FGF21 is a Hormonal Mediator of the Human "Thrifty" Metabolic Phenotype

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Abbreviations

BMI	body mass index
CHO	carbohydrates
CI	confidence interval
CV	coefficient of variation
EE	energy expenditure
FFM	fat free mass
FGF21	fibroblast growth factor 21
FM	fat mass
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
NIH	National Institutes of Health
RQ	respiratory quotient
SPA	spontaneous physical activity
WMD	weight-maintaining diet

Abstract

Fibroblast growth factor 21 (FGF21) regulates energy expenditure (EE) and influences weight change after low-protein overfeeding in rodent models. The change in EE after low-protein overfeeding diet is a predictor of weight change in humans and a feature of the "thrifty" metabolic phenotype. However, there are no studies showing an association between circulating FGF21 and EE in humans. We assessed the changes in plasma FGF21 concentrations after 24 hours of seven dietary interventions with different macronutrient content while in a whole-room indirect calorimeter in 64 healthy subjects with normal glucose regulation. Plasma FGF21 concentration consistently increased by 3-fold only after the two low-protein (3%) overfeeding diets, one high in carbohydrate (75%) and the other high in fat (46%), with larger increases in FGF21 being associated with greater increases in 24-h EE. Subjects with smaller increases in FGF21 after the low-protein high-fat diet gained more weight after six months in free-living conditions. Therefore, the individual predisposition to weight gain over time can be assessed by 24-h overfeeding a low-protein diet and measurements of plasma FGF21 concentrations. Individuals with a blunted FGF21 response to a low-protein diet have a thrifty metabolism and are at risk for future weight gain.

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Introduction

As a result of altered energy homeostasis due to the imbalance between energy intake and expenditure (EE), obesity has become more prevalent and a major public health concern. However, the propensity to weight gain is different among individuals, such that some subjects are more resistant to weight gain when overeating as they appear to be more able to dissipate the excess energy than other individuals who instead are more metabolically "thrifty" (1-4). The inter-individual diversity in susceptibility to weight gain seems to be secondary to genetic factors and to the capacity to increase EE in response to feeding, i.e., the diet-induced thermogenesis (3). The manifestation of metabolic phenotypes can be elucidated more clearly when assessing the individual EE response to extreme and macronutrient-unbalanced dietary interventions (2). Specifically, low-protein (<10%) overfeeding has been shown to most effectively uncover the individual propensity to weight gain (3-5), presumably due to the energetic cost required to maintain body lean mass (3; 4; 6). The underlying hormonal mechanisms by which low-protein overfeeding accentuates inter-individual differences in diet-induced thermogenesis and characterizes the subject-specific inclination to weight gain remain unknown.

We previously determined that the acute (24-hour) EE response to low-protein overfeeding is a feature of the thrifty/spendthrift metabolic phenotypes, where a smaller increase (or even a decrease) in 24-h EE during this diet predicts weight gain (5). Fibroblast growth factor 21 (FGF21) is a relatively newly identified hormone implicated in the regulation of energy homeostasis (7-9). Rodents who are overfed with low-protein diet show FGF21-mediated increases in EE compared to normal-protein diet, and are less likely to gain weight (10-13). In humans, sustained low-protein overfeeding increased plasma FGF21 concentrations after 7 (13) or 28 days (10), although no change in EE was observed in the 28-day study (14). The aim of the current study was to determine

whether FGF21 concentration changes after 24 hours of low-protein overfeeding, and to assess whether FGF21 correlates with the diet-induced change in 24-h EE and free-living weight change. We hypothesized that a reduced capacity to respond to a low-protein overfeeding diet by increasing FGF21 concentrations may be a metabolic feature of the "thrifty" metabolic phenotype, indicating a propensity to weight gain.

