Brief Communication

MOLECULAR METABOLISM

# Circulating FGF21 in humans is potently induced by short term overfeeding of carbohydrates

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## ABSTRACT

**Objective:** Fibroblast-growth factor 21 (FGF21) is thought to be important in metabolic regulation. Recently, low protein diets have been shown to increase circulating FGF21 levels. However, when energy contribution from dietary protein is lowered, other macronutrients, such as carbohydrates, must be increased to meet eucaloric balance. This raises the possibility that intake of a diet rich in carbohydrates may induce an increase in plasma FGF21 levels per se. Here we studied the role of dietary carbohydrates on the levels of circulating FGF21 and concomitant physiologic effects by feeding healthy men a carbohydrate rich diet without reducing protein intake.

**Methods:** A diet enriched in carbohydrates (80 E% carbohydrate; CHO) and a eucaloric control diet (CON) were provided to nine healthy men for three days. The energy intake during the CHO diet was increased (+75% energy) to ensure similar dietary protein intake in CHO and CON. To control for the effect of caloric surplus, we similarly overfed (+75% energy) the same subjects for three days with a fat-rich diet (78 E% fat; FAT), consisting of primarily unsaturated fatty acids. The three diets were provided in random order.

**Results:** After CHO, plasma FGF21 concentration increased 8-fold compared to CON (329 ± 99 vs. 39 ± 9 pg ml$^{-1}$, $p < 0.05$). In contrast, after FAT only a non-significant tendency ($p = 0.073$) to an increase in plasma FGF21 concentration was found. The increase in FGF21 concentration after CHO correlated closely ($r = 0.88$, $p < 0.01$) with increased leg glucose uptake (62%, $p < 0.05$) and increased hepatic glucose production (17%, $p < 0.01$), indicating increased glucose turnover. Plasma fatty acid (FA) concentration was decreased by 68% ($p < 0.01$), supported by reduced subcutaneous adipose tissue HSL Ser$^{660}$ phosphorylation ($p < 0.01$) and perilipin 1 protein content ($p < 0.01$), pointing to a suppression of adipose tissue lipolysis. Concomitantly, a 146% increase in the plasma marker of hepatic de novo lipogenesis C16:1 FA ($p < 0.01$) was observed together with 101% increased plasma TG concentration ($p < 0.001$) in association with CHO and increased plasma FGF21 concentration.

**Conclusion:** Excess dietary carbohydrate, but not fat, leads to markedly increased FGF21 secretion in humans, notably without protein restriction, and affected glucose and lipid homeostais.



**Keywords** FGF21; Diet; Carbohydrates; Lipolysis; Liver

## 1. INTRODUCTION

The hormone fibroblast growth factor 21 (FGF21) was early reported to improve glucose homeostasis and to lower circulating triglycerides in obese and insulin resistant mice [1] and later observed to be important in the metabolic adaptations to fasting and ketosis [2,3]. Now, FGF21 has received increasing interest due to its role in the regulation of energy homeostasis and its potential antidiabetic and lipid-lowering properties [4,5]. Therefore, FGF21 holds promise as a therapeutic target, although intriguingly being elevated in obesity and type 2 diabetes [6,7]. In mice, restriction of essential amino acids [8–10] as well as non-essential amino acids [11] has been shown to increase plasma FGF21 levels. In humans, 28 days restriction of dietary protein content to 6 E%, compared to 15 E% in the baseline diet, was shown to increase plasma FGF21 concentration by 171% [12]. This was, however, under hypercaloric (+39 E%) conditions resulting in a weight gain (+3.16 kg on average). Furthermore, the reduced protein was compensated by increasing the fat intake from 25 to 52 E% and decreasing the carbohydrate intake from 60 to 42 E% [13]. Thus, it is not clear whether the increase in circulating FGF21 obtained in the

