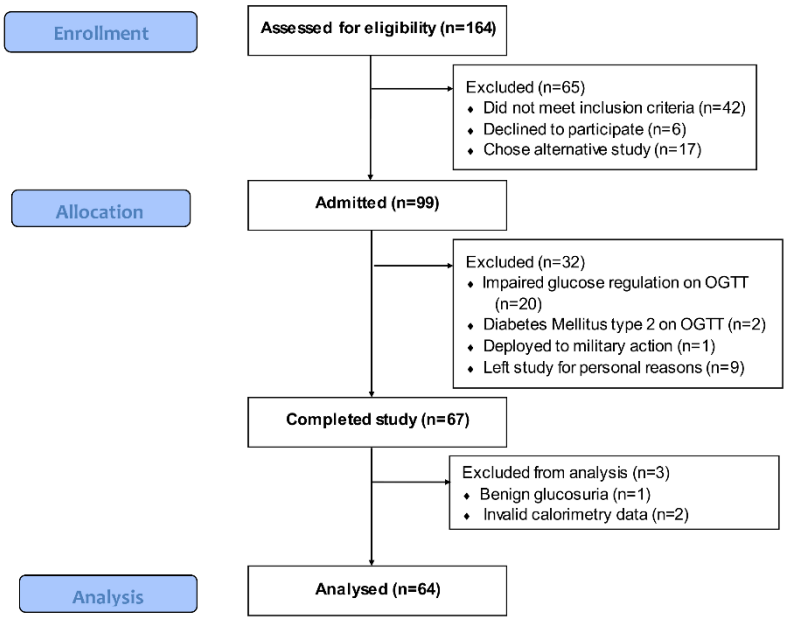
SUPPLEMENTARY DATA

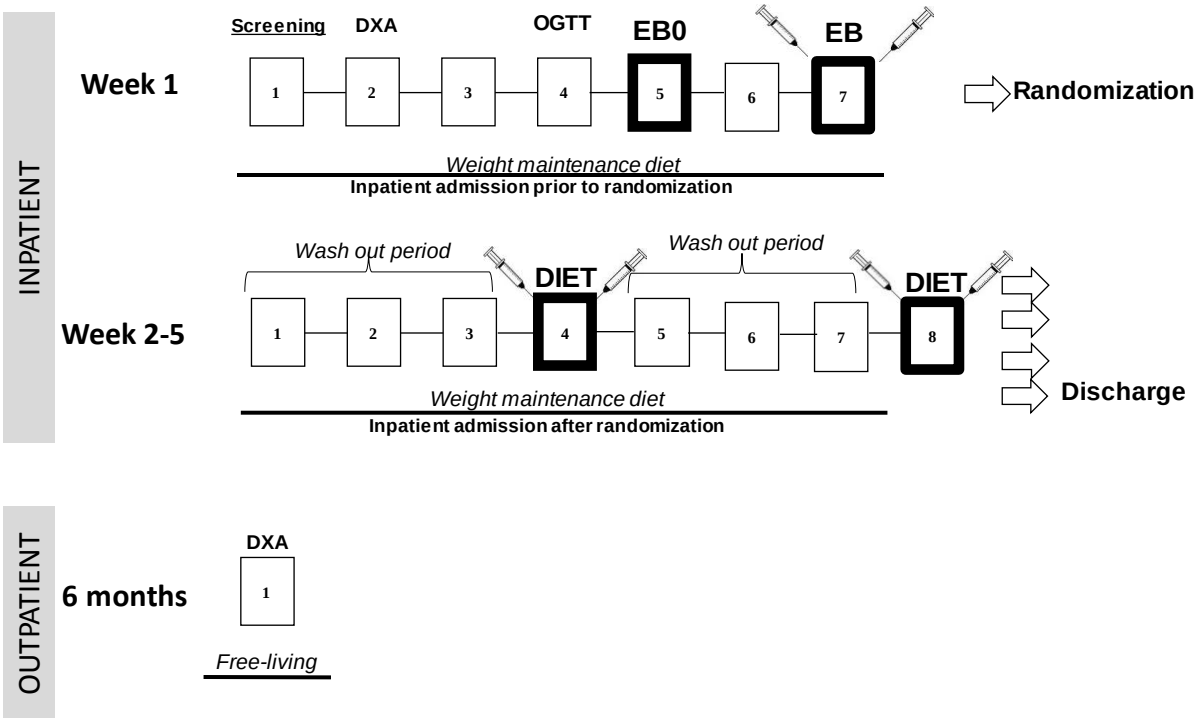
Supplementary Figure 1. Consort flow diagram of the study.



