Protocol (SPIRIT reporting guidelines)

Administrative Information

1. Title: Obesity risk in African American women is determined by a diet-by-phenotype interaction

Trial acronym: CHAMPION (A **C**linical **H**ealth **A**pproach that **M**otivates **P**articipation and **I**nspires **O**thers through **N**utrition)

2a Trial Registration

ClinicalTrials.gov ID NCT03499509

2b Trial registration data set

N/A

3. Protocol Version

Issue Date: June 2019

Authors: Catia Martins, Barbara Gower, Laura Lee Goree

4. Funding

1R01 DK115483-01A1 National Institutes of Health - National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK).

5a Roles and responsibilities: contributorship

Authors contributions

Barbara Gower conceived of the study and is the grant holder. Barbara Gower, Timothy Garvey, Kevin Fontaine, and Gareth Dutton initiated study design. Barbara Gower, Timothy Garvey, Kevin Fontaine, Gareth Dutton, and David Bryan helped with implementation. Catia Martins and

Barbara Gower prepared the statistical plan. All authors contributed to the refinement of the study protocol and approved the final version.

5b Roles and responsibilities: sponsor contact information

Trial Sponsor: National Institutes of Health - National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)

Sponsor's Reference:

Contact Name: Padma Maruvada

Telephone: 301-594-8884

Email: maruvadp@mail.nih.gov

5c Roles and responsibilities: sponsor and funder

This funding source had no role in the design of this study and will not have any role during its execution, analyses, interpretation of the data, or decision to submit results.

5d Roles and responsibilities: committees

Principal investigators: Design and conduct of trial, preparations of protocol and revisions, publication of study reports, agreement of final protocol, reviewing progress of study and if necessary agreeing changes to the protocol to facilitate the smooth running of the study. Barbara Gower, Principal Investigator will oversee participant safety, study design, database integrity, and study conduct.

Holly Wyatt, MD –Professor in the Department of Nutrition Sciences will be appointed as the Independent Safety Monitor to provide independent monitoring and review of the protocol every 6 months.

Introduction

6a Background and rationale

Introduction: The Scientific Premise of this study is that the high level of obesity displayed by African American (AA) women is due to their ability to secrete large amounts of insulin when high glycemic index foods are consumed.

Mechanisms: When AA women eat a diet rich in starchy or sugary food (a "high-glycemic" diet that stimulates insulin secretion), the food that is eaten is stored as fat rather than being burned as fuel.

Existing knowledge: AA women, as a group, are more obese than AA men or Caucasians of either sex. We believe that this is due to the unique biology of AA women. Specifically, AA women secrete a lot of insulin when they eat sugary foods. Because insulin promotes fat storage, this could make the food unavailable to burn as fuel and explain greater body fat in AA women. In fact, AA women have lower energy requirements than white women, and are more energetically efficient. High insulin secretion, however, is only one factor that determines insulin action; another major factor is insulin sensitivity. Our data have shown that over time, weight (fat) gain in obesity-prone AA women is higher in those who are more insulin sensitive, whereas "obesity-resistant" AA women have lower insulin sensitivity. Our data also suggest that the fat-storing actions of insulin in AA women are worsened by a diet that promotes insulin secretion. This diet is called a "high-glycemic diet." Finally, our data have shown that over time weight (fat) gain in AA women is predicted by the combination of insulin sensitivity and the amount of sugar and starch in the diet (called diet "glycemic load").

Need for a trial: We think that AA women will have an easier time losing weight if they eat a "low glycemic" diet. In fact, we have seen in our research that AA women lose 50% more fat with low- vs high-glycemic diet. This project will test this in a larger group of women, under controlled conditions. We will also see if it is easier to keep the weight off with a low glycemic

diet. Additionally, we will determine if insulin sensitivity affects the weight loss response to diet composition. This study will test the effect of high and low glycemic diets for weight loss and weight-loss-maintenance in obese AA women.

6b Background and rationale: choice of comparators

Explanation for choice of comparators:

We will be comparing a low glycemic diet versus a high glycemic diet. These diets were chosen based on our previous research mentioned above.

