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ORIGINAL ARTICLE

Cord blood FGF21 in gestational diabetes and its relationship with postnatal growth

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Abstract
Background/objectives To study whether FGF21 was present in cord blood and explore its relationship with maternal variables and postnatal growth.
Subjects and methods The study included 157 pregnant women at the beginning of the third trimester; 79 with gestational diabetes (GDM), 78 with normal glucose tolerance (NGT), and their offspring. Glucose metabolism was assessed by oral glucose tolerance test. Insulin resistance was assessed by homeostasis model assessment index–insulin resistance (HOMA-IR). FGF21 was determined in maternal plasma drawn at recruitment and in cord blood obtained at delivery. Offspring weight and height was assessed at birth and at 12, 24 and 48 months.
Results Maternal FGF21 was higher in gestational diabetes than in the normal glucose-tolerant group, whereas similar cord blood FGF21 levels were observed in both groups. Lower cord blood FGF21 was strongly positively correlated with maternal circulating levels. This relationship was independent of mother's prepregnancy body mass index (BMI), glucose levels and HOMA-IR. Although maternal FGF21 levels were correlated with prepregnancy BMI and HOMA-IR index, no relationship was observed between FGF21 and birth weight. However, cord blood FGF21 levels were correlated with BMI Zeta Score at 12 and 24 months, and this relationship became stronger when only the NGT group was analyzed.
Conclusion FGF21 is present in human cord blood, and its levels are closely correlated with maternal levels. The association observed between cord blood FGF21 and postnatal BMI may suggest a potential role during intrauterine life that may influence future metabolic imbalance.

Keywords Fibroblast growth factor 21 (FGF21) · Postnatal growth · Diabetes · Gestational · Postnatal development

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Introduction

Fibroblast growth factor 21 (FGF21), a member of the FGF family, has emerged as an important regulator of energy metabolism with beneficial effects on glucose and lipid homeostasis [1]. It is mainly secreted by the liver [2], but also by other metabolically active organs such as the pancreas and adipose tissue [3].FGF21 has a widespread participation in several metabolic pathways, influencing fatty acid oxidation in liver and glucose uptake in adipocytes, with a net effect on triglyceride clearance [4–7].

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Moreover, experimental data reveal a stimulatory effect on β-cell function, improving β-cell survival in rodents [8]. Chronic systemic administration of FGF21 reduces body fat by increasing energy expenditure [9, 10], reverses hepatic steatosis and improves hepatic insulin sensitivity in diet-induced obese mice [10]. In contrast, in humans, FGF21 levels are increased in insulin-resistant morbidities such as obesity [11] and type 2 diabetes [12], and a high plasma level of FGF21 is an independent predictor of type 2 diabetes in epidemiological studies [13]. These data strongly suggest a pattern of FGF21 resistance in the insulin resistance events. Late pregnancy leads to a progressive increase in insulin resistance, involving nutritional, hormonal and inflammatory factors. When β-cell function fails to overcome this exacerbated insulin resistance, gestational diabetes (GDM) appears [14]. Recently, placenta has emerged as an active tissue expressing and secreting FGF21; however, its role in normal pregnancy and in GDM is poorly understood [15, 16]. Further, contradictory data concerning maternal circulating levels of FGF21 have been reported [15–18] Since the metabolic environment during pregnancy is a determinant for adequate fetal and postnatal growth, hormones that participate in glucose metabolism are key molecular targets for identification in the context of impaired glucose utilization, as occurs in GDM. We hypothesized that FGF21 might play a role in metabolic regulation during intrauterine life. To test this hypothesis, we analyzed circulating FGF21 in maternal serum and cord blood of a well-characterized cohort of pregnant women with GDM, and normal glucose tolerance (NGT) counterparts, and their offspring. We also assessed its relationship with anthropometric parameters at birth and in postnatal growth.

