

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: Randomized Trial

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Protocol Amendment History

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/m², obese: ≥30.0 kg/m²). A set of enrollment logs with identifiers in numerical sequence, one for each stratum, were prepared by the data and quality manager (DQM) 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 (± 0.1 g of the target weight for items ≤ 10 g and ± 0.5 g for items > 10 g). 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 START, MID, and END for participants who achieved weight-loss maintenance within ± 2 kg of the START 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.

Prior to weight loss (PRE), 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 \times 100%) was used as a covariate (Horizon A, Hologic Inc., Bedford, MA).

In addition to weight-loss maintenance, we evaluated several biomeasures of carbohydrate intake as markers of dietary compliance using enzymatic assays. Biomeasures include serum triglycerides and HDL-cholesterol (Roche Diagnostics, Indianapolis, IN) and 1,5-anhydroglucitol (Diazyme Laboratories, Poway, CA). 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 intake.⁶

References

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6. 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]

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.

Study Outcomes Corresponding to each Specific Aim (SA) Study Outcomes for Ancillary Studies Covariates and Effect Modifiers	Run-In Phase		Test Phase		Ad libitum Feeding Phase ^a
	PRE	START	MID	END	
	-14 to -12 weeks	-2 to 0 weeks	8 to 10 weeks	18 to 20 weeks	21 to 22 weeks
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 (<i>post hoc</i>)		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 <i>ad libitum</i> 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 (DXA, 4-compartment model) ^b	X	X		X	
Insulin sensitivity and secretion (OGTT) ^b	X	X	X	X	
Fasting glucose (<i>post hoc</i> effect modifier)	X				
Fasting insulin (<i>post hoc</i> effect modifier)	X				
Fasting insulin resistance by HOMA (<i>post hoc</i> effect modifier)	X				
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; DXA, dual-energy x-ray absorptiometry; HOMA, homeostasis model assessment

^a An ad libitum feeding phase followed the Test 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
<ul style="list-style-type: none">• Aged 18 to 65 years (FSU students, faculty, staff, community members)• BMI ≥ 25 kg/m²• 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
<ul style="list-style-type: none">• Change in body weight exceeding $\pm 10\%$ 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 <p><i>Additional exclusion criteria for females</i></p> <ul style="list-style-type: none">• 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 (for a 2,000–kcal target)

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	<i>P</i> ^b Included vs. Excluded
Sex, No. (%)			
Male	35 (29)	14 (33)	0.70
Female	85 (71)	28 (67)	
Ethnicity, No. (%) ^c			
Hispanic	20 (17)	5 (12)	0.62
Race, No. (%) ^c			
White	93 (78)	34 (81)	
Black	12 (10)	5 (12)	0.76
Asian	4 (3)	1 (2)	
Unknown / Other	11 (9)	2 (5)	
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
<i>Possibly or Probably Related to Intervention</i>				
• 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		
<i>Probably or Definitely Related to Assessments</i>				
• Hematoma	3			
• Vasovagal reaction	4		1	1
• Vomiting	2			
• Lightheadedness			1	
<i>Unrelated to Study Participation</i>				
• 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's 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) ^c
0.75	3207	131
0.76	3173	97
0.77	3140	64
0.78	3108	31
0.79	3077	0
0.80	3046	-31
0.81	3016	-61
0.82	2987	-90
0.83	2958	-118

^a These are data for one sample participant on the low-carbohydrate diet whose calculated TEE was 3077 kcal per day (rCO₂ = 22.7).

^b Total energy expenditure (TEE) was calculated from rCO₂ 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	100	20.0	60	20	0.79
5	2100	115	136	105	22	58	20	0.79
10	2200	130	138	110	24	56	20	0.80
15	2300	145	140	115	25	55	20	0.80
20	2400	160	142	120	27	53	20	0.81
25	2500	175	144	125	28	52	20	0.81
30	2600	190	147	130	29	51	20	0.81
35	2700	205	149	135	30	50	20	0.82
40	2800	220	151	140	31	49	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.