Research Design and Methods

Subjects

This is an analysis of data from an ongoing study (clinicaltrials.gov identifier: NCT00523627) aimed to assess whether the 24-h EE responses to fasting and overfeeding predict free-living weight change in healthy, weight-stable individuals (Supplemental Figure 1). On admission to the clinical research unit, subjects were placed on a standard normal-protein weight-maintaining diet (WMD; 50% carbohydrate-CHO, 30% fat, and 20% protein-Pro)(15), adjusted daily by the research dietitian to assure weight stability within 1% of admission weight. The average coefficient of variation (CV) of the volunteers' body weight prior to the dietary interventions was 0.94 kg. All subjects had normal glucose regulation based on OGTT performed after three days on the WMD(16). Body composition was assessed by dual-energy X-ray absorptiometry (DPX-1, Lunar Corp, Madison, Wisconsin, USA) with fat mass (FM) and fat free mass (FFM) calculated from the percentage body fat and weight. Following discharge, forty-eight subjects returned after 6 months [median·(interquartile range)=6.5·(6.1-7.2) months] to assess weight change. All participants provided written informed consent prior to beginning the study. The Institutional Review Board of the NIDDK approved this study.

Dietary interventions

The experimental protocol (Supplemental Figure 2) for dietary manipulation was described previously (17). The assessment of 24-h EE during energy balance was done in two steps. The first eucaloric EE assessment was obtained while subjects resided for 24 hours in a whole-room indirect calorimeter and were provided four balanced meals with total daily energy intake calculated using a unit-specific formula to achieve 24-h energy balance in the confined environment of the calorimeter. Secondly, all subjects had another eucaloric EE assessment inside the calorimeter when the total energy intake of four balanced meals was equal to the 24-h EE value calculated during the first eucaloric EE assessment for precise determination of 24-h EE during energy balance.

Subsequently, volunteers had 24-h EE assessments in the calorimeter in random order and separated by a 3-day washout period on the WMD: 24-h fasting, two low-protein (LowPro/HighFat and LowPro/HighCHO), one high-protein, and three normal-protein overfeeding diets with total energy intake determined by doubling the 24-h EE value obtained during energy balance (Table 2, Supplemental Figure 3).

Metabolic and hormone measurements

The experimental protocol for the assessment of 24-h EE and substrate oxidation inside the whole-room indirect calorimeter was previously described (17; 18). . Carbon dioxide production (VCO_2) and oxygen consumption (VO_2) in liters were calculated every minute and extrapolated to the 24-h interval. The 24-h RQ was calculated as the ratio of 24-h VCO_2 to 24-h VO_2 while 24-h EE was calculated by Lusk's formula (18). Carbohydrate and fat oxidation rates were derived from the 24-h RQ, after accounting for protein oxidation which was estimated from measurement of 24-h urinary nitrogen excretion (18).

Fasting plasma was collected both at entry and at exit from the calorimeter in EDTA-containing tubes and frozen to $-70°C$ for later measurements. FGF21 concentrations were measured by ELISA (R&D Systems, Minneapolis, MN, USA). Intra-assay and inter-assay CVs were 2.5% and 5.2%, respectively.

Statistics

Non-normally distributed FGF21 concentrations were analyzed as $\log_{10}$ values, and results were presented as geometric mean with 95% confidence interval (CI). The change in FGF21 concentration after each diet was assessed by paired t-test. For each subject, all the fasting FGF21 measurements obtained before entering the calorimeter were averaged and used in ANOVA to determine differences according to sex and ethnicity and in correlation analysis with anthropometric characteristics.

Results

Baseline characteristics of the study cohort are presented on Table 1. The fasting FGF21 concentration correlated with anthropometric characteristics (Supplemental Figure 4) and differed by ethnicity, such that on average FGF21 was lower by 60% (CI: 48% to 69%; p<0.0001) in Blacks compared to other ethnicities.