7. Objectives

Specific objectives or hypotheses

1.1 Research hypothesis

AA women with lower S_I and/or higher AIRg will lose more FM when on low-CHO, versus low-fat diet, and that this effect would be modulated by differences in EE (REE, TEE or ME).

2. Study objectives

To examine the independent and interactive effects of S_I, AIRg, and diet with changes in fat mass (FM), resting (REE) and total energy expenditure (TEE), and mechanical efficiency (ME) in AA women with obesity during weight loss.

2.1 Primary objective

To examine the independent and interactive effects of S_I, AIRg, and diet on changes in fat mass in African American women with obesity.

2.2 Secondary objectives

To characterize REE, TEE, and ME following low-CHO, versus low-fat diet.

8. Trial design

The CHAMPION study was designed as a two-arm randomized clinical trial. Randomization (1:1) was performed using a computer-generated randomization scheme prepared with PROC PLAN in SAS Ver. 9.4.

Methods: Participants, interventions, and outcomes

9. Study setting

Data will be collected from healthy, but obese, AA women in the Birmingham, AL, metro area in The United States of America. Study testing will be performed in the Core facilities of the Center for Clinical and Translational Science (CCTS), Nutrition Obesity Research Center (NORC), and Diabetes Research Center (DRC) at UAB.

10. Eligibility criteria:

Participants will provide written, informed consent before the start of the study (see Appendix 1 for sample Informed Consent Form).

Inclusion criteria

Participants eligible for the study must comply with all of the following at randomization:

1. Age 19-65 years
2. African American female
3. BMI 30-45 kg/m^2
4. Sedentary to moderately active
5. Normal menstrual cycle in premenopausal women
6. Normal glucose tolerance based on HbA1c less than 6.0%

Exclusion criteria

1. History of eating disorder

2. Daily use of tobacco (>1 pk/wk)

3. Change in weight greater than 5 lb in the previous 3 months

4. Presence of any condition (e.g. PCOS) or use of any medication (e.g., glucocorticoid) deemed by the project physician to interfere with study outcomes.

11a Interventions: description

Participants will be randomized to either low-fat or low-CHO hypocaloric diets for 10wks, followed by a 4-week weight stabilization period, with all food provided throughout.

Energy requirements will be estimated by multiplying REE, measured with indirect calorimetry, by 1.5 (PA factor), and all participants will receive an individualized dietary prescription throughout the weight loss phase (60% of estimated energy needs or 40% energy deficit) and during the 4-week weight stabilization period (energy balance (EB)). During the weight stable phase, diets will be adjusted if weight is not stable. All the food for the duration of Phases 1 and 2 was delivered to the participants' homes on a weekly basis. The low-fat diet was aligned with the USDA guidelines (http://health.gov/dietaryguidelines/2015/guidelines/), and was comprised of 55% CHO, 20% fat, and 25% protein. The low-CHO diet was comprised of 25% CHO, 55% fat and 25% protein. Both diets emphasized complex over simple CHO, and allowed dairy products, fruits, and vegetables, within the CHO allowance of each diet.

This will be followed by a 6-month weight maintenance phase with dietary prescription only, where participants will provide their own food. There will be monthly peer group support meetings with the dietitian to discuss nutrition education topics (such as food label reading, portion sizes, tips on eating out, etc). Participants will individually report their body weight to the dietitian and turn in an 8-day food record every month for 6 months.

11b Interventions: modifications

On 06/17/2020, the protocol was amended and approved by UAB IRB to minimize in person contact with participants due to COVID. DXA was replaced with an at home creatine dilution test to measure muscle mass. The post intervention (week 14) IVGTT and Mixed Meal Tolerance tests were omitted from the protocol. The Oral Glucose Tolerance Test at screening was replaced with measurement of Hemoglobin A1c finger stick (choice of at home or in person). Food was delivered to participants home via a commercial provider. The REE and Exercise tests were optional, if participants were not comfortable completing these in-person visits. Participants were given the option to complete the TEE visit at home or in-person. The weekly in-person meetings with the dietitian were moved to a Zoom or phone call and the monthly peer group meetings in Phase 3 were moved to Zoom. Food records were emailed and/or discussed via phone call, with food intake and body weight reported.