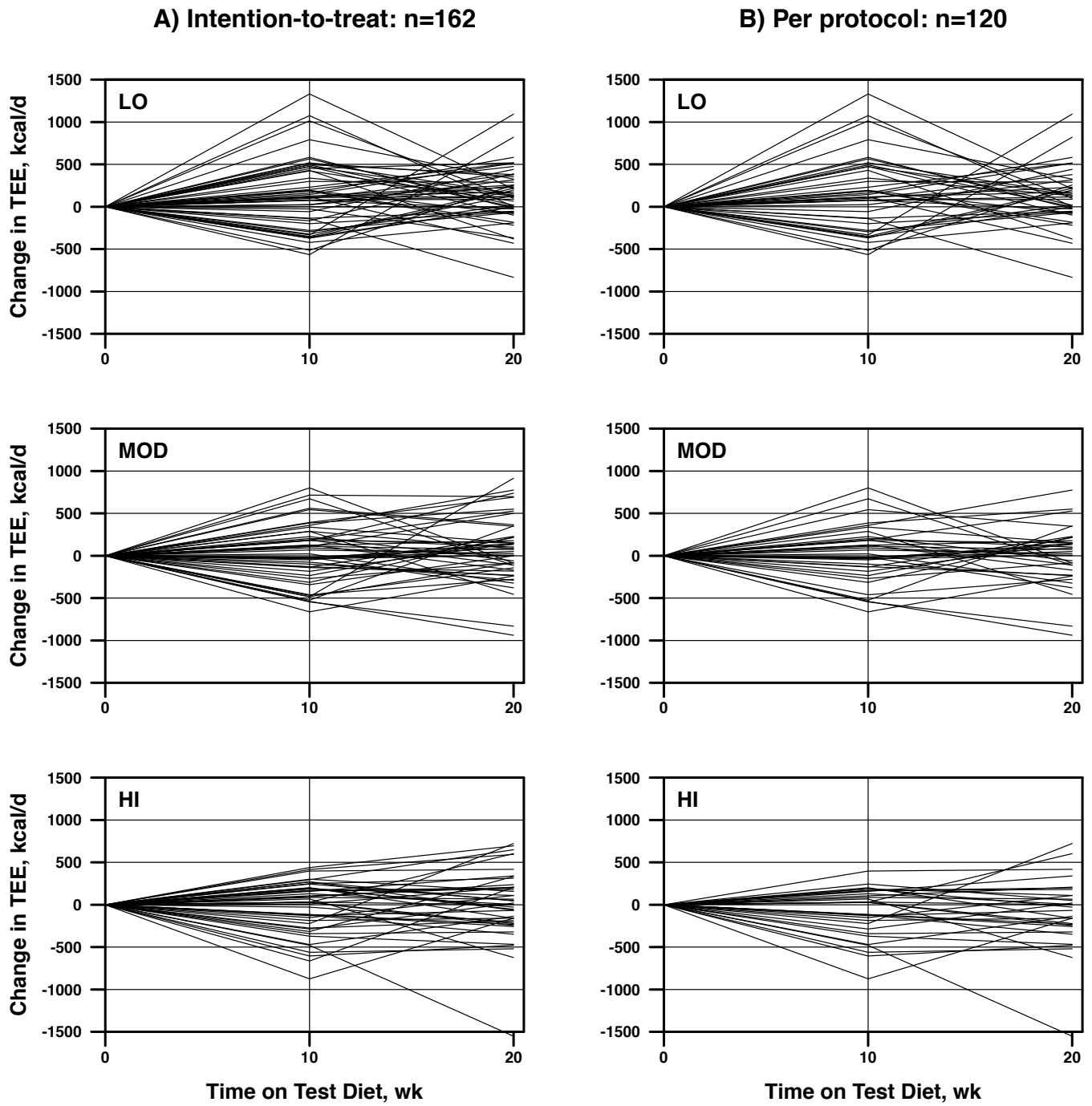
^b Food Quotient (FQ) was calculated using the equation of Black et al.

$$FQ = [\text{Carbohydrate (\%)} \times 1.00] + [\text{Fat (\%)} \times 0.71] + [\text{Protein (\%)} \times 0.81]$$