Abbreviations: OGTT, oral glucose tolerance test.

SUPPLEMENTARY DATA

Supplementary Figure 2. Schematic diagram of the study design.

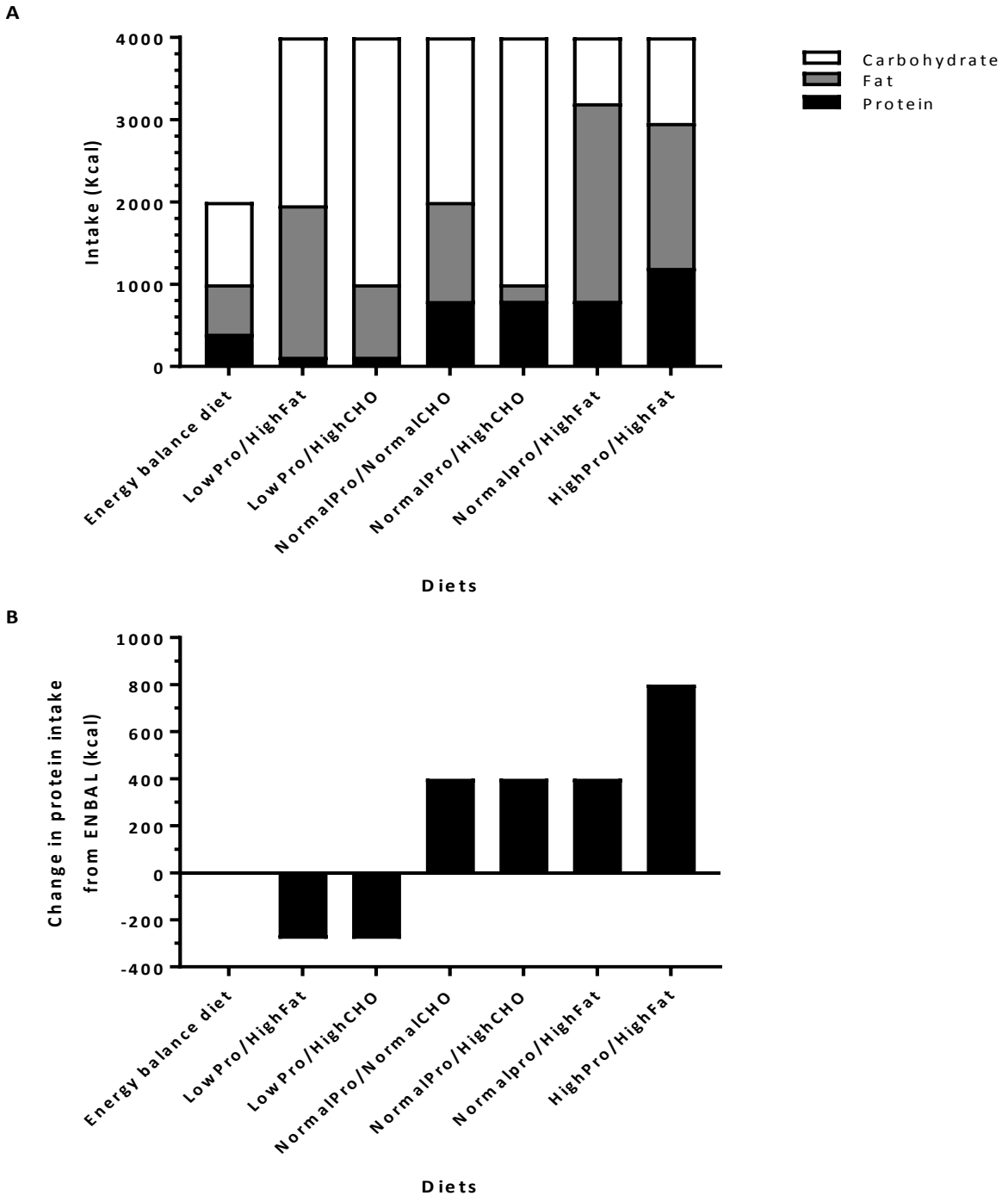


Each square represents 1 day on the clinical research unit. Thicker squares indicate 24-h sessions in the whole-room indirect calorimeter. Syringes represent time points for blood collection.
Abbreviations: DXA, dual-energy X-ray absorptiometry: OGTT, oral glucose tolerance test; EB0, first energy balance or acclimation diet; EB, energy balance diet, where energy intake= 24-h energy expenditure of EB0 assessment; Diet, one of the 7 dietary interventions, all given for 24-h each and with three days of wash-out period between diets: fasting, where only water was provided, and 6 overfeeding diets where dietary intake= 2x24-h EE during EB.

The overfeeding diets included three diets with normal (20%) protein content: 1) standard overfeeding [NormalPro/NormalCHO (n=63)], with 50% carbohydrate and 30% fat, 2) high-carbohydrate [NormalPro/HighCHO (n=63)], with 75% carbohydrate and 5% fat, and 3) high-fat [NormalPro/HighFat (n=63)], with 20% carbohydrate, and 60% fat; two diets with low (3%) protein content: 4) high-fat [LowPro/HighFat (n=63)], with 51% carbohydrate, and 46% fat, and 5) high-carbohydrate [LowPro/HighCHO (n=15)], with 75% carbohydrate, and 22% fat; and one diet with high (30%) protein content: 6) HighPro/HighFat (n=51), with 26% carbohydrate and 44% fat. The LowPro/HighCHO and HighPro/HighFat diets were mutually exclusive, therefore each subject only had EE assessments for five different overfeeding diets during their inpatient stay. Because of technical difficulties or significant return of interventional diets (<95% of total energy content), not all the 64 subjects had valid EE data for all the diets. Specifically, all uneaten food was returned to the metabolic kitchen and EE data for sessions with <95% of food consumed were removed from the analysis.

SUPPLEMENTARY DATA

Supplementary Figure 3. Macronutrient composition of diets.

