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

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ABSTRACT (FOR ONLINE PUBLICATION, PER APPENDIX 1 GUIDELINES)

Objective: To determine the effects of diets varying in carbohydrate-to-fat ratio on total energy expenditure (TEE).

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

Setting: Multi-institutional collaboration conducted at two sites.

Participants: Adults aged 18 to 65 years with BMI ≥25 kg/m^2. From 1,685 individuals who completed telephone screening, 164 were randomized to one of three test diets.

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) for 20 weeks. Test diets were controlled for protein and energy-adjusted to maintain weight loss within ±2 kg.

Main outcomes: The primary outcome was TEE, measured with doubly-labeled water, by Intention-to-Treat (ITT) analysis. Per Protocol analysis included participants who achieved weight-loss maintenance, potentially providing a more precise effect estimate. Main outcomes were pre-specified.

Results: TEE differed by diet in the ITT (n=162, *P*=0.002). Compared to the high-carbohydrate diet, change in TEE (mean, 95% CI) was +91 (-29 to +210) kcal/d greater on the moderate-carbohydrate diet and +209 (+91 to +326) kcal/d greater on the low-carbohydrate diet. In the Per Protocol analysis (n=120, *P*<0.001), the respective differences were +131 (-6 to +267) and +278 (+144 to +411) kcal/d. Among participants in the highest tertile of baseline (pre-weight-loss) insulin secretion, the difference between the low- *vs.* high-carbohydrate diet was +308 or +478

kcal/d (ITT or Per Protocol, respectively, $P<0.004$). Ghrelin, a hormone thought to lower energy expenditure, was significantly lower on the low- *vs.* high-carbohydrate diet ($P=0.004$).

Conclusions: Consistent with the Carbohydrate-Insulin Model, lowering dietary carbohydrate increased energy expenditure during weight-loss maintenance. This metabolic effect may improve the success of obesity treatment, especially among individuals with high insulin secretion.

Trial registration: ClinicalTrials.gov, NCT02068885

WHAT THIS PAPER ADDS (SUMMARY BOX, PER APPENDIX 2 GUIDELINES):

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 dietary 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 effectiveness of obesity treatment.

PRINT ABSTRACT (PER APPRENDIX 3 GUIDELINES)

Study question: Do diets differing in carbohydrate-to-fat ratio affect total energy expenditure (TEE) during weight-loss maintenance, as predicted by the Carbohydrate-Insulin Model of obesity?

Methods: The Framingham State Food Study, (FS)2, is a randomized-controlled feeding study conducted August 2014 to May 2017. Study participants included 164 adults aged 18 to 65 years with BMI ≥25 kg/m^2. Following 12±2% weight loss on the Run-In diet, participants were randomly assigned to high-, moderate-, or low-carbohydrate Test diets (60, 40, or 20% of total energy) for 20 weeks. Test diets were controlled for protein and energy-adjusted to maintain weight loss within ±2 kg. The primary outcome was TEE, measured with doubly labeled water, by Intention-to-Treat analysis (ITT). Per Protocol analysis included participants who achieved weight-loss maintenance, potentially providing a more precise effect estimate. Main outcomes were pre-specified.

Study answer and limitations: TEE differed by diet in the ITT (n=162, *P*=0.002). Compared to the high-carbohydrate diet, change in TEE (mean, 95% CI) was +91 (-29 to +210) kcal/d greater on the moderate-carbohydrate diet and +209 (+91 to +326) kcal/d greater on the low-carbohydrate diet. In the Per Protocol analysis (n=120, *P*<0.001), the respective differences were +131 (-6 to +267) and +278 (+144 to +411) kcal/d. Among participants in the highest tertile of baseline insulin secretion, the difference between the low- *vs.* high-carbohydrate diet was +308 or +478 kcal/d (ITT or Per Protocol, respectively, *P*<0.004). Ghrelin, a hormone thought to lower energy expenditure, was significantly lower on the low- *vs.* high-carbohydrate diet (*P*=0.004). Study limitations involve methodological issues in measurement of energy expenditure, non-compliance, and translation to the clinical setting.

What this study adds: These findings show that a low-carbohydrate diet may increase TEE during weight-loss maintenance, an effect that might improve the effectiveness of obesity treatment.

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Study registration: ClinicalTrials.gov, NCT02068885

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 a high glycaemic-load meal directs metabolic fuels away from oxidation and toward storage in adipose tissue. This physiological state is hypothesized to increase hunger and food cravings,[7] 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-glycaemic load foods, including refined grains and added sugars, in the US,[8 9] Europe, and elsewhere.

This model has been challenged, primarily due to lack of evidence from controlled feeding studies.[10-14] One recent meta-analysis reported no meaningful difference in energy expenditure between low-carbohydrate *vs.* low-fat diets.[11] 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 15-19] 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 through 20 weeks.

METHODS

The study protocol was approved by the Institutional Review Board at Boston Children's Hospital and previously published.[20] 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 with free-living participants.[21] 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). During the Run-In Phase, energy intake was restricted to promote weight loss corresponding to 12±2% of baseline body weight over 9 to 10 weeks. Participants who achieved the targeted weight loss were randomly assigned to high-, moderate-, or low-carbohydrate Test diets (HI, MOD, LO; Table 1) for a 20-week Test Phase. During the Test Phase, energy intake was adjusted periodically to maintain weight loss within ±2 kg of the level achieved prior to randomization. Participants were asked to weigh themselves daily using calibrated Wi-Fi scales (Withings Inc., Cambridge, MA) during both phases. Study outcomes were assessed at baseline (BSL, pre-weight-loss), post-weight-loss (PWL, time 0, pre-randomization), and the midpoint (MID, weeks 8 to 10) and end (END, weeks 18 to 20) of the Test Phase (Figure 1, eTable 1). The study was registered at ClinicalTrials.gov, NCT02068885.

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 prior to BSL (pre-weight-loss) assessments. Additional eligibility criteria are presented in eTable 2 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. (See Supplemental Methods for details on implementation of randomization.)

Dietary Interventions

During the Run-In Phase, macronutrient composition was 45% of total energy from carbohydrate, 30% from fat, and 25% from protein. The targeted macronutrient composition of the Run-In diet reflects ranges considered acceptable by the Institute of Medicine,[22] with protein at the upper end the range to enhance satiety during weight loss.[23] We determined individual energy needs based on resting requirements, estimated using a regression equation,[24 25] with a physical activity factor of 1.5. Energy intake was restricted to 60% of estimated needs. The research team monitored body weight and adjusted the amounts of food provided when necessary to achieve the target weight loss. At the end of the Run-In Phase, we adjusted energy intake to stabilize body weight based on the recent rate of weight loss for each participant (energy intake during weight loss [kcal/day] + (rate of weight loss [kg/day] × 7,700 kcal/kg). During the Test Phase, HI, MOD, and LO varied in carbohydrate (60%, 40%, and 20% of total

energy, respectively) and fat (20%, 40%, and 60%, respectively), with protein fixed at 20% (Table 1). We controlled for protein, in view of its higher thermic effect,[26] to provide a more specific test of the Carbohydrate-Insulin Model. 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. (See Supplemental Methods for detail on menu development, quality control, and strategies to promote adherence.)

Study Outcomes

Pre-specified outcomes included energy expenditure, physical activity, and metabolic hormones, assessed at the time points indicated in Figure 1. Insulin secretion (insulin concentration 30 minutes after oral glucose, INS-30)[27 28] was assessed at BSL 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.[29] 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.[30] The equation of Ravussin et al[31] was used to calculate TEE from rCO_2, with food quotient (FQ) as a proxy for respiratory quotient.[32] We expressed TEE in kcal per kg body weight, then normalized to average post-weight-loss body weight (82 kg) for analysis and reporting. This approach takes into account small changes in body weight that might occur during the Test

Phase, within our definition of weight-loss maintenance (±2 kg of the PWL anchor weight), and thereby improve precision. Some investigators discourage adjustment of TEE for weight because of confounding that would arise from individual differences in relationships between TEE and body weight, body composition, and metabolically active mass.[33] However, this problem, inherent to cross-sectional comparisons between individuals, would not apply to the within-individual comparisons over several months in our study, especially during weight-loss maintenance when these relationships would not change in any meaningful way. We also examined absolute TEE expressed as kcal per day, with and without body weight included as a covariate and obtained similar results. (See Supplemental Methods for details on measurement of body weight, resting energy expenditure (REE) by indirect calorimetry, estimated energy intake, physical activity by accelerometry, skeletal muscle work efficiency by cycle ergometry, oral glucose tolerance testing, and assays of blood samples.)

Statistical Analysis

Sample-size calculations were based on data from a preliminary study.[20] The target of 135 completers provided 80% power, with 5% Type I error, to detect a difference of 237 kcal/d in TEE change between one diet group and the other two. This difference is somewhat smaller than the effect detected in the prior study[34] and consistent with a predicted effect of +50 kcal/d per 10% decrease in the contribution of dietary carbohydrate to total energy intake.[35]

Prior to breaking the randomization blind, 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.[20] 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 pre-weight-loss value at BSL, rather than PWL, was originally specified in the registry as the anchor for calculating change scores, but this error was corrected in an amendment to the IRB protocol, prior to breaking the randomization blind [see Protocol Amendment History in Supplement for details].) The main model was unadjusted except for design factors (study site, cohort, enrollment wave). A fully adjusted model for the primary outcome also included demographic characteristics (sex, race, ethnicity, age); baseline anthropometric measures (BMI, percentage lean mass, percentage weight lost pre-randomization); and pre-weight-loss TEE measured at BSL. 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 for each diet (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[36] permitted us to make the three pairwise diet comparisons (HI vs. LO, HI vs. MOD, MOD vs. LO) with critical *P*-value 0.05 while preserving a maximum familywise 5% Type I error rate.