Reference

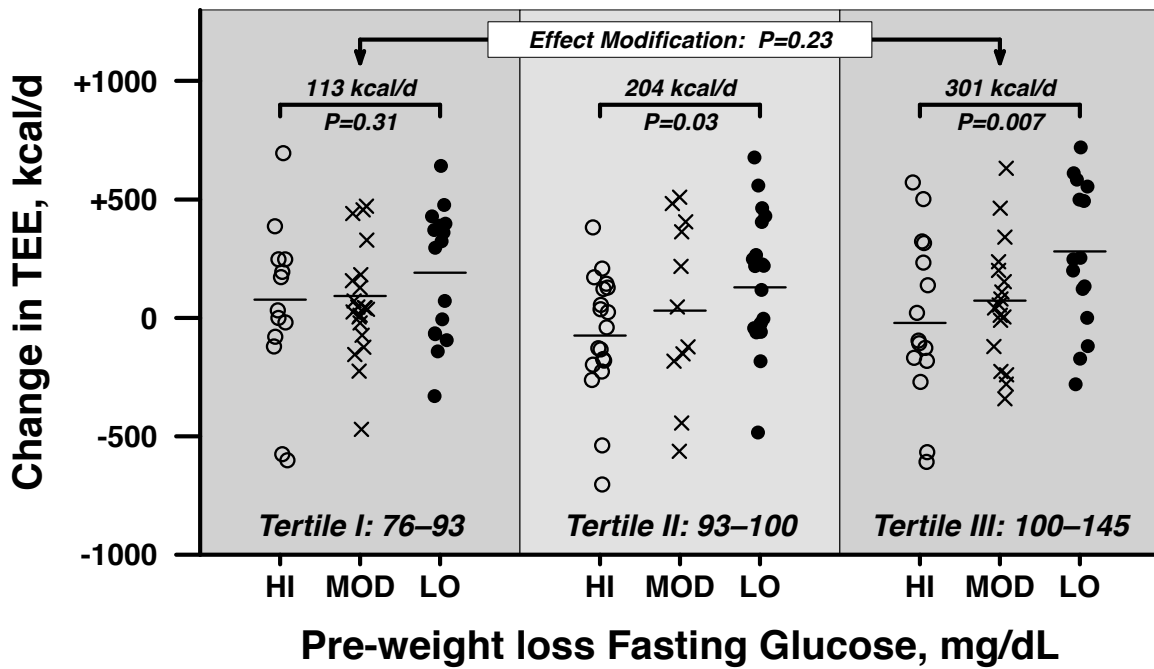
Black AE, Prentice AM, Coward WA. Use of food quotients to predict respiratory quotients for the doubly-labelled water method of measuring energy expenditure. *Hum Nutr Clin Nutr* 1986;40:381-391.

