Fibroblast growth factor-21 serum concentrations are associated with metabolic and hepatic markers in humans

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Abstract

Rather than a traditional growth factor, fibroblast growth factor-21 (FGF21) is considered to be a metabolic hormone. In the current study, we investigated serum FGF21 levels in the self-contained population of Sorbs. Serum FGF21 concentrations were quantified by ELISA and correlated with IGF1 as well as metabolic, renal, hepatic, inflammatory, and cardiovascular parameters in 913 Sorbs from Germany. Moreover, human IGF1 protein secretion was investigated in FGF21-stimulated HepG2 cells. Median FGF21 serum concentrations were 2.1-fold higher in subjects with type 2 diabetes mellitus (141.8 ng/l) compared with controls (66.7 ng/l). Furthermore, nondiabetic subjects with FGF21 levels below the detection limit of the ELISA showed a more beneficial metabolic profile compared with subjects with measurable FGF21. Moreover, FGF21 was significantly lower in female compared with male subjects after adjustment for age and BMI. In multiple regression analyses, circulating FGF21 concentrations remained independently and positively associated with gender, systolic blood pressure, triglycerides, and γ glutamyl transferase whereas a negative association was observed with IGF1 in nondiabetic subjects. Notably, FGF21 significantly inhibited IGF1 secretion into HepG2 cell culture supernatants in preliminary *in vitro* experiments. FGF21 serum concentrations are associated with facets of the metabolic syndrome, hepatocellular function, as well as GH status.

Key Words

- FGF21
- IGF1
- insulin resistance
- liver
- obesity
- Sorbs

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Introduction

Fibroblast growth factor-21 (FGF21) is a member of the FGF family including 22 members (Beenken & Mohammadi 2009). FGF21 is mainly produced by the liver but also by other tissues including white adipose tissue (WAT), skeletal muscle, and pancreatic β cells (Beenken & Mohammadi 2009). FGF21 is regulated by nutritional status and influences glucose and lipid metabolism by central and peripheral mechanisms (Kralisch & Fasshauer 2011). Thus, the protein induces glucose uptake (Kharitonenkov *et al.* 2005) and decreases glucose and lipid concentrations in obese mice (Kharitonenkov *et al.* 2005) and diabetic monkeys (Kharitonenkov *et al.* 2007). Furthermore, it increases energy expenditure resulting in weight loss (Coskun *et al.* 2008) and upregulates fatty acid

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oxidation (Inagaki *et al*. 2007). These results suggest that FGF21 regulates energy balance and improves glucose homeostasis. Furthermore, FGF21 is also considered to be a starvation signal and is additionally involved in the process of 'browning' of WAT (Cantó & Auwerx 2012).

At the cellular level, FGF21 mediates its effects through cell surface receptors composed of the classic four FGF receptor (FGFR) isotypes 1–4 (Zhang *et al*. 2006). FGFRs are tyrosine kinases that are complexed selectively and non-covalently with an essential co-receptor β-Klotho, forming an FGF21-β-Klotho–FGFR complex (Ogawa *et al*. 2007). Expression of FGF21 is controlled by various transcriptional activators such as peroxisome proliferator-activated receptor (PPAR) α in the liver (Badman *et al*. 2007) and PPARγ in the adipocytes (Wang *et al*. 2008).

Several clinical studies investigated the role of FGF21 in human metabolic disease. Thus, high baseline FGF21 serum levels predicted the risk to develop type 2 diabetes mellitus (T2DM) over 5.4 years (odds ratio 1.792; $P<0.01$) together with waist circumference and fasting plasma glucose levels in 1.200 nondiabetic Chinese subjects (Chen *et al*. 2011). Recent data indicate that higher FGF21 serum levels are associated with abnormal fasting glucose and insulin resistance in the Baltimore longitudinal study of aging (Semba *et al*. 2012). Furthermore, a study by Lin *et al*. (2012) suggested that FGF21 potentially regulates insulin secretion in humans. In contrast to the study by Semba *et al*. (2012), FGF21 levels were inversely correlated with changes in serum glucose during an oral glucose tolerance test with lowest FGF21 concentrations after 1 h and a peak at 3 h (Lin *et al*. 2012). This acute response appears to be abolished in patients with impaired glucose tolerance and T2DM (Lin *et al*. 2012). FGF21 concentrations were increased in obese children in comparison with lean controls and were related to leptin and free fatty acids (FFAs; Reinehr *et al*. 2012). Furthermore, FGF21 is considered as a potential marker of nonalcoholic fatty liver disease (NAFLD; Dushay *et al*. 2010, Li *et al*. 2010, Yilmaz *et al*. 2010). Moreover, a correlation of serum FGF21 levels with GH and insulin-like growth factor-1 (IGF1) concentrations is discussed (Inagaki *et al*. 2008, Wu *et al*. 2012). In agreement with this hypothesis, FGF21 levels are negatively associated with IGF1 independent of body fat and insulin resistance in patients with anorexia nervosa (Fazeli *et al*. 2010).