To test for effect modification, we divided the sample into BSL tertiles of INS-30, fasting glucose, and fasting insulin, 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. Concentrations of the hormone (ghrelin, leptin) and triglycerides were log-transformed for analysis and re-transformed to natural units for reporting.

Each analysis was performed in both the full Intention-to-Treat (ITT) sample and a Per Protocol subset comprising those participants who achieved weight-loss maintenance within ±2 kg of the PWL anchor weight during the Test Phase, the latter potentially providing a more precise effect estimate. Following each analysis, we examined residual patterns in order to detect outliers or other departures from assumptions of the statistical model.

Recognizing that estimates of FQ introduce some imprecision when calculating TEE, due in part to uncertainty in estimates of metabolizable energy,[37] we conducted sensitivity analyses to determine how plausible errors in FQ could influence results. To test for selective dropout, we compared baseline characteristics of participants who completed the END assessment with those who did not. To fully assess the influence of missing data (dropouts and unusable data points), we performed an inverse probability-weighted version of the primary analysis,[38] constructing a logistic model for missingness and employing the fitted probabilities to assign 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), 457 were available (94%); for Per Protocol analysis, 337 of 360 (94%). The missing values were attributable to 24 missed doubly labeled water studies (9 MID, 15 END) and

five studies that yielded non-convergent curve fits or implausible parameters (1 PWL, 3 MID, 1 END). Neither the ITT nor the Per Protocol findings changed materially when we applied inverse probability weighting 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.

Patient Involvement

No patients were involved in setting the research question or the outcome measures, nor were they involved in developing plans for design or implementation of the study. No patients were asked to advise on interpretation or writing up of results. Nevertheless, participants received a written summary of their clinically relevant results. We plan to invite study participants to FSU for an oral presentation of findings after publication of the primary outcome. Information may be disseminated to the general public via any media coverage of study findings.

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 weight loss of 12±2% and were randomly assigned to different macronutrient diets for the Test Phase. Characteristics of the randomized sample at BSL are presented in Table 2. Each stratification factor in the randomization (site, sex, age, obesity, Hispanic ethnicity) was balanced across the three diet

groups according to Fisher's exact test (P≥0.28). Retention during the Test Phase was 93% for the MID assessment at 10 weeks and 90% for the END assessment at 20 weeks.

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 weight loss during the Test Phase), 120 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 4). Covariates also did not differ between participants who completed the END assessment and those who did not (data not shown). Body weight tracked strongly during the Test Phase, as indicated by high within-subject correlations from PWL to MID and END (r ≥ 0.99). On average, body weight changed by < 1 kg during the Test Phase, with no significant difference by diet group (*P*=0.43).

As shown in eTable 5, 40 adverse events were recorded for 36 participants throughout the trial. Two serious adverse events were reported involving emergency hospitalization for removal of an intrauterine device (unrelated to study participation) and laparoscopic cholecystectomy (possibly related to study participation). The number of participants (n=13) who had an adverse event or serious adverse event following randomization did not differ by diet group (*P*=0.34).

Total Energy Expenditure

In the ITT analysis (n=162, *P*=0.002), TEE differed significantly by diet (Figure 3A). Compared to HI, change in TEE (mean, 95% CI; normalized to average PWL body weight of 82 kg) was +91 (-29 to +210) kcal/d greater on MOD and +209 (+91 to +326) kcal/d greater on LO. In the

Per Protocol analysis (n=120, $P<0.001$) (Figure 3B), the respective differences were +131 (-6 to +267) and +278 (+144 to +411) kcal/d. These results were similar with full adjustment for all pre-specified covariates: 76 (-42 to 194) kcal/d greater on MOD and 185 (69 to 302) kcal/d greater on LO in the ITT ($P=0.008$); and 111 (-23 to 245) and 249 (117 to 380) kcal/d in the Per Protocol analysis ($P=0.001$). Individual-level change data from PWL through the Test Phase are displayed in eFigure 1. 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 ($P > 0.43$).

In sensitivity analyses, the overall group effect retained statistical significance with a food quotient (FQ) for the LO of 0.81 (ITT) and 0.83 (Per Protocol), compared to 0.79 based on macronutrient composition (Table 1), the value we used to estimate TEE from rCO_2. This finding indicates that the observed effect of diet on TEE is robust to substantial imprecision in estimating FQ (eTable 6) and non-compliance (eTable 7).

The effect of dietary composition on TEE was most pronounced among individuals with high insulin secretion at BSL (Figure 4). Among participants in the highest tertile of INS-30, the difference between LO versus HI was +308 (+101 to +514) kcal/d in the ITT and +478 (+232 to +724) kcal/d in the Per Protocol analysis, with significant effect modification in the latter. Fasting blood glucose and insulin showed a similar but less strong pattern, with individuals in the highest tertiles of these characteristics at baseline showing the largest difference between diet groups (eFigures 2 and 3).

Other Outcomes

We assessed energy intake during the Test Phase among participants in the Per Protocol analysis, providing an estimate of energy requirements during weight-loss maintenance. Although estimates of energy intake are less accurate and precise than TEE[39] (and our methods would tend to selectively underestimate individuals with high energy expenditure, as considered in Supplemental Methods), the results are generally consistent with the TEE findings. Compared to PWL levels, energy intake (kcal/d) changed as follows on the HI, MOD, and LO Test diets, respectively: 139 (-4 to 282), 175 (42 to 308), and 269 (143 to 396), with an overall *P*=0.36. These differences strengthened among participants in the highest tertile of INS-30: 37 (-249 to 323), -24 (-293 to 245), and 340 (132 to 548), with an overall *P*=0.05.

Resting energy expenditure (REE), total physical activity, and moderate- to vigorous-intensity physical activity (MVPA) were marginally higher on the LO (*P*=0.06 to 0.09 in some models), with contrasting within-group changes in some cases; whereas sedentary time and skeletal muscle work efficiency did not differ by diet (Table 3). Ghrelin (ITT and Per Protocol) and leptin (Per Protocol only) differed significantly by diet . Ghrelin showed a steeper decline over the Test Phase on LO compared to HI, and leptin showed a lesser rise.

Process Measures and Biomeasures of Compliance

Attention to treatment fidelity, as previously described,[21] 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 glycaemic 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 glycaemic index, presumably reflecting dietary intakes.[40] As depicted in Figure 5, we found strong differentiation of 1,5-anhydroglucitol among diet groups, ranging from lowest on the LO to highest on the HI ($P<0.001$). Also as expected, triglycerides increased with increasing carbohydrate content ($P<0.001$), whereas HDL-cholesterol decreased ($P<0.001$).

DISCUSSION

Principal Findings and Comparison with Other Studies

In this 20-week controlled feeding trial, we found that TEE was significantly greater on a low-carbohydrate compared to a high-carbohydrate diet with similar protein content. 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[41] and circulating metabolic fuel concentration,[42] results of (FS)2 substantiate several key predictions of the Carbohydrate-Insulin Model. Regardless of the specific mechanisms involved, the study shows that dietary quality can affect energy expenditure independently of body weight, a phenomenon that may be key to obesity treatment, as recently reviewed.[43]

The mean TEE effect we observed (228 kcal/day or about +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,[44] after taking into account

confounding by ongoing weight loss and other sources of bias.[45 46] If this effect were persistent – and we observed no attenuation from 10 to 20 weeks – it would translate into an estimated 10 kg weight loss after 3 years for a typical 30-year-old man with height of 178 cm, baseline weight of 100 kg, and average activity level, assuming no change in energy intake [https://www.niddk.nih.gov/bwp]. If reduction of glycaemic load also decreased hunger and food intake,[3 35] the long-term benefits could be even greater.

The difference in TEE between low- and high-carbohydrate diets among individuals in the highest tertile of insulin secretion was more than double the difference for those with low insulin secretion, highlighting a subgroup that may do particularly well with restriction of total or high-glycaemic load carbohydrates. This finding is consistent with results from an animal study,[47] a cohort study,[48] Mendelian randomization analysis,[49] and clinical trials.[27 28 50] 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.[51] 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 glycaemic 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 glycaemic load diets in some previous intervention studies.[52] Thus, the effects of predisposing risk factors may be attenuated on diets that are generally healthy and specifically low in glycaemic load. In support of this possibility, a high genetic obesity risk score predicted obesity among individuals consuming high-glycaemic load sugary beverages but not among non-consumers.[53]

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 marginally higher on the low-carbohydrate diet (comparisons that may have been underpowered). 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,[54] and changes in the efficiency of movement that we did not capture with cycle ergometry.[55-58]

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,[59 60] providing another mechanistic explanation for our primary outcome. Leptin was also lower on the low-carbohydrate diet, suggesting improvement in leptin sensitivity.[61] Prospective studies have observed that individuals with the greatest declines in leptin following weight loss have the lowest risk for weight regain.[62-64]

Strengths and Limitations

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;[15-18] 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);[65] 4) measurement of TEE by the doubly labeled water method, the gold standard method for studies of free-living people;[29 66] 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.

The study has three main limitations, include potential measurement error, non-compliance, and generalizability. First, 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 maintenance.[32] 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 6). Sensitivity analyses show that the primary outcome remained robust throughout a range of plausible RQ values.

