Effects of a Low-Carbohydrate Diet on Energy Expenditure During Weight Loss Maintenance: A Randomized Feeding Study

Journal:	*BMJ*
Manuscript ID	BMJ.2018.046192
Article Type:	Research
BMJ Journal:	BMJ
Date Submitted by the Author:	25-Jul-2018
Complete List of Authors:	Ebbeling, Cara; Boston Children's Hospital, Medicine; Harvard Medical School, Pediatrics Feldman, Henry; Boston Children's Hospital, Medicine Klein, Gloria; Boston Children's Hospital, Medicine Wong, Julia; Boston Children's Hospital, Medicine Bielak, Lisa; Boston Children's Hospital, Medicine Steltz, Sarah; Boston Children's Hospital, Medicine Luoto, Patricia; Framingham State University Wolfe, Robert; UAMS Medical Center Wong, William; Baylor College of Medicine Ludwig, David; Boston Children's Hospital, Medicine; Harvard Medical School,
Keywords:	Obesity, Nutrition, Low-carbohydrate diet, Metabolism
Note: The following files were submitted by the author for peer review, but cannot be converted to PDF. You must view these files (e.g. movies) online.	
Figure 3.jpg Figure 5.jpg	

Effects of a Low-Carbohydrate Diet on Energy Expenditure During Weight-Loss Maintenance: A Randomized Feeding Study

Cara B Ebbeling, Henry A Feldman, Gloria L Klein, Julia MW Wong, Lisa Bielak, Sarah K Steltz, Patricia K Luoto, Robert R Wolfe, William W Wong, David S Ludwig

Division of Endocrinology, Boston Children's Hospital, Harvard Medical School and New Balance Foundation Obesity Prevention Center, 300 Longwood Avenue, Boston, MA 02115

- Cara B Ebbeling, associate professor
- Gloria L Klein
- Julia MW Wong, instructor
- Lisa Bielak
- Sarah K Steltz
- David S Ludwig, professor

Institutional Centers for Clinical and Translational Research, Boston Children's Hospital, 300 Longwood Avenue, Boston, Massachusetts 02115

- Henry A Feldman, associate professor

Department of Food and Nutrition, Framingham State University, 100 State Street PO Box 9101, Framingham, Massachusetts 01701

- Patricia K Luoto, professor

University of Arkansas for Medical Sciences, 4301 W Markham St, Little Rock, AR 72205

- Robert R Wolfe, professor

USDA/ARS Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, 1100 Bates Street, Houston, TX 77030

- William W Wong, professor

Correspondence to: David S Ludwig, david.ludwig@childrens.harvard.edu

Manuscript length: 4402 words, 3 tables, 5 figures, and an online only supplement

ABSTRACT

Objective: To determine the effects of diets varying in carbohydrate-to-fat ratio on total energy expenditure (TEE), measured using doubly labeled water.

Design: The Framingham State Food Study, (FS)2, was a randomized-controlled feeding study conducted from August 2014 to May 2017. Outcome data were collected by personnel masked to dietary group assignment.

Setting: Multi-institutional collaboration conducted at two sites.

Participants: Healthy adults aged 18 to 65 years with body mass index ≥25 kg/m^2. From 1,685 individuals who completed telephone screening, 164 were randomized to a test diet.

Interventions: Following 12±2% weight loss on the Run-In diet, participants were assigned to high-, moderate-, or low-carbohydrate Test diets (60, 40, or 20% total energy, respectively) for 20 weeks. Test diets were controlled for protein and energy-adjusted to maintain body weight within 2 kg following weight loss.

Main Outcomes: TEE by Intention-to-Treat (ITT) analysis. Per Protocol analysis included participants who achieved weight-loss maintenance. TEE was analyzed in kcal/kg/d and scaled to average post-weight loss body weight (82 kg) for reporting in kcal/d.

Results: TEE differed by diet (n=159, *P*=0.008, ITT). Compared to the high-carbohydrate diet, change in TEE (mean, 95% CI) was +76 (-42 to +194) kcal/d greater on the moderate-carbohydrate diet and +185 (+69 to +302) kcal/d greater on the low-carbohydrate diet. In the Per Protocol analysis (n=118, *P*=0.001), the respective differences were +111 (-23 to +245) and +249 (+117 to +380) kcal/d. Among participants in the highest tertile of baseline insulin secretion, the difference between the low- *vs.* high-carbohydrate diet was +464 (+226 to +701)

kcal/d (P<0.001, Per Protocol). Ghrelin, a hormone thought to lower energy expenditure, was significantly lower on the low- *vs.* high-carbohydrate diet.

Conclusions: Lowering dietary carbohydrate increased energy expenditure independently of body weight. This metabolic effect may facilitate weight-loss maintenance, especially among individuals with high insulin secretion.

Trial Registration: ClinicalTrials.gov, NCT02068885

https://clinicaltrials.gov/ct2/show/NCT02068885

What this paper adds:

Section 1: What is already known on this subject

Energy expenditure declines with weight loss, predisposing to weight regain. However, little is known about how diet composition influences this adaptive metabolic response over the long-term.

Section 2: What this study adds

Our study shows that a low-carbohydrate diet may increase energy expenditure during weight loss maintenance, a metabolic effect that might improve the success of obesity treatment.

INTRODUCTION

Evidence from animal and human studies shows that biological factors strongly influence body weight.[1] With weight loss, hunger increases and energy expenditure decreases – physiological adaptations that defend against long-term weight change.[2] Genetic factors are known to affect body weight, explaining some of the variance in BMI among individuals. However, genetic factors cannot explain why the average person today, compared to 40 years ago, seems to be "defending" a much higher body weight.

According to the Carbohydrate-Insulin Model of obesity,[3-6] the increased ratio of insulin to glucagon concentrations after consumption of high glycemic-load carbohydrates directs metabolic fuels away from oxidation and toward storage in adipose tissue. This physiological state is hypothesized to increase hunger, lower energy expenditure and predispose to weight gain, especially among individuals with inherently high insulin secretion. The Carbohydrate-Insulin Model offers a physiological mechanism for understanding why obesity rates have increased since the 1970s, as dietary fats were replaced with high glycemic-load carbohydrates, including refined grains and added sugars.[7]

This model has been challenged, primarily due to lack of evidence from controlled feeding studies.[8-12] One recent meta-analysis reported no meaningful difference in energy expenditure between low-carbohydrate *vs.* low-fat diets.[9] However, the studies included in that analysis were short term (mostly <2 weeks), whereas the process of adapting to a lower-carbohydrate, higher-fat diet appears to take at least 2 to 3 weeks.[6, 13-17] For this reason, transient effects of macronutrients cannot be distinguished from chronic effects based on existing evidence. The aim of this study was to compare the effects of diets varying in carbohydrate-to-fat ratio on energy expenditure during weight-loss maintenance throughout 20 weeks.

METHODS

The study protocol was approved by the Institutional Review Board at Boston Children's Hospital and previously published.[18] Data were collected on the campus of Framingham State University (FSU, Framingham, MA) between August 2014 and May 2017. A partnership with Sodexo, the food service contractor at FSU, was established for implementing controlled feeding protocols.[19] A satellite feeding site was established at Assabet Valley Regional Technical High School (AV, Marlborough, MA) for the final year of the study. The study was known as the Framingham State Food Study, or (FS)2.

Design

The study was a randomized controlled trial with Run-In and Test Phases (Figure 1). Following collection of baseline (pre-weight loss) data, energy intake was restricted to promote weight loss corresponding to 12±2% of baseline body weight by 10 weeks. Post-weight loss (PWL, time 0) data were collected at the end of the Run-In Phase for participants who achieved the targeted weight loss. These participants were randomly assigned to high-, moderate-, or low-carbohydrate diets (HI, MOD, LO) for a 20-week Test Phase (Table 1). Participants weighed themselves daily using calibrated Wi-Fi scales (Withings Inc., Cambridge, MA). Study outcomes were assessed at the midpoint (MID, weeks 8 to 10) and end (END, weeks 18 to 20) of the Test Phase.

Participants

Men and women aged 18 to 65 years, with a body mass index (calculated as weight in kilograms divided by height in meters squared) of 25 or higher and body weight less than 160 kg, were

screened for participation. Additional eligibility criteria are presented in eTable 1 in Supplement. For each of three cohorts, recruitment occurred during the spring semester prior to the respective academic year (August to May) of study participation. Participants provided written informed consent at the time of enrollment. The stipend for participation was $3,280 over the course of the study, and meals were valued at $3,220, for total compensation of $6,500.

Implementation of Randomization

Participants who successfully completed the Run-In Phase were eligible for randomization. A blocked randomization design was employed to ensure close balance among the three diet arms at any point in the study. The randomization was stratified by feeding site (FS U, AV), sex (male, female), 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, 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 under supervision of the Lead Biostatistician, using specialized software developed for that purpose. The diet assignments were randomly permuted within blocks of 3, 6, and 9, and the blocks themselves were randomly permuted. Each upcoming assignment thus was unpredictable, preventing any deliberate or inadvertent bias on the part of those conducting enrollment.

Master randomization lists, one for each of 32 strata, were prepared with a numerical sequence of identifiers. The master randomization list was prepared by the Data and Quality Manager (DQM), with supervision from the Lead Biostatistician, using specialized software developed for that purpose. The software accounted for the three treatment arms, two feeding

sites, and four stratification factors (sex, ethnicity-race, age, BMI) each with two levels. Each identifier on the master randomization list was assigned to one of the three treatment arms.

The assignment list was kept in the private custody of the DQM and maintained in a secure shared drive folder accessible only to the DQM and an assigned back-up staff member trained in randomization. The DQM, after confirming eligibility criteria (including adequate weight loss during the Run-In Phase) with the Study Director, assigned the next available randomization ID according to the participant's stratum. Randomization lists were maintained in an Excel spreadsheet, and assignments were made electronically. Randomization occurred in waves (*i.e.*, groups of participants randomized at a time) for each cohort. The DQM relayed the diet assignment of each participant to intervention staff by email. Randomization was done in an at Boston Children's Hospital.