To date, no study with sufficient statistical power has included gender, renal function, metabolic, hepatic, and vascular risk markers combined in its analysis of FGF21 serum concentration. Therefore, the aim of our cross-sectional study was to assess the complex interaction of these clinical parameters, as well as GH status, with FGF21 levels in a large well-characterized sample.

Materials and methods

Subjects

All subjects are part of a sample from an extensively phenotyped self-contained population of Sorbs from Eastern Germany described in more detail recently (Tonjes *et al*. 2009, 2010, 2012). At present, about 1000 Sorbs are enrolled in the study. Nine hundred and thirteen subjects were available for the present analyses. To exclude any secondary effects, associations with glucose and insulin levels were assessed only in the subgroup of nondiabetic subjects ($n=812$) defined according to the American Diabetes Association criteria (American Diabetes Association 2010). The study was approved by the local ethics committee and all subjects gave written informed consent before taking part in the study.

Phenotyping

Phenotyping included collection of anthropometric data (weight, height, BMI, waist-to-hip ratio (WHR), waist-to-height ratio (WHtR), and body impedance analysis (BIA)) and a 75 g oral glucose tolerance test. Furthermore, homeostasis model assessment of insulin resistance (HOMA-IR), Stumvoll index, and quantitative insulin sensitivity check (Quicky) index were calculated as described previously (Matthews *et al*. 1985, Stumvoll *et al*. 2001, Chu *et al*. 2003). Renal function was assessed as glomerular filtration rate (GFR) estimated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (Levey *et al*. 2009). Ultrasound sensor (10 MHz; GE Healthcare, Inc., München, Germany) was used to measure the intima-media thickness (IMT) of the common carotid arteries. After three measurements, IMT values at each side were averaged. BIA was performed using BIA-2000-S (Data Input GmbH, Darmstadt, Germany) and evaluated using the software Nutri3 (Data Input GmbH).

Assays

Blood samples were taken after an overnight fast. Serum insulin was measured with AutoDELFIA Insulin assay (PerkinElmer Life and Analytical Sciences, Turku, Finland). The assay sensitivity was 3.0 pmol/l. The interassay coefficient of variance (%) was between 3.37 and

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6.13% for concentrations between 71 and 867 pmol/l. FGF21 serum concentrations were determined with a commercially available ELISA (Biovendor, Modrice, Czech Republic) according to the manufacturer's instructions. The sensitivity of FGF21 ELISA was 7 ng/l. The degree of precision of ELISA system in terms of intra-assay coefficient of variance (%) was between 2.0 and 20.8% and that of interassay was between 2.9 and 12.0%. Spike recovery and linearity were in a range of 94.3–105.6 and 101.0–109.9% respectively. Furthermore, the ELISA was specific for human FGF21 and did not cross-react with human FGF19, human FGF23, bovine, cat, dog, goat, hamster, horse, monkey mouse pig, rat, and sheep sera. All serum samples had two freeze–thaw cycles before quantification of FGF21 serum concentrations. According to the manufacturer's instructions, no decline of human FGF21 was observed in serum and plasma samples even after five freeze–thaw cycles. Each commercial FGF21 ELISA included two quality controls (high and low). For all ELISA assessments performed in our study, FGF21 values for these two quality controls were within the range given by the manufacturer. Serum creatinine, total, HDL, LDL cholesterol, triglycerides (TGs), urea, albumin, alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), alkaline phosphatase (aP), gamma glutamyl transferase (γGT), TSH, and C-reactive protein (CRP) were measured by standard laboratory methods in a certified laboratory. Serum IGF1 concentrations were measured by commercially available automated two-site chemiluminescent immunometric assays (Immulite 2000, Siemens Healthcare Diagnostics GmbH, Bad Nauheim, Germany). The intra-assay coefficient of variance (%) was between 3.1 and 4.4% and that of interassay was between 5.7 and 6.6%.