Research design and methods

Subjects

One hundred and fifty-seven pregnant women with GDM ($n = 79$) and with NGT ($n = 78$), and their offspring were included in the study. Women were recruited at the time of the 100 g oral glucose tolerance test (OGTT) between 26 and 30 weeks of pregnancy and were followed until delivery. Diagnosis of GDM was carried out according to the Spanish diabetes in pregnancy guidelines that followed the National Data Group Criteria [19, 20]. In this study, the participants, matched for age and prepregnancy body mass index (BMI), fulfilled the following criteria at the end of pregnancy: (1) a singleton pregnancy, (2) accurate gestational age confirmed by an ultrasound examination before 20 weeks of gestation, (3) the absence of fetal anomalies identified at birth, (4) normal glucose tolerance or GDM diagnosed before 30 weeks of pregnancy, and (5) no pre-existing DM, inflammatory or chronic diseases, or current use of drugs known to affect carbohydrate metabolism. This study was performed at the Hospital Universitari de Tarragona Joan XXIII. The study protocol was approved by the Hospital Research Ethics Committee, and all patients gave written informed consent before participating in the study. All patients with GDM were initially treated with diet and supplemented with insulin as required. Maternal and cord blood samples were obtained at recruitment and at delivery, respectively. Cord blood was not viable for the analysis in eight patients, one offspring of NGT subjects and seven offspring of GDM subjects. For gene expression and immunoblot analysis, we also collected umbilical cord tissue and umbilical cord blood from four additional women.

Upon inclusion, demographic and historical information was collected by an interviewer administering a questionnaire that included patient demographics, personal medical information and information regarding the current and previous pregnancies. BMI was calculated using the formula BMI = weight (in kg)/height (in m)^2. Increase in BMI was calculated by the formula BMI Gain = [final BMI]−[prepregnancy BMI].

Neonatal length and weight were determined using a measuring board to the nearest 0.1 cm and a calibrated scale to the nearest 10 g. Triceps, biceps, subscapular and flank skinfold thickness were measured with a Holtain skinfold caliper (Chasmors Ltd, London, UK) from the left side at least three times until a consistent and stable reading was obtained. The sum of the four skinfolds (SSF) was used to estimate neonatal adiposity.

Weight and height at 12, 24 and 48 months were gathered by chart review of the electronic medical records. Both measures were routinely collected at well-child visits. The child's BMI Z-Score (ZS) for gender and age was calculated based on Spanish growth charts [21].

Laboratory assays

Maternal blood samples were obtained at the time of the OGTT after an overnight fast, and cord blood from umbilical vein and tissue samples were obtained at the time of delivery. Serum was immediately separated by centrifugation, and umbilical cord tissues were washed twice or more with phosphate-buffered saline (PBS). All samples were processed within one hour and stored at −80° C until analysis. Total circulating FGF21 levels were measured using a commercially available ELISA kit (BioVendor, Czech Republic) with an intra-assay coefficient of variation of 3.55 and inter-assay 3.8 %. The assay sensitivity was

7 ng/mL, and no cross-reactivity with human FGF19 and human FGF23 has been observed. Serum fasting glucose, insulin, triglycerides, total cholesterol and high-density lipoprotein were determined by standard enzymatic methods. Insulin resistance was estimated using homeostasis model assessment index-insulin resistance (HOMA-IR) [22].

Gene expression analysis

Total RNA was extracted from cord tissue using the RNeasy Lipid Tissue Midi Kit (Qiagen Science, Hilden, Germany). Total RNA quantity was measured at 260 nm, and purity was assessed by the OD260/OD280 ratio. One microgram of RNA was retrotranscribed with random primers using the Reverse Transcription System (Applied Biosystems, Foster City, CA, USA). Quantitative gene expression was analyzed by real-time PCR (qPCR) on a 7900HT Fast Real-Time PCR System using TaqMan® Gene Expression Assays (Applied Biosystems).The following genes were evaluated: FGFR1 (Hs 01092738_m1), FGFR4 (Hs00999691_m1) and β-Klotho (KLB) (Hs 00545621_m1). Results were calculated using the comparative Ct method (2-ΔΔCt) and expressed relative to the expression of the housekeeping gene 18S rRNA (Hs 03928985).

Immunoblot analysis

Human serum albumin (HAS) and the major subclasses of gamma globulin (IgG) from cord blood serum (CBS) were removed using the Pierce Albumin/IgG Removal Kit (Pierce Biotechnology, Boston, MA, USA). Cord blood serum was separated on SDS-PAGE gels, transferred to immobilon membranes, blocked and incubated with an antibody to FGF21 (Abcam, Cambridge, UK). Immunoreactive band (a band of approximately 24 kDa, FGF21 predicted molecular weight is 22 kDa) was visualized using Supersignal West Femto chemiluminescent substrate (Pierce Biotechnology, Boston, MA, USA), and images were captured using the VersaDoc imaging system and Quantity One software (Bio-Rad, Hercules, CA, USA).

