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# **Gene-Environment Interaction on Adiposity, Glycemic, Lipids Changes and Bone Health: Study Protocol with Statistical Analysis**

## **1. Objective**

The purpose of this study is to test the interactions between the genes and diet interventions varying in macronutrient intake on glycemic, waist circumference, body weight, visceral fat, lipids changes and bone health. In the POUNDS LOST, all of the 811 participants DNA was extracted from the buffy coat fraction of centrifuged blood using the QIAmp blood kit (Qiagen, Chatsworth, CA). Single nucleotide polymorphisms were genotyped using the OpenArray™ SNP genotyping system (BioTrove, Woburn, MA). The study is only accepting participants in the Boston, Massachusetts or Baton Rouge, Louisiana area. For further enrollment information in Boston or Baton Rouge, see Eligibility Criteria or Design Narrative.

Besides, the investigators integrated novel pathway analyses in large cohorts and comprehensive genetic analyses on long-term weight loss and mechanisms in randomized diet intervention trials would provide very important evidence to unravel the etiology of obesity, and have significant public health and clinical implications. Establishing relationship between genetic variants and diets in determining weight change will help identify individuals at high risk for obesity especially when adherent to specific diet.

The epidemic of obesity has become a major public health problem. Obesity is a multifactorial abnormality that has a genetic basis but requires environmental influences to manifest. Common-form obesity is underpinned by both environmental

and genetic factors. Recent genome-wide association studies have identified several genes convincingly related to obesity risk. Testing gene-environment interaction is a relatively new field. Evidence from association studies and intervention trials continues to mount, indicating that genetic components may modify lifestyle effects on the development of obesity. However, these findings are at most preliminary. The interplay between genetic and environmental components may facilitate the choice of more effective and specific measures for obesity prevention based on the personalized genetic make-up.

## **2. Design and Methods**

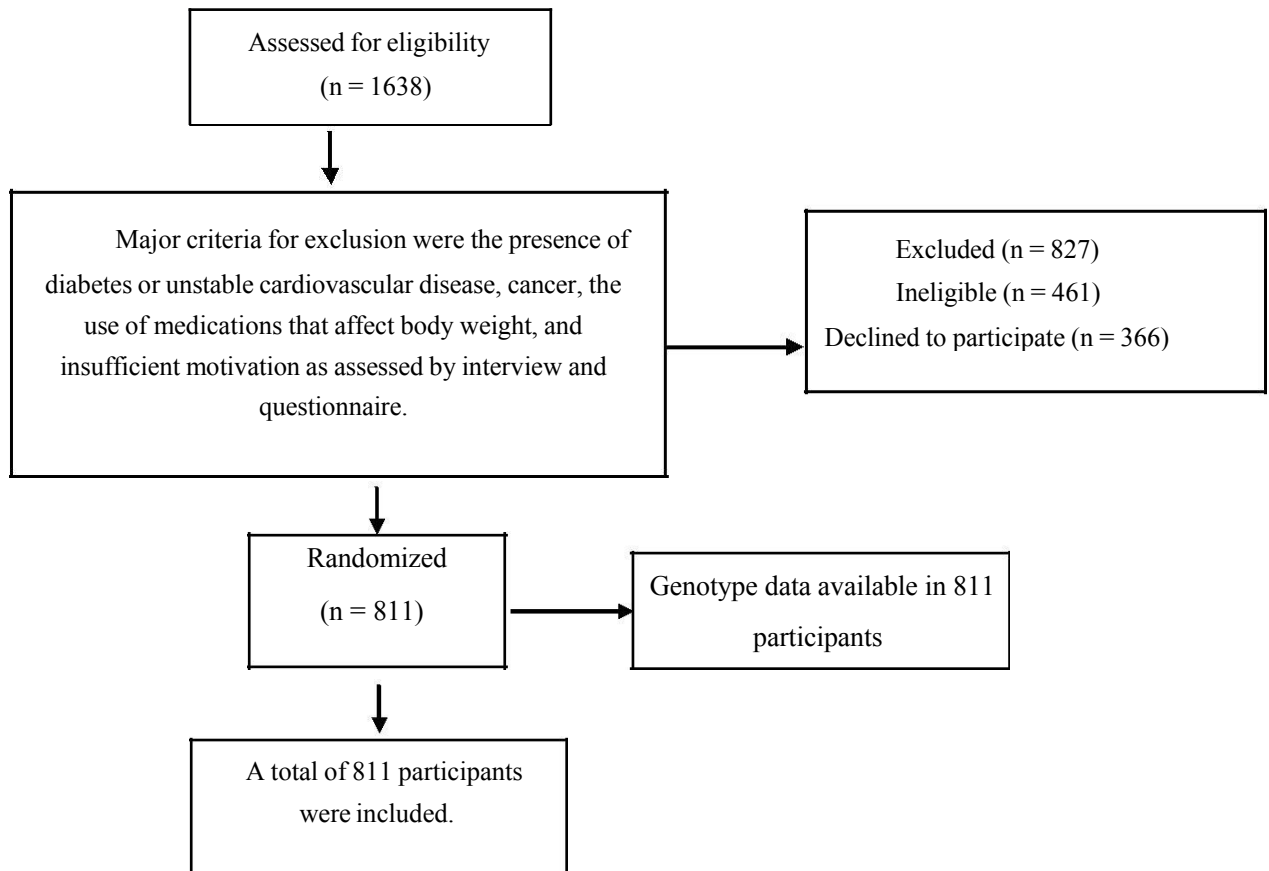
The investigators aimed to examine whether the genetic variation affected glycemic, waist circumference, body weight, visceral fat, lipids, and bone mineral density (BMD) changes in response to weight-loss dietary intervention varying in macronutrient intake (target percentages of energy derived from fat, protein, and carbohydrate in the 4 diets were as follows 20%, 15% and 65%; 20%, 25%, and 55%; 40%, 15%, and 45%; and 40%, 25%, and 35%).

