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Serum fibroblast growth factor 21 levels in gestational diabetes mellitus in relation to insulin resistance and dyslipidemia

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Abstract

Fibroblast growth factor 21 (FGF21) has beneficial effects on glucose homeostasis and insulin sensitivity. In the current study, we investigated serum concentrations of FGF21 in patients with gestational diabetes mellitus (GDM) as compared with healthy pregnant controls matched for gestational age and fasting insulin. Fibroblast growth factor 21 was determined by enzyme-linked immunosorbent assay in control (n = 80) and GDM (n = 40) patients and correlated to clinical and biochemical measures of renal function, glucose and lipid metabolism, as well as inflammation in both groups. Median maternal serum FGF21 concentrations were not significantly different in subjects with GDM (97.5 ng/L) as compared with healthy pregnant controls (102.9 ng/L). Fibroblast growth factor 21 significantly and positively correlated with markers of insulin resistance (increased homeostasis model assessment of insulin resistance, decreased adiponectin) and dyslipidemia (increased triglycerides, decreased high-density lipoprotein cholesterol) in univariate and multivariate analyses. Furthermore, FGF21 serum levels were highest in patients in the third tertile of homeostasis model assessment of insulin resistance. Fibroblast growth factor 21 is independently associated with markers of insulin resistance and an adverse lipid profile but is not dysregulated in GDM if patients are matched with controls for fasting insulin.

1. Introduction

Gestational diabetes mellitus (GDM) is a serious complication in pregnancy. It is characterized by glucose intolerance with onset or first recognition during pregnancy [1]. Both mother and newborn have a significantly increased future risk for metabolic and cardiovascular disease as a consequence of GDM [2,3].

The pathogenesis of GDM has been better elucidated in recent years, and mechanisms similar to obesity-associated type 2 diabetes mellitus (T2DM) have been described. Thus, insulin resistance and a limitation in the pancreatic β-cell reserve contribute to the development of both GDM and T2DM. Furthermore, adipocyte-secreted factors—so-called adipokines—that influence insulin sensitivity might play an important role in both disease states. Here, lower levels of the insulin-sensitizing and vasoprotective adipokine adiponectin have been found in GDM in several independent studies [4-6]; and lower adiponectin concentrations in early pregnancy predicted GDM [7]. Interestingly, a positive association between circulating adiponectin and β-cell function existed in pregnant women [8]. Furthermore, several studies have described dysregulation of the appetite-suppressive adipokine leptin in GDM [9-11].

Recently, fibroblast growth factor 21 (FGF21) was introduced as a secreted protein that is expressed by different organs and tissues including adipose tissue, liver, pancreas,

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and muscle and improves glucose tolerance [12-15]. Thus, FGF21 significantly stimulated glucose uptake in 3T3-L1 fat cells [12]. Furthermore, FGF21 improved pancreatic β-cell function and survival by activation of p44/42 mitogen-activated protein kinase [16]. Fibroblast growth factor 21–transgenic animals were resistant to diet-induced obesity [12], and therapeutic administration of FGF21 reduced plasma glucose concentrations in *ob*/*ob* and *db*/*db* mice [12] as well as in rhesus monkeys [17]. Furthermore, systemic administration of FGF21 for 2 weeks in diet-induced obese and *ob*/*ob* mice lowered their mean body weight by 20% predominantly via a reduction in adiposity [18]. Down-regulation of FGF21 in the liver resulted in the development of fatty liver disease and dyslipidemia [19]. In addition, 3 independent articles suggested that FGF21 is an important mediator of the metabolic effects of peroxisome proliferator–activated receptors α agonists [19-21].

In contrast to other adipocyte-secreted proteins including adiponectin and leptin, concentrations of circulating FGF21 have not been evaluated so far in GDM. In the current study, we therefore sought to investigate for the first time whether maternal FGF21 levels are altered in GDM and might be linked to clinical and biochemical measures of renal function, glucose, and lipid metabolism, as well as inflammation.

2. Subjects and methods

2.1. Subjects

Forty women with GDM and 80 pregnant controls matched for gestational age and fasting insulin were recruited for the study. *Gestational diabetes mellitus* was defined as at least 1 elevated plasma glucose value during a 75-g, 2-hour oral glucose tolerance test (oGTT) according to the criteria of the Austrian Diabetes Association with the following threshold glucose concentrations: fasting, at least 95 mg/dL; 1 hour, at least 180 mg/dL; and 2 hours, at least 155 mg/dL [22]. Body mass index (BMI) was calculated as weight before pregnancy divided by squared height and ranged from 15.6 to 38.2 kg/m^2 in the study population. Patients were between 18 and 45 years old. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as described recently [23]. Patients with renal diseases, preeclampsia, and generalized inflammation were excluded from the study. The study was approved by the local Ethics Committee, and all patients gave written informed consent before taking part in the study.