Also relating to measurement error, some investigators recently proposed a novel reason why doubly labeled water methodology – used extensively in nutrition research for decades – would bias comparisons of diets varying in macronutrient ratio. According to this argument, presented in a *post-hoc* analysis of an observational pilot study,[67] increased de novo lipogenesis on a high-carbohydrate diet could trap deuterium, leading to an artifactually lower TEE with doubly-labeled water compared to whole room calorimetry. The origin of this concern involves studies of pigs consuming an extremely high carbohydrate diet during their most rapid growth phase, when body fat may increase by several hundred grams per day.[68] In this exceptional scenario, significant error could arise in estimates of TEE; however, statistical extrapolation predicted no error during weight maintenance. During weight maintenance in humans, rates of hepatic de novo lipogenesis are low on whole food, low-sugar diets with carbohydrate intake up

to 75% (substantially higher than our high-carbohydrate diet, which was also low in sugar).[69 70] Even with consumption of 50% excess carbohydrate, hepatic de novo lipogenesis was < 5g/d.[71]

Some[72] but not all[73] studies report significant lipogenesis in adipose tissue, but it is not known how dietary composition might differentially affect this phenomenon. Indeed, adipocyte lipogenesis appears poorly responsive to changes in dietary carbohydrate[74 75] and high intakes of carbohydrate may not affect adipose gene expression or lipogenic activity during weight stability[76 77] or after weight loss,[78] as opposed to massive overfeeding.[79 80] Moreover, a carefully controlled validation study reported that the doubly labeled water method was more accurate than whole room calorimetry, which tends to underestimate adaptive thermogenesis.[81] With 10% weight gain or loss, doubly labeled water determination of TEE (but not whole room calorimetry) corresponded closely with titration of energy requirements, suggesting that changes in metabolism following major perturbations in adipose mass do not confound isotopic measurements. Thus, any bias of dietary composition on the accuracy of doubly labeled water methodology during weight maintenance is highly speculative and unlikely to be meaningful. Additional evidence for the validity of our primary outcome derives from the effect modification by insulin secretion, as there would be no reason why any systematic error in TEE should co-segregate with insulin secretion status in the hypothesized direction.

Regarding the second 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.[21] Despite these efforts, we recognize that some non-compliance may have occurred, especially among individuals whose weight deviated beyond the pre-specified definition of weight-loss maintenance. 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 >20% (ITT) or > 40% (Per Protocol) additional energy from foods with macronutrients reflecting the high-carbohydrate diet (and substantially more if the additional foods had an intermediate macronutrient composition) (eTable 7). Furthermore, the primary outcome was strengthened in the Per Protocol analysis, including only individuals who maintained weight loss throughout the Test Phase. The Per Protocol analysis should provide a more accurate estimate of the true diet effects by excluding participants with objective evidence of non-compliance.

Translation of findings from feeding studies to public health recommendations comprises the third limitation. However, aspects of study design improve generalizability, 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.[82-87] But inferences about efficacy from these trials are limited by characteristically poor long-term compliance and lack of differentiation in dietary intake between groups, reflecting the difficulty of behavior change in the modern food environment. Furthermore, our study cannot prove that changes in carbohydrate-to-fat ratio alone mediate study findings. Although we constructed Test diets as similar as possible (e.g., controlling for protein content, amount of non-starchy vegetables, the ratio of saturated to total fat), unrecognized dietary factors could have contributed to the observed effects. This possibility, of relevance to translation, requires exploration in future mechanistically-oriented research.

Conclusions and Policy Implications

Dietary composition appears to affect energy expenditure independently of body weight. A low-glycaemic load, high-fat diet may facilitate weight-loss maintenance beyond the conventional focus on restricting energy intake and encouraging physical activity. Additional research is warranted to examine the effects of low-glycaemic load diets on body weight, with control of energy intake; to compare diets aiming to reduce glycaemic 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; 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; and to explore the mechanisms relating dietary composition to energy expenditure. If metabolic benefits of reduced glycaemic-load diets are confirmed, development of appropriate behavioral and environmental interventions would be necessary for optimal public health translation.

Data Sharing: The full dataset is available at Open Science Framework (https://osf.io/rvbuy/).

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Table 1. Dietary Energy and Macronutrient Composition for Test Diets, Calculated Daily Averages (per 2,000 kcal)[a]

Variable	HI		MOD		LO	
Energy (kcal)		2001		2001		2001
Carbohydrate (g)		304.8		204.7		104.9
Carbohydrate (%) [b]		59.2%		39.7%		20.3%
Glycaemic index [c]		49.5		45.5		29.9
Glycaemic load (g) [c]		134.7		80.5		28.4
Fat (g)		47.8		91.7		136.7
Fat (%) [b]		20.9%		40.1%		59.6%
Saturated fat (%) [d]		5.9		13.7		20.9
Monounsaturated fat (%) [d]		8.2		15.9		25.1
Polyunsaturated fat (%) [d]		5.3		8.6		11.3
Protein (g)		102.3		103.8		103.5
Protein (%) [b]		19.9%		20.2%		20.1%
Fiber (g)		32.7		27.9		22.2
FQ [e]		0.90		0.85		0.79

[a] 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] Glycaemic index for each day was calculated by summing the weighted values for each food item: Σ(glycaemic index for food item × proportion of total net carbohydrate contributed by the item).[88] Glycaemic load was calculated as the product of the glycaemic index and net carbohydrate for the day: (glycaemic index/100 × net carbohydrate).[89]

[d] Percent of total energy. The target for saturated fat was 35% of total fat (equating to 7%, 14%, and 21% of total energy for HI, MOD, and LO, respectively). The remainder of the total fat target (20%, 40%, 60% of total energy) was distributed between mono- and polyunsaturated fat. The sum of saturated, monounsaturated, and polyunsaturated fat does not equal total fat because data regarding fat type were missing for some foods.

[e] Food Quotient (FQ) was calculated using the equation of Black *et al.*[32]
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, Hispanic, No. (%) [a]	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/ d	2915 (686)	3030 (788)	3110 (680)
REE, mean (SD), kcal/ d	1654 (318)	1751 (387)	1695 (331)
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)
Skeletal Muscle Work Efficiency at 10 W (%)	11.1 (2.5)	10.3 (2.6)	11.1 (3.6)
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)
Abnormal Fasting Blood Glucose, No (%) [f]	18 (33)	19 (36)	16 (28)
Fasting Glucose, mean (SD), mg/dL	97 (9)	97 (9)	99 (11)
Fasting Insulin, mean (SD), µIU/mL	13.9 (6.8)	15.6 (10.3)	19.7 (21.1)

[a] Ethnicity and race were determined by self-report using fixed categories.
[b] Total activity was quantified based on triaxial counts, representing a composite vector magnitude of three orthogonal planes (vertical, anteroposterior, mediolateral).[90] 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.*[91]
[d] Sedentary time was defined as <100 counts per minute for vertical axis counts.[90]
[e] Lean body mass does not include bone mineral content.
[f] Fasting blood glucose ≥100 mg/dL.

Table 3. Primary and Secondary Outcomes Involving Energy Expenditure, Physical Activity, and Metabolic Hormones [a]

Variable Analysis	Dietary Intervention Group	N	Pre-Randomization, PWL, Mean (SE)	Change: AV[MID, END] – PWL		
				Mean (95% CI)	*P* between groups	*P* HI *vs.* LO [b]
TEE, kcal/d (*primary*)						
Intention-to-Treat	HI	54	2640 (62)	-19 (-104 to 66)	0.002	<0.001
	MOD	52	2504 (65)	71 (-12 to 155)		
	LO	56	2713 (64)	190 (109 to 270)		
Per Protocol	HI	38	2711 (77)	-102 (-201 to -2)	<0.001	<0.001
	MOD	39	2577 (72)	29 (-64 to 123)		
	LO	43	2758 (70)	176 (87 to 265)		
REE, kcal/d						
Intention-to-Treat	HI	54	1603 (24)	34 (10 to 57)	0.47	0.22
	MOD	51	1576 (25)	46 (23 to 69)		
	LO	56	1615 (24)	54 (32 to 76)		
Per Protocol	HI	38	1601 (28)	20 (-8 to 48)	0.18	0.08
	MOD	38	1597 (27)	28 (2 to 54)		
	LO	43	1608 (26)	53 (28 to 78)		
Total physical activity, counts/d, thousands [c]						
Intention-to-Treat	HI	54	476.6 (23.3)	-26.3 (-52.0 to -0.6)	0.13	0.28
	MOD	52	463.8 (24.9)	-42.4 (-67.7 to -17.1)		
	LO	55	495.8 (23.9)	-6.9 (-31.0 to 17.1)		
Per Protocol	HI	38	493.2 (28.8)	-29.1 (-59.0 to 0.7)	0.17	0.39
	MOD	39	481.3 (27.0)	-48.3 (-76.3 to -20.3)		
	LO	42	521.3 (26.3)	-11.6 (-38.2 to 14.9)		
MVPA, min/d [d]						
Intention-to-Treat	HI	54	31.6 (2.6)	-3.6 (-6.3 to -0.9)	0.09	0.14
	MOD	52	31.3 (2.7)	-4.8 (-7.5 to -2.1)		
	LO	55	30.0 (2.6)	-0.9 (-3.4 to 1.6)		
Per Protocol	HI	38	33.4 (3.0)	-4.3 (-7.4 to -1.1)	0.06	0.08
	MOD	39	33.0 (2.8)	-5.2 (-8.1 to -2.2)		
	LO	42	32.2 (2.8)	-0.5 (-3.3 to 2.3)		
Sedentary time, min/d [e]						
Intention-to-Treat	HI	54	592.1 (14.2)	8.6 (-7.7 to 25.0)	0.12	0.34
	MOD	52	604.7 (14.8)	20.9 (4.8 to 37.0)		
	LO	55	597.0 (14.6)	-2.3 (-17.6 to 13.0)		
Per Protocol	HI	38	593.6 (17.2)	2.1 (-17.7 to 22.0)	0.31	0.80
	MOD	39	611.0 (16.1)	21.4 (2.8 to 40.0)		
	LO	42	589.4 (15.7)	5.6 (-12.0 to 23.1)		
Skeletal Muscle Work Efficiency at 10 Watts (%) [f]						
Intention-to-Treat	HI	53	12.2 (0.3)	-0.1 (-0.8 to 0.5)	0.66	0.37
	MOD	51	11.7 (0.4)	-0.0 (-0.6 to 0.6)		
	LO	55	12.2 (0.3)	0.3 (-0.3 to 0.9)		
Per Protocol	HI	38	12.1 (0.4)	-0.1 (-0.9 to 0.6)	0.46	0.28
	MOD	38	11.9 (0.4)	-0.0 (-0.7 to 0.6)		
	LO	42	12.2 (0.4)	0.5 (-0.2 to 1.1)		