The primary outcome is to test the interactions between the genes and diet interventions varying in macronutrient intake on body weight changes. The secondary outcome is to test the interactions between the genes and diet interventions varying in macronutrient intake on glycemic, waist circumference, visceral fat, lipids, and BMD changes. Understanding gene–diet interactions in relation to weight loss holds great promise for delivering more efficient prevention and treatment on obesity and related metabolic disorders.

We assessed overall genetic variation by calculating a genetic risk score (GRS). The GRS was calculated based on the SNPs by summing risk allele' s numbers, and weighted by their effect sizes ( $\beta$  -coefficients) derived from genome-wide association meta-analyses data (Supplementary Table 1). The GRS was computed using the equation:  $GRS = (\beta_1 \times SNP1 + \beta_2 \times SNP2 + \dots + \beta_n \times SNPn) \times (n/\text{sum of the } \beta \text{ -coefficients})$ , where  $\beta$  is the  $\beta$  -coefficient of each SNP for higher levels of fasting glucose, SNP1, SNP2  $\dots$  and SNP n indicate the number of risk alleles (0, 1 or 2) for each SNP.

The Hardy-Weinberg equilibrium and comparison of categorical variables at baseline were assessed with chi-square test. Differences in continuous variables according to tertiles of the GRS(genetic risk score) at baseline were tested using general linear models, with adjustment for age, sex, and race. The investigators used general linear models to test changes in adiposity, glycemic and lipids traits in high-fat and low-fat diets groups according to tertiles of the GRS. Covariates adjustment included age, sex, race, physical activity, smoking, alcohol, seasonal variation, and the baseline value for the respective outcome in model. Moreover, to analyze the potential interactions between GRS and diet interventions, an interaction product term of GRS-diet was included in the models. As a secondary analysis, with the use of time as a repeated variable, linear mixed models were used to test the GRS effect on the trajectory of changes in adiposity, glycemic and lipids traits by including a GRS-by-time interaction term. Because our analysis is hypothesis-driven and primarily focused on the GRS (rather than individual SNPs), we did not adjust for multiple testing. The level of significance for all tests was set to  $P < 0.05$ . Statistical analyses were performed with SAS version 9.2 (SAS Institute, Inc., Cary, NC).

### 3. Participant Flow



**Recruitment Details:** The included participants were a group of 811 overweight or obese individuals with ages and BMI being 30-70y and 25-40 kg/m<sup>2</sup>, respectively. The major exclusion criteria were the presence of diabetes treated with oral medications or insulin, unstable cardiovascular disease, cancer, the use of medications that influence body weight and inadequate motivation as estimated by an interview and questionnaire.

#### **Arm/Group Information and title:**

diet 1: diet with moderate in fat (40% energy) and protein(15%)

diet 2: diet with moderate in fat (40% energy) and protein(25%)

diet 3: diet with low in fat (20% energy) and protein(15%)

diet 4: diet with low in fat (20% energy) and protein(25%)

**Arm/Group Description :**

diet 1: The study tests the effectiveness for weight loss and weight maintenance of four diets differing in macronutrient composition: moderate in fat (40% energy) with two different protein levels (15% and 25%), and low in fat (20% energy), also with 15% and 25% protein levels.

diet 2: The study tests the effectiveness for weight loss and weight maintenance of four diets differing in macronutrient composition: moderate in fat (40% energy) with two different protein levels (15% and 25%), and low in fat (20% energy), also with 15% and 25% protein levels.

diet 3: The study tests the effectiveness for weight loss and weight maintenance of four diets differing in macronutrient composition: moderate in fat (40% energy) with two different protein levels (15% and 25%), and low in fat (20% energy), also with 15% and 25% protein levels.

diet 4: The study tests the effectiveness for weight loss and weight maintenance of four diets differing in macronutrient composition: moderate in fat (40% energy) with two different protein levels (15% and 25%), and low in fat (20% energy), also with

15% and 25% protein levels.