2.2. Assays

Blood was drawn after an overnight fast, and none of the women was in labor at the time of the blood sampling. Insulin concentrations were determined with a 2-site chemiluminescent enzyme immunometric assay for the Immulite automated analyzer (Diagnostic Products, Los Angeles, CA). Fibroblast growth factor 21 (Biovendor, Modrice, Czech Republic), adiponectin (Mediagnost, Reutlingen, Germany), and leptin (Mediagnost) were determined with commercial enzyme-linked immunosorbent assays according to the manufacturers' instructions. Serum creatinine, cholesterol, triglycerides (TG), and C-reactive protein (CRP) were measured by standard laboratory methods in a certified laboratory.

2.3. Statistical analysis

SPSS software version 11.5 (SPSS, Chicago, IL) was used for statistical analyses. Differences between groups were assessed by Mann-Whitney *U* test or Kruskal-Wallis test as indicated in the figure and table legends. Correlation analysis was performed using the Spearman rank correlation method. To identify independent relationships and adjust the effects of covariates, multivariate linear regression analyses were performed. Distribution was tested for normality using Shapiro-Wilk *W* test, and nonnormally distributed parameters were logarithmically transformed before multivariate analysis. A P value $< .05$ was considered as statistically significant in all analyses.

3. Results

3.1. FGF21 serum levels are not altered in GDM patients matched for gestational age and fasting insulin

The clinical characteristics of the subgroups studied (control, GDM) are summarized in Table 1. All continuous

Table 1
Baseline characteristics of the study population

	Control	GDM
n	80	40
FGF21 (ng/L)	102.9 ± 144.9	97.5 ± 166.1
Age (y)	28 ± 5	33 ± 10*
BMI (kg/m^2)	22.3 ± 7.0	24.9 ± 4.9
SBP (mm Hg)	125 ± 16	121 ± 23
DBP (mm Hg)	75 ± 13	71 ± 17
Gestational age at blood sampling (d)	198 ± 39	205 ± 30
Glucose 0 h (mmol/L)	4.2 ± 0.4	4.5 ± 0.9*
Glucose 1 h (mmol/L)	7.5 ± 1.6	10.3 ± 1.6*
Glucose 2 h (mmol/L)	6.2 ± 1.8	9.0 ± 2.3*
FI (pmol/L)	56.5 ± 39.3	60.3 ± 37.1
HOMA-IR	1.4 ± 1.0	1.6 ± 1.3
Creatinine (μmol/L)	49 ± 12	46 ± 11
TG (mmol/L)	2.1 ± 1.4	2.2 ± 1.3
Cholesterol (mmol/L)	6.3 ± 1.8	6.6 ± 2.2
HDL cholesterol (mmol/L)	1.9 ± 0.5	1.7 ± 0.7
LDL cholesterol (mmol/L)	3.7 ± 1.6	3.8 ± 1.9
Leptin (μg/L)	23.1 ± 11.7	24.9 ± 13.9
Adiponectin (mg/L)	7.0 ± 3.9	7.3 ± 5.6
CRP (mg/L)	4.3 ± 4.5	4.2 ± 4.9

Values for median ± interquartile range are shown. DBP indicates diastolic blood pressure; FI, fasting insulin; LDL, low-density lipoprotein; SBP, systolic blood pressure.

* $P < .05$ as compared with control as assessed by Mann-Whitney *U* test.

variables are given as median ± interquartile range. Maternal serum FGF21 concentrations were not significantly different in subjects with GDM (97.5 ± 166.1 ng/L) as compared with healthy pregnant controls (102.9 ± 144.9 ng/L) (Table 1). Because patients were matched for gestational age and fasting insulin, both parameters were similar between the 2 groups. Patients with GDM were significantly older (33 ± 10 years) as compared with control subjects (28 ± 5 years) ($P < .05$) (Table 1). Fasting plasma glucose and 1- and 2-hour glucose values during 75-g oGTT were significantly higher in GDM patients as compared with controls ($P < .001$) (Table 1). In contrast, measures of adiposity (BMI), lipid metabolism (TG, cholesterol), inflammation (CRP), and renal function (creatinine) were not significantly different between the 2 groups (Table 1). When patients were divided by extent of insulin resistance into HOMA-IR tertiles, FGF21 serum levels were significantly higher in the second and third tertile as compared with the first tertile ($P < .05$) (Fig. 1).