On average, FGF21 concentrations greatly increased after the two low-protein overfeeding diets with a nearly threefold increase both after the LowPro/HighFat (+297%, CI: 254% to 347%] and LowPro/HighCHO (+326%, CI: 234% to 456%) overfeeding (Figure 1A-1B). The individual increases in FGF21 observed after these two low-protein overfeeding diets were correlated in the same subject (r=0.78, p<0.001, Supplemental Figure 5). Conversely, FGF21 concentrations decreased following 24-h fasting (−34%, CI: −21% to −44%) and all normal-protein overfeeding

diets (Table 2), with the largest decrease after high-protein overfeeding (−75%, CI: −66% to −81%). A greater increase in FGF21 concentration after LowPro/HighFat overfeeding was associated with greater increase in 24-h EE (r=0.34, p=0.008, Figure 2A; in men only: r=0.31, p=0.03), but not during any normal-protein overfeeding diet or fasting (p=0.32, Supplemental Figure 6). There were no associations between the change in FGF21 after LowPro/HighFat overfeeding and 24-h RQ, macronutrient oxidation rates or substrate balance (all p>0.05).

Despite a wide variability in weight change (SD=4.7 kg), on average body weight was stable after 6 months (mean±SD, 0.8±4.7 kg, p=0.26). A greater increase in plasma FGF21 concentration after LowPro/HighFat overfeeding at baseline was associated with weight loss at the follow-up visit (r=−0.36, p=0.01; R^2=12.9%, Figure 2B; in men only: r=−0.41, p=0.008), such that a 100 ng/mL increase in FGF21 after LowPro/HighFat overfeeding was associated with an average weight change of −0.9 kg (CI: −1.5 to −0.2) at follow-up. Similarly, the change in 24-h EE during LowPro/HighFat overfeeding, but not during any other dietary intervention (all p>0.05), was inversely associated with weight change (r=−0.30, p=0.04). However, in multivariate analysis only the change in FGF21 after LowPro/HighFat overfeeding (p=0.04), but not the concomitant change in 24-h EE (p=0.16), was the only predictor of weight change independently of age (p=0.21), sex (p=0.77), and ethnicity (p=0.12).

Discussion

We aimed to test whether FGF21 mediates the change in 24-h EE observed during low-protein overfeeding, as we had previously shown that this diet identifies a metabolic phenotype resistant to weight gain. Circulating FGF21 concentrations increased acutely (i.e., after 24 hours) and consistently after two different overfeeding diets both with low-protein content (3%), while decreasing after fasting and other normal/high-protein overfeeding diets. Importantly, the increase

in plasma FGF21 concentration following low-protein overfeeding was associated with diet-related changes in 24-h EE, where a greater increase in FGF21 concentration was associated with a higher increase in EE. We further determined that the extent of the increase in FGF21 concentration following low-protein high-fat overfeeding was associated with weight change at 6 months, indicating that a decreased capability to increase FGF21 concentration in response to low-protein overfeeding is a hormonal feature of the thrifty metabolic phenotype inclined to gain weight over time.

The "thrifty" and "spendthrift" metabolic phenotypes hypothesis has evolved over the years from a theorized genotype that led to insulin overproduction due to food consumption favoring adipose storage ([19]), to a focus on the energy conservation in face of repeated famine or overeating ([5]; [20]; [21]). These human metabolic phenotypes can be described by the individual ability to increase or decrease EE in an energetically restricted (fasting) or unrestricted (overeating) setting. While the extent of EE increase during overfeeding is highly dependent on the macronutrient composition of the diet ([22]), certain diets such as low-protein overfeeding appear more likely to uncover these metabolic phenotypes associated with weight change ([5]). In the present study including healthy subjects with normal glucose regulation, FGF21 concentrations increased in nearly all subjects by approximately threefold after one day of low-protein overfeeding, in line with what reported in previous studies ([10]; [13]). Importantly, as the degree of this increase correlated with the dietary-related EE, we have identified one of the hormonal mediators of the EE response to this diet that characterizes the thrifty metabolic phenotype prone to weight gain. In addition, the ability to increase circulating FGF21 after low-protein overfeeding was associated with less weight gain or weight loss 6 months following subjects return to their routine activities, and explained 12.9% of the inter-individual variance in free-living weight change, a value much higher than that of

established metabolic determinants of weight change such as lower 24-h EE (2.5%) and higher RQ (5.8%) during energy balance ([23](#); [24](#)).