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

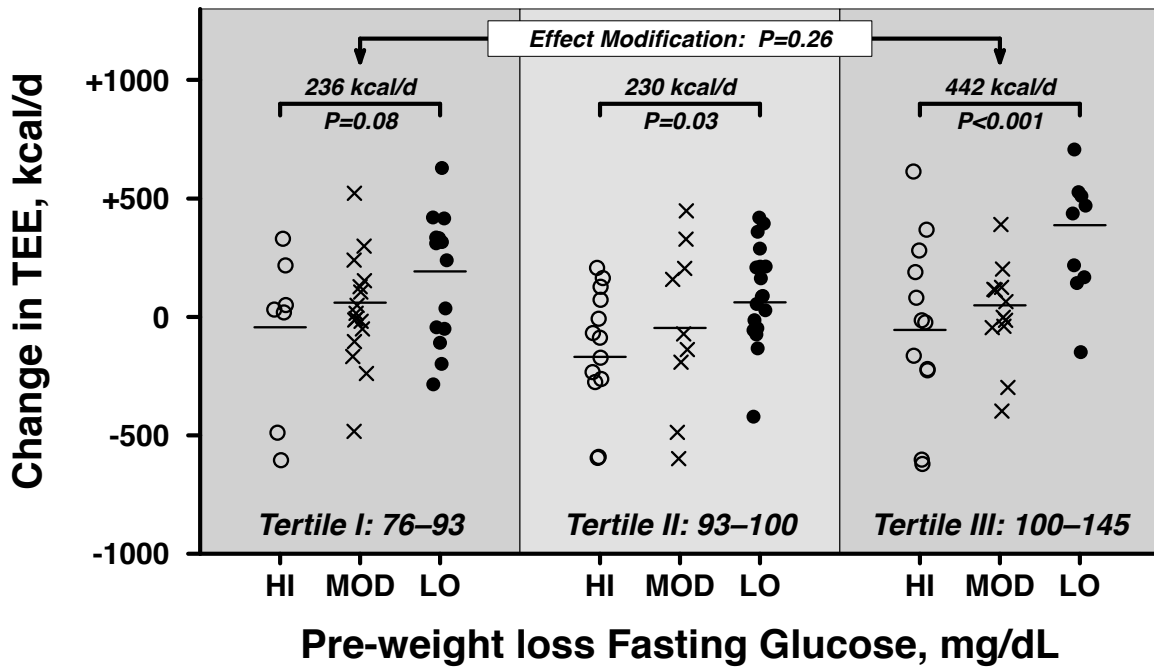


eFigure 2. Effect Modification of Total Energy Expenditure, the Primary Outcome, by