11c Interventions: adherance

Strategies used to improve adherence to intervention protocols included weekly phone calls with participants to report previous week's specific food intake, report current body weight, and troubleshooting/support from a Registered Dietitian. Procedures for monitoring adherence include the measurement of respiratory quotient by indirect calorimetry, body weight and composition by bioelectrical Impedance Analysis and blood draw for the quantification of β-hydroxybutyrate, insulin, glucose, triglycerides, and cholesterol.

11d Interventions: concomitant care

Participants will be prohibited from participating in other weight loss programs (diet, exercise, and/or medications that induce weight loss). Participants will be instructed to continue their usual physical activity while in the study.

12. Outcomes

Main outcome measures**:** S_I and AIRg will be measured at baseline with an intravenous glucose tolerance test, body composition with bioimpedance at baseline and Wk10. REE and body composition will also be measured at week 5. REE, TEE, and mechanical efficiency (ME) will be measured with indirect calorimetry, doubly labeled water, and a submaximal bike test, respectively, at baseline and Wk14.

13. Participant timeline

Participants were enrolled between June 2019 and August 2023. Participants will complete 10 weeks of hypocaloric diet, 4 weeks of weight stabilization, and 6 months of weight loss maintenance. See figure 1.

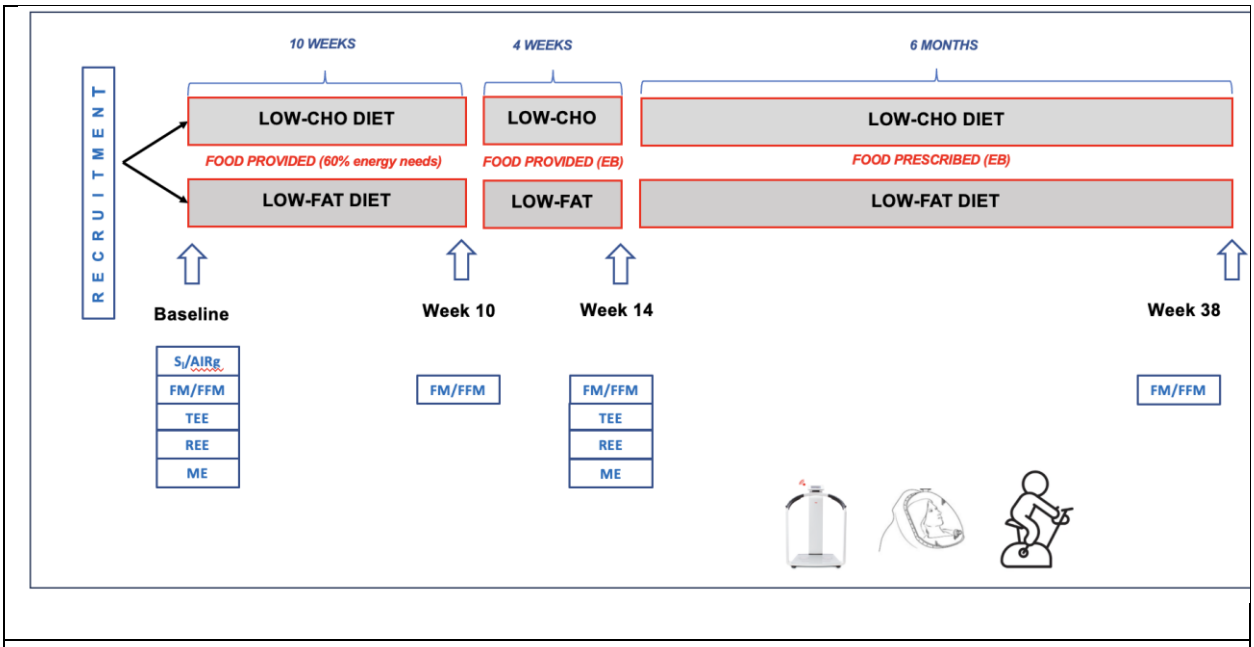

Fig. 1 Schematic diagram of the study design.