Ghrelin, pg/mL and % change [g]						
Intention-to-Treat	HI	54	693.2 (51.4)	-4.9 (-8.4 to -1.2)	0.02	0.004
	MOD	51	640.1 (49.9)	-8.7 (-12.0 to -5.3)		
	LO	56	598.2 (45.4)	-11.8 (-14.8 to -8.6)		
Per Protocol	HI	38	689.5 (63.0)	-5.9 (-10.1 to -1.5)	0.02	0.007
	MOD	38	620.6 (52.8)	-8.0 (-11.8 to -4.0)		
	LO	43	603.0 (49.0)	-13.5 (-16.9 to -10.0)		
Leptin, ng/mL and % change [g]						
Intention-to-Treat	HI	54	10.9 (1.6)	34.2 (21.8 to 47.7)	0.07	0.06
	MOD	51	9.8 (1.5)	34.8 (22.6 to 48.2)		
	LO	56	9.6 (1.4)	17.9 (7.7 to 29.1)		
Per Protocol	HI	38	11.8 (2.2)	47.6 (33.9 to 62.8)	0.009	0.005
	MOD	38	8.6 (1.5)	42.0 (29.4 to 55.8)		
	LO	43	9.0 (1.5)	21.9 (11.7 to 33.0)		

[a] Means and changes were constructed and compared from repeated-measures analysis of variance, unadjusted except for structural design variables (study site, cohort, enrollment wave).

[b] The test comparing HI vs. LO is equivalent to a test for linear trend across the three diets, for which carbohydrate content was uniformly spaced.

[c] Total activity was quantified based on triaxial counts, representing a composite vector magnitude of three orthogonal planes (vertical, anteroposterior, mediolateral).[90] Average accelerometer wear time (mean, SD) was 14.9 (1.2) hours per day.

[d] Moderate- to vigorous-intensity physical activity was quantified using vertical axis count thresholds of Troiano *et al.*[91]

[e] Sedentary time was defined as <100 counts per minute for vertical axis counts.[90]

[f] Efficiency is expressed as the percentage ratio of power generated (with conversion of Watts to kcal/min using a factor of 0.01433) to energy expenditure above resting (kcal/min).[55 56] Data not collected at MID. Change: END – PWL. There were no significant group effects at 10W (presented here), 25W, and 50W.

[g] 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

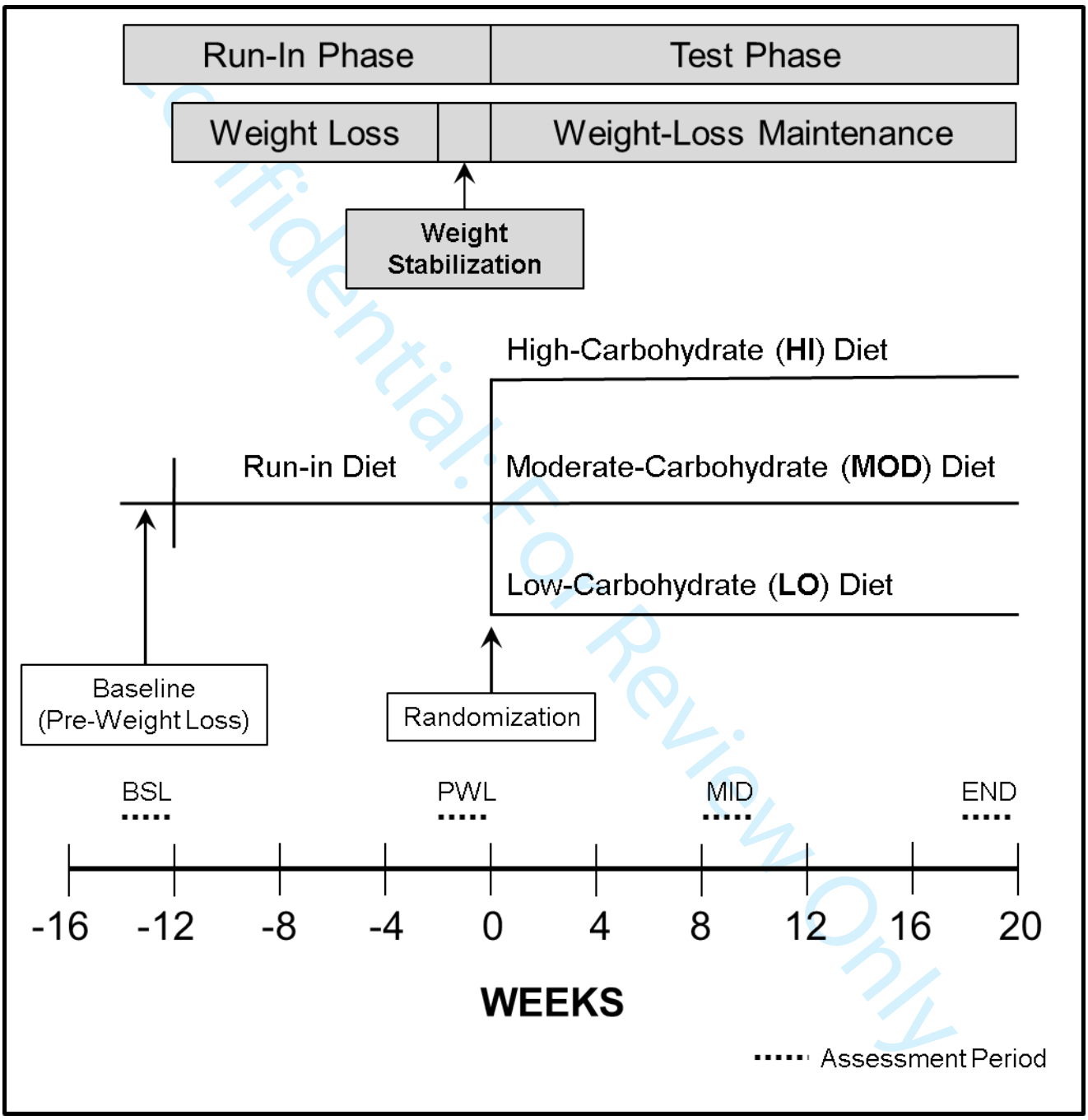
Figure 1. Study Design (BSL, Baseline; PWL, Post-Weight Loss; MID, Midpoint of Test Phase; END, End of Test Phase)