Statistical analysis

Data were analyzed with SPSS software version 15.0 (IBM, Armonk, USA). The one-sample Kolmogorov–Smirnov test was performed to verify the normal distribution of the quantitative variables. Normally distributed data are expressed as mean ± SD, whereas variables with a skewed distribution are represented as the median [Q25–Q75]. Categorical variables are reported as number (percentages). Student's *t* test, the Mann–Whitney *U* test and the Kruskal–Wallis test were used to compare the mean values of continuous variables normally distributed as required. To analyze differences in nominal variables between groups, we used the Chi-square test. Spearman's correlation coefficient was used to analyze the univariate correlation between FGF21 and clinical and metabolic parameters.

Non-normally distributed parameters were logarithmically (lg) transformed before multivariate analyses. Analysis of covariance was used to test for differences between sexes in mean adjusted for GDM lg cbFGF21. Stepwise multiple linear regression analysis was used to investigate the model which best explained maternal and cord blood circulating FGF21 concentrations. The variables selected to enter into stepwise regression were those that correlated significantly in the univariate analysis or which may be potentially involved. Logistic regression analysis was used in calculating the association of the odds ratio (OR) for the association with GDM in subjects with raised baseline FGF21 (second and third tertiles) compared with those with low FGF21 (lowest tertile reference group), with additional adjustment for factors known to be associated with GDM diagnosis. A P value of <0.05 was considered statistically significant in all analyses.

Results

Clinical and metabolic characteristics of the population studied

Clinical and metabolic data of the studied population are shown in Table 1. Maternal FGF21 (mFGF21) levels were higher in GDM than in NGT pregnant women, whereas similar cord blood FGF21 (cbFGF21) concentrations were found in both groups. Moreover, circulating mFGF21 levels were significantly higher than cbFGF21 levels (83.16 [47.22–156.60] vs 55.11 [49.94–69.39] pg/mL; $P < 0.001$). Similar birth weight and BMI ZS at 12, 24 and 48 months were observed in both groups.

In the GDM group, no differences in mFGF21 and cbFGF21 levels were observed between women treated with insulin compared with women only treated with diet (data not shown).

To test the predictive value of FGF21 as a biomarker of GDM, we classified pregnant women according to mFGF21 divided in tertiles. We observed that women with GDM belonged more frequently to the second and highest tertile group (38 and 39.2 % vs 29.5 and 26.9 %, respectively; $P = 0.021$). We also developed a logistic regression

Table 1 Clinical and Analytical characteristics of the population studied

	NGT (78)	GDM (79)	P
Maternal age (years)	30.82 ± 4.78	32.15 ± 5.09	0.093
Gestational week (n)	27 (26–28.5)	27 (27–29)	0.856
Pregestational BMI (kg/m^2)	24.88 ± 5.17	25.6 ± 4.80	0.318
Gain in BMI (kg/m^2)	5.05 ± 2.11	3.52 ± 2.12	**<0.001**
Glucose 0′ (mg/dL)	81.14 ± 7.31	85.73 ± 12.13	**0.005**
Glucose 60′ (mg/dL)	152.15 ± 25.72	211.37 ± 29.87	**<0.001**
Glucose 120′ (mg/dL)	119.62 ± 23.24	184.30 ± 23.44	**<0.001**
Glucose 180′ (mg/dL)	97.80 ± 21.28	134.98 ± 30.82	**<0.001**
Insulin (mUI/L)	8.32 (5.83–13.48)	9.85 (6.87–14.58)	0.153
Insulin Treatment n (%)		31 (39.24)	
HOMA-IR	1.49 (1.15–2.96)	2.07 (1.32–3.37)	0.095
Triglycerides (mg/dL)	176.53 ± 58.01	192.46 ± 62.33	0.099
Total cholesterol (mg/dL)	260.19 ± 43.15	254.93 ± 43.45	0.430
HDL cholesterol (mg/dL)	73.49 ± 12.89	71.82 ± 13.28	0.430
Maternal FGF21 (pg/mL)	71.96 (38.28–144.40)	95.59 (61.95–164.12)	**0.021**
Cord blood FGF21 (pg/mL)	55.12 (50.80–69.04)	54.94 (49.30–70.15)	0.593
Cord blood insulin (mu/L)	3.61 (2.30–6.79)	4.87 (2.98–9.90)	0.053
Birth weight (g)	3,288.26 ± 483.848	3,256.01 ± 472.778	0.673
Sum four skinfolds	15.64 ± 2.64	14.71 ± 2.44	**0.029**
Gestational age delivery (weeks)	40 (38–41)	39 (38–40)	**0.039**
Male sex n (%)	30 (38.46)	45 (56.90)	**0.020**
BMI ZS at birth	−0.5,083 ± 1.12014	−0.5554 ± 1.19883	0.800
BMI ZS 12 months	−0.8845 ± 0.90760	−0.6105 ± 0.83394	0.092
BMI ZS 24 months	−0.6869 ± 1.11248	−0.4898 ± 1.13823	0.356
BMI ZS 48 months	−0.5261 ± 1.29468	−0.3361 ± 1.10776	0.420