Hepatocyte culture and analysis of IGF1 protein secretion

Human HepG2 cells (American Type Culture Collection, Rockville, MD, USA) were cultured in DMEM–high-glucose medium (DMEM-H, PAA, Pasching, Austria) supplemented with 10% fetal bovine serum (PAA). Equivalent numbers of HepG2 cells starved in DMEM-H for 24 h were treated with 250 ng/ml recombinant FGF21 (R&D Systems, Wiesbaden, Germany; treatment group) or PBS (control group) for 24 h. Quantification of human IGF1 protein secretion into HepG2 cell culture supernatants was performed with a commercially available ELISA (R&D Systems) according to the manufacturer's instructions. The sensitivity of the ELISA was 0.026 ng/ml. The degree of precision of the ELISA system in terms of intra-assay coefficient of variance (%) was between 3.5 and 4.3% and that of interassay was between 7.5 and 8.3%. Spike recovery and linearity were in a range of 97–102 and 92–108% respectively.

Statistical analysis

PASW software version 18.0.1 (SPSS, Chicago, IL, USA) was used in all statistical analyses. Distribution was tested for normality using Shapiro–Wilk *W* test and non-normally distributed parameters were naturally logarithmically transformed before analyses. For patients with no measurable FGF21 concentration, we used half (3.5 ng/l) of the sensitivity of the FGF21 ELISA (7 ng/l) in group comparisons between controls and T2DM. Differences between two groups were assessed by the Mann–Whitney *U* test for continuous parameters and by the χ^2 test for gender followed by Bonferroni adjustment for multiple testing. Analyses were adjusted for age and gender and in a second model additionally for BMI to exclude that differences were mainly driven by body weight variation. Correlations were performed using the Spearman's rank correlation method followed by Bonferroni adjustment for multiple testing. Multivariate regression analyses were performed including all parameters with highly significant correlations in the univariate analysis ($P \leq 0.001$) as covariates. In case of parameters strongly related to each other, one representative covariate was included in the model. A *P* value of <0.05 was considered as statistically significant in all analyses.

Results

Study subjects

Baseline characteristics of nondiabetic and diabetic subjects are summarized in Table 1. All continuous variables are given as median (interquartile range). Serum FGF21 levels were significantly increased in subjects with T2DM (141.8 (174.4) ng/l; $n=101$) compared with nondiabetic controls (66.7 (117.8) ng/l; $n=812$) after adjustment for gender and age ($P<0.001$). To exclude any secondary effects, subsequent analyses were performed only in nondiabetic subjects (Table 1). In nondiabetic subjects, circulating FGF21 levels were below the limit of detection of the FGF21 ELISA in 142 subjects. Interestingly, subjects with FGF21 concentrations below the limit of detection showed a more beneficial metabolic profile compared with subjects with measurable FGF21 (Table 1). Thus, the group with non-detectable FGF21

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Table 1 Baseline characteristics