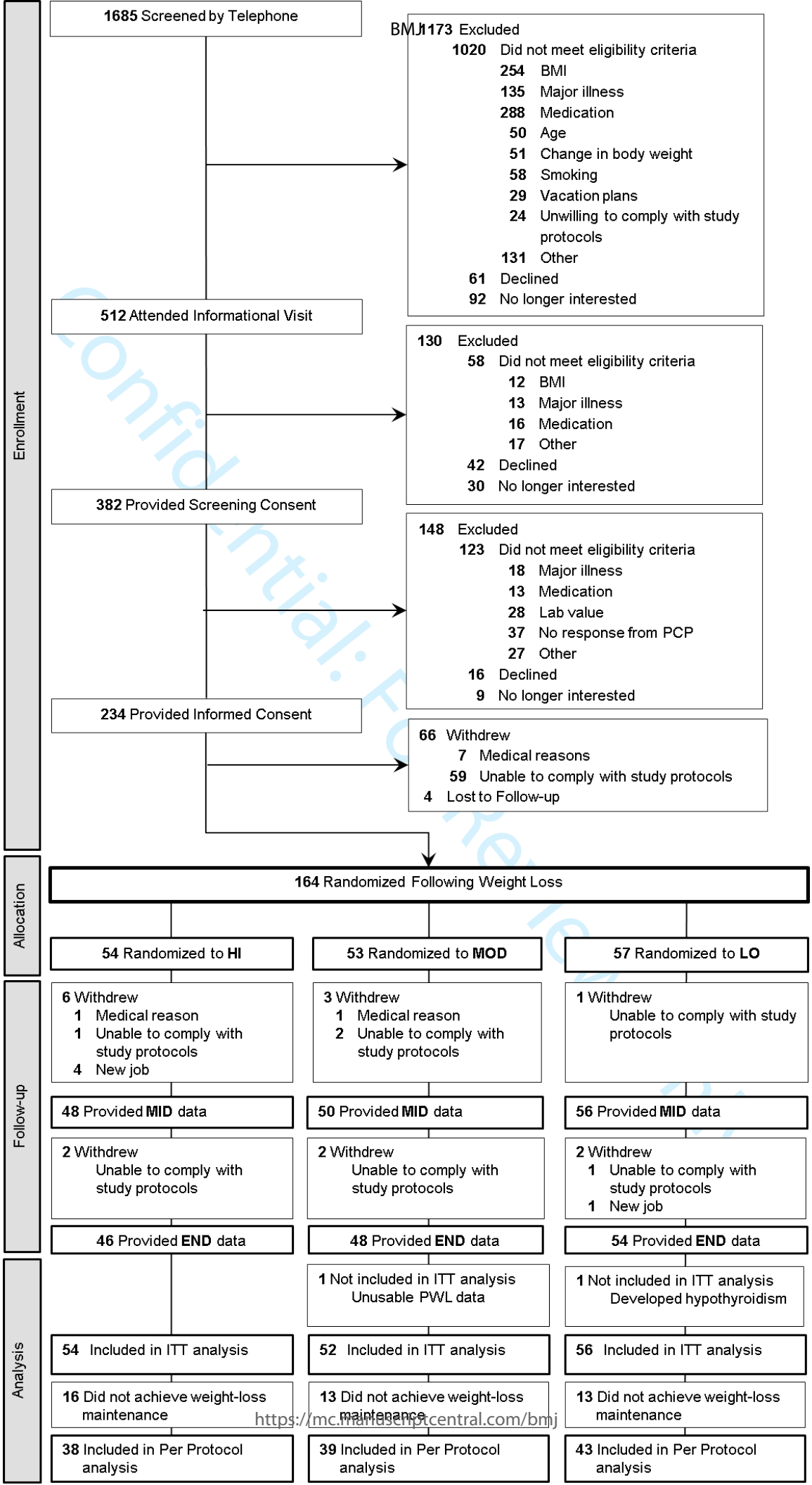
Figure 2. Participant Flow

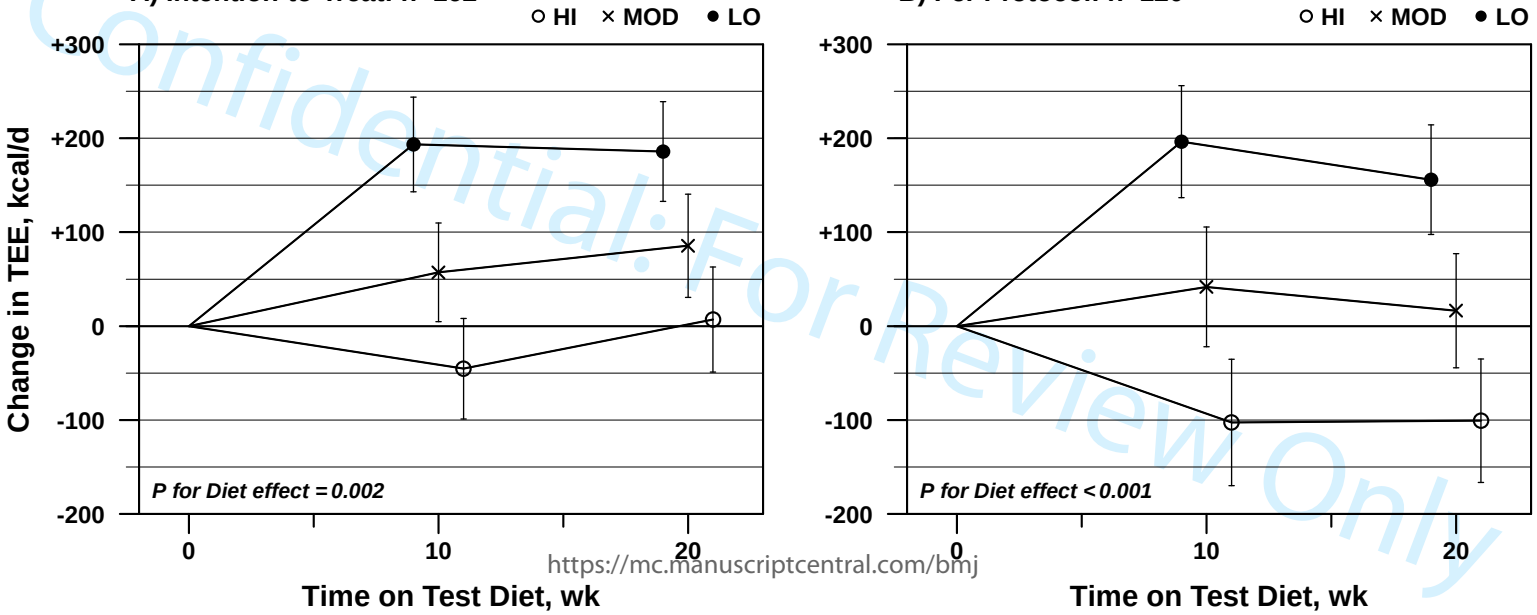
Figure 3. Change in TEE, the Primary Outcome, in the (A) Intention-to-Treat and (B) Per Protocol Analyses. Data expressed as mean ± SE. *P* tests uniformity of Test Phase change (Av[MID, END] - PWL) across diet groups.

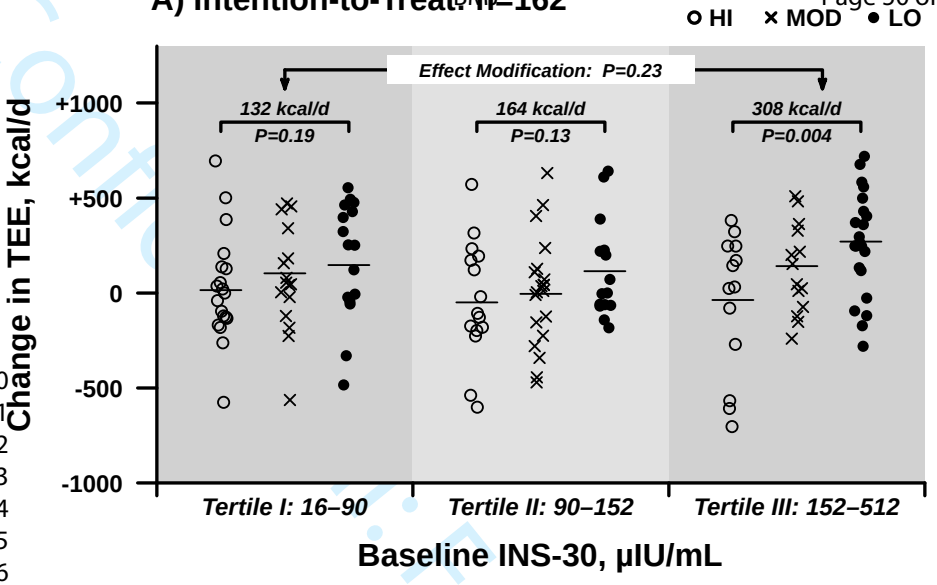
Figure 4. Effect Modification by Baseline Insulin-30 in the (A) Intention-to-Treat and (B) Per Protocol Analyses. Pre-weight-loss body weight differed by tertiles (Tertile I, 83.8 kg; Tertile II, 92.8 kg; Tertile III 98.4 kg, $P<0.001$ in the ITT). Change in body weight during the Test Phase did not differ by tertile ($P=0.08$) or across diet groups ($P=0.43$).

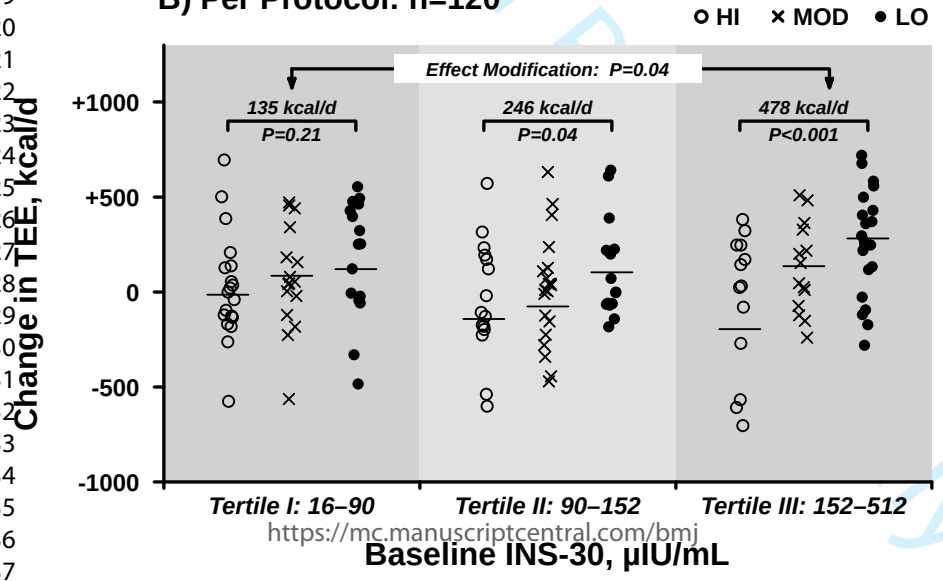
Figure 5. Biomeasures of Compliance. A) Intention to Treat; B) Per Protocol. Measures include 1,5-Anhydroglucitol, mean baseline 17 µg/mL; Triglycerides, mean baseline 78 mg/dL (retransformed); and HDL-cholesterol, men baseline 48 mg/dL. Data expressed as mean ± SE. *P* tests uniformity of Test Phase change (Av[MID, END] - PWL) across diet groups.