The biological mechanisms by which FGF21 may increase diet-induced EE during low-protein overfeeding in humans are not known but could involve UCP2 and UCP3 as FGF21 treatment of cultured human cardiomyocytes increases expression of UCP2 and UCP3 ([25](#)). Notably, the significant intra-subject variance (25%, Supplemental Figure 7) in FGF21 concentrations suggests that the capacity of increasing FGF21 in response to low-protein diet is an individual-specific characteristic, perhaps genetically determined, which could partly explain the lower FGF21 concentrations found in Blacks.

Our study has some limitations. First, we have a relatively small representation of women, therefore our results may need to be validated in a larger female cohort. However, sensitivity analyses including only men provided similar results. Second, we do not have assessment of free-living food intake, physical activity, or fitness during the follow-up period, thus we were not able to assess whether the association between FGF21 concentration and weight change was independent of these factors. Nevertheless, subjects were recruited as being weight-stable for six months before admission, and on average weight did not change after 6 months, thus indicating that no substantial changes in free-living physical activity or food intake took place during the follow-up period.

In conclusion, we have identified a thrifty metabolic phenotype that can be characterized by reduced FGF21 response after 24 hours of low-protein overfeeding, and that confers susceptibility to weight gain. Furthermore, we have found that the increase in FGF21 after low-protein overfeeding is correlated with the diet-induced change in 24-h EE and, ultimately, with weight change. The present results are important in the context of our current obesogenic environment

that includes the widespread overexposure to low-protein dietary options that are highly palatable, easily overeaten, and inexpensive, such as sodas, ice creams, doughnuts, etc. We speculate that exogenous FGF21 therapy may help metabolically thrifty individuals to prevent weight gain or achieving greater weight loss during obesity interventions. This may be useful for preventing and treating obesity in some people genetically prone to obesity and its complications.

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Tables

Table 1. Baseline characteristics of the study group.

	All subjects (n=64)	Women (n=12)	Men (n=52)
Age (year)	37 ± 10 (18, 54)	33 ± 8 (20, 45)	38 ± 10 (18, 54)
Ethnicity	14 BLK, 19 WHT, 11 HSP, 20 NAM	5 BLK, 4 WHT, 1 HSP, 2 NAM	9 BLK, 15 WHT, 10 HSP, 18 NAM
Body weight (kg)	78.5 ± 12.2 (47.5, 107.8)	74.4 ± 16.8 (47.5, 107.8)	79 ± 11 (56, 105)
BMI (kg/m²)	26.2 ± 3.9 (17.8, 39.1)	26.9 ± 5.8 (17.8, 39.1)	26 ± 3 (18, 37)
Body fat (%)	27.6 ± 10.0 (6.9, 53.8)	40.4 ± 8.4 (24.2, 53.8)	25 ± 8 (6.9, 38) [*]
FM (kg)	22.1 ± 10.0 (4.9, 56.9)	31.2 ± 13.0 (13.6, 56.9)	20 ± 8 (4.9, 36) [*]
FFM (kg)	56.4 ± 9.3 (33.9, 79.4)	43.2 ± 4.9 (33.9, 50.9)	59 ± 7 (47, 79.4) [*]
24-h EE (kcal/day)	2038 ± 283 (1502, 2810)	1802 ± 223 (1502, 2290)	2094 ± 268 (1573, 2810) [*]
24-h RQ (ratio)	0.87 ± 0.03 (0.80, 0.93)	0.86 ± 0.03 (0.81, 0.91)	0.87 ± 0.03 (0.80, 0.93)
Fasting glucose (mg/dL)	92.0 ± 5.07 (80.0, 99.0)	91.0 ± 3.3 (86.5, 97.0)	92.3 ± 5.4 (80, 99)
2-h OGTT glucose (mg/dL)	103.8 ± 19.9 (65, 138)	104.2 ± 16.5 (80, 130)	103.7 ± 20.8 (65, 138)
Fasting plasma FGF21 (pg/mL)	128.8: 105.8 to 156.9 (13.0, 492.9)	119.0: 70.6 to 200.5 (22.4, 288.2)	131.2: 105.4 to 163.4 (13.0, 492.9)

Data are presented as mean ± standard deviation (minimum, maximum), except for FGF21 where values are presented as geometric mean with its 95% CI (minimum, maximum). Fasting plasma FGF21 concentration was calculated as the average of all pre-diet fasting measurements.