Dietary Interventions

During the Run-In Phase, the macronutrient composition was 45% of total energy from carbohydrate, 30% from fat, and 25% from protein. Energy intake was restricted to 60% of estimated needs. The research team monitored body weight and made adjustments in amounts of food provided when necessary to achieve the target weight loss.

During the Test Phase, HI, MOD, and LO varied in carbohydrate (60%, 40%, and 20% of total energy) and fat (20%, 40%, and 60%), while controlled for protein (20%). Calculations using Food Processor Nutrition Analysis Software (ESHA Research Inc., Salem, OR) indicated that calculated macronutrient composition was within 1% of the targets for carbohydrate and fat and 0.2% for protein (Table 1). The relative amounts of added sugar (15% of total carbohydrate), saturated fat (35% of total fat), and sodium (3000 mg per 2000 kcal) were held constant across

diets. Based on regression of body weight (g) on time (days), a slope ≥ 15 g per day over 14 days indicated the need to adjust energy intake to achieve weight stability within ±2 kg of the PWL anchor weight.

Study Outcomes

Pre-specified outcomes included energy expenditure, physical activity, and metabolic hormones. Baseline insulin secretion (insulin concentration 30 minutes after oral glucose, INS-30)[20 21] was assessed to test for effect modification predicted by the Carbohydrate-Insulin Model. Outcome data were collected by personnel masked to dietary group assignment.

Total energy expenditure (TEE, primary outcome) was assessed using doubly labeled water methodology.[22] Participants provided two pre-dose spot urine samples on separate days and seven post-dose samples at regular intervals over a 14-day assessment period. Isotopic enrichments of urine samples were measured in duplicate by Gas-Isotope-Ratio Mass Spectrometry at the USDA/ARS Children's Nutrition Research Center.[23] Body weight was measured using a calibrated electronic scale (BWB-800S, Tanita, Arlington Heights, IL) every time a participant provided a urine sample. We averaged these weight measurements, expressed TEE in kcal per kg body weight for analysis, and then normalized TEE to average post-weight loss body weight (82 kg) for reporting. We expressed TEE per kg per day to take into account changes in body weight that might occur during the Test Phase, and thereby improve precision. We also examined absolute TEE expressed as kcal per day, with body weight included as a covariate, in secondary analyses.

The equation of Ravussin et al[24] was used to calculate TEE from rCO_2, with food quotient (FQ) as a proxy for respiratory quotient.[25] Recognizing that estimates of FQ involve

some imprecision, due in part to uncertainty when calculating metabolizable energy,[26] sensitivity analyses (described below) were conducted to determine how plausible errors could influence results.

Resting energy expenditure (REE) was assessed after a 12-hour overnight fast using a metabolic cart (TrueOne 2400, Parvo Medics, Sandy, UT). When measurements averaged over 20 minutes on two separate mornings were not within 10%, a third measurement was obtained on another morning. The mean of the two closest measurements was used as the best estimate of REE and expressed as kcal per kg body weight.

Physical activity was measured by accelerometry over seven days using a triaxial accelerometer placed on the right hip (wGT3x-BT, Actigraph LLC, Pensacola, FL). The ActiLife Data Analysis Platform (version 6.13.3, ActiGraph LLC, Pensacola, FL) was used to calculate daily physical activity (total counts), minutes of moderate- to vigorous-intensity physical activity (MVPA), and minutes of sedentary time.[27 28]

A blood sample was drawn after a 12-hour overnight fast for determination of metabolic hormones. Plasma and serum samples were stored at −80°C in the Biobank Core Laboratory at Boston Children's Hospital. Enzyme-linked immunosorbent assays were used to measure plasma ghrelin (Linco Research, St. Louis, MO) and serum leptin (R&D Systems, Minneapolis, MN) in the Clinical and Epidemiological Research Laboratory (CERLab) at Boston Children's Hospital.

At baseline (pre-weight loss), each participant had an oral glucose tolerance (75-g dose of dextrose). Blood was collected 30 minutes after the dose for determination of INS-30, quantified by electrochemiluminescence immunoassay (Roche Diagnostics, Indianapolis, IN) in the CERLab. Baseline body composition was measured by dual-energy x-ray absorptiometry, and

percentage lean mass (lean soft tissue mass/total body mass × 100%) was used as a covariate (Horizon A, Hologic Inc., Bedford, MA).

Statistical Analysis

Sample-size calculations were based on data from a preliminary study.[18] The target of 135 completers was chosen to provide 80% power to detect a difference of 237 kcal/d in TEE change between one diet arm and the other two, with 5% Type I error.

The primary outcome measure, TEE, was derived from a nonlinear decay model fitted jointly to urinary disappearance curves of stable oxygen and hydrogen isotopes following oral administration of the doubly labeled water.[18] We used the jackknife technique to smooth the parameter estimates and discarded a small number of incomplete or poorly fitting curves, deviant data points, and implausible values.

The pre-specified analytic framework for the primary outcome was repeated-measures analysis of variance spanning three time points (PWL, MID, END), with diet assignment as a three-level independent variable (HI, MOD, LO). The model was adjusted for demographic characteristics (sex, race, ethnicity, age); design factors (study site, cohort, enrollment wave); baseline anthropometric measures (BMI, percentage lean mass, percentage weight lost pre-randomization); and baseline level of the outcome variable. An unstructured covariance matrix provided maximum flexibility in modeling correlation within-subject over time. From parameters of the fitted model, we constructed the mean test-phase change in TEE (covariate-adjusted change between PWL and weeks 10 and 20, the latter two averaged) and tested the hypothesis that this change was uniform across diets, using a 2-df F-test with critical *P*-value 0.05. When this hypothesis was rejected, the principle of closed testing[29] permitted us to make

each pairwise diet comparison with critical *P*-value 0.05 while preserving a maximum familywise 5% Type I error rate for the set of four comparisons.

To test for effect modification, we divided the sample into baseline INS-30 tertiles, added appropriate interaction terms to the repeated-measures model, and constructed contrasts to test for linear trend across tertiles for the diet differences in change during the Test Phase. Secondary outcomes (REE, physical activity, hormone levels) were analyzed similarly to TEE. The hormone levels were log-transformed for analysis and re-transformed to natural units for reporting.

Each analysis was performed in both the full Intention-to-Treat sample and a Per Protocol subset comprising those participants who maintained their weight within ±2 kg of the PWL anchor weight during the Test Phase. Following each analysis, we examined residual patterns in order to detect outliers or other departures from assumptions of the statistical model.

We conducted sensitivity analysis to determine the robustness of our findings to alternative values for the food quotient parameter assumed in calculating TEE. To test for selective dropout, we compared baseline characteristics of participants who completed the END assessment with those who did not. To assess fully the influence of missing data (excluded participants, dropouts, and unusable data points) we performed an inverse probability-weighted version of the primary analysis,[30] constructing a logistic model for missingness based on the covariates listed above and employing the fitted probabilities as weights in the primary analysis. We used SAS software for all computations (SAS Institute Inc., Cary, NC).

Missing Data and Quality of Fit

Two randomized participants were excluded from all analyses: one who developed a disqualifying medical condition (*i.e.*, hypothyroidism) and one who provided unreliable doubly labeled water data at PWL and then withdrew prior to notification of diet assignment. Of 486 potential TEE values for use in the primary repeated-measures analysis (162 participants × 3 time points), 448 were available (92%); for Per Protocol analysis, 331 of 360 (92%). The missing values were attributable to 24 missed doubly labeled water studies) (9 MID, 15 END) and eight studies that yielded non-convergent curve fits or implausible parameters (3 pre-weight-loss baseline, 1 PWL, 3 MID, 1 END). Neither the Intention-to-Treat nor the Per Protocol findings changed materially when we applied inverse probability weighting, based on the covariate list, to compensate for the missing data. For secondary outcomes, the percentage of non-missing values varied between 94% (REE, physical activity) and 95% (hormones). Residual patterns showed a satisfactory fit to the repeated-measures model in all cases, with no extreme outliers or pathological distributions.

RESULTS

Participants

The flow of participants through the trial is shown in Figure 2. From a total of 1685 screened, we enrolled 234 participants for the Run-In Phase. Of these, 164 achieved an initial weight loss of 12±2% and were randomly assigned to different macronutrient diets for the Test Phase. Retention during the Test Phase was 94% for the MID assessment at 10 weeks and 90% for the END assessment at 20 weeks. Baseline characteristics are presented in Table 2.

During the Run-In Phase, mean weight loss for randomly assigned participants was 9.6 (SD, 2.5) kg, corresponding to 10.5 (SD, 1.7) % of baseline body weight. At week 0, body

weight did not differ across dietary intervention groups (P=0.18). Among the randomly assigned participants (for whom energy intake was adjusted as needed to maintain body weight during the Test Phase), 118 had data for the primary outcome and remained within the targeted ±2 kg of their PWL anchor weight, comprising the Per Protocol group. Covariates did not differ between these participants and those who did not achieve weight-loss maintenance, except for age which had marginal significance (eTable 2). Covariates also did not differ between participants who completed the END assessment and those who did not (data not shown).

Throughout the trial, 40 adverse events were recorded for 38 participants. Of these, 15 were possibly or probably related to the intervention (e.g., constipation; food allergy, aversion, or intolerance; gastroenteritis; mood changes; increased blood cholesterol levels based on primary care evaluation; possible gall bladder disease; possible hypoglycemia), 12 were probably or definitely related to assessments (e.g., hematoma, vasovagal reaction, vomiting, lightheadedness), and 13 were deemed unrelated to study participation. Two serious adverse events were reported involving emergency hospitalization for removal of an intrauterine device and laparoscopic cholecystectomy (the latter was possibly related to study participation). The number of participants who had an adverse event following randomization did not differ by diet group (P=0.34).