Fasting Glucose in the Intention-to-Treat and Per Protocol Analyses

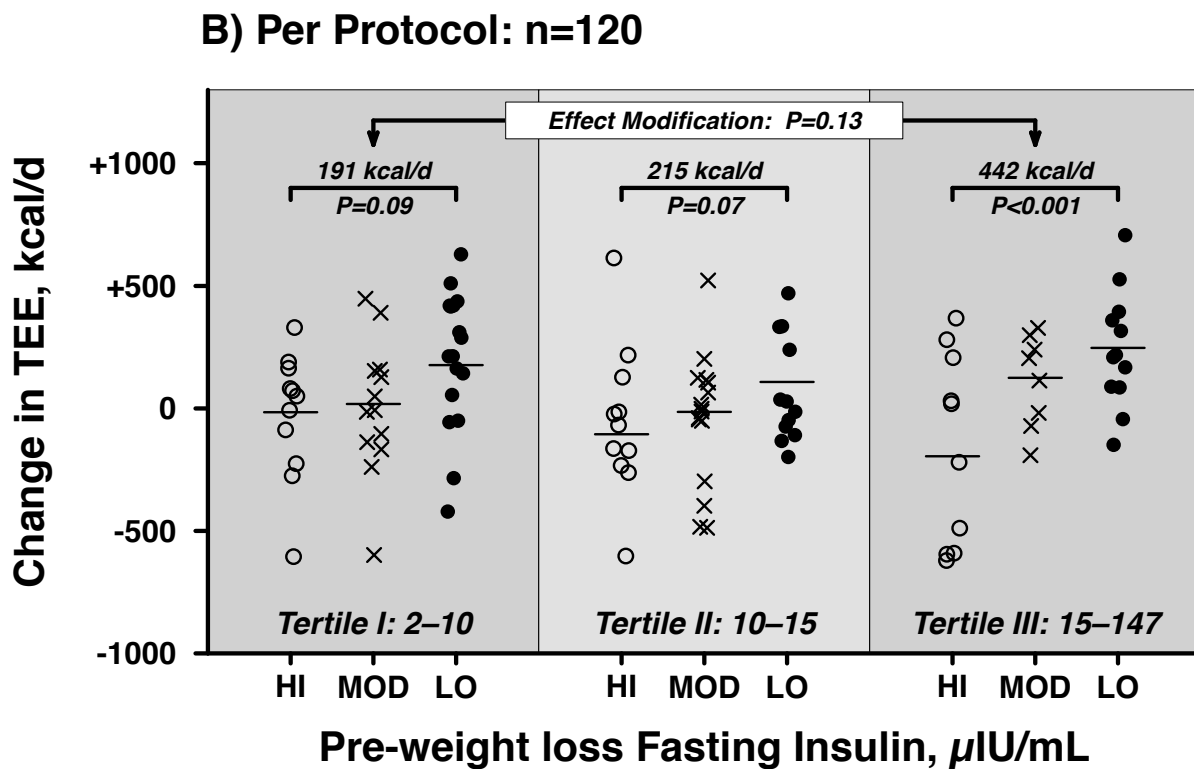
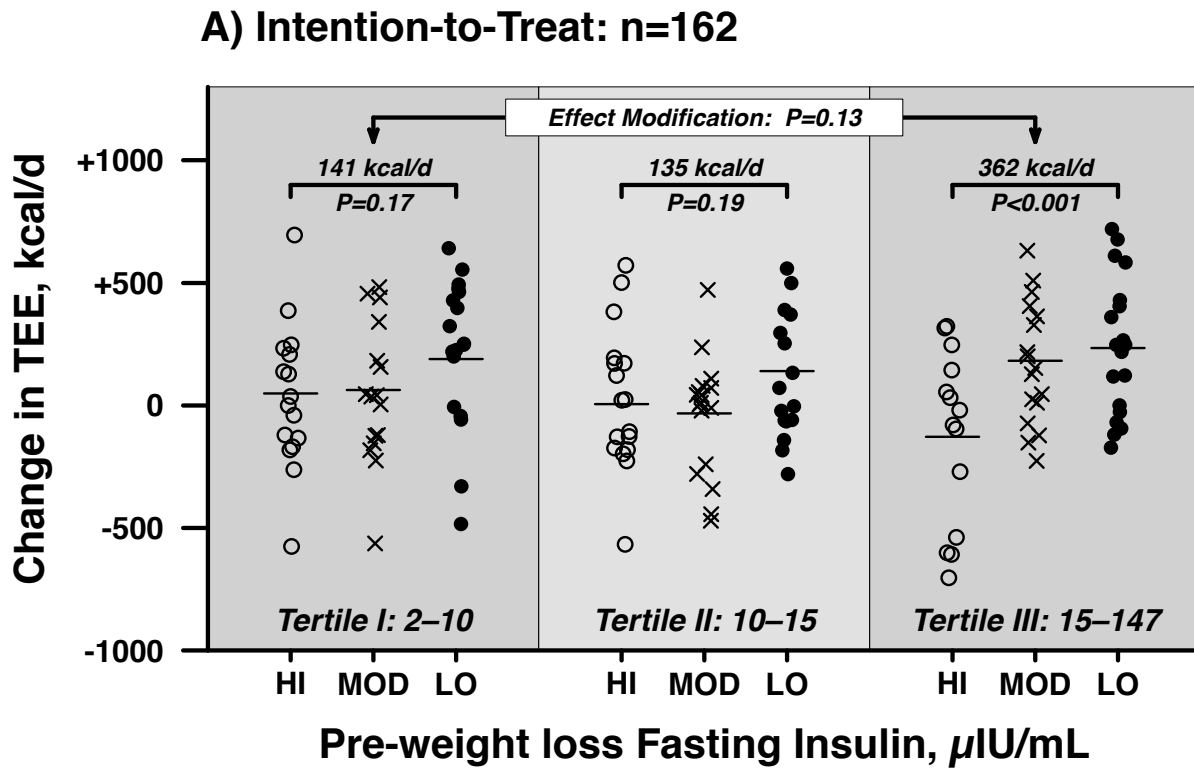
A) Intention-to-Treat: n=162