3.2. Univariate correlations

Serum FGF21 concentrations were significantly and positively associated with fasting insulin, HOMA-IR, and TG (Table 2). Furthermore, a significant negative correlation existed between FGF21 on one hand and high-density lipoprotein (HDL) cholesterol as well as adiponectin on the other hand (Table 2). In contrast, circulating FGF21 levels were not correlated with age, BMI, blood pressure, gestational age at blood sampling, fasting glucose, 1- and 2-hour glucose during 75-g oGTT, creatinine, total and LDL cholesterol, leptin, and CRP (Table 2).

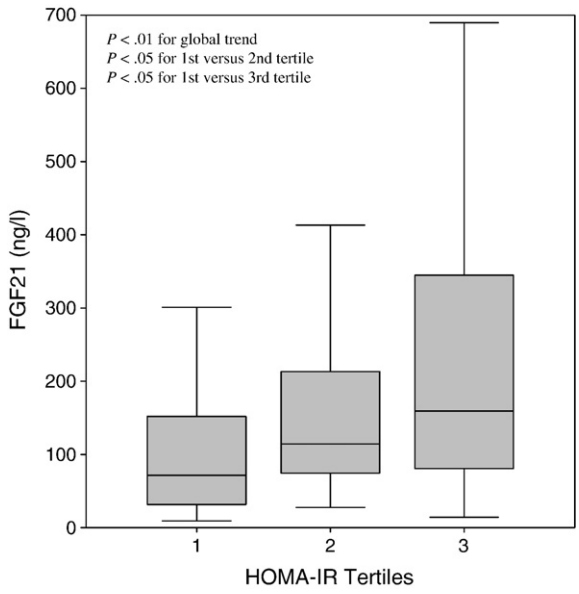

Fig. 1. Fibroblast growth factor 21 serum levels are increased in insulin resistance. Box plot graphs for HOMA-IR tertiles are shown. Statistical significance for global trend was tested by Kruskal-Wallis test. Differences between groups were assessed by Mann-Whitney U test with Bonferroni adjustment for multiple testing.

Table 2
Univariate correlations with serum FGF21 concentrations; r and P values are given

	r	P
Age (y)	−0.040	.663
BMI (kg/m^2)	0.141	.131
SBP (mm Hg)	0.147	.118
DBP (mm Hg)	0.103	.277
Gestational age at blood sampling (d)	−0.182	.101
Glucose 0 h (mmol/L)	0.137	.136
Glucose 1 h (mmol/L)	0.038	.693
Glucose 2 h (mmol/L)	0.030	.755
FI (pmol/L)	0.288	.002*
HOMA-IR	0.326	<.001*
Creatinine (μmol/L)	0.146	.113
TG (mmol/L)	0.414	<.001*
Cholesterol (mmol/L)	−0.091	.323
HDL cholesterol (mmol/L)	−0.303	.001*
LDL cholesterol (mmol/L)	−0.144	.119
Leptin (μg/L)	0.176	.054
Adiponectin (mg/L)	−0.295	.001*
CRP (mg/L)	−0.015	.870

* Significant correlation as assessed by Spearman correlation method.

3.3. Multivariate correlations

In multiple regression analysis, the association between FGF21 serum concentrations on one hand and TG, HDL cholesterol, and HOMA-IR on the other hand persisted after adjustment for age (Table 3, model 1). When circulating insulin-sensitizing adiponectin instead of HOMA-IR was included in multivariate analysis, this adipokine remained independently associated with serum FGF21 concentrations (Table 3, model 2).