Table 2. Plasma FGF21 concentrations before and after each dietary intervention.

Diet	Pre-diet FGF21 (pg/mL)	Post-diet FGF21 (pg/mL)	Change in FGF21 (pg/mL)	Fold change (ratio)	P-value
24-h fasting (n=64)	124.8 (101.6 to 153.4)	82.6 (70.2 to 97.3)	−65.0 (−87.8 to −42.2)	0.66 (0.56 to 0.79)	**<0.0001**
Energy balance (n=64)	97.6 (76.8 to 124.1)	85.5 (68.2 to 107)	−25.0 (−42.9 to 7.2)	0.88 (0.75 to 1.02)	0.10
NormalPro/NormalCHO overfeeding (n=63)	119.3 (90.8 to 156.7)	68.3 (52.4 to 89)	−72.0 (−94.7 to −49.3)	0.57 (0.47 to 0.70)	**<0.0001**
NormalPro/HighCHO overfeeding (n=63)	123.3 (100.5 to 151.2)	99.8 (79 to 126)	−20.6 (−39.0 to −2.1)	0.81 (0.72 to 0.92)	**0.001**
NormalPro/HighFat overfeeding (n=63)	126.2 (102.7 to 155.2)	63.7 (50.9 to 79.6)	−72.5 (−89.0 to −56.0)	0.50 (0.43 to 0.59)	**<0.0001**
HighPro/HighFat overfeeding (n=51)	125.4 (93.3 to 165.6)	31.7 (23.5 to 42.8)	−121.1 (−148.0 to −94.3)	0.25 (0.19 to 0.34)	**<0.0001**
LowPro/HighFat overfeeding (n=63)	121.6 (96.1 to 153.9)	361.4 (303.2 to 430.8)	+278.7 (226.5 to 331.0)	2.97 (2.54 to 3.47)	**<0.0001**
LowPro/HighCHO overfeeding (n=15)	146.7 (98.1 to 219.5)	461.8 (312.8 to 681.6)	+214.8 (128.6 to 333.4)	3.26 (2.34 to 4.56)	**<0.0001**

Pre-diet and post-diet plasma FGF21 concentrations are expressed as geometric means with 95%

CI. The absolute changes in FGF21 concentrations (pg/mL) are reported as arithmetic means

with 95% CI. Fold changes (95% CI) were calculated by exponentiating the average difference between post-diet minus pre-diet FGF21 concentrations both expressed as $\log_{10}$ values. P-values were calculated by paired t-test analysis of $\log_{10}$ FGF21 values.

Macronutrient composition of diets: 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.

Figure legends

Figure 1. Plasma FGF21 concentrations prior to and following 24-h dietary interventions.

Panel A. Plasma FGF21 concentrations before (white bars) and after (black bars) each dietary intervention. Bars represent geographic means with 95% CI.

Panel B. Individual changes in plasma FGF21 concentration after each dietary intervention. Red lines represent arithmetic means with 95% CI.

Macronutrient composition of diets: 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.

Figure 2. Relationships between the change in plasma FGF21 concentration after 24-h low-protein high-fat overfeeding and the percent change in 24-h energy expenditure from energy balance (Panel A) and free-living weight change after 6 months (Panel B).
The macronutrient composition of low-protein high-fat overfeeding diet was: 51% carbohydrate, 46% fat, 3% protein. The percent change in 24-h energy expenditure was calculated as: (24-h $EE_{\text{overfeeding diet}} - $ 24-h $EE_{\text{energy balance}}$) / 24-h $EE_{\text{energy balance}} \times 100$. One subject found to have impaired fasting glucose on the oral glucose tolerance test and one subject with benign glycosuria were excluded from these analyses as these conditions are known to affect 24-h EE. Associations were quantified by the Pearson's correlation index.