	Non-type 2 diabetes mellitus				
	Subjects (above the limit of detection)	Subjects (below the limit of detection)	P1	**Type 2 diabetes mellitus**	**P2**
n	670	142		101	
FGF21 (ng/l)	88.9 (117.7)	3.5 (0)	<0.001[a,b]	141.8 (174.4)	<0.001[a,b]
Age (years)	47.7 (22.1)	39.6 (19.6)	<0.001[b]	65.8 (16.8)	<0.001[b]
Gender (f/m)	385/285	100/42	0.005	59/42	0.064
SBP (mmHg)	135.0 (22)	127.3 (19)	0.001[a]	151.0 (26)	<0.001[a,b]
DBP (mmHg)	83.3 (13)	78.5 (11)	0.001[a]	86.0 (14)	0.424
Height (m)	1.69 (0.13)	1.69 (0.13)	0.338	1.65 (0.16)	0.554
Weight (kg)	76 (20)	69 (17)	<0.001[a,b]	82 (21)	<0.001[b]
BMI (kg/m^2)	26.5 (5.8)	24.0 (4.0)	<0.001[a,b]	30.3 (7.4)	<0.001[b]
Fat mass (%)	20.1 (10.7)	16.4 (6.5)	<0.001[a,b]	25.3 (14.1)	0.001
Waist circumference (cm)	91 (19)	82 (14)	<0.001[a,b]	101 (17)	<0.001[b]
Hip circumference (cm)	102 (10)	99 (8)	<0.001[a,b]	107 (12)	0.013[a]
WHR	0.87 (0.14)	0.82 (0.11)	<0.001[a,b]	0.96 (0.13)	<0.001[a,b]
WtHR	0.54 (0.11)	0.48 (0.08)	<0.001[a,b]	0.60 (0.11)	<0.001[b]
Glucose 0 h (mmol/l)	5.32 (0.65)	5.00 (0.46)	<0.001[a,b]	7.36 (2.49)	<0.001[a,b]
Glucose 30 min (mmol/l)	8.52 (2.31)	7.95 (1.57)	0.007[a]	11.74 (2.04)	<0.001[a,b]
Glucose 120 min (mmol/l)	5.22 (2.32)	4.78 (1.68)	0.256	11.91 (6.27)	<0.001[a,b]
Insulin 0 h (pmol/l)	33.8 (27.6)	25.6 (19.3)	<0.001[a,b]	52.5 (45.5)	<0.001[a,b]
Insulin 30 min (pmol/l)	252 (172)	223 (173)	0.012[a]	219 (153)	0.190
Insulin 120 min (pmol/l)	125 (159)	118 (117)	0.673	279 (292)	<0.001[a,b]
HOMA-IR	1.09 (1.00)	0.81 (0.61)	<0.001[a,b]	2.42 (2.44)	<0.001[a,b]
Stumvoll index	0.10 (0.04)	0.12 (0.02)	<0.001[a,b]	0.07 (0.04)	<0.001[a,b]
Quicki index	0.44 (0.07)	0.47 (0.07)	<0.001[a,b]	0.39 (0.06)	<0.001[a,b]
Cholesterol (mmol/l)	5.34 (1.30)	5.14 (1.24)	0.188	5.25 (1.35)	<0.001[a,b]
HDL cholesterol (mmol/l)	1.58 (0.53)	1.79 (0.57)	<0.001[a,b]	1.33 (0.48)	<0.001[a,b]
LDL cholesterol (mmol/l)	3.41 (1.30)	3.11 (1.12)	0.085	3.35 (1.15)	<0.001[a,b]
TG (mmol/l)	1.13 (0.74)	0.78 (0.47)	<0.001[a,b]	1.64 (1.00)	<0.001[a,b]
Creatinine serum (μmol/l)	71 (18)	68 (14)	0.764	69 (25)	0.175
GFR (ml/min per 1.73 m^2)	97.9 (21.2)	103.9 (18.8)	0.587	90.2 (25.0)	0.294
Urea (mmol/l)	4.7 (1.7)	4.6 (1.7)	0.002[a]	5.6 (2.0)	0.004[a]
Protein serum (g/l)	72.8 (5.3)	72.6 (5.5)	0.648	72.3 (5.2)	0.641
Creatinine urine (μmol/l)	12 129 (8713)	10 967 (8852)	0.103	10 842 (8581)	0.718
Protein urine (mg/l)	80.8 (82.0)	66.1 (72.0)	0.219	92.5 (72.1)	0.477
Albumin urine (mg/l)	8.1 (8.9)	7.1 (6.2)	0.248	14.2 (28.2)	0.493
ALAT (μkat/l)	0.37 (0.25)	0.29 (0.14)	<0.001[a,b]	0.46 (0.26)	0.008
ASAT (μkat/l)	0.44 (0.15)	0.40 (0.12)	0.021[a]	0.48 (0.16)	0.346
aP (μkat/l)	1.05 (0.42)	0.90 (0.34)	<0.001[a,b]	1.20 (0.44)	0.002[a]
γGT (μkat/l)	0.32 (0.29)	0.22 (0.12)	<0.001[a,b]	0.51 (0.52)	0.003
IMT right (mm)	0.63 (0.20)	0.57 (0.10)	0.070	0.83 (0.27)	0.001[a]
IMT left (mm)	0.63 (0.17)	0.57 (0.13)	0.278	0.82 (0.24)	0.003[a]
IGF1 (ng/ml)	159.9 (79.3)	176.7 (77.6)	0.291	120.0 (59.7)	0.993
TSH (mU/l)	1.52 (1.2)	1.7 (1.3)	0.297	1.33 (1.0)	0.049[a]
CRP (mg/l)	1.2 (1.9)	0.5 (1.3)	0.016[a]	2.5 (3.5)	<0.001[b]