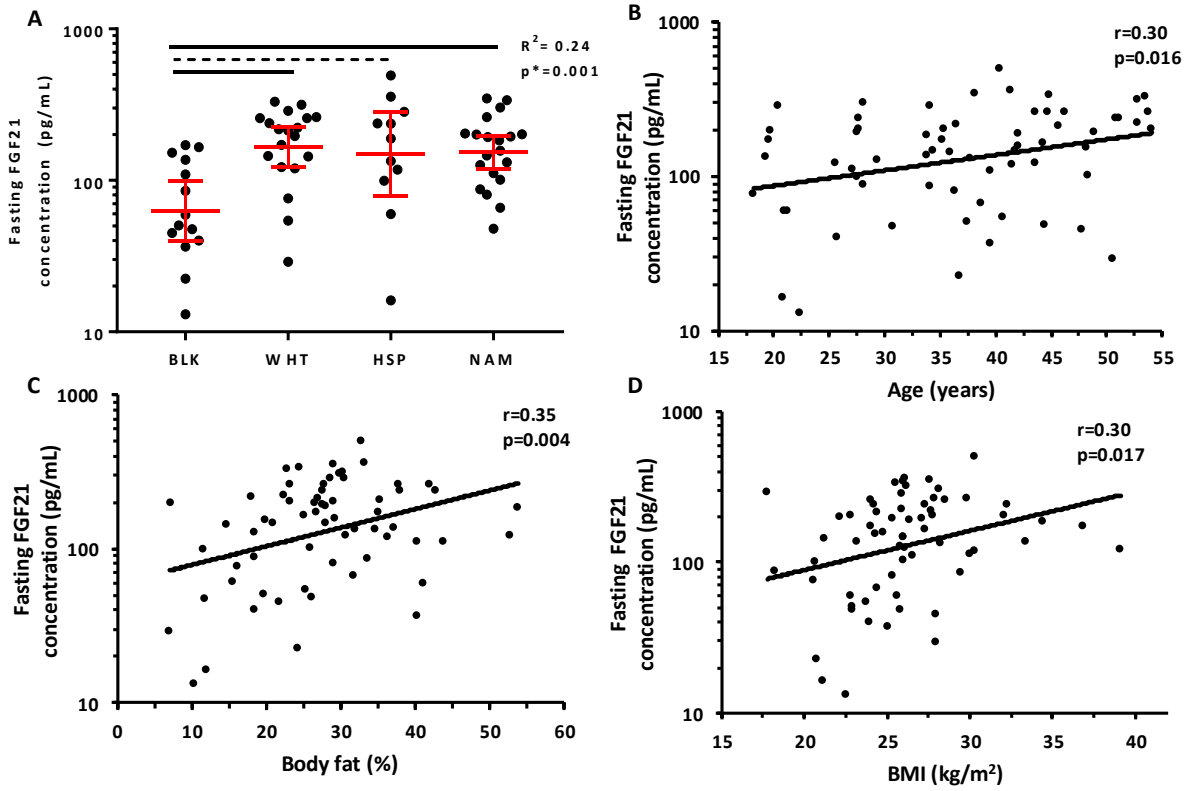


Total intake (Panel A) and change in protein intake from the eucaloric diet (Panel B) based on an energy balance diet of 2000 kcal/day. The total intake of all overfeeding diets is double the total intake of the energy balance diet.

Macronutrient composition of each diet: energy balance diet and NormalPro/NormalCHO overfeeding diet: 50% carbohydrate, 30% fat, 20% protein; NormalPro/HighCHO overfeeding diet: 75% carbohydrate, 5% fat, 20% protein; NormalPro/HighFat overfeeding diet: 20% carbohydrate, 60% fat, 20% protein; HighPro/HighFat overfeeding diet: 26% carbohydrate, 44% fat, 30% protein; LowPro/HighFat overfeeding diet: 51% carbohydrate, 46% fat, 3% protein; and LowPro/HighCHO overfeeding diet: 75% carbohydrate, 22% fat, 3% protein.

SUPPLEMENTARY DATA

Supplementary Figure 4. Relationships between baseline plasma FGF21 concentration and demographic and anthropometric characteristics.