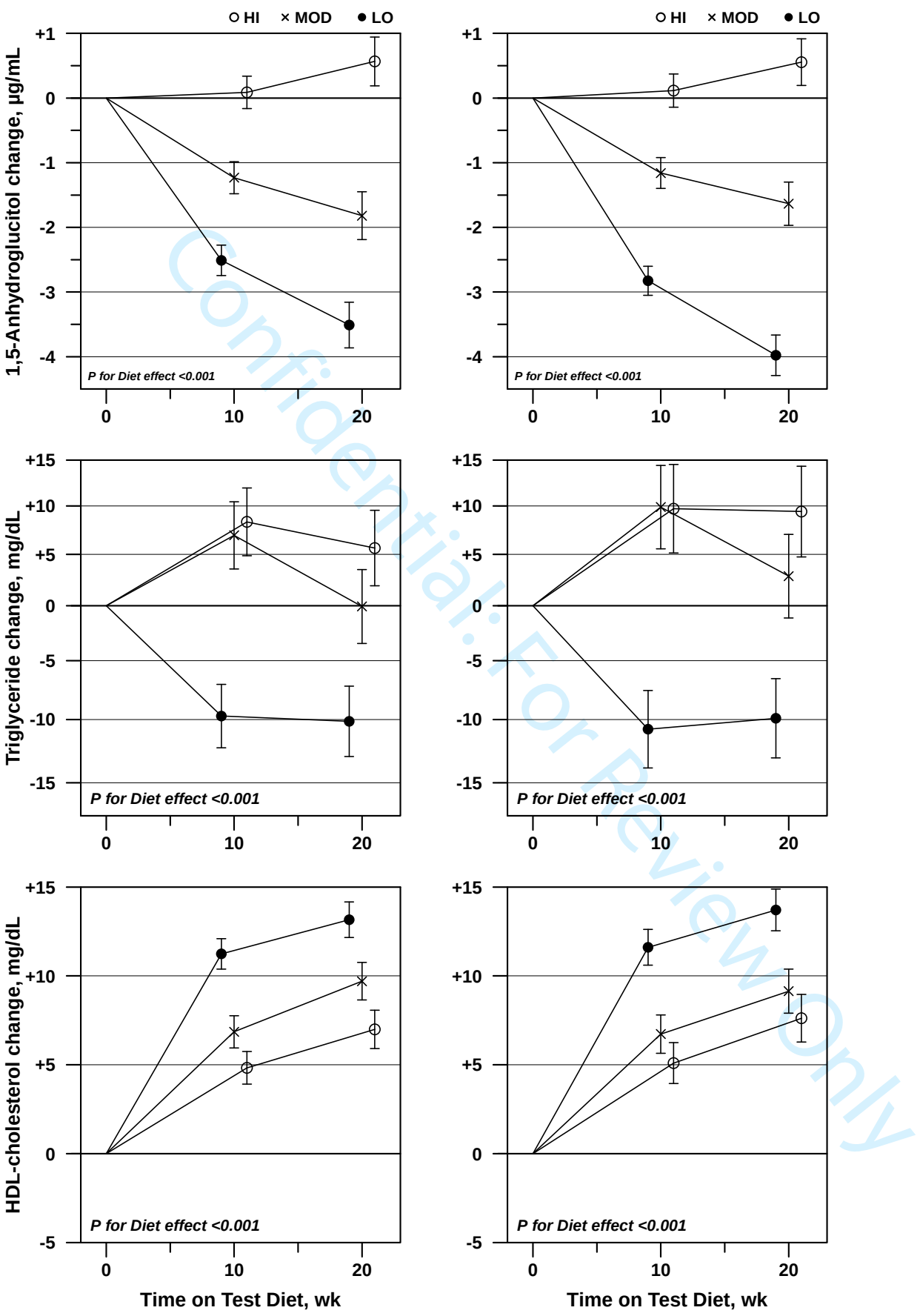







A) Intention-to-Treat: n=162
HI
MOD
LO
Effect Modification: P=0.23
132 kcal/d
P=0.19
164 kcal/d
P=0.13
308 kcal/d
P=0.004
+1000
+500
0
-500
-1000
Change in TEE, kcal/d
Tertile I: 16–90
Tertile II: 90–152
Tertile III: 152–512
Baseline INS-30, µIU/mL


B) Per Protocol: n=120
HI
MOD
LO
Effect Modification: P=0.04
135 kcal/d
P=0.21
246 kcal/d
P=0.04
478 kcal/d
P<0.001
+1000
+500
0
-500
-1000
Change in TEE, kcal/d
Tertile I: 16–90
Tertile II: 90–152
Tertile III: 152–512
Baseline INS-30, µIU/mL



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

Supplemental Methods

Implementation of Randomization

Participants who successfully completed the Run-In Phase were eligible for randomization. The randomization was stratified by feeding site (FSU, 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). A set of enrollment logs with identifiers in numerical sequence, one for each stratum, were prepared by the Data and Quality Manager at Boston Children's Hospital, along with diet assignment lists, identical to the enrollment logs but with an added computer-generated random choice of diet. To ensure close balance among the three diet groups at any point in the study while preserving unpredictability, the diet assignments were randomly permuted within blocks of 3, 6, and 9, and the blocks themselves were randomly permuted.

The diet assignment lists were kept in a secure electronic folder accessible only to staff responsible for randomization. The DQM, after confirming eligibility criteria with the Study Director, assigned the next available randomization identifier to eligible participants according to stratum.

Dietary Interventions – Additional Details

We developed cycle menus for the two study phases. There were 42 meals (14 breakfasts, 14 lunches, 14 dinners), and 14 snacks incorporated into three 1-week cycle menus during the Run-In Phase. Another 42 meals and 14 snacks for each of the different macronutrient diets, totaling 126 meals and 42 snacks, were incorporated into six 1-week cycle menus during the Test Phase. We used many of the same foods, in differing amounts, across Test 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 and contained consistent sources of protein (eTable 3). We provided extra *ad libitum* snacks, reflecting the macronutrient composition of respective Test diets, to participants who continued to lose weight and had difficulty consuming large meals.

All menu items were weighed within narrow tolerance limits (±0.1g of the target weight for items ≤10g and ±0.5g for items >10g). Participants were asked to eat at least one supervised meal per day, Monday through Friday, in a dining area at FSU or AV under the supervision of research staff. Other meals were packaged for take-out. For supervised meals, weights of leftover menu items were recorded in an online study portal; for take-out meals, participants were asked to record the proportion of each provided menu item consumed using a form in the portal that was pre-populated with daily menus. We instructed participants to consume only foods and beverages provided for the research study and, if desired, up to three servings per day of specified non-caloric items (*e.g.*, beverages containing artificial sweeteners, caffeinated beverages, packets of artificial sweeteners, gum or mints containing artificial sweeteners).

Strategies to encourage adherence included monthly group workshops, weekly educational handouts posted in the dining area, personalized notes, and special activities during major holidays or events. Participants also received individualized quarterly progress reports indicating weight loss or weight-loss maintenance, depending on study phase. The presence of study dietitians in the dining area allowed for frequent communication and direct observation of dietary intake during on-site meals. Individual counseling sessions to address adherence issues were conducted in-person in a private space at FSU or AV, or by telephone.

Study Outcomes – Additional Details

Body weight was measured using a calibrated electronic scale (BWB-800S, Tanita, Arlington Heights, IL) every time a participant provided a urine sample.

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.

Energy intake data were compiled for the two-week doubly-labeled water assessment periods at PWL, MID, and END for participants who achieved weight-loss maintenance within ±2 kg of the PWL anchor weight.

We calculated average intake in kcal/d based on provided energy, weights of leftover menu items following supervised meals, and estimated proportions of menu items consumed (as recorded by participants in the portal) following take-out meals and snacks. Due to reliance on self-report for documenting consumption of take-out meals and snacks, our estimates of energy intake lack precision and accuracy compared to TEE.[1] Moreover, we did not fully capture energy intake from *ad libitum* snacks provided to participants who continued to lose weight and had difficulty consuming large meals (see above), possibly leading to selective underestimation of energy intake among those with higher TEE. While recognizing these limitations, we included energy intake as a *post hoc* outcome to evaluate consistency with TEE data.

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.[2 3]

We conducted graded cycle ergometry to measure skeletal muscle work efficiency, according to published methods.[4 5] Following a 10-minute warm-up period, participants pedaled at 60 rpm against graded resistance to generate power corresponding to 10W, 25W, and 50W in 4-minute stages. We measured oxygen uptake and carbon dioxide production using a metabolic cart (TrueOne 2400, Parvo Medics, Sandy, UT) and converted oxygen consumption to energy expenditure based on respiratory exchange ratio. Skeletal muscle work efficiency at each grade was calculated as power generated (with conversion of W to kcal/min using a factor of 0.01433) per increase in energy expenditure above resting (kcal/min). We instructed participants to fast for 5 hours prior to the cycling test.

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 test (75-g dose of dextrose). Blood was collected for analysis of insulin and glucose. Fasting insulin and insulin at 30 minutes (INS-30) were quantified by electrochemiluminescence immunoassay (Roche Diagnostics, Indianapolis, IN) and fasting blood glucose was measured enzymatically using the hexokinase method (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).