ALAT, alanine aminotransferase; aP, alkaline phosphatase; ASAT, aspartate aminotransferase; CRP, C-reactive protein; DBP, diastolic blood pressure; FFA, free fatty acids; FG, fasting glucose; FGF21, fibroblast growth factor-21; FI, fasting insulin; GFR, glomerular filtration rate; γGT, gamma glutamyl transferase; IGF, insulin-like growth factor; IMT, intima-media thickness; SBP, systolic blood pressure; TG, triglyceride; WHR, waist-to-hip ratio; WtHR, waist-to-height ratio; values for median (interquartile range) or total number are shown. Differences between groups were assessed by the Mann–Whitney *U* test for continuous parameters and by the χ^2 test for gender. Age and gender distribution were tested without adjustment. All other analyses were adjusted for age and gender and in a second model additionally for BMI to exclude that differences were mainly driven by body weight variation. *P* values are presented for model 1 (adjustment for age and gender).
[a]Significant differences after additional adjustment for BMI (model 2).
[b]Differences (model 1) remaining significant after Bonferroni adjustment for multiple testing. P1 indicates *P* value of comparison between nondiabetic subjects with and without detectable FGF21 levels; P2 indicates *P* value of comparison between nondiabetic and diabetic subjects.

showed significantly lower blood pressure and TG, higher HDL cholesterol levels, as well as better insulin sensitivity (Table 1). These differences remained significant after adjustment for age, gender, and BMI (Table 1). FGF21 was significantly lower in female (median 85.0 (107.8) ng/l, $n=385$) compared with male (median 92.6 (133.2) ng/l, $n=285$) subjects after adjustment for age and BMI ($P=0.029$).

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Univariate correlations

Univariate correlations were performed only in subjects with detectable FGF21 concentrations (n=670). Here, serum FGF21 levels were positively associated with age, blood pressure, parameters of obesity (weight, BMI, fat mass, waist circumference, hip circumference, WHR, and WtHR), parameters of insulin and glucose metabolism (fasting glucose, fasting insulin, and HOMA-IR), dyslipidemia (total cholesterol, LDL-cholesterol, and TG), liver function (ALAT, ASAT, aP, and γGT), and IMT (Table 2). Moreover, serum FGF21 concentrations were negatively associated with Stumvoll index, Quicki index, HDL cholesterol, as well as with renal function (GFR) and IGF1 (Table 2). By contrast, FGF21 levels did not show an association with height, serum creatinine, urine protein, urine albumin, thyroid function (TSH), and CRP (Table 2).

Table 2 Univariate correlations

	r	*P*
Age (years)	0.200	<0.001[a,b]
SBP (mmHg)	0.277	<0.001[a,b]
DBP (mmHg)	0.225	<0.001[a,b]
Height (m)	−0.037	0.345
Weight (kg)	0.131	0.001[a,b]
BMI (kg/m^2)	0.177	<0.001[a,b]
Fat mass (%)	0.122	0.002[a]
Waist circumference (cm)	0.241	<0.001[a,b]
Hip circumference (cm)	0.088	0.022[a]
WHR	0.249	<0.001[a,b]
WtHR	0.246	<0.001[a,b]
Glucose 0 h (mmol/l)	0.152	<0.001[a,b]
Glucose 30 min (mmol/l)	0.206	<0.001[a,b]
Glucose 120 min (mmol/l)	0.103	0.009[a]
Insulin 0 h (pmol/l)	0.130	0.001[a,b]
Insulin 30 min (pmol/l)	0.107	0.006[a]
Insulin 120 min (pmol/l)	0.096	0.014[a]
HOMA-IR	0.148	<0.001[a,b]
Stumvoll index	−0.201	<0.001[a,b]
Quicki index	−0.148	<0.001[a,b]
Cholesterol (mmol/l)	0.099	0.011[a]
HDL cholesterol (mmol/l)	−0.148	<0.001[a,b]
LDL cholesterol (mmol/l)	0.104	0.007[a]
TG (mmol/l)	0.327	<0.001[a,b]
Creatinine serum (μmol/l)	0.063	0.106
GFR (ml/min per 1.73 m^2)	−0.131	0.001[a,b]
Urea (mmol/l)	0.000	0.996
Protein serum (g/l)	−0.002	0.950
Creatinine urine (μmol/l)	0.016	0.685
Protein urine (mg/l)	0.011	0.780
Albumin urine (mg/l)	0.045	0.249
ALAT (μkat/l)	0.142	<0.001[a,b]
ASAT (μkat/l)	0.153	<0.001[a,b]
aP (μkat/l)	0.152	<0.001[a,b]
γGT (μkat/l)	0.271	<0.001[a,b]
IMT right (mm)	0.221	<0.001[a,b]
IMT left (mm)	0.219	<0.001[a,b]
IGF1 (ng/ml)	−0.226	<0.001[a,b]
TSH (mU/l)	0.016	0.704
CRP (mg/l)	0.067	0.084

Univariate correlations with serum FGF21 concentrations in all subjects with measurable FGF21 (n=670); *r* and *P* values are given.
[a]Significant correlation as assessed by Spearman's correlation method.
[b]Correlations remaining significant after Bonferroni adjustment for multiple testing. Abbreviations are indicated in Table 1.