**Period(s):** a 2-year randomized multicenter clinical trial .

**Period Title:** A 2-year randomized multicenter clinical trial

**Started:** September 2003

**Completed:** December 2010

#### **4. Baseline Characteristics**

**Arm/Group Information:** Participants were randomly distributed to one of four diets that composed a two-by-two factorial design: 1) a low-fat, average-protein diet (20% fat, 15% protein, and 65% carbohydrate, 2) a low-fat, high-protein diet (20% fat, 25% protein, and 55% carbohydrate), 3) a high-fat, average-protein diet (40% fat, 15% protein, and 45% carbohydrate), and 4) a high-fat, high- protein diet (40% fat, 25% protein, and 35% carbohydrate).

#### **Arm/Group Description :**

diet 1: The study tests the effectiveness for weight loss and weight maintenance of four diets differing in macronutrient composition: moderate in fat (40% energy) with two different protein levels (15% and 25%), and low in fat (20% energy), also with 15% and 25% protein levels.

diet 2: The study tests the effectiveness for weight loss and weight maintenance of four diets differing in macronutrient composition: moderate in fat (40% energy) with two different protein levels (15% and 25%), and low in fat (20% energy), also with 15% and 25% protein levels.

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diet 4: The study tests the effectiveness for weight loss and weight maintenance of four diets differing in macronutrient composition: moderate in fat (40% energy) with two different protein levels (15% and 25%), and low in fat (20% energy), also with 15% and 25% protein levels.

**Overall Number of Baseline Participants: 811**

Baseline Measure Information: After an overnight fast, body weight, height and waist circumference were measured in the morning on 2 nonconsecutive days at baseline, 6 months, and 2 years.

**5. Outcome Measures**

**Outcome Measure Information:** Anthropometric measures were collected at baseline and 6, and 2 y according to standard protocols. BMI was calculated by dividing weight by the squared height of a participant. Dual-energy X-ray absorptiometry (DXA) scans were performed on a random subset of 50% of participants to assess body fat and BMD at baseline, 6 mo, and 2 y. Fasting blood samples were collected at baseline and 6 and 24 mo. Analyses for glucose and insulin were done from aliquoted serum at the Pennington Laboratory



with an immunoassay with chemiluminescent detection on an Immukite analyzer (Diagnostics Products Corp). Triglyceride, TC, LDL cholesterol, and HDL cholesterol were measured by using the Beckman Synchron CX7 analyzer (Beckman Coulter).

Genotyping has been described in detail previously. Briefly, DNA was extracted by using a QIAmp Blood Kit (Qiagen). Polymorphisms were genotyped by using the OpenArray SNP Genotyping System (BioTrove). The genotype success rate and replication concordance were >99%.

Outcomes included changes at 6 and 24 mo (determined as the difference from baseline) for anthropometric measures (weight, waist circumference, and BMI) and at 6 mo and 2 y for DXA body fat (total body fat, lean mass, and trunk fat) and BMD measures. The body-composition outcomes were selected to depict various adipose depots (ie, abdominal or central adiposity by using waist circumference and overall body fat by using BMI). The other endpoints for this study were changes in glycemic traits including fasting glucose, fasting insulin, insulin resistance and insulin sensitivity over the intervention. Homeostasis model assessment (HOMA) models were used to estimate insulin resistance (HOMA-IR), insulin sensitivity (HOMA-S), and  $\beta$ -cell function (HOMA-B),<sup>12,13</sup> which were calculated by the following equations:

$$\begin{aligned}\text{HOMA-IR} &= (\text{fasting insulin } (\mu\text{U ml}^{-1}) \times \text{fasting glucose } (\text{mmol l}^{-1}))/22.5, \\ \text{HOMA-S} &= 22.5/(\text{fasting insulin } (\mu\text{U ml}^{-1}) \times \text{fasting glucose } (\text{mmol l}^{-1})) \text{ and} \\ \text{HOMA-B} &= (20 \times \text{fasting insulin } (\mu\text{U ml}^{-1})) / (\text{fasting glucose } (\text{mmol l}^{-1}) - 3.5).\end{aligned}$$

#### **Outcome Measure Type and Title :**

The primary outcome is to test the interactions between the genes and diet interventions varying in macronutrient intake on body weight changes.

The secondary outcome is to test the interactions between the genes and diet interventions varying in macronutrient intake on glycemic, waist circumference, visceral fat and lipids changes.

**Outcome Measure Time Frame:**

measured at Year 2

**Overall Number of Participants Analyzed:**

811

**Measure Type:**

Mean

**Measure of Dispersion/Precision:**

Standard Deviation

**Unit of Measure:** Body weight: weight in kilograms

glycemic changes: mg/dL for fasting glucose, uU/mL for fasting insulin

waist circumference changes: cm

visceral fat changes:(%)

lipids changes: mg/dL for Total cholesterol, HDL cholesterol, LDL cholesterol and

Triglycerides