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*Abbreviations:* ATGL, adipose triglyceride lipase; AMPK, AMP-activated kinase; BCA, bicinchoninic acid; BM, body mass; BMI, body mass index; CHO, carbohydrate-rich diet; ChREBP, carbohydrate-responsive element binding protein; CON, control diet; FA, fatty acid; CHO, fat-rich diet; FGF21, fibroblast growth factor 21; GLUT4, glucose transporter 4; HSL, hormone sensitive lipase; LM, leg mass; $VO_2$peak, maximal oxygen consumption; PKA, protein kinase A; $R_a$, rate of appearance; $R_d$, rate of disappearance; TG, triacylglycerol; VLDL, very low density lipoprotein

Received October 4, 2016 • Revision received November 7, 2016 • Accepted November 9, 2016 • Available online xxx

http://dx.doi.org/10.1016/j.molmet.2016.11.001

 

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study by Laeger et al. resulted from protein restriction per se, alterations in carbohydrate and fat intake, or as a result of the weight gain. Our recent human study demonstrated that 7 days of a eucaloric protein diluted diet (9.0 E% protein) increased plasma FGF21 concentration by 500% compared to a control diet with 20.2 E% dietary protein [11]. Together, these findings have led to speculations that dietary protein restriction induces FGF21 secretion and thereby enhances metabolic health [12,14].

Studies in rodents have also implied a role of carbohydrates in FGF21 induction. Hence, 12 h carbohydrate feeding (provided after 24 h of fasting) was shown to induce FGF21 mRNA in rat liver [15]. Furthermore, monosaccharide-induced increases in FGF21 gene expression in hepatocytes and mouse liver were mediated via the transcription factor ChREBP [16–18]. This is supported by the observation that FGF21 content in plasma was not increased in ChREBP knockout mice in response to various sugars [19]. In addition, a recent study in mice, providing 25 diets with varying macronutrient composition, showed an additive effect of high-carbohydrate feeding to the induction of circulating FGF21 with low-protein feeding [20]. Even though it has been shown in humans that single monosaccharide intake briefly increased plasma FGF21 concentration [21], the role of dietary carbohydrates in an ordinary diet (including mainly polysaccharides, but also di- and monosaccharides) without protein restriction in humans is not known. Despite reports demonstrating that starvation and ketogenic diets induce FGF21 in mice, an increase in circulating FGF21 concentration is not detected until after 7–10 days of starvation in humans [22,23], and the reported effects of ketogenic diets are inconsistent [22,24]. Hence, in the context of dietary interventions the regulation of FGF21 may vary between mouse and man.

The aim of the present study was to evaluate the role of dietary carbohydrates in the regulation of circulating FGF21, without restricting protein intake. We furthermore aimed to investigate whether a potential dietary regulation of circulating FGF21 would have an impact on glucose and lipid homeostasis in humans. For this purpose, we performed a highly controlled randomized three day cross-over dietary intervention study in healthy men. In order to maximize substrate provision and hence metabolic flux, in particular to the liver but also in peripheral tissues, and even more importantly to avoid a decrease in protein intake, the carbohydrate-rich diet was provided in excess of eucaloric energy requirements. To control for the effect of caloric excess, subjects also ingested a similar hypercaloric fat-rich diet in which fat consisted primarily of unsaturated fatty acids.