Process Measures and Biomeasures of Compliance

Attention to treatment fidelity, as previously described,[19] encompassed differentiation and consistency in designing the diets (Table 1) and integrity in preparing the diets. To monitor integrity of the intervention, we did spot weight checks, comparing actual with target weights of

menu items and documenting that 98% were within ±5 g (a level of deviation that would not compromise macronutrient differentiation).

In addition to weight loss maintenance, we evaluated several biomeasures of carbohydrate intake as markers of dietary compliance. Serum 1,5-anhydroglucitol is inversely associated with glycemic excursions when blood glucose exceeds the renal threshold, as a result of competition with glucose for reabsorption in the proximal tubules. However, in the absence of diabetes, 1,5-anhydroglucitol is directly associated with both total carbohydrate and glycemic index, presumably reflecting dietary intakes.[31] As depicted in Figure 3, we found strong differentiation of 1,5-anhydroglucitol among diet groups, ranging from lowest on the LO to highest on the HI (P<0.0001). Also as expected, triglycerides increased with increasing carbohydrate content (P<0.0001), whereas HDL-cholesterol decreased (P<0.0001).

Total Energy Expenditure

In the Intention-to-Treat analysis (n=159), total energy expenditure (TEE) differed significantly by diet (Figure 4A). Compared to HI, change in TEE (mean, 95% CI; normalized to average PWL body weight of 82 kg) was 76 (-42 to 194) kcal/d greater on MOD and 185 (69 to 302) kcal/d greater on LO. In the Per Protocol analysis (n=118, P=0.001) (Figure 4B), the respective differences were 111 (-23 to 245) and 249 (117 to 380) kcal/d. Findings from both analyses remained materially unchanged with inverse probability weighting to compensate for missing data or when examining absolute TEE expressed in kcal per day. TEE did not change significantly within any diet group between 10 and 20 weeks (P > 0.48).

In sensitivity analyses, the overall group effect retained statistical significance with a food quotient (FQ) as high as 0.82, compared to 0.79 based on actual macronutrient composition

(and an even higher FQ in pairwise comparisons between the LO and HI diets). This finding indicates that the observed effect of diet on TEE is robust to substantial imprecision in estimating FQ (eTable 3) and non-compliance (eTable 4).

The effect of dietary composition on TEE was most pronounced among individuals with high insulin secretion (Figure 5). Among participants in the highest tertile of INS-30 in the Per Protocol analysis, TEE was 464 (95% CI, 226 to 701) kcal/d higher for LO compared to HI. This pattern was weaker but qualitatively similar in the ITT analysis 304 (95% CI, 103 to 505) kcal/d.

Other Outcomes

Resting energy expenditure (REE), total physical activity, moderate- to vigorous-intensity physical activity (MVPA), and sedentary time did not differ significantly by diet in the Per Protocol (Table 3), or ITT (eTable 5), analysis. Ghrelin and leptin differed significantly by diet in the Per Protocol analysis (Table 3). Ghrelin showed a steeper decline over the Test Phase on LO compared to the HI (-13.6% *vs*. -6.0%, *P*=0.006), and leptin showed a lesser rise (22.1% *vs*. 46.7%, *P*=0.006).

DISCUSSION

In this 5-month controlled feeding trial, we found that TEE was significantly greater on a low-carbohydrate compared to a high-carbohydrate diet with similar protein content, independent of body weight. In addition, insulin secretion at baseline may modify individual response to this diet effect. Taken together with preliminary reports involving activation of brain areas involved in food cravings[32] and circulating metabolic fuel concentration,[33] results of (FS)2 substantiate several key predictions of the Carbohydrate-Insulin Model.

The mean TEE effect we observed (about 200 kcal/day or +50 kcal/day for every 10% decrease in energy from dietary carbohydrate) is comparable to that obtained by isotopic methods over 1-month intervention periods in a previous randomized cross-over study with 21 adults[34] and in a non-randomized cross-over study with 17 men,[35] after taking into account confounding by ongoing weight loss and other factors.[36] If this effect were persistent – and we observed no attenuation from 10 to 20 weeks – it would translate into an estimated 20-lb weight loss after 3 years for a typical 30-year-old man with height of 5'10", baseline weight of 200 lb, and average activity level, assuming no change in energy intake [https://www.supertracker.usda.gov/bwp/index.html].

The difference in TEE between low- and high-carbohydrate diets among individuals in the highest tertile of insulin secretion (464 kcal/day) was triple the difference for those with lower insulin secretion, highlighting a subgroup that may do particularly well with restriction of total or high-glycemic load carbohydrates. This finding is consistent with results from an animal study,[37] a cohort study,[38] Mendelian randomization analysis,[39] and clinical trials.[20, 21, 40] In contrast, the recent DIETFITS trial reported no effect modification by insulin secretion or genetic factors among 609 overweight adults assigned to Healthy Low-Fat *vs.* Healthy Low-Carbohydrate diets for 12 months.[41] However, in that study, which relied on nutrition education and behavioral counseling, all participants were instructed to "minimize or eliminate refined grains and added sugars and maximize intake of vegetables" and other minimally processed foods. Probably for this reason, the reported glycemic load of the Healthy Low-Fat Diet was very low for a diet that is by nature higher in total carbohydrate, and similar to the value for the lowest glycemic load diets in some previous intervention studies.[42] Thus, the effects of predisposing risk factors may be attenuated on diets that are generally healthy and specifically low in

glycemic load. In support of this possibility, a high genetic obesity risk score predicted obesity among individuals consuming sugary beverages but not among non-consumers.[43]

Similar to our prior cross-over study,[34] the difference in TEE between diets was not primarily attributable to REE or physical activity level, which were minimally higher on the low-carbohydrate diet. Other potentially contributory components of energy expenditure include thermic effect of food, brown adipose tissue activity, autonomic tone, nutrient cycling, fidgeting and related non-exercise activity thermogenesis,[44] and changes in the efficiency of movement.[45-48]

A change in metabolism is suggested by hormonal responses to diet. Ghrelin, produced primarily in the stomach, was significantly lower on the low-carbohydrate diet, a novel finding. Beyond effects on hunger, ghrelin has been reported to lower energy expenditure and promote fat deposition,[49 50] providing another mechanistic explanation for our primary outcome. Leptin was also lower on the low-carbohydrate diet, suggesting improvement in leptin sensitivity.[51] Prospective studies have observed that individuals with the greatest declines in leptin following weight loss have the lowest risk for weight regain.[52-54]

As one of the largest and longest controlled feeding studies among free living participants, (FS)2 has several strengths: 1) sufficient intervention duration to avoid confounding by transient metabolic adaptations to changes in macronutrient content;[13-16] 2) power to achieve a relatively precise effect estimate for the primary outcome; 3) biomeasures demonstrating substantial and sustained differentiation between diets (findings not characteristically observed in trials relying on nutrition education and behavioral counseling);[55] 4) measurement of TEE by the doubly labeled water method, the gold standard method for studies of free-living people;[22, 56] 5) control for dietary protein and body weight, minimizing confounding by other potentially

significant influences on TEE; and 6) design of diets to reflect realistic and healthful examples of their respective macronutrient compositions.

Study limitations include potential measurement error, non-compliance, and generalizability. Measurement of TEE by the doubly labeled water method involves several assumptions, most notably that FQ (reflecting dietary composition) equals respiratory quotient (RQ, reflecting the ratio of macronutrients oxidized). This assumption is generally valid during weight stability.[25] Reassuringly, potential errors in estimation of FQ would have only a modest effect, with a 0.01 shift in FQ equating to ~1% change in TEE (eTable 3). Sensitivity analyses show that the primary outcome remained robust throughout a range of plausible RQ values. Although *de novo* lipogenesis could theoretically confound isotopic determination of body water, rates would be much too low across a wide range of macronutrient composition during weight stability to affect TEE in any meaningful way.[57, 58] Evidence for effect modification by insulin secretion provides additional reassurance for the validly of the primary outcome, as there would be no reason why any systematic error in TEE would segregate by insulin secretion status.

Regarding another limitation, we considered our protocol too long to be logistically practical or financially feasible for an inpatient setting. Instead, we provided participants fully prepared meals, and implemented strategies to promote compliance with the assigned diets.[19] Despite these efforts, we recognize that some non-compliance may have occurred, especially among individuals whose weight deviated beyond the pre-specified range. However, this issue unlikely presented a threat to study integrity because our sensitivity analysis showed robustness to substantial degrees of non-compliance. The primary outcome would have remained statistically significant if participants in the low-carbohydrate group consumed up to 40% additional calories from foods with macronutrients reflecting the high-carbohydrate diet (and

even more if the additional foods had an intermediate macronutrient composition) (eTable 4). Furthermore, the primary outcome was strengthened in the Per Protocol analysis, including only individuals who maintained weight loss throughout the Test Phase – opposite what would have happened if non-compliance contributed to the observed diet effect. The Per Protocol analysis should provide a more accurate estimate of the true diet effects than the Intention-to-Treat analysis, by excluding participants with objective evidence of non-compliance.

As with any feeding study, translation of our findings to public health recommendations comprises a third limitation. However, aspects of study design improve generalizability to some degree, including provision of food in the pragmatic setting of a university in collaboration with a food service contractor. More broadly, these results must be reconciled with the long-term weight-loss trials relying on nutrition education and behavioral counseling that find only a small advantage for low-carbohydrate *vs*. low-fat diets according to several recent meta-analyses.[59-64] But inferences about efficacy from these trials are limited by characteristically poor long-term compliance and lack of differentiation between diet arms, reflecting the difficulty of behavior change in the modern food environment. If metabolic benefits of reduced glycemic-load diets are confirmed in other mechanistically-oriented research, behavioral and environmental interventions would be necessary for optimal public health translation.

In conclusion, dietary composition appears to affect energy expenditure independently of body weight. A low-glycemic load, high-fat diet may facilitate weight loss maintenance beyond the conventional focus on restricting calorie intake and encouraging physical activity. Additional research is warranted to examine the effects of low-glycemic load diets on body weight, with control of calorie intake; to compare diets aiming to reduce glycemic index at prevailing carbohydrate levels (*e.g.*, the DIETFITS lower-fat diet) *vs.* restricting total carbohydrate; to

explore subgroup susceptibility based on insulin secretion and other biological factors; and to determine whether severe carbohydrate restriction (*e.g.*, with a ketogenic diet) confers unique advantages for obesity or specific conditions such as type 2 diabetes.

