ANOVA with covariate-adjusted residual variance estimates taken from our pilot data. As a conservative, minimal-impact alternative, pictured in the inset panel of Fig. 3, 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 Fig. 3 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 participants, as observed in a previous study [18]. Our proposed sample size, 45 completers per diet arm (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 a previous study [18], 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. As indicated in Fig. 2, we exceeded the recruitment target of 150 participants and had more completers than anticipated.

9. Discussion

Preventing weight regain following weight loss is a critical public health issue. Most randomized controlled trials of different macronutrient diets have focused on the period of active weight loss [4–6]. This protocol paper describes a novel study in which we compared diets during an extended period of weight-loss maintenance, directing attention to the potential effect of macronutrient composition on metabolic adaptations that may influence propensity for weight regain. We implemented a feeding protocol, in contrast to most previous studies using nutrition education and dietary counseling [4–6], and conducted the study in a community setting rather than a hospital-based clinical research center. We collaborated with food service personnel, including professional chefs, in designing cycle menus that allowed for strict control of macronutrient composition while paying attention to palatability. Chefs prepared foods with appropriate spices and garnishes so that study meals had the look and taste of restaurant meals, rather than institutional food or classic research diets. Intervention staff provided support to study participants during supervised mealtimes.

When designing this feeding study and conceptualizing translation of science to practice, we considered internal and external validity. With regard to internal validity, we provided food to participants throughout the study to 1) ensure differentiation among test diets, 2) discourage consumption of non-study foods that could contaminate the dietary interventions, and 3) reduce inter- and intra-subject variability in dietary intake that could lead to confounding. We recognize that provision of all foods does not represent the “real world,” thereby limiting external validity (generalizability). Nevertheless, our purpose was to examine biological effects of different macronutrient diets, providing the scientific foundations for future effectiveness research. Establishing external validity is a multi-step process, requiring several trials over time with: intervention strategies ranging from controlled feeding to nutrition education and dietary counseling. Designing and evaluating dietary interventions, with the ultimate goal of translating results to public health messages, likely will involve collaboration with the food industry to ensure that diets found to be most healthful are palatable and readily available to consumers. By moving the study outside of a hospital setting and collaborating with the hospitality industry (Sodexo), we have laid the foundation for future dietary intervention studies emphasizing external validity.

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