Bold values indicate correlations with a P value of < 0.05

Data are mean ± standard deviation or median (interquartile range)

NGT Normal glucose tolerance, *GDM* Gestational diabetes mellitus, *BMI ZS* Body mass index Z-Score

model in which the dependent variable was diagnosis of GDM and mFGF21 tertiles; HOMA-IR, maternal age and prepregnancy BMI were introduced as covariates. Maternal FGF21 was independently associated with the diagnosis of GDM (Exp B: 1.617 IC95 %: 1.065–2.454; $P = 0.014$). Women in the second tertile and third tertile of mFGF21 had an OR: 2.577 (1.148–5.786; $P = 0.022$) and OR: 2.606 (1.127–6.030; $P = 0.025$) of the association with GDM compared with subjects in the lowest tertile of mFGF2, respectively.

Immunoblot analysis of cord blood FGF21 and gene expression of its receptor in cord tissue

In order to confirm the presence of FGF21 in cord blood, an immunoblot analysis was performed in four independent samples. FGF21 reactivity could be clearly demonstrated in three of the four patients (Fig. 1a). We also analyzed the expression of genes encoding FGFR1, FGFR4 and the co-receptor ß-Klotho, in cord tissue. FGF21 exerts its action through binding preferentially to FGFR1 and requires the interaction of the receptor with the scaffold protein ß-Klotho [23, 24]. Although found to a lesser extent than in adipose tissue, we observed that both FGFR1 and ß-Klotho were expressed in umbilical cord tissue, whereas FGFR4 was undetectable (Fig. 1b).

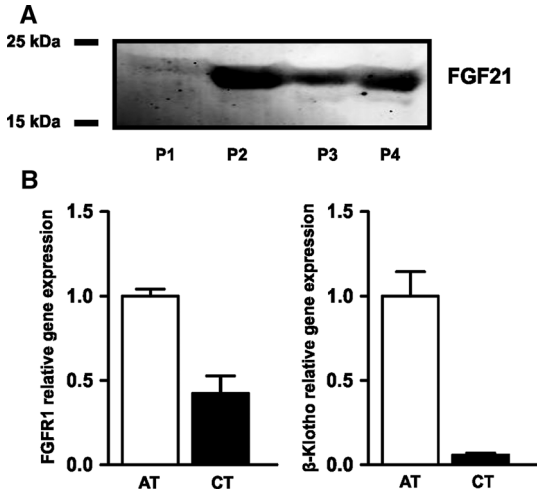

Fig. 1 **a**) FGF21 protein expression was analyzed by Western blot in cord blood serum from four subjects (P1–P4). One preterm (P1) and three at term (P2–P4). **b**) FGFR1 and β-klotho mRNA expression in visceral adipose tissue (AT) and cord tissue (CT)

Table 2 Significant spearman correlation coefficients observed between maternal FGF21 concentrations and some clinical and analytical parameters

	Whole Group		NGT		GDM	
	R	P	R	P	R	P
Pregravid BMI	**0.182**	**0.023**	0.054	0.637	**0.302**	**0.007**
Glucose 60	**0.227**	**0.004**	0.170	0.137	0.139	0.222
Glucose 120	**0.198**	**0.013**	0.130	0.255	0.040	0.724
Glucose 180	**0.197**	**0.013**	0.067	0.558	0.168	0.138
Insulin	**0.234**	**0.001**	0.117	0.307	**0.335**	**0.003**
Triglycerides	**0.241**	**0.002**	**0.248**	**0.029**	0.212	0.061
HDL cholesterol	−0.109	0.177	**−0.236**	**0.037**	0.039	0.736
HOMA-IR	**0.238**	**0.03**	0.135	0.239	**0.321**	**0.004**

Bold values indicate correlations with a P value of < 0.05

NGT Normal glucose tolerance, *GDM* Gestational diabetes mellitus

Relationship between maternal FGF21 (mFGF21) levels with some clinical and metabolic parameters

mFGF21 was positively correlated with HOMA-IR index and insulin and glucose concentrations after the glucose load. Furthermore, a positive association was observed with prepregnancy BMI and maternal triglycerides concentrations. Correlation coefficients are shown in Table 2. To ascertain the influence of glucose tolerance on these relationships, GDM and NGT groups were analyzed separately.

