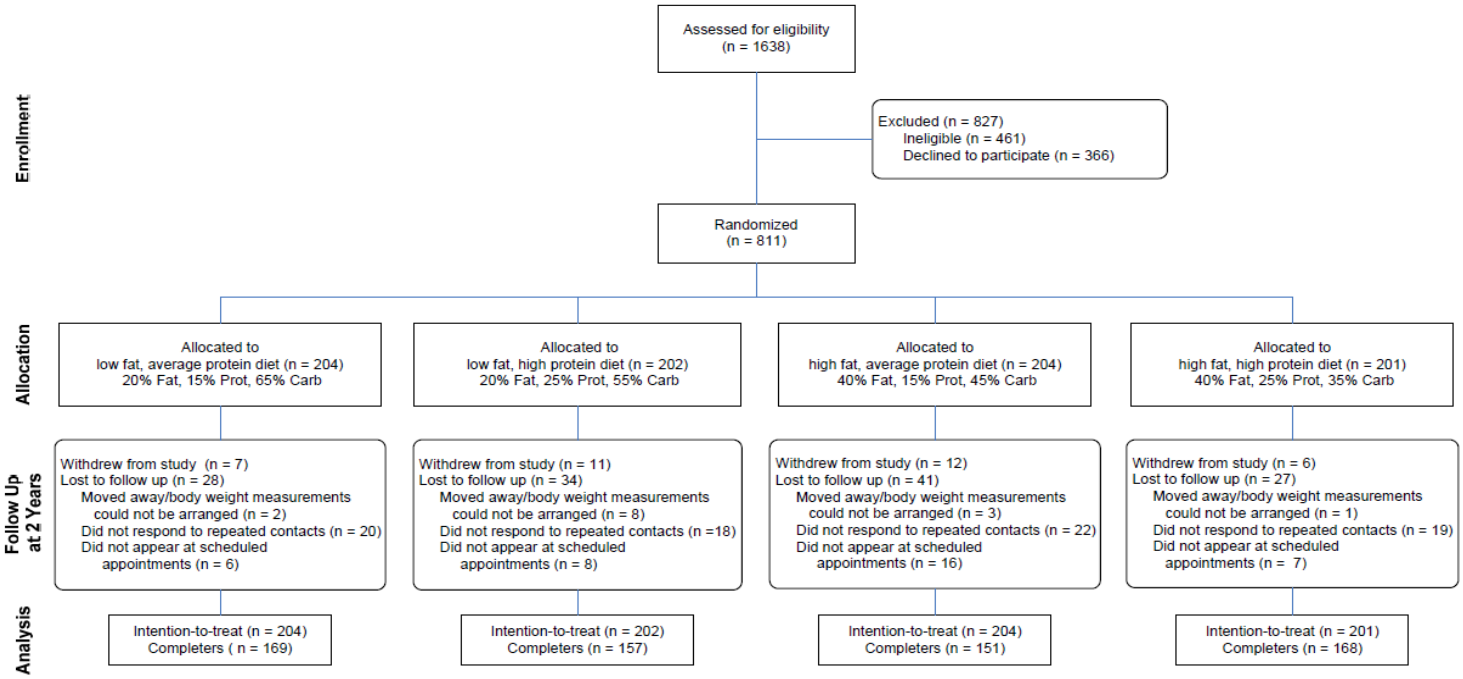
Perfluoroalkyl Substances and Changes in Body Weight and Resting Metabolic Rate in The POUNDS Lost Trial

Overview of Study Design

The POUNDS LOST Trial is a randomized, double-blinded clinical trial that examined the effects of four calorie-restricted diets on weight loss. The four diet groups were: 1) Low-fat, average-protein (20% fat, 15% protein, 65% carbohydrate, 2) Low-fat, high-protein (20% fat, 25% protein, 55% carbohydrate), 3) High-fat, average-protein (40% fat, 15% protein, 45% carbohydrate), and 4) High-fat, high-protein (40% fat, 25% protein, 35% carbohydrate). Moreover, diet prescription for each participant represented a 750 kcal/day deficit from estimated energy needs, which were calculated based on measured resting energy expenditure. To facilitate blinding, the diets used similar foods in different proportions. Participants, investigators, and staff were blind to diet assignment throughout the intervention. The duration of intervention was 2 years, based on the consideration that in clinical trials weight loss is typically the greatest within 6-12 months after the initiation of dietary interventions and is followed by a steady weight regain. The details have been documented previously (*N Engl J Med* 2009;360:859-73).