Multivariate correlations

Multivariate regression analysis revealed that gender, SBP, TG, γGT, and IGF1 remained independently associated with circulating FGF21 levels after adjustment for age, WHR, Stumvoll index, HDL cholesterol, GFR, and right IMT ($P<0.05$) (Table 3). This multivariate linear regression model explained 20% of the FGF21 serum variation. The associations between FGF21 serum levels, IGF1, and γGT are depicted in Fig. 1. Here, highest FGF21 levels were observed in the subgroup with lowest IGF1 and highest γGT levels (Fig. 1).

FGF21 is a suppressor of IGF1 protein secretion *in vitro*

As FGF21 was negatively and independently associated with IGF1 in our study population (Table 3 and Fig. 1), we elucidated in preliminary experiments whether FGF21 might directly influence IGF1 expression in a human hepatocyte model *in vitro*. Therefore, serum-starved human HepG2 cells were treated with 250 ng/ml recombinant FGF21 for 24 h and IGF1 protein secretion into supernatants was quantified by ELISA. Interestingly, in four independent experiments, FGF21 significantly inhibited IGF1 secretion into the HepG2 cell culture supernatants from 0.089 ng/ml (basal) to 0.062 ng/ml ($P<0.05$) (Fig. 2). Total protein content in the supernatants was not significantly different between control and FGF21-treated cells (data not shown).

Discussion

In the current study, we demonstrate for the first time that FGF21 is negatively and independently associated with IGF1 in unselected nondiabetic subjects. A negative association between FGF21 and IGF1 has also been observed in patients with anorexia nervosa independent from body fat and insulin resistance (Fazeli *et al*. 2010). It is interesting to note in this context that FGF21 inhibits IGF1 production in a human hepatocyte cell model *in vitro* in our study. However, it needs to be emphasized that dose–response, time-course, and signaling experiments are necessary to more thoroughly establish the link between FGF21 and IGF1 secretion from hepatocytes. Our results

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Table 3 Multivariate linear regression analysis (dependent variable: FGF21)

Independent variable	β	P
Age (years)	−0.106	0.181
Gender	−0.114	0.035[a]
SBP (mmHg)	0.113	0.011[a]
WHR	0.112	0.060
Stumvoll index	0.041	0.375
HDL cholesterol (mmol/l)	−0.010	0.822
TG (mmol/l)	0.223	<0.001[a]
GFR (ml/min)	0.020	0.694
IMT right (mm)	−0.005	0.925
γGT (μkat/l)	0.170	<0.001[a]
IGF1 (ng/ml)	−0.170	<0.001[a]

Multivariate linear regression analysis between FGF21 (dependent variable; n=670 nondiabetic subjects with measurable FGF21) and age, gender, SBP, WHR, Stumvoll index, HDL cholesterol, TG, GFR, IMT right, γGT, and IGF1. β-Coefficients and P values are given. Abbreviations are indicated in Table 1. [a]Significant correlation.

suggest that FGF21 directly and negatively impacts IGF1 production. This hypothesis is further supported by recent independent data from Inagaki *et al*. (2008). The authors demonstrate convincingly that FGF21 reduces levels of active form of STAT5, a major mediator of GH action, in the liver of mice *in vivo*. FGF21-mediated suppression of STAT5 is accompanied by decreases in the expression of its target genes including IGF1 (Inagaki *et al*. 2008). Furthermore, FGF21 induces hepatic expression of IGF1 binding protein 1 and suppressor of cytokine signaling 2, both of which impair GH signaling (Inagaki *et al*. 2008). In agreement with a GH signaling-suppressive effect, chronic FGF21 treatment significantly inhibits growth in mice (Inagaki *et al*. 2008). Furthermore, these findings are in accordance with the investigations in FGF21 knockout animals (Kubicky *et al*. 2012). Here, FGF21-deficient mice exhibit greater body and tibial growth during starvation compared with WT littermates, and daily injections of recombinant human FGF21 in a subgroup of food-restricted knockout mice prevent this phenotype (Kubicky *et al*. 2012). Interestingly, GH binding and GH receptor expression are reduced in the liver and in the growth plate of food-restricted compared with *ad libitum*-fed WT mice whereas they are similar between food-restricted and *ad libitum*-fed FGF21-deficient animals. Taking these results into consideration, our present data are in accordance with the hypothesis that FGF21 and IGF1 are not only significantly and negatively associated but that FGF21 directly downregulates IGF1 as well as impairs GH signaling in the liver.