Relationship between cord blood FGF21 (cbFGF21) levels and clinical/metabolic parameters

cbFGF21 levels were higher in girls compared to boys (56.07 [51.48–89.75] vs 54.19 [48.45–61.47]; $P = 0.004$); this difference persisted after adjusting for GDM diagnosis. As differences among both groups were observed in sex, BMI gain and gestational age at delivery, we then used sex-, BMI gain- and gestational age at delivery-adjusted cbFGF21 concentrations in the univariate analysis.

In the whole group, we observed that cbFGF21 levels were positively associated with mFGF21 concentrations and negatively associated with total and HDL cholesterol levels. Also, cbFGF21 concentrations were positively associated with BMI ZS at 12 and 24 months of age. Correlation coefficients of the whole group and the two groups considered separately are shown in Table 3.

Multiple linear relationships

To verify independent associations found in the univariate analysis, stepwise multiple linear regression analysis was performed. HOMA-IR index and GDM diagnosis were the two variables independently associated to mFGF21. The model explained up to 9.5 % of the variance of mFGF21 levels. cbFGF21 was independently related to maternal FGF21 and negatively related to prepregnancy BMI. The model explained up to 36 % of the variance of cbFGF21 (Table 4).

Discussion

This report is the first to demonstrate the detection of FGF21 in human fetal cord blood. Additionally, FGF21 levels at birth appear to correlate with postnatal growth. There is little information about the role of FGF21 during fetal life. Our results are consistent with previous reports in mice that found lower levels of FGF21 in the fetus and at birth compared to adults [25] and with a recent report showing FGF21 expression in interscapular and visceral adipose tissue in human neonates [26].Notwithstanding the

Table 3 Significant Spearman correlation coefficients observed between sex-, gestational age at delivery- and BMI gain-adjusted cord blood FGF21 concentrations with some clinical and analytical parameters

	Whole group		NGT		GDM	
	R	P	R	P	R	P
mFGF21	**0.529**	**<0.001**	**0.511**	**<0.001**	**0.578**	**<0.001**
Total cholesterol	**−0.163**	**0.047**	−0.156	0.174	−0.160	0.179
HDL cholesterol	**−0.172**	**0.038**	−0.224	0.051	−0.106	0.385
Triglycerides	0.105	0.208	0.143	0.216	0.053	0.656
BMI ZS at birth	0.133	0.106	0.033	0.777	0.195	0.100
BMI ZS 12 months	**0.192**	**0.044**	**0.361**	**0.005**	−0.030	0.832
BMI ZS 24 months	**0.212**	**0.029**	**0.355**	**0.007**	0.035	0.814
BMI ZS 48 months	0.163	0.103	**0.371**	**0.004**	−0.188	0.228

Bold values indicate correlations with a P value of < 0.05

NGT Normal glucose tolerance, *GDM* Gestational diabetes mellitus, *mFGF*21 maternal FGF21, *BMI ZS* Body mass index Z-Score

Table 4 Multivariate stepwise linear regression analysis with maternal and cord blood FGF21 dependent variables

	Covariates	Standardized Beta	P
Maternal FGF21[a]	HOMA-IR	0.265	0.001
R^2: 9.5	GDM	0.158	0.044
Cord blood	Maternal FGF21	0.616	<0.001
FGF21[b] R^2: 36.4	Prepregnancy BMI	−0.189	0.008

Variables logarithmically transformed before the analysis: maternal FGF21, cord blood FGF21 and HOMA-IR

R^2 adjusted R^2

[a] Covariates included for selection in the model: HOMA-IR, GDM, triglycerides, HDL cholesterol, total cholesterol, maternal age and prepregnancy BMI

[b] Covariates included for selection in the model: GDM, prepregnancy BMI, birth weight, sum skinfolds, gestational age at delivery, maternal FGF21, neonatal sex and BMI gain

fact that FGF21 is expressed and released by placenta [15, 16] and its expression in human neonates [26], surprisingly, a recent work failed to detect measurable levels of FGF21 in cord blood from healthy and GDM pregnant women [15]. In contrast, we have found FGF21 in cord blood from the umbilical vein, albeit at a lower concentration than in maternal blood during pregnancy, although a close correlation was observed between the two. Although the ELISA kits in the two studies were from different commercial vendors, both use polyclonal antibodies against total FGF21. We postulate that differences in the sample collection could explain the discrepancies observed since in the Nitert study, cord blood was collected from mixed arterial and venous origin, whereas in our study, it came from the umbilical vein that carries oxygen and placental substances to the fetus. This finding is in accordance with the specific placental origin of this factor, although fetal production from other tissues, such as brown adipose tissue (BAT), cannot be discarded.