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eTable 1. Study Outcomes, Covariates and Effect Modifiers – Outcomes (pre-specified unless indicated as *post-hoc*) in bold are presented in the current manuscript; others will be the focus of future manuscripts.

	Run-In Phase		**Test Phase**		***Ad libitum***
	BSL	PWL	MID	END	**Feeding Phase** [a]
	-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)					
SA#1 (Related to energy expenditure)					
TEE (primary)	X	X	X	X	
REE	X	X	X	X	
Physical activity	X	X	X	X	
Estimated energy intake (*post hoc*)		X	X	X	
SA#2 (Related to chronic disease risk factors)					
Insulin sensitivity and secretion (OGTT) [b]	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 (Related to mechanisms)					
Skeletal muscle work efficiency (cycle ergometry)	X	X		X	
Body composition (4-compartment model) [b]	X	X		X	
Insulin sensitivity and secretion (OGTT) [b]	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, **ghrelin**	X	X	X	X	
Adiponectin (total, high-molecular weight)	X	X	X	X	
Metabolomics profile (saved samples)	X	X	X	X	
Gut microbiome (saved samples) [c]	X	X		X	
SA#4 (Related to hunger and *ad libitum* food intake) [a]					
Body weight					X
Study Outcomes for Ancillary Studies					
Lipoprotein particle subfraction distribution [d]	X	X		X	
Sleep [d]	X	X	X	X	
Psychological health [c]	X	X		X	
Cognition [c]	X	X		X	
Weight bias (hypothesis-generating questions) [c]	X	X		X	
Postprandial metabolic fuels [c]			10-15 weeks		
Adipocyte biology [c]		X	10-15 weeks		
Brain activity [c]				14-20 weeks	
Covariates and Effect Modifiers					
Sex	X				
Ethnicity	X				
Race	X				
Age	X				
Body weight, BMI [b]	X	X	X	X	
Body composition (DEXA, 4-compartment model) [b]	X	X		X	
Insulin sensitivity and secretion (OGTT) [b]	X	X	X	X	
Fasting glucose (*post hoc* effect modifier)					
Fasting insulin (*post hoc* effect modifier)					
Obesity-related genes [c]	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] An ad libitum feeding phase followed the Test diet phase to assess change in body weight as a proxy measure of hunger.

[b] Assessed as outcomes and covariates.

[c] Assessed only for participants who opted-in.

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

eTable 2. 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 3. Sample Test Diet Menu (per 2,000 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

eTable 4. Comparison of Covariates between Participants Included vs Excluded in the Per Protocol Analyses

Characteristic	Included (N=120)	Excluded (N=42) [a]	*P* [b] Included *vs.* Excluded
Sex, No. (%)			0.70
Male	35 (29.2)	14 (33.3)	
Female	85 (70.8)	28 (66.7)	
Ethnicity, No. (%) [c]			0.62
Hispanic	20 (16.7)	5 (11.9)	
Race, No. (%) [c]			0.76
White	93 (77.5)	34 (81.0)	
Black	12 (10.0)	5 (11.9)	
Asian	4 (3.3)	1 (2.4)	
Unknown / Other	11 (9.2)	2 (4.8)	
Age, mean (SD), y	39.6 (13.9)	34.4 (15.0)	0.05
Weight loss, mean (SD), (% of baseline)	10.4 (1.6)	10.7 (2.0)	0.25
BMI, mean (SD), kg/m^2	32.0 (4.4)	33.5 (6.0)	0.08
TEE, mean (SD), kcal/d	2984 (721)	3146 (720)	0.22
Lean body mass (% of total mass) [d]	56.5 (6.3)	56.6 (5.1)	0.98

[a] Participants not maintaining weight loss within ±2 kg of the post-weight loss anchor were excluded from the Per Protocol analyses.
[b] Testing for equal proportions by Fisher exact test or equal means by Student t-test.
[c] Ethnicity and race were determined by self-report using fixed categories.
[d] Lean body mass does not include bone mineral content.

eTable 5. Adverse Events by Dietary Intervention Group [a]

Adverse Event	Number of Occurrences			
	Pre-Randomization	Post-Randomization		
		HI	MOD	LO
Possibly or Probably Related to Intervention				
• Constipation	1			
• Food allergy, aversion, or intolerance	4	1	2	2
• Gastroenteritis	1			
• Mood changes	1			
• Increased blood cholesterol	1			
• Possible gall bladder disease	1			
• Possible hypoglycemia	1			
• Laproscopic cholecystectomy (Serious Adverse Event)		1		
Probably or Definitely Related to Assessments				
• Hematoma	3			
• Vasovagal reaction	4		1	1
• Vomiting	2			
• Lightheadedness			1	
Unrelated to Study Participation				
• Migraine	1			
• Bone fracture	1		1	
• High blood pressure	1			
• Ankle sprain	1			
• Testing to rule out meningitis	1			
• Possible post-viral lactose intolerance	1			
• Food intolerance	1			
• Hypertension (new prescription medication)	1			
• Urinary tract infection	1			
• Back pain				2
• Pericarditis			1	
• Removal of intrauterine device (Serious Adverse Event)		1		
Number of Participants with Post-Randomization Event [b]		2	6	5

[a] Two participant each had 2 adverse events (1 pre-randomization,1 post-randomization). One participant had 3 adverse events (2 pre-randomization, 1 post randomization) and 1 serious adverse event (post-randomization).

[b] Fisher Exact Test for comparison by diet group, P=0.34 (N.B., one participant in HI had 2 post-randomization events).

eTable 6. 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 7. 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

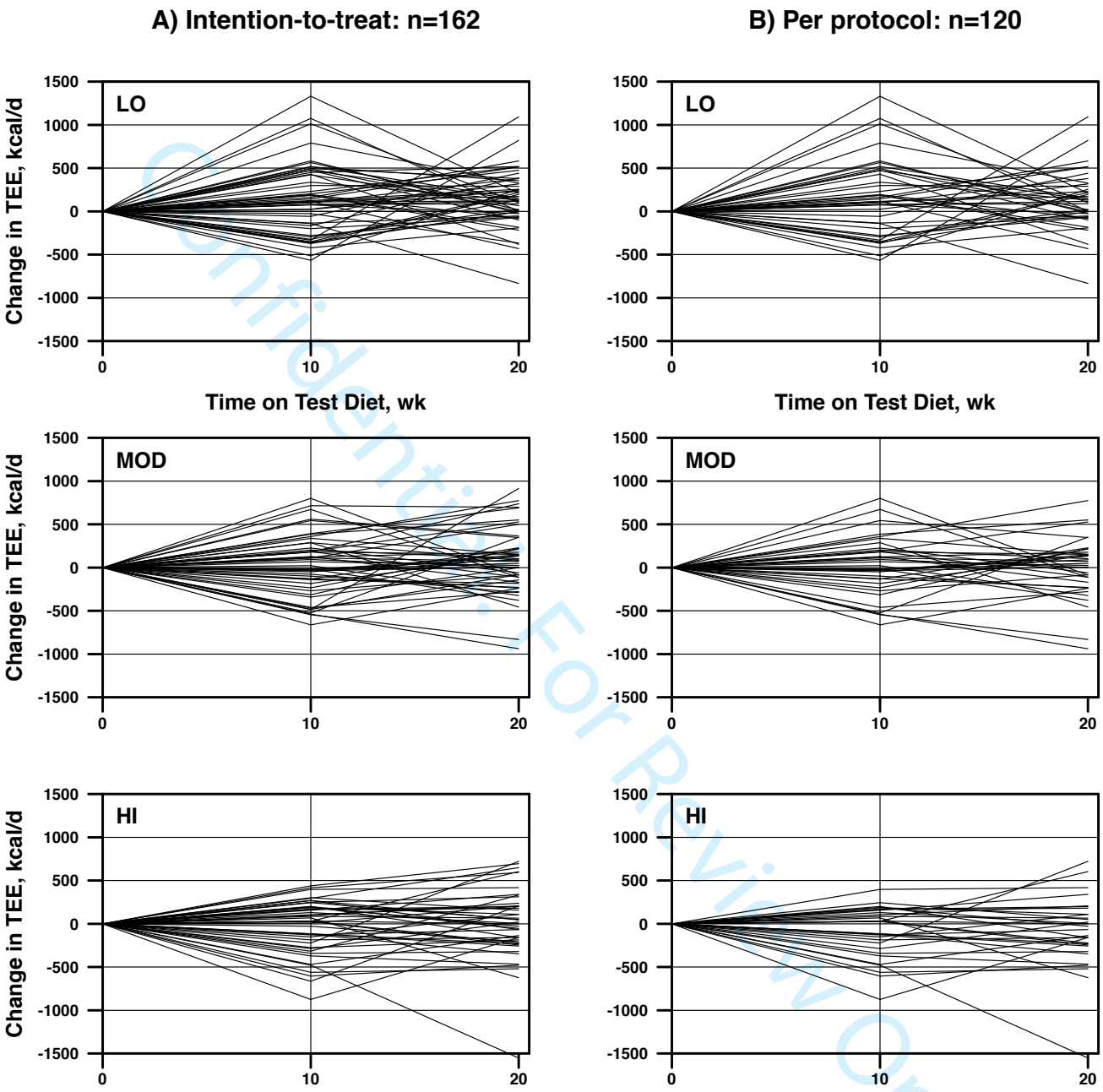
[a] *Question*: How much non-compliance would need to occur on the Low-Carbohydrate Diet to reach various FQ thresholds, assuming that the extra food consumed contained a macronutrient distribution similar to the High-Carbohydrate Diet? For a 2,000-kcal diet, noncompliance of 5% (100 kcal) would equate 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]