Data Access, Responsibility, and Analysis: The full database will be made available upon publication of this article on Open Science Framework (https://osf.io/rvbuy/).

Funding: This work was conducted with grants from the NuSI (made possible by gifts from the Laura and John Arnold Foundation and Robert Lloyd Corkin Charitable Foundation), New Balance Foundation, Many Voices Foundation, and Blue Cross Blue Shield. Dr Ludwig was supported by a mid-career mentoring award from the National Institute of Diabetes and Digestive and Kidney Diseases (K24DK082730). NuSI monitored study progress and was given an opportunity to comment on the manuscript. The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; approval of the manuscript; and decision to submit the manuscript for publication. The content of this article is solely the responsibility of the authors and does not necessarily represent the official views of the study sponsors.

Author Contributions: CBE, Principal Investigator (designed study, secured funding, interpreted data, wrote first draft of manuscript); HAF, Co-Investigator, Biostatistician (designed study, analyzed and interpreted data); GLK, Study Director (acquired data); JMWW, Associate Study Director (calculated diets, acquired data); LB, Nutrition Research Manager (calculated diets, managed dietary intervention); SKS, Data and Quality Manager (performed randomization, monitored quality control of data acquisition); PKL, Study Director for Framingham State

University (maintained partnerships); RRW, Consultant (provided expertise on stable isotope modeling); WWW, Co-Investigator (conducted isotope-ratio mass spectroscopy); DSL, Principal Investigator (designed study, secured funding, interpreted data, wrote first draft of manuscript). CBE and DSL affirm that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained. CBE, HAF and DSL had access to all of the data (including statistical reports and tables) in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Acknowledgements: We thank the following individuals: study participants for their time and commitment to advancing science; study nurses who conducted oral glucose tolerance tests; Framingham State University students who served as research assistants; consultants Helen Rasmussen (Tufts University), Janis Swain (Brigham and Women's Hospital), and Jeff Volek (The Ohio State University); study safety officer Michael Agus (Boston Children's Hospital); Boston Children's Hospital research staff Teri Allers, Rachel Apone, Michaela Barhite, Gary Bradwin, Carissa Burton, Emily Caplan, Nicole Chenard, Kimberly Chin, Courtenay Devlin, Jillian Fergione, Maria Khan, Lesley Levitt, Amanda Gonsalves, Veronica Gragnano, Tessa Graham, Shannon Hourigan, Bridget Hron, Paul Lakin, Meghan Leary, Ethan Litman, Catherine Matero, Marlee McGurl, Carly Milliren, Anne Nero, Victoria Ravenelle, Lisa Robinson, Adrienne Salisbury, Megan Sandman, Amanda Scott, Linda Seger-Shippee, Kim Shimy, Kaitlyn Shannon, Rachael Sharpe, Caitlin Shaughnessy, Daniele Skopek, Tram Anh Tran, Benjamin Trapp, and Emma van Emmerik; Framingham State University employee Denise Harney; Sodexo employees David Garufi, Renae Gauvin, Carolyn Holland, Darline Hughes, Eric

Johnson, Lauren Stone, and Denise Stultz; Assabet Valley Regional Technical High School employees Danielle Gervais, Jon Thering, Kelsey Viera, and Dina Wiroll; Baylor College of Medicine employee Zhensheng Chen; and SetPoint Health staff David Blackburn, Rob O'Neill, and Kristina Spellman for designing a study web portal.

We also thank faculty and staff of Boston Children's Hospital, Framingham State University, and Sodexo who helped in establishing protocols as members of the Administrative, Recruitment, Assessment, and Intervention Workgroups; Framingham State University leadership Rita Colucci, Susanne Conley, Dale Hamel, Jonathan Lee, Daniel Magazu, and Linda Vaden-Goad for support throughout study design and execution; Sodexo leadership Ralph Eddy, Elizabeth Olson, and Beth Winthrop for help planning and conducting the dietary interventions; Assabet Valley Regional Vocational School District leadership Ernest F. Houle and Kristopher Luoto for the opportunity to establish a satellite feeding site on campus; and Jeffrey Flier (Harvard Medical School) and Steven Heymsfield (Pennington Biomedical Research Center) for critical review of the manuscript.

REFERENCES

1. Maclean PS, Bergouignan A, Cornier MA, et al. Biology's response to dieting: the impetus for weight regain. *Am J Physiol Regul Integr Comp Physiol* 2011;301(3):R581-600. doi: 10.1152/ajpregu.00755.2010 [published Online First: 2011/06/17]
2. Leibel RL, Rosenbaum M, Hirsch J. Changes in energy expenditure resulting from altered body weight. *N Engl J Med* 1995;332(10):621-8.
3. Ludwig DS. The glycemic index: physiological mechanisms relating to obesity, diabetes, and cardiovascular disease. *JAMA* 2002;287(18):2414-23.
4. Ludwig DS, Friedman MI. Increasing adiposity: consequence or cause of overeating? *Jama* 2014;311(21):2167-8. doi: 10.1001/jama.2014.4133
5. Taubes G. The science of obesity: what do we really know about what makes us fat? An essay by Gary Taubes. *BMJ* 2013;346:f1050. doi: 10.1136/bmj.f1050
6. Ludwig DS, Ebbeling CB. The Carbohyrate-Insulin Model of obesity: beyond “calories in, calories out”. *JAMA Internal Medicine* 2018;In Press
7. Ludwig DS. Lowering the Bar on the Low-Fat Diet. *Jama* 2016;316(20):2087-88. doi: 10.1001/jama.2016.15473 [published Online First: 2016/09/30]
8. Hall KD. A review of the carbohydrate-insulin model of obesity. *Eur J Clin Nutr* 2017;71(3):323-26. doi: 10.1038/ejcn.2016.260 [published Online First: 2017/01/12]
9. Hall KD, Guo J. Obesity Energetics: Body Weight Regulation and the Effects of Diet Composition. *Gastroenterology* 2017;152(7):1718-27 e3. doi: 10.1053/j.gastro.2017.01.052 [published Online First: 2017/02/15]
10. Howell S, Kones R. "Calories in, calories out" and macronutrient intake: The Hope, Hype, and Science of Calories. *Am J Physiol Endocrinol Metab* 2017:ajpendo 00156 2017. doi: 10.1152/ajpendo.00156.2017 [published Online First: 2017/08/03]
11. Schwartz MW, Seeley RJ, Zeltser LM, et al. Obesity Pathogenesis: An Endocrine Society Scientific Statement. *Endocrine Reviews* 2017;38:1-30.
12. Bosy-Westphal A, Hagele F, Nas A. Impact of dietary glycemic challenge on fuel partitioning. *Eur J Clin Nutr* 2017;71(3):327-30. doi: 10.1038/ejcn.2016.230 [published Online First: 2016/12/03]
13. Owen OE, Caprio S, Reichard GA, Jr., et al. Ketosis of starvation: a revisit and new perspectives. *Clin Endocrinol Metab* 1983;12(2):359-79. [published Online First: 1983/07/01]
14. Vazquez JA, Adibi SA. Protein sparing during treatment of obesity: ketogenic versus nonketogenic very low calorie diet. *Metabolism* 1992;41(4):406-14.
15. Yang MU, Van Itallie TB. Composition of weight lost during short-term weight reduction. Metabolic responses of obese subjects to starvation and low-calorie ketogenic and nonketogenic diets. *J Clin Invest* 1976;58(3):722-30. doi: 10.1172/JCI108519 [published Online First: 1976/09/01]
16. Phinney SD, Bistrian BR, Wolfe RR, et al. The human metabolic response to chronic ketosis without caloric restriction: physical and biochemical adaptation. *Metabolism* 1983;32(8):757-68. [published Online First: 1983/08/01]
17. Ludwig DS, Ebbeling CB. The Carbohydrate-Insulin Model of Obesity: Beyond "Calories In, Calories Out". *JAMA Intern Med* 2018 doi: 10.1001/jamainternmed.2018.2933 [published Online First: 2018/07/05]