Interestingly, in our study, higher levels of FGF21 were observed in GDM compared with NGT subjects despite the fact that no relationship with glucose concentrations was observed when the groups were considered separately. Data on FGF21 levels in pregnancy are controversial; some studies have found increased levels in GDM compared to NGT pregnant women [16, 17], whereas others have failed to find any difference between both groups [15, 18, 27]. In agreement with previous studies in pregnant women [17, 18], FGF21 levels were related to insulin resistance index and they were also related to BMI, as has been demonstrated in non-pregnant subjects [11, 13]. Intriguingly, this association was found at the expense of the GDM group, although prepregnancy BMI and HOMA-IR were similar in both groups. This observation is in line with data reported by Stepan et al. in non-diabetic pregnant women [28] and with the well-known increased risk of metabolic syndrome in women with previous GDM [29]. Accordingly, they were also unable to find any correlation between FGF21 circulating concentrations with HOMA-IR whereas, in a result similar to ours, a negative correlation with triglycerides levels was observed [28]. We are aware that our study design does not permit us to infer causality, but one is tempted to speculate that women with a pregravid FGF21 resistance state may be more prone to develop GDM during pregnancy. The association observed in our study between mFGF21 circulating levels and the presence of GDM, with independence of the other well-known confounders, would support this hypothesis. Our study, which included a large number of participants compared with other studies, may provide a partial explanation for the variability of this protein during dysglycemia observed in pregnancy.

Of note, our data suggest that there is a sex difference in FGF21 levels at birth; however, the reason for this is unclear. To the best of our knowledge, there have been no described differences in FGF21 levels in adult life, in relation to sex. Sex dimorphism has been observed previously with hormones derived principally from adipose tissue and have been related to differences in adiposity. The absence of a relation of cbFGF21 concentrations to birth weight or the sum of skinfolds, a surrogate metric of neonatal adiposity, in our study makes it unlikely that subtle changes in body composition underlie the sex differences observed. Androgen levels in cord blood may also be a factor influencing cbFGF21, but unfortunately these hormones were not available to measure in our study.

It is worth mentioning that postnatal but not fetal growth was associated with cbFGF21 levels, and notably, these associations were observed only in the NGT group. Moreover, the associations between FGF21 and BMI ZS were observed after adjusting for gestational age at delivery, sex and BMI gain during pregnancy. FGF21 is known to promote BAT activity, and the BAT-mediated thermogenic energy expenditure may protect against obesity. Considering that FGF21 could be involved in the recruitment of inducible brown adipocytes during development, we would expect an inverse relationship between postnatal BMI and FGF21 concentrations. In contrast, we have observed that cord blood FGF21 concentrations were related to BMI ZS. Since FGF21 is able to cross the blood brain barrier and may be able to act on the central nervous system and influence energy metabolism, our results allow us to hypothesize that FGF21 resistance, which has been postulated in obese human adults, perhaps starts during intrauterine life. The lack of association in offspring born to GDM patients may indicate that a hyperglycemic intrauterine environment could influence the effect of FGF21 later in life, reducing its usefulness as a marker of

postnatal growth in this group. However, the observational design of this study prohibits definitive conclusions at this point. Only one previous longitudinal study performed in children observed that FGF21 levels were increased in obese children and decreased after weight loss, suggesting that the elevation of FGF21 was a result and not just a cause [30]. In contrast, our findings suggest that cbFGF21 may be considered a potential fetal biomarker of postnatal growth in normal glucose-tolerant women.

The strengths of our study include the prospective measurement of maternal and umbilical cord blood FGF21 and neonatal anthropometry, and its relatively large number of mother–child dyads, which have provided the power required to allow statistical analysis and to have available child anthropometric data up to 4 years. However, this study does not permit us to discard a potential effect of fetal FGF21 in the modulation of normal growth by itself, or through other unknown mediators. Some limitations should also be considered. Differences by ethnicity in maternal glucose metabolism and neonatal weight have been previously described [31–33]. In this study, women included were European Mediterranean origin so we cannot rule out that these findings may differ in other populations with different ethnic backgrounds.

In summary, we show that FGF21 is increased in GDM pregnant women with a close relationship with prepregnancy BMI, suggesting a potential link between the two. Moreover, we describe the first detection of FGF21 in human cord blood and demonstrate that it correlates strongly with maternal circulating FGF21. The association observed between cbFGF21 concentration and children BMI ZS from 1 to 4 years may suggest that FGF21 may be considered as a potential biomarker of postnatal growth in healthy pregnancy. To confirm these data, studies that involved a larger cohort of subjects and longer follow-up are needed.