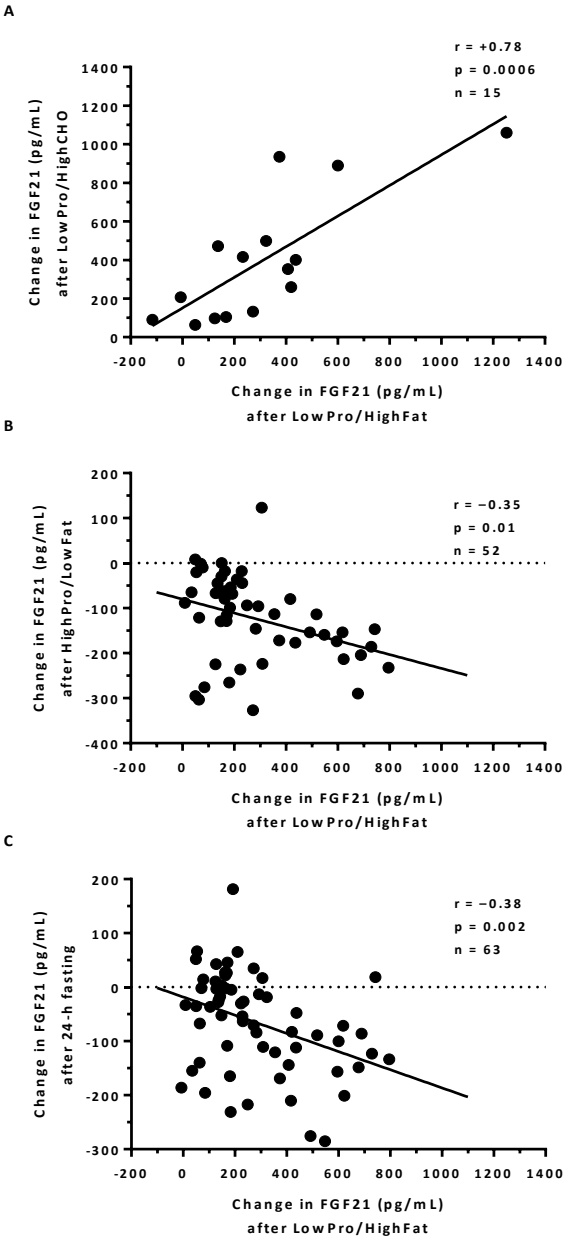
The average of all FGF21 plasma concentrations measured before each dietary intervention is reported on the y-axis on a logarithmic scale. The coefficient of variation and intraclass correlation coefficient of fasting FGF21 concentrations obtained before each dietary intervention were 9% and 0.70 ($p<0.001$), respectively. There was no effect of storage time on FGF21 concentrations ($p=0.97$).

FGF21 concentration by ethnicity (Panel A), using ANOVA with Tukey-Kramer adjustment of the least square means for multiple comparison, where the continuous line represents $p<0.005$ and the intermittent line, $p=0.018$. Red lines represent geometric means with 95% CI. Pearson's correlation coefficient between plasma FGF21 concentration and age (Panel B), percentage body fat (Panel C), and body mass index (BMI; Panel D). Abbreviations: BLK, Black, WHT, White; HSP, Hispanic; NAM, Native American. *P value for overall difference across ethnicities by ANOVA.

Fasting FGF21 concentration positively correlated with age ($r=0.30$, $p=0.015$), BMI ($r=0.30$, $p=0.018$), percentage body fat ($r=0.35$, $p=0.004$), and FM ($r=0.32$, $p=0.0009$), but not with FFM ($p=0.39$). Only percentage body fat [an increase by 98.0 pg/mL or 43% (15% to 77%) per 10% body fat difference, $p=0.001$] and ethnicity [Blacks had on average lower concentration by 120 pg/mL or 53% (29% to 68%) compared to other ethnicities, $p=0.0004$], but not gender ($p=0.12$), were independent determinants of fasting FGF21 concentration (total $R^2=41\%$).

SUPPLEMENTARY DATA

Supplementary Figure 5. Relationships between the change in plasma FGF21 concentrations after low-protein high-fat overfeeding and other overfeeding diets with altered protein content or 24-h fasting.