18. Ebbeling CB, Klein GL, Luoto PK, et al. A randomized study of dietary composition during weight-loss maintenance: Rationale, study design, intervention, and assessment. *Contemp Clin Trials* 2018;65:76-86. doi: 10.1016/j.cct.2017.12.004 [published Online First: 2017/12/14]
19. Wong JMW, Bielak L, Eddy RG, et al. An academia-industry partnership for planning and executing a community-based feeding study. *Curr Dev Nutr* 2018;In press
20. Ebbeling CB, Leidig MM, Feldman HA, et al. Effects of a low-glycemic load vs low-fat diet in obese young adults: a randomized trial. *Jama* 2007;297(19):2092-102.
21. Hron BM, Ebbeling CB, Feldman HA, et al. Relationship of insulin dynamics to body composition and resting energy expenditure following weight loss. *Obesity (Silver Spring)* 2015;23(11):2216-22. doi: 10.1002/oby.21213
22. Wong WW, Roberts SB, Racette SB, et al. The doubly labeled water method produces highly reproducible longitudinal results in nutrition studies. *J Nutr* 2014;144(5):777-83. doi: 10.3945/jn.113.187823 [published Online First: 2014/02/14]
23. 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(5):905-13. doi: 10.1093/ajcn/45.5.905 [published Online First: 1987/05/01]
24. Ravussin E, Harper IT, Rising R, et al. Energy expenditure by doubly labeled water: validation in lean and obese subjects. *Am J Physiol* 1991;261(3 Pt 1):E402-9. doi: 10.1152/ajpendo.1991.261.3.E402 [published Online First: 1991/09/01]
25. 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(5):381-91. [published Online First: 1986/09/01]
26. Sanchez-Pena MJ, Marquez-Sandoval F, Ramirez-Anguiano AC, et al. Calculating the metabolizable energy of macronutrients: a critical review of Atwater's results. *Nutr Rev* 2017;75(1):37-48. doi: 10.1093/nutrit/nuw044 [published Online First: 2016/12/16]
27. Troiano RP, Berrigan D, Dodd KW, et al. Physical activity in the United States measured by accelerometer. *Med Sci Sports Exerc* 2008;40(1):181-8. doi: 10.1249/mss.0b013e31815a51b3 [published Online First: 2007/12/20]
28. Chomistek AK, Yuan C, Matthews CE, et al. Physical Activity Assessment with the ActiGraph GT3X and Doubly Labeled Water. *Med Sci Sports Exerc* 2017;49(9):1935-44. doi: 10.1249/MSS.0000000000001299 [published Online First: 2017/04/19]
29. Bender R, Lange S. Adjusting for multiple testing--when and how? *J Clin Epidemiol* 2001;54(4):343-9. [published Online First: 2001/04/12]
30. Seaman SR, White IR. Review of inverse probability weighting for dealing with missing data. *Stat Methods Med Res* 2013;22(3):278-95. doi: 10.1177/0962280210395740 [published Online First: 2011/01/12]
31. Juraschek SP, Miller ER, 3rd, Appel LJ, et al. Effects of dietary carbohydrate on 1,5-anhydroglucitol in a population without diabetes: results from the OmniCarb trial. *Diabet Med* 2017;34(10):1407-13. doi: 10.1111/dme.13391 [published Online First: 2017/06/03]
32. Holsen L, Cerit H, Lennerz B, et al. Hypothalamic and Nucleus Accumbens Cerebral Blood Flow Vary as a Function of Long-Term Carbohydrate-To-Fat Ratio Diets. *Neuropsychopharmacology (Abstract, American College of Neuropsychopharmacology 56th Annual Meeting, Palm Springs)* 2017;42:S150-1.
33. Shimy K, Feldman HA, Klein GL, et al. A Mechanistic Examination of Dietary Composition on Metabolic Fuel Availability. *Hormone Research in Paediatrics (Abstract, 10th*

International Joint Meeting of Pediatric Endocrinology; Washington DC) 2017;88(Suppl 1):S337.
34. Ebbeling CB, Swain JF, Feldman HA, et al. Effects of dietary composition on energy expenditure during weight-loss maintenance. *JAMA* 2012;307(24):2627-34. doi: 1199154 [pii]10.1001/jama.2012.6607 [published Online First: 2012/06/28]
35. 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(2):324-33. doi: 10.3945/ajcn.116.133561
36. Ludwig DS, Ebbeling CB. Raising the bar on the low-carbohydrate diet. *Am J Clin Nutr* 2016;104(5):1487-88. doi: 10.3945/ajcn.116.142182 [published Online First: 2016/11/03]
37. Pawlak DB, Kushner JA, Ludwig DS. Effects of dietary glycaemic index on adiposity, glucose homoeostasis, and plasma lipids in animals. *Lancet* 2004;364(9436):778-85.
38. Chaput JP, Tremblay A, Rimm EB, et al. A novel interaction between dietary composition and insulin secretion: effects on weight gain in the Quebec Family Study. *Am J Clin Nutr* 2008;87(2):303-9.
39. Astley CM, Todd JN, Salem RM, et al. Genetic Evidence That Carbohydrate-Stimulated Insulin Secretion Leads to Obesity. *Clin Chem* 2018;64(1):192-200. doi: 10.1373/clinchem.2017.280727 [published Online First: 2018/01/04]
40. Pittas AG, Das SK, Hajduk CL, et al. A low-glycemic load diet facilitates greater weight loss in overweight adults with high insulin secretion but not in overweight adults with low insulin secretion in the CALERIE Trial. *Diabetes Care* 2005;28(12):2939-41. doi: 28/12/2939 [pii] [published Online First: 2005/11/25]
41. Gardner CD, Trepanowski JF, Del Gobbo LC, et al. Effect of Low-Fat vs Low-Carbohydrate Diet on 12-Month Weight Loss in Overweight Adults and the Association With Genotype Pattern or Insulin Secretion: The DIETFITS Randomized Clinical Trial. *Jama* 2018;319(7):667-79. doi: 10.1001/jama.2018.0245 [published Online First: 2018/02/22]
42. Larsen TM, Dalskov SM, van Baak M, et al. Diets with high or low protein content and glycemic index for weight-loss maintenance. *N Engl J Med* 2010;363(22):2102-13. doi: 10.1056/NEJMoa1007137 [published Online First: 2010/11/26]
43. Qi Q, Chu AY, Kang JH, et al. Sugar-sweetened beverages and genetic risk of obesity. *N Engl J Med* 2012;367(15):1387-96. doi: 10.1056/NEJMoa1203039 [published Online First: 2012/09/25]
44. Levine JA, Eberhardt NL, Jensen MD. Role of nonexercise activity thermogenesis in resistance to fat gain in humans. *Science* 1999;283(5399):212-4. [published Online First: 1999/01/08]
45. 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(5):R1259-66. doi: 10.1152/ajpregu.00397.2011 [published Online First: 2011/09/16]
46. 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(1):R79-88. doi: 10.1152/ajpregu.00053.2009 [published Online First: 2009/11/06]
47. 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(12):3579-86. doi: 10.1172/JCI25977 [published Online First: 2005/12/03]

48. 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(1):R183-92. doi: 10.1152/ajpregu.00474.2002 [published Online First: 2003/03/01]
49. Cummings DE, Foster-Schubert KE, Overduin J. Ghrelin and energy balance: focus on current controversies. *Curr Drug Targets* 2005;6(2):153-69. [published Online First: 2005/03/22]
50. Mihalache L, Gherasim A, Nita O, et al. Effects of ghrelin in energy balance and body weight homeostasis. *Hormones (Athens)* 2016;15(2):186-96. doi: 10.14310/horm.2002.1672 [published Online First: 2016/07/05]
51. Ye Z, Liu G, Guo J, et al. Hypothalamic endoplasmic reticulum stress as a key mediator of obesity-induced leptin resistance. *Obes Rev* 2018;19(6):770-85. doi: 10.1111/obr.12673 [published Online First: 2018/03/08]
52. Crujeiras AB, Goyenechea E, Abete I, et al. Weight regain after a diet-induced loss is predicted by higher baseline leptin and lower ghrelin plasma levels. *J Clin Endocrinol Metab* 2010;95(11):5037-44. doi: 10.1210/jc.2009-2566 [published Online First: 2010/08/20]
53. Erez G, Tirosh A, Rudich A, et al. Phenotypic and genetic variation in leptin as determinants of weight regain. *Int J Obes (Lond)* 2011;35(6):785-92. doi: 10.1038/ijo.2010.217 [published Online First: 2010/11/03]
54. Mavri A, Stegnar M, Sabovic M. Do baseline serum leptin levels predict weight regain after dieting in obese women? *Diabetes Obes Metab* 2001;3(4):293-6. [published Online First: 2001/08/25]
55. Sacks FM, Bray GA, Carey VJ, et al. Comparison of weight-loss diets with different compositions of fat, protein, and carbohydrates. *N Engl J Med* 2009;360(9):859-73. doi: 10.1056/NEJMoa0804748
56. Lam YY, Ravussin E. Analysis of energy metabolism in humans: A review of methodologies. *Mol Metab* 2016;5(11):1057-71. doi: 10.1016/j.molmet.2016.09.005 [published Online First: 2016/11/08]
57. Schwarz JM, Neese RA, Turner S, et al. Short-term alterations in carbohydrate energy intake in humans. Striking effects on hepatic glucose production, de novo lipogenesis, lipolysis, and whole-body fuel selection. *J Clin Invest* 1995;96(6):2735-43. doi: 10.1172/JCI118342 [published Online First: 1995/12/01]
58. Parks EJ, Krauss RM, Christiansen MP, et al. Effects of a low-fat, high-carbohydrate diet on VLDL-triglyceride assembly, production, and clearance. *J Clin Invest* 1999;104(8):1087-96. doi: 10.1172/JCI6572 [published Online First: 1999/10/19]
59. Bueno NB, de Melo IS, de Oliveira SL, et al. Very-low-carbohydrate ketogenic diet v. low-fat diet for long-term weight loss: a meta-analysis of randomised controlled trials. *The British journal of nutrition* 2013;110(7):1178-87. doi: 10.1017/S0007114513000548 [published Online First: 2013/05/09]
60. Johnston BC, Kanters S, Bandayrel K, et al. Comparison of weight loss among named diet programs in overweight and obese adults: a meta-analysis. *Jama* 2014;312(9):923-33. doi: 10.1001/jama.2014.10397 [published Online First: 2014/09/04]
61. Mancini JG, Filion KB, Atallah R, et al. Systematic Review of the Mediterranean Diet for Long-Term Weight Loss. *Am J Med* 2016;129(4):407-15 e4. doi: 10.1016/j.amjmed.2015.11.028 [published Online First: 2016/01/02]

62. Mansoor N, Vinknes KJ, Veierod MB, et al. Effects of low-carbohydrate diets v. low-fat diets on body weight and cardiovascular risk factors: a meta-analysis of randomised controlled trials. *The British journal of nutrition* 2016;115(3):466-79. doi: 10.1017/S0007114515004699
63. Sackner-Bernstein J, Kanter D, Kaul S. Dietary Intervention for Overweight and Obese Adults: Comparison of Low-Carbohydrate and Low-Fat Diets. A Meta-Analysis. *PLoS One* 2015;10(10):e0139817. doi: 10.1371/journal.pone.0139817
64. Tobias DK, Chen M, Manson JE, et al. Effect of low-fat diet interventions versus other diet interventions on long-term weight change in adults: a systematic review and meta-analysis. *Lancet Diabetes Endocrinol* 2015;3(12):968-79. doi: 10.1016/S2213-8587(15)00367-8 [published Online First: 2015/11/04]