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Conflict of interest Ana Megia, Pilar Gil-Lluís, Silvia Näf, Victoria Ceperuelo-Mallafre, José Miguel González-Clemente, Gemma Llauradó, Catalina Nuñez-Roa, Kelly Roche, Mónica Ballesteros, Rosa-Elena Yañez, Sonia Fernandez-Veledo and Joan Vendrell declare that they have no conflict of interest.

Ethical standard Study was approved by the Hospital Research Ethics Committee.

Statement of human and animal rights All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008.

Statement of informed consent Informed consent was obtained from all patients for being included in the study.

References

1. Kharitonenkov A, Shiyanova TL, Koester A, Ford AM, Micanovic R, Galbreath EJ et al (2005) FGF-21 as a novel metabolic regulator. J Clin Invest 115(6):1627–1635
2. Nishimura T, Nakatake Y, Konishi M, Itoh N (2000) Identification of a novel FGF, FGF-21, preferentially expressed in the liver. Biochem Biophys Res Commun 1492:203–206
3. Fon Tacer K, Bookout AL, Ding X, Kurosu H, John GB, Wang L et al (2010) Comprehensive expression atlas of the fibroblast growth factor system in adult mouse. Mol Endocrinol 24(10):2050–2064
4. Inagaki T, Dutchak P, Zhao G, Ding X, Gautron L, Parameswara V et al (2007) Endocrine regulation of the fasting response by PPARalpha-mediated induction of fibroblast growth factor 21. Cell Metab 5(6):415–425
5. Badman MK, Pissios P, Kennedy AR, Koukos G, Flier JS, Maratos-Flier E (2007) Hepatic fibroblast growth factor 21 is regulated by PPARalpha and is a key mediator of hepatic lipid metabolism in ketotic states. Cell Metab 5(6):426–437
6. Lundäsen T, Hunt MC, Nilsson L-M, Sanyal S, Angelin B, Alexson SEH et al (2007) PPARalpha is a key regulator of hepatic FGF21. Biochem Biophys Res Commun 360(2):437–440
7. Arner P, Pettersson A, Mitchell PJ, Dunbar JD, Kharitonenkov A, Rydén M (2008) FGF21 attenuates lipolysis in human adipocytes—a possible link to improved insulin sensitivity. FEBS Lett 582(12):1725–1730
8. Wente W, Efanov AM, Brenner M, Kharitonenkov A, Köster A, Sandusky GE et al (2006) Fibroblast growth factor-21 improves pancreatic beta-cell function and survival by activation of extracellular signal-regulated kinase 1/2 and Akt signaling pathways. Diabetes 55(9):2470–2478
9. Coskun T, Bina HA, Schneider MA, Dunbar JD, Hu CC, Chen Y et al (2008) Fibroblast growth factor 21 corrects obesity in mice. Endocrinology 149(12):6018–6027
10. Xu J, Lloyd DJ, Hale C, Stanislaus S, Chen M, Sivits G et al (2009) Fibroblast growth factor 21 reverses hepatic steatosis, increases energy expenditure, and improves insulin sensitivity in diet-induced obese mice. Diabetes 58(January):250–259
11. Zhang X, Yeung DCY, Karpisek M, Stejskal D, Zhou Z, Liu F et al (2008) Serum FGF21 levels are increased in obesity and are independently associated with the metabolic syndrome in humans. Diabetes 57(May):1246–1253
12. Chavez A, Molina-Carrion M, Abdul-Ghani M, Folli F, DeFronzo R, Tripathy D (2009) Circulating fibroblast growth factor-21 is elevated in impaired glucose tolerance and type 2 diabetes and correlates with muscle and hepatic insulin resistance. Diabetes Care 32:1542–1546
13. Chen C, Cheung B, Wang Y, Law L, Leung K, Wat N et al (2011) High plasma level of fibroblast growth factor 21 is an independent predictor of type 2 diabetes. Diabetes Care 34:11–13