## 2. METHODS

### 2.1. Subjects and diets

Nine men were recruited for the study, which was approved by the Copenhagen Ethics Committee (KF 01 261127) and performed in accordance with the Declaration of Helsinki. Informed written consent was received from each participant prior to study inclusion. All subjects were healthy, moderately physically active, and with no family history of diabetes. This study is part of a larger project aiming at elucidating the effects of different types of diets on metabolism in nine male volunteers (unpublished). In the present study, these subjects ingested a hypercaloric carbohydrate-rich diet (CHO) (80 E% carbohydrate, 11 E % protein, 9 E% fat) and a eucaloric control diet (CON) (62 E% carbohydrates, 14 E% protein, 24 E% fat), reflecting their habitual diet. A hypercaloric high-fat diet, consisting primarily of unsaturated FA (FAT) (10 E% carbohydrate, 12 E% protein, 78 E% fat) was also provided in order to serve as control for the caloric excess. The macronutrient composition of CHO, FAT, and CON are summarized in Supplemental Table S1. All diets were provided for three days in a randomized cross-over design, with each intervention separated by at least three weeks. Before each three-day intervention, all subjects consumed the eucaloric control diet for 5 days to ensure the same conditions before each intervention. All food items were weighed and prepared in the metabolic kitchen. The daily menus were delivered to the subject, who ingested them at home. The CHO diet consisted of carbohydrate-rich food items as bread, pasta, cereals, corn, jam, and juice, with high to moderate glycemic index. The diet was mainly comprised of polysaccharides, with 19% refined sugar and the ratio between glucose and fructose was 1:1 (Supplemental Table S3 and S4). In the FAT diet, mono- and polyunsaturated fatty acids both comprised 34 E% (Supplemental Table S1).

To evaluate the training status of the participants, maximal oxygen uptake was measured by an incremental exercise test on a Monark Ergomedic 839E bicycle ergometer (Monark, Sweden). Body composition and leg mass (LM) were determined using dual-energy X-ray absorptiometry (Lunar DPX-IQ DEXA Scanner, Lunar Corporation, USA) after 4 h of fasting. The nine male subjects were 23 ± 3 (mean ± SD) years old, with a body mass index (BMI) of 23.7 ± 1.7 kg $m^{-2}$ and maximal oxygen uptake ($VO_2$peak) of 52 ± 4 ml $kg^{-1}$ $min^{-1}$.

The daily energy requirements were individually determined from weighed dietary registrations and calculations of energy requirements (WHO/FAO/UNU). The three days CHO and FAT diets were provided in excess of eucaloric energy requirements, with a +75% increase in daily energy intake. Hence, the subjects ingested 13.7 ± 0.2 MJ during the CON diet and according to the hypercaloric energy provision 24.0 ± 0.4 MJ during the experimental diets (Table S1).

### 2.2. Experimental protocol

After three days on the experimental diets or the control diet, subjects ingested a light standardized breakfast (1.6 MJ) at 5 A.M at home (Supplemental Table S5). Subjects arrived at the institute at 7.30 A.M, and, after a period of supine rest, teflon catheters were inserted in the femoral artery and vein under local anesthesia. Then, a [6,6-$^2H_2$] glucose tracer infusion (0.055 mg $kg^{-1}$ $min^{-1}$) was administered in order to measure hepatic glucose production. The infusion was initiated by a bolus injection of [6,6-$^2H_2$] glucose (3.203 mg $kg^{-1}$). After 120 min, blood samples were obtained from the femoral artery and vein. Femoral arterial blood flow was determined simultaneously with the arterio-venous blood sampling by a laser ultra-sound Doppler technique (Philips iU22, ViCare Medical, Denmark) [25]. In order to study molecular metabolism in adipose tissue, biopsies were obtained from the subcutaneous periumbilical adipose tissue, immediately frozen in liquid nitrogen and stored at −80 °C. All blood and tissue samples were collected 6 h after the breakfast meal, when subjects were in the post-absorptive state.

### 2.3. Plasma analyses

Plasma glucose concentration was measured on an ABL615 (Radiometer Medical A/S, Denmark), and insulin concentration was measured by an enzyme-linked immunosorbent assay (ELISA) (ALPCO, USA). The plasma concentrations of FA (NEFA C kit, Wako Chemicals GmbH, Germany) and triacylglycerol (TG) (GPO-PAP kit, Roche Diagnostics, Germany) were measured using enzymatic colorimetric methods (Hitachi 912 automatic analyzer, Boehringer, Germany). Plasma cholesterol concentration was measured by a fluorometric method (Roche Diagnostics, Germany). Plasma epinephrine and norepinephrine concentrations were determined by radioimmunoassay (2-CAT $^{125}$I RIA kit, Labor Diagnostika, Germany).



 