Table 1. Dietary Energy and Macronutrient Composition for Test Diets, Daily Averages, Target *vs*. Actual (per 2,000 kcal)

Variable	HI		MOD		LO	
	Target	Actual [a]	Target	Actual [a]	Target	Actual [a]
Energy (kcal)	2000	2001	2000	2001	2000	2001
Carbohydrate (g)	300	304.8	200	204.7	100	104.9
Carbohydrate (%) [b]	60%	59.2%	40%	39.7%	20%	20.3%
Fat (g)	44	47.8	89	91.7	133	136.7
Fat (%) [b]	20%	20.9%	40%	40.1%	60%	59.6%
Protein (g)	100	102.3	100	103.8	100	103.5
Protein (%) [b]	20%	19.9%	20%	20.2%	20%	20.1%
Glycemic load (g)	–	134.7	–	80.5	–	28.4
Fiber (g)	35	32.7	30	27.9	25	22.2
FQ [c]	0.90	0.90	0.85	0.85	0.79	0.79

[a] Actual values were calculated using Food Processor Nutrition Analysis Software (ESHA Research Inc., Salem, OR).
[b] Percent of energy from macronutrients takes into account digestibility for some foods.
[c] Food Quotient (FQ) was calculated using the equation of Black et al [25].
FQ = [Carbohydrate (%) × 1.00] + [Fat (%) × 0.71] + [Protein (%) × 0.81]

Table 2. Baseline (Pre-Weight-Loss) Characteristics of Study Participants by Dietary Intervention Group (N=164)

Characteristic	HI (n=54)	MOD (n=53)	LO (n=57)
Sex, No. (%)			
Male	12 (22.2)	17 (32.1)	20 (35.1)
Female	42 (77.8)	36 (67.9)	37 (64.9)
Ethnicity, No. (%) [a]			
Hispanic	8 (14.8)	7 (13.2)	10 (17.5)
Race, No. (%) [a]			
White	44 (81.5)	41 (77.4)	43 (75.4)
Black	4 (7.4)	7 (13.2)	6 (10.5)
Asian	2 (3.7)	2 (3.8)	1 (1.8)
Unknown / Other	4 (7.4)	3 (5.7)	7 (12.3)
Age, mean (SD), y	39.8 (15.1)	37.3 (14.9)	37.1 (13.3)
Weight, mean (SD), kg	88.4 (16.6)	94.8 (19.7)	91.2 (17.9)
Weight loss, mean (SD), (% of baseline)	10.6 (1.7)	10.5 (1.8)	10.3 (1.6)
Height, mean (SD), cm	166.7 (9.0)	167.9 (11.2)	168.5 (9.8)
BMI, mean (SD), kg/m^2	31.7 (4.3)	33.5 (5.3)	32.0 (4.8)
TEE, mean (SD), kcal/kg/d	32.8 (4.5)	32.1 (5.5)	34.4 (6.3)
REE, mean (SD), kcal/kg/d	18.8 (1.9)	18.6 (2.3)	18.7 (1.8)
Physical Activity, mean (SD)			
Total PA, counts/d, thousands [b]	510.0 (172.1)	509.1 (146.4)	525.2 (182.4)
MVPA, min/d [c]	26.4 (19.4)	27.7 (19.5)	29.7 (19.8)
Sedentary time, min/d [d]	567.2 (91.0)	591.8 (105.4)	566.1 (97.1)
Ghrelin, mean (SD), pg/mL	648.6 (293.7)	530.0 (281.0)	558.2 (288.3)
Leptin, mean (SD), ng/mL	31.3 (16.4)	30.6 (19.0)	27.5 (16.4)
Body Composition, mean (SD)			
Body fat mass (% of total mass)	41.4 (5.5)	41.1 (7.3)	40.0 (5.8)
Lean body mass (% of total mass) [e]	56.0 (5.3)	56.3 (7.0)	57.3 (5.6)

[a] Ethnicity and race were determined by self-report using fixed categories.
[b] Average accelerometer wear time (mean, SD) was 14.8 (1.3) hours per day.
[c] Moderate- to vigorous-intensity physical activity was quantified using count thresholds of Troiano *et al* [27].
[d] Sedentary time included intervals with <100 counts per minute [28].
[e] Lean body mass does not include bone mineral content.

Table 3. Energy Expenditure and Physical Activity, Per Protocol Analyses [a]

Variable	Dietary Intervention Group	N	Pre-Randomization, PWL, Mean (SE)	Change: AV[MID, END] – PWL		
				Mean (95% CI)	*P* between groups	*P* HI *vs.* LO
TEE, kcal/kg/d	HI	37	32.8 (0.6)	-1.1 (-2.3 to 0.1)	0.001	<0.001
	MOD	39	32.9 (0.6)	0.3 (-0.8 to 1.4)		
	LO	42	33.4 (0.6)	2.0 (0.9 to 3.0)		
REE, kcal/kg/d	HI	38	19.5 (0.2)	0.3 (-0.1 to 0.6)	0.20	0.09
	MOD	38	19.6 (0.2)	0.3 (0.0 to 0.7)		
	LO	43	19.5 (0.2)	0.6 (0.3 to 0.9)		
Total physical activity, counts/d, thousands [b]	HI	38	484.6 (19.7)	-30.3 (-60.0 to -0.5)	0.21	0.36
	MOD	39	506.6 (18.5)	-46.1 (-74.1 to -18.1)		
	LO	42	504.7 (17.8)	-11.6 (-38.2 to 14.9)		
MVPA, min/d [c]	HI	38	33.4 (2.4)	-4.2 (-7.3 to -1.0)	0.06	0.09
	MOD	39	33.5 (2.2)	-5.2 (-8.1 to -2.2)		
	LO	42	28.3 (2.1)	-0.5 (-3.3 to 2.3)		
Sedentary time, min/d [d]	HI	38	604.4 (12.8)	4.1 (-15.8 to 24.0)	0.40	0.92
	MOD	39	588.7 (12.0)	20.4 (1.8 to 39.1)		
	LO	42	594.6 (11.5)	5.5 (-12.1 to 23.2)		
Ghrelin, pg/mL, % change [e]	HI	38	627.5 (19.3)	-6.0 (-10.2 to -1.6)	0.02	0.006
	MOD	38	654.3 (18.6)	-8.2 (-12.0 to -4.3)		
	LO	43	634.8 (17.0)	-13.6 (-17.0 to -10.1)		
Leptin, ng/mL, % change [e]	HI	38	9.5 (0.6)	46.69 (33.2 to 61.5)	0.01	0.006
	MOD	38	8.8 (0.5)	42.57 (30.0 to 56.3)		
	LO	43	9.6 (0.6)	22.1 (12.0 to 33.1)		

[a] Means and changes were constructed from repeated-measures analysis of variance, adjusted for sex, age, race, ethnicity, percentage weight loss during Run-In, and pre-weight loss values of body-mass index, percentage lean mass, and the outcome variable.

[b] Total activity was quantified based on triaxial counts, representing a composite vector magnitude of three orthogonal planes (vertical, anteroposterior, mediolateral).[28] Average accelerometer wear time (mean, SD) was 14.9 (1.2) hours per day.

[c] Moderate-to-vigorous intensity physical activity was quantified using vertical axis count thresholds of Troiano et al [27].

[d] Sedentary time was defined as <100 counts per minute for vertical axis counts [28].

[e] Hormone levels were log-transformed for analysis. Adjusted mean ± SE are retransformed to natural units: exp(mean log) ± exp(mean log)×(exp(SE log)–1). Change is expressed as a percentage: 100% × (exp(change in log)–1).

FIGURE LEGENDS

Figure 1. Study Design (PWL, Post-Weight Loss; MID, Midpoint of Test Phase; END, End of Test Phase)

Figure 2. Participant Flow

Figure 3. Biomeasures of Compliance. A) 1,5-Anhydroglucitol, mean baseline 17 µg/mL; B) Triglycerides, mean baseline 78 mg/dL (retransformed); C) HDL-cholesterol, men baseline 48 mg/dL. Data expressed as mean ± SE. (o) High-carbohydrate; (x) Moderate-carbohydrate, (•) Low-carbohydrate

Figure 4. Change in TEE (kcal/kg/d) During the Test Phase in the Intention-to-Treat (A) and Per Protocol (B) Analyses. Data expressed as mean ± SE. (o) High-carbohydrate; (x) Moderate-carbohydrate, (•) Low-carbohydrate. (See text for results normalized to average post-weight loss body weight of 82 kg.)

Figure 5. Effect Modification by Baseline INS-30 (Per Protocol Analysis, N=118). TEE was analyzed in kcal/kg/d and normalized to average post-weight loss body weight (82 kg) for reporting in kcal/d. (o) High-carbohydrate, (x) Moderate-carbohydrate, (•) Low-carbohydrate; (—) mean.