14. Sample size

This study was powered for differences in FM loss between the two diet groups. Our published data indicate that AA (both men and women) lose more FM when consuming a LG vs HG diet over 16 weeks (1). Subgroup analysis within only the AA women (n=17), for only the 8-week hypocaloric phase, indicated that the women lost more FM with the LG diet [4.5 ± 1.0 (SEM) kg] vs the HG diet [2.0 ± 0.9 (SEM) kg] at a significance level of P=0.113. Based on these numbers, with 74 women (37/ group) we would have 80% power to detect a difference of 2.5 kg in FM loss between diets.

15. Recruitment

We will recruit from the local communities (UAB, city of Birmingham, and Jefferson and Shelby counties) via radio, newspaper, web-based advertisement, television advertisement, Research Match, UAB Reporter, word of mouth, and posting of flyers.

Methods: Assignment of interventions

16a Allocation: sequence generation

After baseline testing, participants were randomized to one of two diets (low-glycemic, LG or high glycemic, HG) using a computer generated randomization scheme prepared with PROC PLAN in SAS Ver. 9.4.

16b Allocation concealment mechanism

Randomizations were placed in sealed sequentially numbered envelopes until interventions were assigned. All participants who give consent for participation, who fulfill the inclusion criteria, and complete baseline testing will be randomized. Participants will be enrolled by eligibility from the study team (principal investigator, coordinators, and dietitians). Study Coordinator will open sequentially numbered envelopes for diet intervention. The study dietitians will reveal the diet intervention to participants, along with a detailed diet introduction and explanation.

17a Blinding (masking)

No blinding. Since it was necessary to reveal the diet assignment to the study dietitian and the participant for appropriate diet introduction and education to the participant, this study will not involve blinding.

17b Blinding (masking): emergency unblinding

N/A

Methods: Data collection, management, and analysis

18a Data collection plan

All measurements will be done in the Core facilities of the Center for Clinical and Translational Science (CCTS), Nutrition Obesity Research Center (NORC), and Diabetes Research Center (DRC) at UAB. Participants will be instructed to avoid strenuous physical

activity (PA) the day prior to testing, and all PA on the morning of testing. All S_I tests will be performed in the Clinical Research Unit (CRU) at UAB's CCTS after an overnight fast of 12 h. The following measurements will be performed at baseline and end of weeks 10 and 14, unless otherwise stated.

S_I will be assessed at baseline only using the intravenous glucose tolerance test (IVGTT) and minimal modeling, as previously described (2), but using 300 mg of glucose (20 % dextrose)/kg of body weight. AIRg will be calculated as the incremental insulin area-under-the-curve from minutes 0–10 following glucose injection using the trapezoidal method.

Body composition (FM and fat-free mass) will be determined by bioimpedance analysis (BIA) (Seca 514 mBCA, Seca GmHb & Co. KG, Hamburg, Germany).

Resting Energy Expenditure and substrate utilization will be determined in the CCTS/NORC/DRC Core Laboratory by indirect calorimetry (TrueOne 2400, Parvo Medics, Sandy, UT). Participants will be tested in the fasted (10h) condition following a 30-min supine rest at baseline and week 14.

Total Energy Expenditure will be measured through the CCTS/NORC/DRC Core Laboratory by doubly-labeled water (DLW), at baseline and week 14, using a protocol based on established procedures (3). A FQ of 0.85 will be used at baseline to reflect a standard omnivorous diet, while at week 14, the FQ of the intervention diets will be used (4). FQ will be calculated using the equation of Black et al, FQ = (CHO% x 1.00) + (fat% x 0.71) + (protein% x 0.81) (4).

Mechanical efficiency will be assessed using a submaximal cycling test, in the fasted state, at baseline and week 14. Following a familiarization period, participants cycled at 60 RPM at 10, 20 and 50 watts of power in 4-minute increments. Ventilation, oxygen uptake, carbon dioxide production, respiratory exchange ratio, and heart rate were obtained throughout. Net skeletal muscle work efficiency will be expressed as the ratio of power generated (kcal/min) to the change in EE above REE (kcal/min) at each power output (5).

Laboratory analyses. Blood samples will be collected in the fasting state and concentrations of glucose, βHB, total cholesterol, HDL-cholesterol and triglycerides measured using a SIRRUS analyzer (Stanbio, Boerne, TX), and insulin using a TOSOH A1A-900 immunoassay analyzer (TOSOH Bioscience, South San Francisco, CA) in the CCTS/DRC Core Laboratory. LDL-cholesterol will be calculated using the Friedewald formula (6).