In the current study, we demonstrate that FGF21 serum levels are positively associated with hepatic enzymes including ASAT, ALAT, aP, and γGT. Furthermore, the association between FGF21 and γGT remains independent in multivariate analysis. As γGT is a surrogate parameter of fatty liver degeneration, our results are in accordance with the hypothesis that circulating FGF21 is positively associated with metabolic liver disease including NAFLD (Tyynismaa *et al*. 2010). In agreement with this notion, FGF21 upregulation has also been demonstrated in recent independent studies using selected NAFLD patient populations (Dushay *et al*. 2010, Li *et al*. 2010, Yan *et al*. 2011, Shen *et al*. 2012). One potential mechanism contributing to FGF21 upregulation in NAFLD is hepatic FGF21 resistance (Fisher *et al*. 2010, Shen *et al*. 2012). In an elegant study, Fisher *et al*. (2010) demonstrate that FGF21 signaling is attenuated in liver and WAT of diet-induced obese mice, with a fourfold increase in FGF21 serum levels compared with control animals. It needs to be elucidated in future studies whether FGF21 can be used as a biomarker for NAFLD and whether this cytokine is a causal factor of this condition. One limitation of the currently available clinical studies is the lack of liver biopsies to reliably differentiate NAFLD from nonalcoholic steatohepatitis.

In this study, we demonstrate that FGF21 is positively and independently correlated with facets of the metabolic syndrome including obesity, insulin resistance, hypertension, and dyslipidemia in nondiabetic subjects.

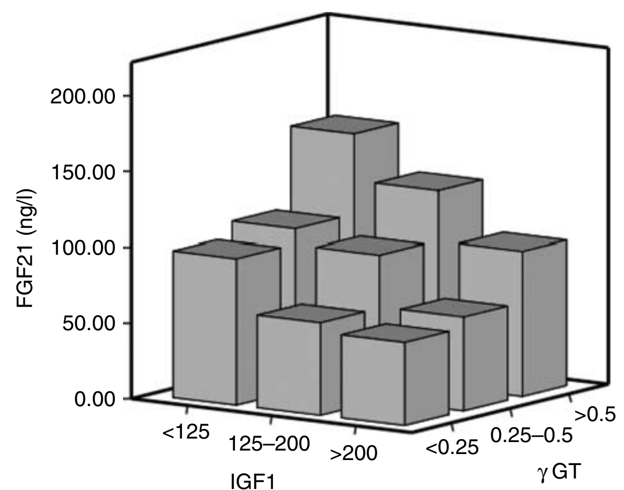

Figure 1
Relationship between serum FGF21, IGF1, and γGT. FGF21 serum levels are depicted depending on IGF1 (low: 0–125 ng/ml; medium: 125–200 ng/ml; and high: >200 ng/ml) and γGT (low: 0–0.25 μkat/l; medium: 0.25–0.5 μkat/l; and high: >0.5 μkat/l) values. Only nondiabetic subjects with FGF21 values above the detection limit of the FGF21 ELISA were included (total n=670).

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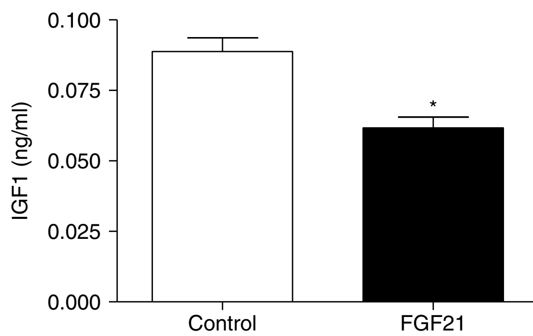

Figure 2
FGF21 downregulates IGF1 protein secretion. Human HepG2 cells were serum deprived overnight before FGF21 (250 ng/ml) was added. After 24 h, IGF1 protein levels were determined in the cell supernatants as described in Materials and Methods section. Results are the mean ± S.E.M. of four independent experiments. *Significant ($P < 0.05$) regulation.