14. Catalano PM, Hauguel-De Mouzon S (2011) Is it time to revisit the Pedersen hypothesis in the face of the obesity epidemic? Am J Obstet Gynecol 204(6):479–487
15. Nitert MD, Barrett HL, Kubala MH, Scholz Romero K, Denny KJ, Woodruff TM et al (2014) Increased placental expression of fibroblast growth factor 21 in gestational diabetes mellitus. J Clin Endocrinol Metab 99(4):E591
16. Tan BK, Sivakumar K, Bari MF, Vatish M, Randeva HS (2013) Lower cerebrospinal fluid/plasma fibroblast growth factor 21 (FGF21) ratios and placental FGF21 production in gestational diabetes. PLoS One 8(6):e65254
17. Wang D, Zhu W, Li J, An C, Wang Z (2013) Serum concentrations of fibroblast growth factors 19 and 21 in women with gestational diabetes mellitus: association with insulin resistance, adiponectin, and polycystic ovary syndrome history. PLoS One 8(11):e81190
18. Stein S, Stepan H, Kratzsch J, Verlohren M, Verlohren H-J, Drynda K et al (2010) Serum fibroblast growth factor 21 levels in gestational diabetes mellitus in relation to insulin resistance and dyslipidemia. Metabolism 59(1):33–37
19. GEDE. Grupo Español de Diabetes y Embarazo (2006) Guía asistencial de diabetes mellitus y embarazo (3ª edición). Av en Diabetol 22:73–87
20. National Diabetes Data Group (1979) Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes 28:1039–1057
21. Sobradillo B, Aguirre A, Aresti U, Bilbao A, Fernandez-Ramos C, Lizárraga A, et al. (2014) Curvas y Tablas de Crecimiento. Estudios longitudinal y transversal. Bilbao: Fundación Faustino Orbegozo. http://www.aepap.org/pdf/f_orbegozo_04.pdf. Accessed 25 June 2014
22. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC (1985) Homeostasis model assessment: insulin resistance and beta cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28:412–419
23. Yang C, Jin C, Li X, Wang F, McKeehan WL, Luo Y (2012) Differential specificity of endocrine FGF19 and FGF21 to FGFR1 and FGFR4 in complex with KLB. PLoS One 7(3):e33870
24. Suzuki M, Uehara Y, Motomura-Matsuzaka K, Oki J, Koyama Y, Kimura M et al (2008) BetaKlotho is required for fibroblast growth factor (FGF) 21 signaling through FGF receptor (FGFR) 1c and FGFR3c. Mol Endocrinol 22(4):1006–1014
25. Hondares E, Rosell M, Gonzalez FJ, Giralt M, Iglesias R, Villarroya F et al (2011) Hepatic FGF21 expression is induced at birth via PPARα in response to milk intake and contributes to thermogenic activation of neonatal brown fat. Cell Metab 11(3):206–212
26. Hondares E, Gallego-Escuredo JM, Flachs P, Frontini A, Cereijo R, Goday A et al (2014) Fibroblast growth factor-21 is expressed in neonatal and pheochromocytoma-induced adult human brown adipose tissue. Metabolism 63(3):312–317
27. Xu L, Ping F, Yin J, Xiao X, Xiang H, Ballantyne CM et al (2013) Elevated plasma sparc levels are associated with insulin resistance, dyslipidemia, and inflammation in gestational diabetes mellitus. PLoS One 8(12):e81615
28. Stepan H, Kley K, Hindricks J, Kralisch S, Jank A, Schaarschmidt W et al (2013) Serum levels of the adipokine fibroblast growth factor-21 are increased in preeclampsia. Cytokine 62(2):322–326
29. Noctor E, Crowe C, Carmody LA, Kirwan B, O'Dea A, Glynn LG, et al (2014) ATLANTIC-DIP: prevalence of metabolic syndrome and insulin resistance in women with previous gestational diabetes mellitus by international association of diabetes in pregnancy study groups criteria. Acta Diabetol. doi:10.1007/s00592-014-0621-z
30. Reinehr T, Woelfle J, Wunsch R, Roth CL (2012) Fibroblast growth factor 21 (FGF-21) and its relation to obesity, metabolic syndrome, and nonalcoholic fatty liver in children: a longitudinal analysis. J Clin Endocrinol Metab 97(6):2143–2150
31. Savona-Ventura C, Vassallo J, Marre M, Karamanos BG (2012) Hyperglycaemia in pregnancy in Mediterranean women. Acta Diabetol 49(6):473–480
32. Ignell C, Shaat N, Ekelund M, Berntorp K (2013) The impact of ethnicity on glucose homeostasis after gestational diabetes mellitus. Acta Diabetol 50(6):927–934
33. Sletner L, Nakstad B, Yajnik CS, Mørkrid K, Vangen S, Vårdal MH et al (2013) Ethnic differences in neonatal body composition in a multi-ethnic population and the impact of parental factors: a population-based cohort study. PLoS One. 8(8):e73058