18b Data collection plan: retention

Participant retention will be promoted by weekly communications from the study dietitians and reminder communications from the study coordinators for upcoming in-person testing visits.

19. Data management

Confidentiality will be maintained by coding all data with the subject's identification number. Computerized data will be stored in the Department of Nutrition Sciences database. Paper data will be stored in the Department of Nutrition Sciences in a locked room in a file cabinet. Only qualified research personnel will have access to the database containing the subject information. All subject data that are entered into statistical analyses and publication reports will refer to individuals only by number rather than name.

All records pertaining to medical history, participation in this study, and data collected will be kept strictly confidential and will be recorded only by study number, to protect participant privacy. Only study investigators and the UAB IRB will have access to this data. Information regarding participant demographics, body composition, metabolic outcomes, or blood outcome measures will be shared and labeled with the study number, a unique identifier, and the date of acquisition.

20a Statistics: outcomes

Statistical analysis will be performed with SPSS version 29 (SPSS Inc., Chicago, IL), and data presented as mean ± SD (or SEM). Statistical significance will be set at $P < 0.05$, unless otherwise specified, and normality of variables was assessed by visual inspection of histogram and Shapiro-Wilk test. Participants will be included in the analysis if they have S_I data at baseline, and body composition at baseline and week 10.

To test the hypothesis that AA women with obesity randomized to a low-CHO diet lose more FM than those randomized to a low-fat diet, we will analyze data from Phase 1 with generalized linear models (GLM). FM at week 10 will be the dependent variable, baseline FM, diet group, and S_I (and/or AIR) the covariates. Finally, to test the hypothesis that S_I (and/or) modulates the response to diet, a "diet x S_I" interaction term will be added to the models; i.e., $\Delta FM = \beta_0 + \beta_1 \text{ baseline FM} + \beta_2 \text{ baseline } S_i + \beta_3 \text{ diet group} + \beta_4 \text{ diet group} * S_i + e_1$.

To test the hypothesis that AA women with obesity randomized to a low-CHO diet maintain a higher TEE and REE, and a lower ME, than those randomized to a low-fat diet, we analyzed data from the end of Phase 2 with GLM. TEE, REE, or ME (week 14) were the dependent variables; baseline TEE, REE, or ME, diet group, and S_I (and/or AIRg) the covariates. For all

outcomes, we tested for a phenotype-by-diet interaction by adding relevant interaction terms to the model; e.g., $\Delta\text{TEE (week 14} - \text{baseline)} = \beta_0 + \beta_1 \text{ baseline TEE} + \beta_2 \text{ baseline } S_i + \beta_3 \text{ diet group} + \beta_4 \text{ diet group} * S_i + e_1$.

We will also test for menopausal status as potential predictors of the independent variables of interest, and include this variable in the models if necessary. The primary analyses will treat S_I as a continuous variable and test for independent and interactive effects of S_I and diet in statistical models. For sub-group analyses the median S_I will be used to designate status: high vs low S_I.

20b Statistics: additional analyses

N/A

20c Statistics: analysis population and missing data

N/A

Methods: Monitoring

21a Data monitoring: formal committee

Holly Wyatt, MD –Professor in the Department of Nutrition Sciences is appointed as the Independent Safety Monitor to provide independent monitoring and review of the protocol every 6 months.

21b Data monitoring: interim analysis

Description of interim analyses and stopping guidelines:

22. Harms

If an individual indicates discomfort or the desire to discontinue testing, tests will be terminated immediately. During routine visits and telephone calls, the study coordinator will query

participants for any adverse events since their last contact with study personnel. In the case of an adverse event during times of testing, a study physician will be on call. The physician will provide medical supervision for this study. All adverse events will be reported to the UAB IRB and reviewed by the investigative team. Should any of these individuals and/or the IRB identify that any adverse event may be related to the study protocol, testing will be suspended, and the protocol will be amended accordingly.

23. Auditing

A progress report will be submitted annually to the sponsor and to UAB IRB.