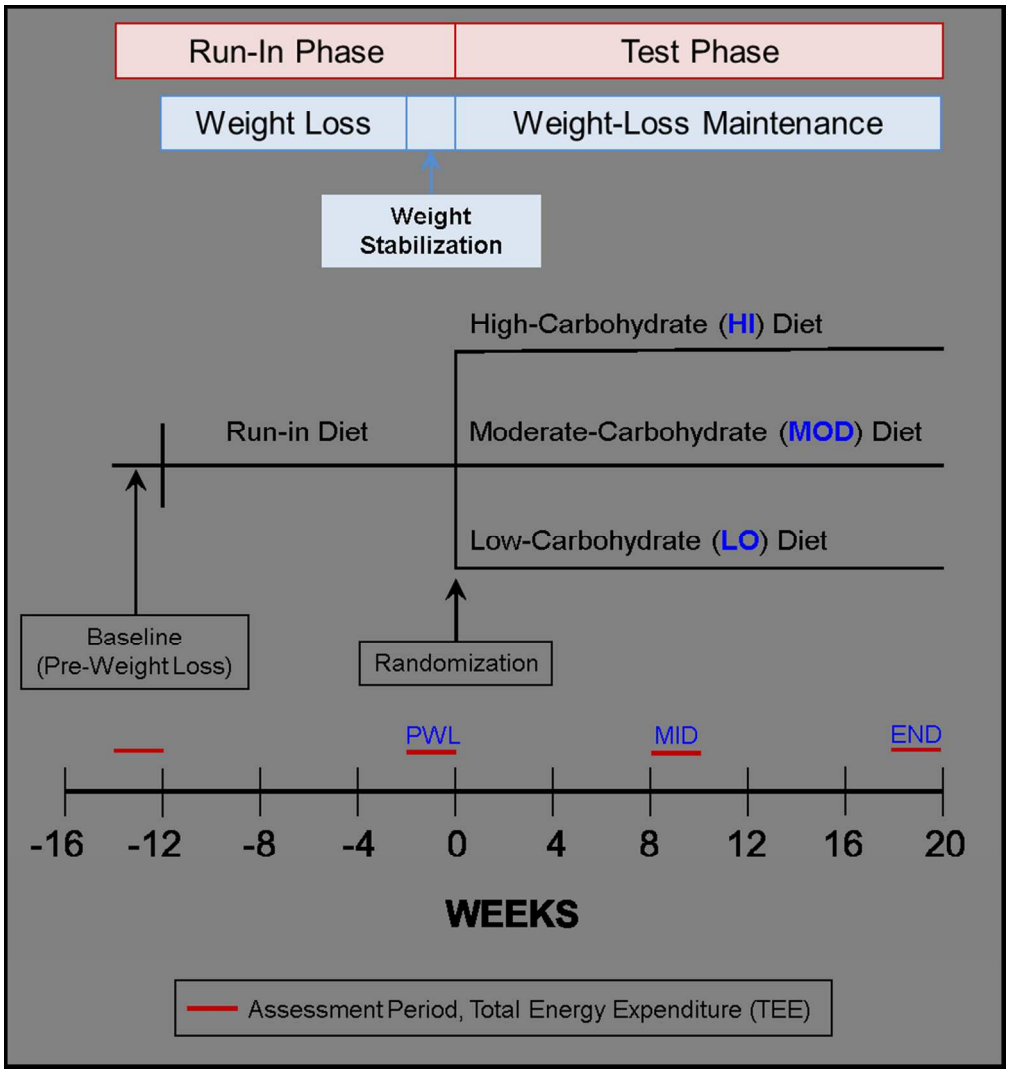

Figure 1. Study Design (PWL, Post-Weight Loss; MID, Midpoint of Test Phase; END, End of Test Phase)

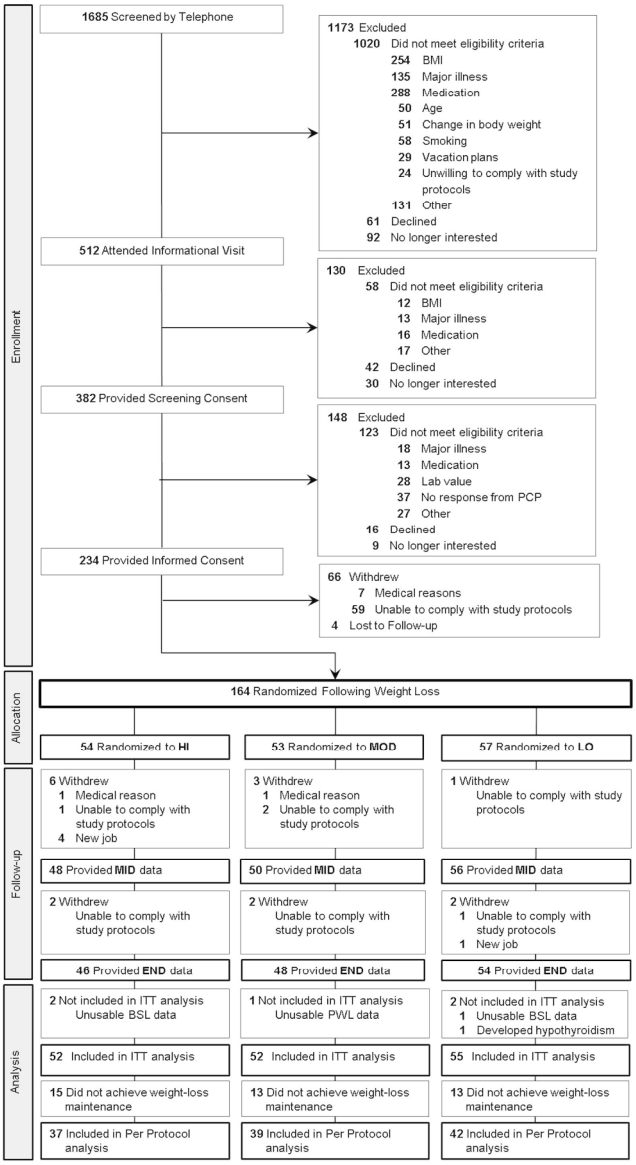

Figure 2. Participant Flow

190x254mm (300 x 300 DPI)

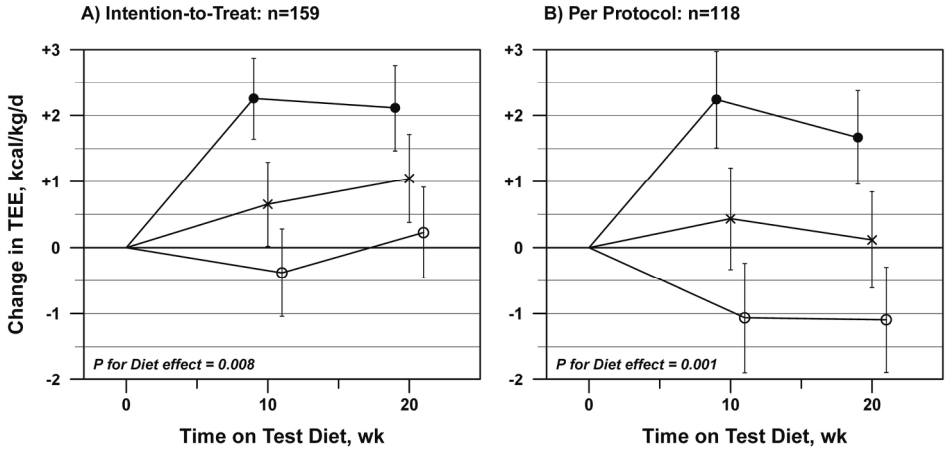

Figure 4. Change in TEE (kcal/kg/d) During the Test Phase in the Intention-to-Treat (A) and Per Protocol (B) Analyses. Data expressed as mean ± SE. (o) High-carbohydrate; (x) Moderate-carbohydrate, (•) Low-carbohydrate. (See text for results normalized to average post-weight loss body weight of 82 kg.)

94x45mm (600 x 600 DPI)

Supplementary Online Content

Ebbeling CB, Feldman HA, Klein GL, Wong JMW, Bielak L, Steltz SK, Luoto PK, Wong WW, Wolfe RR, Ludwig, DS. Effects of a Low-Carbohydrate Diet on Energy Expenditure During Weight-Loss Maintenance: A Randomized Feeding Study

Table of Contents

eTable 1. Participant 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. • 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).
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). • 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.

eTable 2. Comparison of Covariates between Participants Designated for Inclusion *vs.* Exclusion in the Primary Per Protocol Analyses

Characteristic	Included (N=122)	Excluded (N=42) [a]	*P* Included *vs.* Excluded
Sex, No. (%)			
Male	35 (28.7)	14 (33.3)	0.56
Female	87 (71.3)	28 (66.7)	
Ethnicity, No. (%) [b]			
Hispanic	20 (16.4)	5 (11.9)	0.62
Race, No. (%) [b]			
White	94 (77.1)	34 (81.0)	
Black	12 (9.8)	5 (11.9)	0.82
Asian	4 (3.3)	1 (2.4)	
Unknown / Other	12 (9.8)	2 (4.8)	
Age, mean (SD), y	39.3 (14.0)	34.4 (15.0)	0.05
Weight loss, mean (SD), (% of baseline)	10.4 (1.5)	10.7 (2.0)	0.23
BMI, mean (SD), kg/m^2	32.0 (4.3)	33.5 (6.0)	0.08
TEE, mean (SD), kcal/kg/d	33.3 (5.8)	32.4 (4.7)	0.37
Lean body mass (% of total mass) [c]	56.6 (6.3)	56.6 (5.1)	>0.99

[a] Participants not maintaining weight loss within ±2 kg of the post-weight loss anchor were excluded from the Per Protocol analyses.
[b] Ethnicity and race were determined by self-report using fixed categories.
[c] Lean body mass does not include bone mineral content.

eTable 3. Potential Effect of Imprecision in Estimating Food Quotient (FQ) on Calculated TEE[a]

FQ	Calculated TEE kcal/d [b]	Difference from TEE Calculated using FQ=0.79 (low-carbohydrate diet)	Sensitivity (%) for 0.01 shift in FQ [c]
0.75	3207	131	1.07
0.76	3173	97	1.05
0.77	3140	64	1.04
0.78	3108	31	1.02
0.79	**3077**	**0**	**0.00**
0.80	3046	-31	1.00
0.81	3016	-61	0.98
0.82	2987	-90	0.97
0.83	2958	-118	0.95

[a] These are data for one sample participant on the low-carbohydrate diet whose calculated TEE was 3077 kcal per day (rCO_2 = 22.7).
[b] Total energy expenditure (TEE) was calculated from rCO_2 using the equation of Ravussin et al.
[c] According to the Ravussin equation: TEE = constant × (1.2321 + 3.815/FQ).
It follows that: $\partial \log TEE/\partial FQ = (1/(1.2321 + 3.815/FQ)) \times -3.815/FQ^2 = -1.0085$ for FQ=0.79.
An increase of 0.01 in FQ thus results in a change of –0.010085 in logTEE. That's a relative change of 100% × (exp(–0.010085)–1), which comes out to –1% almost exactly.