Participant Flow



Specific Aims (07/01/2014)

In this proposed project, we will examine effects of PFASs on weight change among 811 men and women who participated in the well-designed and rigorously-conducted Prevention of Obesity Using Novel Dietary Strategies (POUNDS) LOST trial. This two-year trial examined the effects of reduced-calorie diets on weight loss and showed that reduced calories, rather than macronutrient compositions, led to weight loss and maintenance. Rich resources in this unique study, including repeated blood samples and data on measured body weight, waist circumference, body fat composition, biomarkers, diet, and gene expression in adipose tissue, will enable us to evaluate the effects of PFASs on body weight in a comprehensive and efficient manner.

Specifically, we will examine the following specific aims:

1. To determine the relationship between PFASs and weight change

Hypothesis 1.1: Higher plasma concentrations of PFOA and PFOS at baseline are associated with less weight loss at 6 months since baseline and with greater weight regain between 6 months and 2 years.

Hypothesis 1.2: Higher plasma concentrations of PFOA and PFOS at baseline are associated with less loss and greater regain of total fat mass, visceral fat, waist circumference, and hepatic fat.

2. To determine PFASs in relation to biological factors involved in body weight regulation

Hypothesis 2.1: Higher plasma concentrations of PFOA and PFOS at baseline are associated with less favorable changes in adipokines and related factors (leptin, soluble leptin receptor, adiponectin, and retinol-binding protein-4) and thyroid hormones (thyroid-stimulating hormone, total and free triiodothyronine [T3], and total and free thyroxine [T4]) during weight loss and weight regain.

3. To determine PFASs in relation to gene expression profiles in adipose tissue

Hypothesis 3.1: Higher plasma concentrations of PFOA and PFOS are associated with altered gene expression profiles at baseline that are consistent with PFASs' detrimental effects on energy metabolism.

Secondary Aims: We will explore 1) the association between PFASs and changes in blood lipids, 2) demographic, lifestyle, and dietary predictors of PFAS concentrations, and 3) effect modifications by dietary interventions, age, ethnicity, gender, and genetics on the association between PFASs and weight change.

The POUNDS LOST Trial provides an unparalleled opportunity not only for us to evaluate the effects of PFAS exposures on weight change but also to help shed light on potential pathways in a prospective manner. This prospective study has the potential to further our understanding of the role of PFASs in the etiology of adiposity in humans. The unique focus on weight loss and weight regain in a clinical trial setting will help provide direct evidence on whether PFASs should be targeted as part of a comprehensive obesity prevention and intervention strategy.

APPROACH

Participants recruitment, screening, baseline measurements, and randomization. The details have been documented previously (*N Engl J Med* 2009;360:859-73). Briefly, people who responded to recruitment were interviewed by phone to describe the study and to ascertain eligibility. Those interested and potentially eligible attended two screening visits at the clinical sites. During the first screening visit, informed consent was obtained. There were measurements of height, weight, blood pressure, urinary microalbumin, and TSH. Eligible participants were given a 5-day food diary to complete at home, and a pedometer to measure activity for 7 days. Participants attended a second screening visit 7-28 days later. Baseline nutrient intake was determined from the 5-day diet records. Other baseline measurements were obtained after the screening visits. Randomization assignments to one of 4 diet groups were generated by the data manager at the coordinating center, upon request of a study dietitian, after confirming, by computer program, that all screening activities had occurred, that the participant met all eligibility criteria, and that all required baseline data had been collected. Diet group assignments were stratified by site with varying block sizes to ensure a balance at each site. **Implementation.** After a participant was randomized, the data manager contacted the assigned dietitian to schedule the first individual visit consisting of an orientation and counseling session on the assigned diet. **Dietary teaching**. The participants were encouraged to attend all group sessions which were held 3 out of 4 weeks during the first 6 months, and 2 out of 4 weeks during 6 to 24 months; and individual sessions held every 8 weeks for the entire 24 months. Structured meal plans were provided based on the American Dietetic Association (ADA) exchange system. Daily meal plans in 2-week blocks were given to the participants. The participants were taught to follow the meal plans exactly so that they could achieve the nutrient goals. **Anthropometric Measurements and Other Body Fatness Assessments.** All measurements were performed in the morning before breakfast, and participants were instructed to urinate and wear a hospital gown before taking these measurements. **Body Weight** was measured using calibrated hospital scales on two nonconsecutive days at baseline, 6 and 24 months. **Blood and Urine Sample Collection in POUNDS LOST Trial**. Fasting blood samples and 24-hour urine samples were collected at baseline and at 6 and 24 months. **Measurement of Plasma PFASs.** PFASs were analyzed in plasma by a sensitive and reliable method based on on-line solid phase extraction (SPE) and

liquid chromatography (LC) coupled to a triple quadropole mass spectrometer (MS/MS) as developed in Norway, with minor modifications. **Measurement of Thyroid Hormones.** TSH, total and free T4, and total and free T3 in plasma were measured by a competitive electrochemiluminescence immunoassay on the Roche E Modular system (Roche Diagnostics, Indianapolis, IN). **Adipose Tissue Biopsy, RNA Extraction, and Gene Expression**. Gene expression was measured by direct hybridization using the Illumina HT-12v3 expression beadchip (Illumina, San Diego, CA).