Ethics and dissemination

24. Research ethics approval

The study is approved by UAB IRB (IRB-300001324) and annual progress reports will be sent to UAB IRB during the full duration of the study.

25. Protocol amendments

On 06/17/2020, the protocol was amended and approved by UAB IRB to minimize in person contact with participants due to COVID. DXA was replaced with an at home creatine dilution test to measure muscle mass. The post intervention (week 14) IVGTT and Mixed Meal Tolerance tests were omitted from the protocol. The Oral Glucose Tolerance Test at screening was replaced with measurement of Hemoglobin A1c finger stick (choice of at home or in person). Food was delivered to participants home via a commercial provider. The REE and Exercise tests were optional, if participants were not comfortable completing these in-person visits. Participants were given the option to complete the TEE visit at home or in-person. The weekly in-person meetings with the dietitian were moved to a Zoom or phone call and the

monthly peer group meetings in Phase 3 were moved to Zoom. Food records were emailed and/or discussed via phone call, with food intake and body weight reported.

26a Consent

The study coordinator will obtain informed consent from trial participants. Potential participants who met the inclusion criteria for the study will be asked to review the consent form and if interested, to contact Study Coordinators. If an individual is eligible to join the study, a screening measurement will be scheduled. During this visit, the project coordinator will review the consent form and obtain informed consent. The consenting process is confidential, and will take place in a private room.

26b Consent: ancillary studies

N/A

27. Confidentiality

Confidentiality is maintained by coding all data with the subject's identification number. Computerized data will be stored in the Department of Nutrition Sciences database. Paper data will be stored in the Department of Nutrition Sciences in a locked room in a file cabinet. Only qualified research personnel have access to the database containing the subject information. All subject data that are entered into statistical analyses and publication reports will refer to individuals only by number rather than name.

All records pertaining to medical history, participation in this study, and data collected will be kept strictly confidential and will be recorded only by study number, to protect participant privacy. Only study investigators and the UAB IRB will have access to this data. Information regarding participant demographics, body composition, metabolic outcomes, or blood outcome

measures will be shared and labeled with the study number, a unique identifier, and the date of acquisition.

28. Declaration of interests

All study investigators and other personnel will complete conflict of interest disclosure training and forms.

29. Data access

Only those listed on the study protocol, as approved by UAB IRB will have access to study data.

30. Ancillary and post trial care

UAB and NIDDK will not provide any payment if participants are harmed as a result of taking part in this study. If such harm occurs, treatment will be provided. However, this treatment will not be provided free of charge.

31a Dissemination policy: trial results

Study investigators will report study analyses and results via publications. Participants will receive individual clinical test results after completion of the study.

31b Dissemination policy: authorship

Authorship eligibility guidelines and any intended use of professional writers

31c Dissemination policy: reproducible research

Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code

The protocol and results will be posted on Clinicaltrials.gov website.

Appendices

32. Informed consent materials

33. Biological specimens

DNA will be collected for admixture analysis and analysis of SNPs related to beta cell function.

1. Gower BA, Goss AM. A lower-carbohydrate, higher-fat diet reduces abdominal and intermuscular fat and increases insulin sensitivity in adults at risk of type 2 diabetes. J Nutr. 2015;145(1):177s-83s.

2. Gower BA, Bergman R, Stefanovski D, Darnell B, Ovalle F, Fisher G, et al. Baseline insulin sensitivity affects response to high-amylose maize resistant starch in women: a randomized, controlled trial. Nutr Metab (Lond). 2016;13:2.

3. Goran MI, Carpenter WH, McGloin A, Johnson R, Hardin JM, Weinsier RL. Energy expenditure in children of lean and obese parents. American Journal of Physiology. 1995;31:E917-E24.

4. Black AE, Prentice AM, Coward WA. Use of food quotients to predict respiratory quotients for the doubly-labelled water method of measuring energy expenditure. Hum Nutr Clin Nutr. 1986;40(5):381-91.

5. Rosenbaum M, Goldsmith R, Bloomfield D, Magnano A, Weimer L, Heymsfield S, et al. Low-dose leptin reverses skeletal muscle, autonomic, and neuroendocrine adaptations to maintenance of reduced weight. J Clin Invest. 2005;115(12):3579-86.

6. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry. 1972;18(499):502.