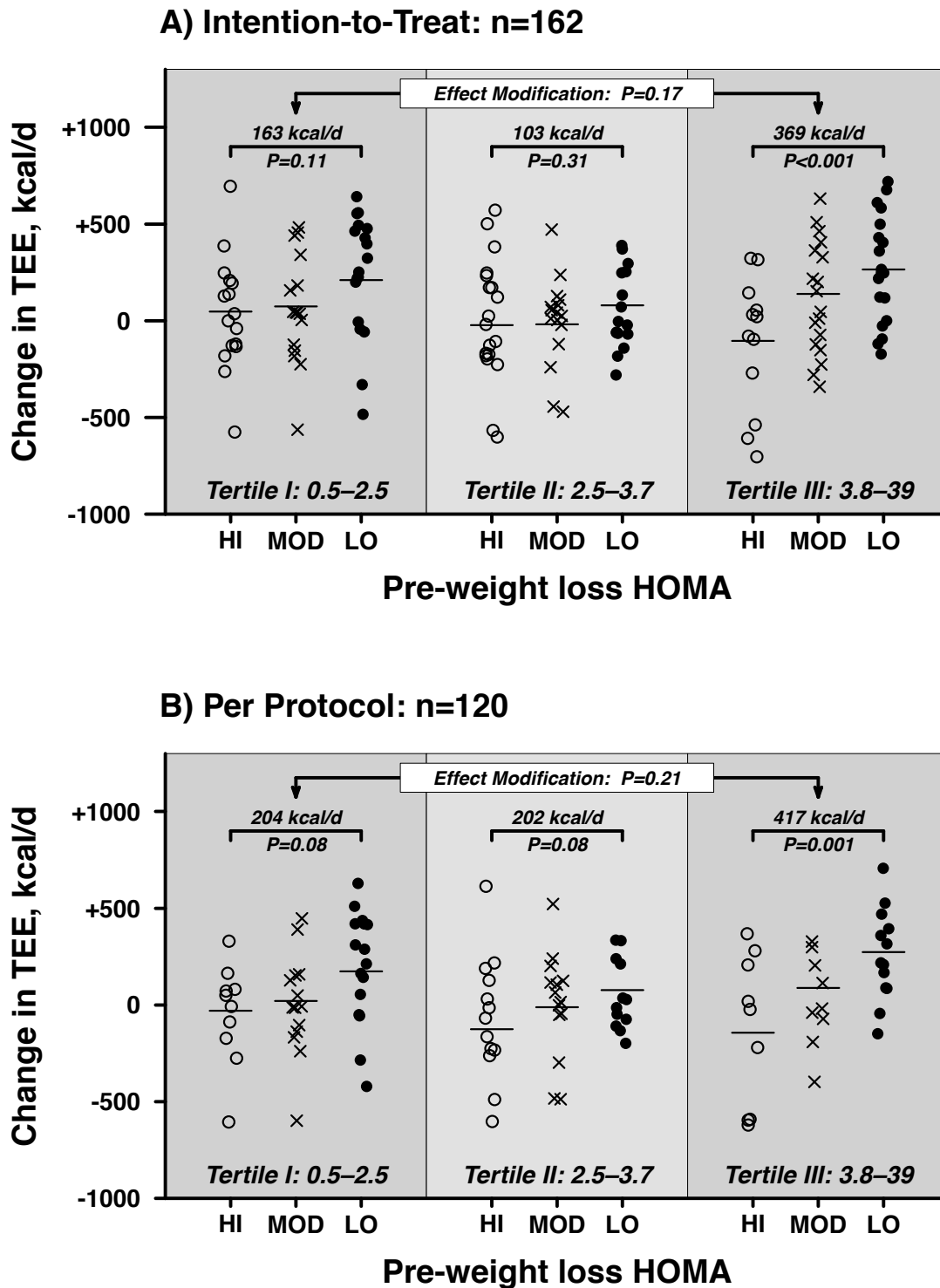
B) Per Protocol: n=120



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



eFigure 4. Effect Modification of Total Energy Expenditure, the Primary Outcome, by Fasting Insulin Resistance in the Intention-to-Treat and Per Protocol Analyses. Fasting insulin resistance was calculated by Homeostasis Model Assessment (HOMA) according to the methods Matthews et al.



Reference

Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28:412-419.

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	<ul style="list-style-type: none"> • 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 start of the Test Diets (START, <i>immediately prior randomization</i>), rather than the pre-weight loss baseline (PRE) as the anchor for evaluating change over time. Original plan was erroneous for the reasons outlined below. <ul style="list-style-type: none"> – 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 PRE measurement was obtained 3 to 4 months prior to initiation of the Test diets. – The PRE 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, <i>Dietary Composition and Energy Expenditure During Weight-Loss Maintenance</i>). – 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 PRE 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).

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2016.07.07	2016.07.06	Screening: 2016.04.29 Trial: 2016.07.06	<ul style="list-style-type: none"> • 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 $\leq 425\text{lb}$ (193kg)" based on the upper limit of the DXA instrumentation to now be "weight $\leq 350\text{lb}$ (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 $\geq 350\text{lb}$.
2016.05.06	2016.04.29	Screening: 2016.04.29 Trial: 2016.04.29	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • 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

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2015.03.23	2015.03.12	Screening: 2015.03.12 Trial: 2015.03.12	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • 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	