Reference

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-E409.

eTable 4. Effects of Noncompliance on Estimated Food Quotient (FQ) for the Low-Carbohydrate Diet[a]

Noncompliance (%)	Energy (kcal)	Carbohydrate (g)	Fat (g)	Protein (g)	Carbohydrate (%)	Fat (%)	Protein (%)	Calculated FQ [b]
0	2000	100	133.3	100	20.0	60.0	20	0.79
5	2100	115	135.5	105	21.9	58.1	20	0.79
10	2200	130	137.7	110	23.6	56.3	20	0.80
15	2300	145	139.9	115	25.2	54.8	20	0.80
20	2400	160	142.1	120	26.7	53.3	20	0.81
25	2500	175	144.3	125	28.0	52.0	20	0.81
30	2600	190	146.5	130	29.2	50.7	20	0.81
35	2700	205	148.7	135	30.4	49.6	20	0.82
40	2800	220	150.9	140	31.4	48.5	20	0.82

[a] *Question*: How much non-compliance would need to occur on the Low-Carbohydrate Diet to reach the FQ threshold of 0.82 (above which the primary outcome in the Per Protocol analysis would lose statistically significance), assuming that the extra food consumed contained a macronutrient distribution similar to the High-Carbohydrate Diet? For a 2,000-kcal diet, noncompliance of 5% (100 kcal) would equate to an additional 15 g carbohydrate, 5 g protein, and 2.2.g fat.

[b] Food Quotient (FQ) was calculated using the equation of Black et al.
FQ = [Carbohydrate (%) × 1.00] + [Fat (%) × 0.71] + [Protein (%) × 0.81]

Reference

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.

eTable 5. Study Outcomes, Intention-to-Treat Analyses[a]

Variable	Dietary Intervention Group	N	Pre-Randomization PWL, Mean (SE)	Change: AV[MID, END] – PWL		
				Mean (95% CI)	*P* between groups	*P* HI *vs.* LO
TEE, kcal/kg/d	HI	52	32.2 (0.6)	-0.1 (-1.1 to 1.0)	0.008	0.002
	MOD	52	31.7 (0.6)	0.9 (-0.2 to 1.9)		
	LO	55	32.9 (0.6)	2.2 (1.2 to 3.2)		
REE, kcal/kg/d	HI	54	19.5 (0.1)	0.4 (0.1 to 0.7)	0.49	0.24
	MOD	51	19.3 (0.2)	0.6 (0.3 to 0.8)		
	LO	56	19.6 (0.1)	0.6 (0.4 to 0.9)		
Total PA, counts/d, thousands [b]	HI	54	480.3 (16.6)	-28.2 (-53.9 to -2.6)	0.16	0.23
	MOD	52	481.7 (17.3)	-40.6 (-66.0 to -15.3)		
	LO	55	488.4 (17.0)	-6.9 (-31.1 to 17.2)		
MVPA, min/d [c]	HI	54	31.9 (1.9)	-3.6 (-6.3 to -0.9)	0.09	0.15
	MOD	52	31.4 (2.0)	-4.8 (-7.5 to -2.2)		
	LO	55	27.4 (2.0)	-0.9 (-3.4 to 1.6)		
Sedentary time, min/d [d]	HI	54	595.8 (10.5)	9.7 (-6.6 to 26.1)	0.16	0.29
	MOD	52	589.2 (11.0)	19.1 (3.0 to 35.3)		
	LO	55	598.6 (10.8)	-2.4 (-17.7 to 13.0)		
Ghrelin, pg/mL, % change [e]	HI	54	606.1 (16.2)	-5.0 (-8.5 to -1.3)	0.02	0.004
	MOD	51	649.2 (18.1)	-8.9 (-12.2 to -5.4)		
	LO	56	613.16 (16.7)	-11.9 (-8.5 to -1.3)		
Leptin, ng/mL, % change [e]	HI	54	10.2 (0.5)	33.7 (21.6 to 47.0)	0.08	0.06
	MOD	51	9.5 (0.5)	35.1 (22.9 to 48.5)		
	LO	56	10.5 (0.6)	18.0 (7.9 to 29.1)		

[a] Means and changes were constructed from repeated-measures analysis of variance, adjusted for sex, age, race, ethnicity, percentage weight loss during Run-In, and pre-weight loss values of body-mass index, percentage lean mass, and the outcome variable.

[b] Total activity was quantified based on triaxial counts, representing a composite vector magnitude of three orthogonal planes (vertical, anteroposterior, mediolateral).[22] Average accelerometer wear time (mean, SD) was 14.8 (1.3) hours per day.

[c] Moderate-to-vigorous intensity physical activity was quantified using vertical axis count thresholds of Troiano et al.

[d] Sedentary time was defined as <100 counts per minute for vertical axis counts, according to Chomistek et al.

[e] Hormone levels were log-transformed for analysis. Adjusted mean ± SE are retransformed to natural units: exp(mean log) ± exp(mean log)×(exp(SE log)–1). Change is expressed as a percentage: 100% × (exp(change in log)–1).

Reference

Troiano RP, Berrigan D, Dodd KW, Masse LC, Tilert T, and McDowell M. Physical activity in the United States measured by accelerometer. *Med Sci Sports Exerc.* 2008;40(1):181-8.

Chomistek AK, Yuan C, Matthews CE, Troiano RP, Bowles HR, Rood J, et al. Physical Activity Assessment with the ActiGraph GT3X and Doubly Labeled Water. *Med Sci Sports Exerc.* 2017;49(9):1935-44.

Dietary Composition and Energy Expenditure during Weight-Loss Maintenance

Protocol Amendment History

Final Pre-Analysis Protocol available at Open Science Framework: https://osf.io/t7abx/

Prepared: 2014.06.26
Updated: 2017.09.29

Current Version

Protocol: 2017.06.14
Consent (Screening): 2016.04.29
Consent (Trial): 2016.07.06

Dates			Amendment Summary
Amendment	**Protocol Version**	**Consent Version**	
2017.09.06	2017.06.14	**Screening:** 2016.04.29 **Trial:** 2016.07.06	- Submitted Final Data Analysis Plan (version 2017.06.14) to IRB, prior to receiving the primary outcome data and breaking the randomization blind. Key changes: Expressed TEE per kg body weight; Specified Week 0 (PWL, *time of randomization*), rather than the start of the baseline (BSL) period as the anchor for evaluating time course. - Clarified study procedures (No changes to study design or procedures) - Clarified protocol sections: Assessment of Outcomes and Statistical Methods for consistency in wording with the Data Analysis Plan and Methods Manuscript (No changes to study design)
2016.07.07	2016.07.06	**Screening:** 2016.04.29 **Trial:** 2016.07.06	- Added ancillary study to the ongoing trial to assess implicit, explicit, and internalized weight bias. The Research Team will be conducting exploratory analyses to provide hypothesis-generating data to inform the design of future studies. In the Consent Form, the instruments (i.e. questionnaires) to assess weight bias will be optional to subjects participating in the main study. There are 4 instruments that will be completed electronically with a BCH iPad and keyboard. The instruments are as follows: Implicit Associations Test (IAT), Obese Persons Trait Survey (OPTS), Weight Bias Internalization Scale (WBIS), and Beliefs About Obese Persons Scale (BAOP). - The Research Team will ask for each participant's permission to take a full face photographic image for uploading to their profile in the Study Portal (HIPAA secure patient monitoring Website). The Consent Forms have been modified accordingly. - Modified the "telephone screening for provisionally eligibility" form to clarify the wording of questions we ask to potentially interested participants. - Updated the inclusion criterion of "weight ≤425lb (193kg)" based on the upper limit of the DXA instrumentation to now be "weight ≤350lb (159kg)" based on the upper weight limit of the cycle ergometer. Of the enrolled participants to date, we have not enrolled anyone who met eligibility criteria and was also ≥ 350lb.

Dates			Amendment Summary
Amendment	Protocol Version	Consent Version	
2016.05.06	2016.04.29	**Screening:** 2016.04.29 **Trial:** 2016.04.29	- Clarifications (minor) to the Screening Consent Form - Clarifications (minor) to the Trial Consent Form - Establish New Study Feeding Site at Assabet Valley Regional Technical High School (AV) - Revisions to Consent Forms, Recruitment Material, Screening Scripts and Case Report Forms to accommodate new study feeding site at AV - Addition of a Palatability Questionnaire - Modifications to main study protocol
2015.08.03	2015.07.30	**Screening:** 2015.07.30 **Trial:** 2015.07.30	- Added a questionnaire to collect information about participant health, medical symptoms, and fitness level in advance of exercise training - Will link the Wi-Fi scales to a secure patient monitoring website (SetPoint Health) to monitor subject weights. SetPoint Health website will also be used to track food intake on all subjects - Clarified what food will be provided on each of the 3 test diets - Editorial changes to the Consent Form
2015.04.17	2015.04.15	**Screening:** 2015.04.15 **Trial:** 2015.04.15	- Clarified instructions regarding eating more, if hungry, during Ad Libitum (Free Eating) Phase. The protocol, Trial Consent Form, and telephone script were updated to reflect this clarification - Updated the Screening Consent Form to include a previously approved change to recruit participants from the greater Framingham community
2015.03.23	2015.03.12	**Screening:** 2015.03.12 **Trial:** 2015.03.12	- Research Team will screen and enroll members of the greater Framingham community; these participants will be denoted as “community-based participants” and will receive the same compensation as non-residents, faculty, and staff - Recruitment materials included to recruit community-based participants - Third cohort added to reach 150 participant enrollment goal
2014.11.02	2014.10.28	**Screening:** 2014.06.26 **Trial:** 2014.06.26	- Revised diet plan - Safety Officer changed from Dr. Joseph Majzoub to Dr. Michael Agus - Changed wording for Pre-Randomization Assessment to "Post-Weight Loss Assessment" - No Consent Form changes were required.
	2014.06.26	**Screening:** 2014.06.26 **Trial:** 2014.06.26	