4. Discussion

In the current study, circulating levels of FGF21 are determined for the first time in pregnant patients. We show that FGF21 is not significantly different between GDM

Table 3
Multivariate linear regression analyses between FGF21 (dependent variable) and age, TG, HDL cholesterol, and HOMA-IR (model 1), as well as between FGF21 (dependent variable) and age, TG, HDL cholesterol, and adiponectin (model 2)

Dependent variable: FGF21			
Model	Independent variable	β	P
Model 1	Age	−0.011	.899
	TG	0.326	<.001*
	HDL cholesterol	−0.188	.038*
	HOMA-IR	0.202	.020*
Model 2	Age	0.001	.993
	TG	0.344	<.001*
	HDL cholesterol	−0.181	.044*
	Adiponectin	−0.175	.039*

β Coefficients and P values are given.
* Significant correlation.

patients and healthy pregnant controls who are matched for gestational age and fasting insulin. However, FGF21 serum concentrations show a strong and positive association with metabolic and vascular risk factors including insulin resistance (increased HOMA-IR, decreased adiponectin) and adverse lipid profile (increased TG, decreased HDL cholesterol) in univariate and multivariate analysis. In accordance with these findings, circulating FGF21 is significantly higher in patients in the second and third tertile of HOMA-IR as compared with the first tertile. These results indicate that FGF21 is linked to metabolic and vascular risk factors in pregnant women but is probably not a causal factor in the pathogenesis of GDM independent of insulin resistance.

In the current study, GDM is diagnosed as soon as 1 glucose value during oGTT exceeds its threshold according to the criteria of the Austrian Diabetes Association. In central European women, the use of these more stringent criteria for the diagnosis of GDM detects more large-for-gestational age neonates with hypoglycemia and mothers with impaired postpartum glucose metabolism than the World Health Organization criteria [22]. Furthermore, Kautzky-Willer and coworkers [22] demonstrate convincingly that women with 1 abnormal value during oGTT do not differ from those with 2 abnormal values in their obstetric outcome. In our study, FGF21 levels are not significantly different between control subjects (102.9 ± 144.9 ng/L, n = 80), GDM patients with 1 glucose value greater than the threshold (100.6 ± 118.3 ng/L, n = 25), and GDM subjects with 2 or all 3 glucose values greater than the threshold (80.6 ± 311.4 ng/L, n = 15) during oGTT. It needs to be emphasized in this context that GDM patients and controls are matched for fasting insulin in the current study. Because circulating FGF21 positively correlates with fasting insulin, it is likely that FGF21 serum levels would be higher in GDM patients as compared with controls if both patient populations would not be matched for fasting insulin. Along this line, serum concentrations of insulin-sensitizing adiponectin would probably be decreased in GDM subjects as compared with controls if the 2 groups were not matched for fasting insulin.

Our findings appear paradoxical because various animal studies suggest that FGF21 is a potent metabolic regulator with multiple beneficial effects on obesity and glucose metabolism [12,16,17,19-21]. However, our data are in good agreement with a recent study by Zhang and coworkers [24] in which serum FGF21 concentrations were quantified in 232 nonpregnant subjects from the community-based Hong Kong Cardiovascular Risk Factor Prevalence Study. Here, circulating FGF21 is significantly and positively associated with fasting insulin and TG, whereas a negative correlation exists with HDL cholesterol in multivariate analyses [24], in agreement with our findings. Similar to our data, FGF21 is positively correlated with fasting insulin and inversely related to serum adiponectin in control subjects and patients with anorexia nervosa [25]. In this study, adiponectin remains an independent predictor of circulating FGF21 [25]. Furthermore, fasting insulin is an independent predictor of plasma FGF21 in normal subjects and in newly diagnosed patients with T2DM [26]. Moreover, Galman and coworkers [27] demonstrate convincingly that hypertriglyceridemic nondiabetic patients have 2-fold elevated FGF21 levels that further increase by 28% during fenofibrate treatment. Taking these studies and our results into consideration, paradoxical up-regulation of FGF21 might be a compensatory mechanism to improve glucose metabolism when insulin resistance and an adverse lipid profile are present. It is interesting to note in this context that these metabolic parameters are significantly influenced in diabetic rhesus monkeys by FGF21 treatment [17]. Thus, FGF21 induces a significant decrease in fasting insulin and TG, whereas HDL cholesterol and adiponectin are increased [17]. Alternatively, insulin resistance and/or dyslipidemia might cause resistance to FGF21, leading to compensatory up-regulation of this antidiabetic protein. Clearly, more work is needed to better elucidate the pathophysiologic significance of FGF21 up-regulation when insulin resistance and dyslipidemia are present.

Taken together, our results suggest that FGF21 is independently associated with insulin resistance and dyslipidemia in pregnant women similar to results in nonpregnant populations. Prospective studies are needed to better elucidate the role of FGF21 in metabolic and cardiovascular disease.

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