Statistical Analysis

To ensure the validity of study results, we will restrict our analysis to 714 participants who completed 6-month follow-up for aims related to weight-loss, and 645 participants who finished the 2-year follow-up for aims related to weight regain. Among these participants, body weight and other body fatness indices were measured, and no imputed data will be included. Before a statistical analysis is conducted, we will perform preliminary data processing, which includes univariate distribution assessments for all PFAS levels and all other continuous variables using density estimation (average shifted histograms or log-spline models) and outlier detection (generalized extreme studentized deviates). Non-parametric multivariate summaries will be obtained as well (e.g., by two-dimensional average-shifted histograms). All reported P values will be two-sided. Statistical analyses will be performed primarily using SAS 9.3 (SAS Institute Inc., Cary, NC).

1. Linear regression analyses for examining PFAS levels in relation to weight loss, loss of body fat, reduction of waist circumference, and change in biomarkers between baseline and 6 months. We will first calculate Pearson correlation coefficients to determine the strength of correlations of PFAS concentrations with weight loss, as well as changes in body fat content, adipokines, thyroid hormones, and blood lipids. To minimize the influence of outliers and to detect any non-linear associations, as a secondary analysis we will use multivariate linear regression (SAS PROC GLM) to examine the linear trend of the biomarkers across quintiles of PFAS concentrations. Weight loss or other study outcomes will be entered into the model as dependent variables; quintiles of PFAS concentrations as well as study sites, age, gender, ethnicity, education levels, household income, marital status, menopause status (women only), post-menopausal hormone use (women only), smoking status, dietary intervention arms, alcohol consumption, BMI, physical activity, physical and mental status, and REE at baseline, plus compliance (number of sessions attended through 6 months), will be entered as independent variables. Least-square means of dependent variables will be calculated for each quintile of PFAS concentrations. Robust estimators of variance for these means will be calculated to allow for deviance from the assumption of normally distributed dependent variables. P values for linear

trend will be estimated by entering the median value of each quintile of PFAS levels into the model as a continuous variable.

We will run a series of stepwise models to explore the proportion of variance in weight loss that is explained by compliance, obesity risk factors, and PFASs. We will first examine the variance explained by compliance, and then will explore the proportion of remained variance that is explained by obesity risk factors and the BMI-predicting genetic score. Finally, we will examine whether PFASs explain a significant proportion of the remaining variance not explained by these aforementioned factors. The proportion explained by a factor(s) is measured by r2, which is the difference of model sum of squares between models with and without the factor(s) divided by the error sum of squares of the model without the factor(s).

Because blood samples were collected only at baseline and at 6 and 24 months, we will use the same method to examine baseline PFAS concentrations in relation to changes of adipokines, thyroid hormones, and blood lipids assessed between 6 months and 2 years, when most participants regained weight. For the same reason, this method will be applied for analysis examining baseline PFAS concentrations in relation to change of body fat mass, visceral fat, and hepatic fat between 6 months and 24 months.

2. Linear mixed model analyses for modeling PFAS levels in relation to change in weight and waist circumference between 6 and 24 months. We will use linear mixed model analyses (SAS PROC MIXED) to model baseline PCB concentrations in relation to the change of body weight between 6 and 24 months. We will investigate whether more complex correlation structures are necessary as well as use a robust empirical variance estimator that yields valid inferences even in the presence of a misspecified covariance structure for the longitudinal response. In this model, we are able to examine whether PFASs are associated with body weight and whether the rate of weight change over 18-month (6 to 24 months) follow-up differs by PFAS levels. In these analyses, we will adjust for study sites, age, gender, ethnicity, education levels, household income, marital status, smoking status, dietary intervention arms, and alcohol consumption at the trial baseline, as well as menopause status (women only), post-menopausal hormone use (women only), BMI, physical activity, physical and mental status, and REE at 6 months, plus compliance (number of sessions attended between 6 and 24 months). We will investigate the appropriateness of the linearity assumption both on time and on PFAS concentrations by considering higher-order parametric functions of time (and the corresponding interactions with PFAS levels) as well as exposure categorized into a binary variable using the median as the cutpoint.

3. Analysis plan for evaluating the association of PFASs with gene expression profile in adipose tissue. Adipose tissue gene expression data will first be adjusted for known technical and clinical covariates, as well as unknown latent variables using surrogate variable analysis. Then, these data will be assessed on the gene-level and gene set-level for association with PFAS exposures using approaches similar to those described above with correction for multiple testing.

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