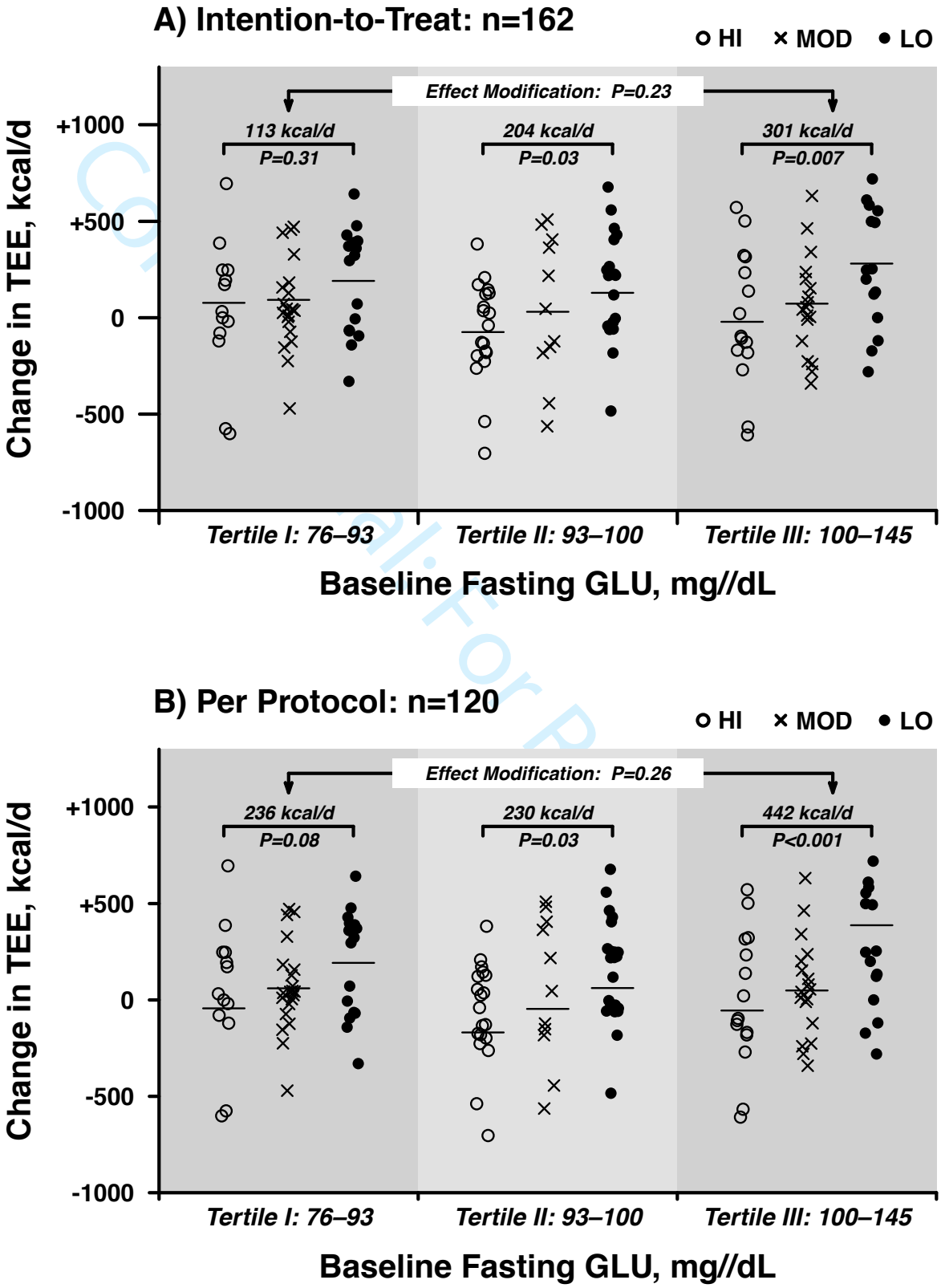
Reference

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.

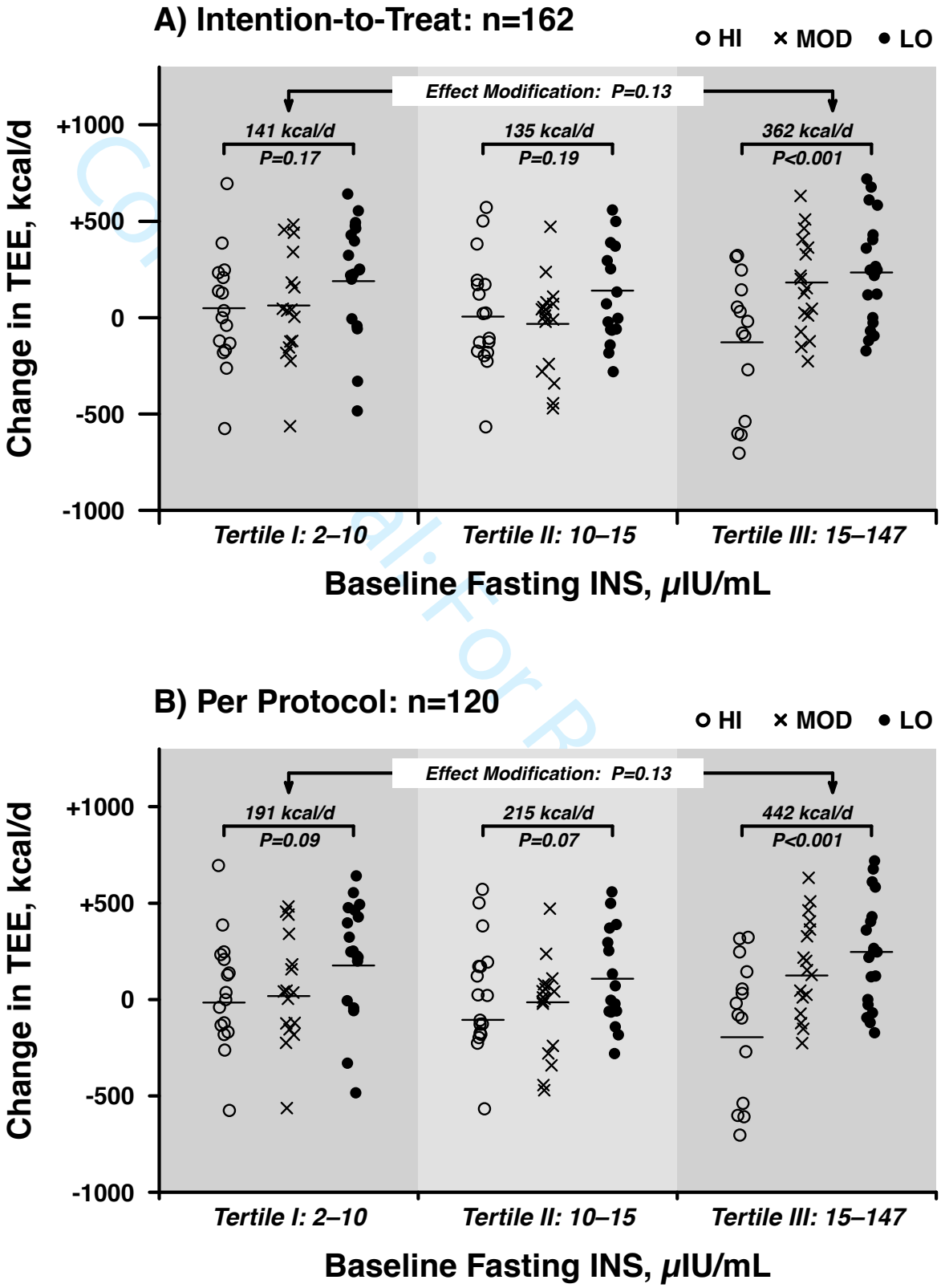
eFigure 1. Individual Data for Total Energy Expenditure, the Primary Outcome, in the Intention-to-Treat and Per Protocol Analyses



eFigure 2. Effect Modification of Total Energy Expenditure, the Primary Outcome, by Fasting Glucose in the Intention-to-Treat and Per Protocol Analyses



eFigure 3. Effect Modification of Total Energy Expenditure, the Primary Outcome, by Fasting Insulin in the Intention-to-Treat and Per Protocol Analyses



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
IRB 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 and obtained approval 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 pre-weight loss baseline (BSL) as the anchor for evaluating change over time. Original plan was erroneous for the reasons outlined below. – As a general rule, anchor data should be collected as close to the time of randomization as possible, to decrease error introduced by any time-varying confounder. The pre-weight loss BSL measurement was obtained 3 to 4 months prior to initiation of the Test diets. – The BSL measurement is strongly confounded by weight loss, whereas the specific aim is to examine TEE during weight-loss maintenance (consistent with the registered title of the protocol, *Dietary Composition and Energy Expenditure During Weight-Loss Maintenance*). – The stated purpose of the Run-In Phase is to produce 12% weight loss, changing biological state (i.e., creating a predisposition to weight regain) to test the study hypotheses. Thus, it would be inconsistent with study aims and methodologically inappropriate to use the BSL measurement as a precise and accurate anchor for evaluating change in TEE in response to the Test diets. Doing so would necessitate a substantially larger number of participants (and increased cost) to account for the additional imprecision, with no scientific benefit. • 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).

Dates			Amendment Summary
Amendment	Protocol Version	Consent Version	
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.
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

Dates			Amendment Summary
Amendment	**Protocol Version**	**Consent Version**	
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	