In agreement with this notion, circulating FGF21 is increased in T2DM compared with nondiabetic controls in our study population. Furthermore, nondiabetic subjects with FGF21 levels below the detection limit of the FGF21 ELISA show a more beneficial metabolic profile compared with subjects with detectable FGF21 concentrations. These data strongly suggest that FGF21 increases with deteriorating metabolic control. Our data are in accordance with recent findings from independent groups. Thus, circulating FGF21 levels in obese subjects are significantly higher compared with lean individuals (Zhang *et al*. 2008). Furthermore, serum FGF21 correlates positively with adiposity, fasting insulin, and TG and negatively with HDL-cholesterol after adjusting for age and BMI similar to our results (Zhang *et al*. 2008). Moreover, FGF21 is independently associated with insulin resistance and increased in T2DM in adults from the Baltimore longitudinal study of aging (Semba *et al*. 2012). In addition, Chen *et al*. (2011) demonstrate an independent association between serum FGF21 levels on one hand and TG, LDL-cholesterol, and SBP on the other hand in Chinese individuals using multiple linear regression analysis. Consistent with these findings, FGF21 significantly and positively correlates with markers of insulin resistance and dyslipidemia in univariate and multivariate analyses in gestational diabetes mellitus patients in a study from our group (Stein *et al*. 2010). Mechanistically, elevated FFAs might contribute to FGF21 upregulation when facets of the metabolic disease are present (Mai *et al*. 2010). To date, the physiological significance for increased FGF21 in metabolic disease is unclear. Paradoxical upregulation of FGF21 might be a compensatory mechanism to improve glucose metabolism when obesity, hypertension, insulin resistance, and an adverse lipid profile are present. Alternatively, the metabolic syndrome might cause resistance to FGF21 leading to compensatory upregulation of this antidiabetic cytokine as proposed by Fisher *et al*. (2010). Clearly, more work is needed to better elucidate the pathophysiological significance of FGF21 upregulation in metabolic disease states.

Despite the independent association between FGF21 and metabolic parameters, FGF21 is not independently correlated with IMT, a surrogate parameter of atherosclerosis. To our knowledge, IMT has not been included in studies on circulating FGF21 so far. In contrast to our findings, FGF21 concentrations are increased in coronary heart disease patients (Lin *et al*. 2010). However, coronary heart disease and control groups were not matched for blood pressure, HOMA-IR, and inflammation in this study (Lin *et al*. 2010). Taking these findings into consideration, it is tempting to speculate that FGF21 is an independent predictor of metabolic but not vascular disease.

Interestingly, the current study population does not show an independent association of circulating FGF21 with renal function assessed by serum creatinine and GFR. These findings are in contrast to a recent report suggesting that FGF21 serum levels are progressively increased from the early to end stages of chronic kidney disease and are associated with renal function in a Chinese cohort (Lin *et al*. 2011). Furthermore, median FGF21 concentrations are >15-fold higher in hemodialysis patients compared with subjects with a GFR >50 ml/min (Stein *et al*. 2009). Moreover, serum creatinine positively and GFR negatively predict circulating FGF21 in multiple regression analyses in control subjects (Stein *et al*. 2009). Similarly, Han *et al*. (2010) demonstrated that FGF21 levels were eightfold higher in 72 nondiabetic patients receiving peritoneal dialysis compared with controls. Our findings are in agreement with the hypothesis that circulating FGF21 is increased only in more severe stages of renal dysfunction.

Recent studies indicate that FGF21 might mediate glucagon effects. Thus, hepatic FGF21 expression is upregulated by hepatic glucagon receptor activation in a manner that is further augmented by fatty acids. Furthermore, FGF21 preserves β-cell function and survival by stimulating p44/42 mitogen-activated protein and Akt signaling pathways. Moreover, FGF21 lowers glucagon in mice (Iglesias *et al*. 2012). Unfortunately, glucagon could not be quantified within our study, and, therefore, the relationship between FGF21 and glucagon could not be determined.

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Strengths of our study include the high number of subjects, the broad range of phenotypical features, as well as the genetically homogeneous, self-contained population of Sorbs. A limitation of the study is the cross-sectional design and, therefore, causality cannot be established. Furthermore, our clinical study by necessity only measures circulating FGF21. Therefore, our study does not provide information on whether local FGF21 production in liver and adipose tissue is associated with circulating FGF21, as well as with facets of the metabolic syndrome.

Taken together, we demonstrate that FGF21 serum concentrations are associated with facets of the metabolic syndrome, hepatocellular function, as well as GH status. Additional studies are necessary to better elucidate the physiological significance of these findings.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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