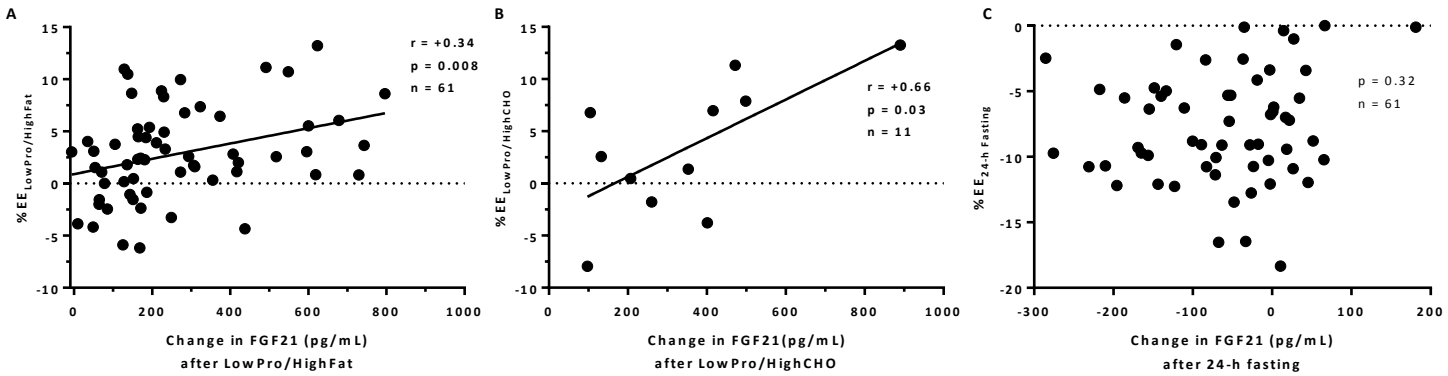


Positive association between the change in plasma FGF21 concentrations following low-protein high-fat overfeeding diet and low-protein high-carbohydrate diet (Panel A). Inverse relationship between the change in plasma FGF21 concentration after low protein-high-fat overfeeding and after both high-protein overfeeding diet (Panel B) and 24-h fasting (Panel C). Associations were quantified by the Pearson's correlation index. The association between low-protein high-fat and low-protein high-carbohydrate overfeeding diets remained significant when subjects with impaired fasting glucose (n=3) were removed from the analysis (r=0.65, p=0.03, n=12).

Diets composition: HighPro/HighFat overfeeding diet: 26% carbohydrate, 44% fat, 30% protein; LowPro/HighFat overfeeding diet: 51% carbohydrate, 46% fat, 3% protein; and LowPro/HighCHO overfeeding diet: 75% carbohydrate, 22% fat, 3% protein.

SUPPLEMENTARY DATA

Supplementary Figure 6. Relationships between the percent change in 24-h energy expenditure from energy balance during two different low-protein overfeeding diets and 24-h fasting vs. the concomitant change in plasma FGF21 concentration.

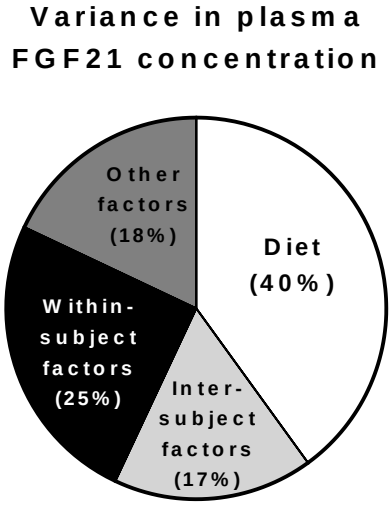


The percent change in 24-h energy expenditure (%EE, y-axis) was calculated as: (24-h $EE_{overfeeding\ diet}$ − 24-h $EE_{energy\ balance}$) / 24-h $EE_{energy\ balance}$ ×100. Associations were quantified by the Pearson's correlation index.

Diets composition: LowPro/HighFat overfeeding diet: 51% carbohydrate, 46% fat, 3% protein; and LowPro/HighCHO overfeeding diet: 75% carbohydrate, 22% fat, 3% protein; 24-h Fasting: only water permitted.

SUPPLEMENTARY DATA

Supplementary Figure 7. Determinants of plasma FGF21 concentration.



Pie chart illustrating the different factors determining the variance in plasma FGF21 concentration. The variance explained by each determinant was quantified by the interclass correlation coefficient obtained from a linear mixed model of plasma FGF21 concentration (logarithmic values to correct skewness of data distribution) across all the dietary interventions accounting for repeated measures using a compound symmetry covariance structure. A total of 57% of the variance in plasma FGF21 concentration was accounted by diet (40%, $p<0.0001$) along with between-subject characteristics including body fat and ethnicity (inter-subject variance = 17% in aggregate, $p<0.0001$), while ¼ of variance was accounted by subject-specific factors (intra-subject variance, 25%, $p<0.0001$) and the remaining 18% was unrelated to these factors.