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

Running title. Cord blood FGF21 in gestational diabetes

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**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 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 birthweight. 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.

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). 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

126 followed the National Data Group Criteria (19,20). In this study the participants, matched for age and
127 prepregnancy body mass index (BMI), fulfilled the following criteria at the end of pregnancy: 1) a
128 singleton pregnancy, 2) accurate gestational age confirmed by an ultrasound examination before 20 weeks
129 of gestation, 3) the absence of fetal anomalies identified at birth and, 4) normal glucose tolerance or GDM
130 diagnosed before 30 weeks of pregnancy, 5) no preexisting DM, inflammatory or chronic diseases, or
131 current use of drugs known to affect carbohydrate metabolism. This study was performed at the Hospital
132 Universitari de Tarragona Joan XXIII. The study protocol was approved by the Hospital Research Ethics
133 Committee and all patients gave written informed consent before participating in the study. All patients
134 with GDM were initially treated with diet and supplemented with insulin as required. Maternal and cord
135 blood samples were obtained at recruitment and at delivery, respectively. Cord blood was not viable for
136 the analysis in 8 patients, 1 offspring of NGT subjects and 7 offspring of GDM subjects. For gene
137 expression and immunoblot analysis, we also collected umbilical cord tissue and umbilical cord blood
138 from four additional women.
139
140 Upon inclusion, demographic and historical information was collected by an interviewer administering a
141 questionnaire that included patient demographics, personal medical information and information
142 regarding the current and previous pregnancies. BMI was calculated using the formula BMI = weight (in
143 kilograms)/height (in meters)$^2$. Increase in BMI was calculated by the formula BMI Gain= [final BMI]-
144 [prepregnancy BMI].
145
146 Neonatal length and weight were determined using a measuring board to the nearest 0.1 cm and a
147 calibrated scale to the nearest 10 g. Triceps, biceps, subscapular and flank skinfold thickness were
148 measured with a Holtain skinfold caliper (Chasmors Ltd, London UK) from the left side at least three
149 times until a consistent and stable reading was obtained. The sum of the four skinfolds (SSF) was used to
150 estimate neonatal adiposity.
151
152 Weight and height a 12, 24 and 48 months were gathered by chart review of the electronic medical
153 records. Both measures were routinely collected at well-child visits. The child's BMI Z-Score (ZS) for
154 gender and age was calculated based on Spanish growth charts (21).
155 ***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 -80º 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 260nm 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). Quantitative gene expression was analysed 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). 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) and images were captured using the VersaDoc imaging system and Quantity One software (Bio-Rad, Hercules, CA).

***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 $X^2$ 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 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*=0.14).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 4 independent samples. FGF21 reactivity could be clearly demonstrated in three of the four patients (Figure 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 (Figure 1B).

***Relationship between maternalFGF21 (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***

cbFG21 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 cbFG21levels 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 FGF21expression in interscapular and visceral adipose tissue in human neonates (26).Notwithstanding the 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 FGF21in 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 accord 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). 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 (29). 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, 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(30–32). 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.

**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.

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**Figure 1**

**A)** FGF21 protein expression was analyzed by western blot in cord blood serum from 4 subjects (P1-P4). One preterm (P1) and 3 at term (P2-P4)

**B)** FGFR1 and β-klotho mRNA expression in visceral adipose tissue (AT) and cord tissue (CT)

480 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) | 3288.26±483.848 | 3256.01±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 | -0.5083±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 |

481 Data are mean±standard deviation or median (interquartile range). NGT: Normal glucose tolerance.

482 GDM: Gestational diabetes mellitus. BMI ZS: Body mass index Z Score

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** |

NGT: Normal glucose tolerance. GDM: Gestational diabetes mellitus.

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 |

NGT: Normal glucose tolerance. GDM: Gestational diabetes mellitus. mFGF21: 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* | HOMA-IR | 0.265 | 0.001 |
| $R^2$: 9.5 | GDM | 0.158 | 0.044 |
| Cord blood FGF21† | Maternal FGF21 | 0.616 | <0.001 |
| $R^2$: 36.4 | Prepregnancy BMI | -0.189 | 0.008 |

$R^2$: adjusted $R^2$; * covariates included for selection in the model: HOMA-IR, GDM, triglycerides, HDL cholesterol, total cholesterol, maternal age and prepregnancy BMI. † Covariates included for selection in the model: GDM, prepregnancy BMI, birth weight, sum skinfolds, gestational age at delivery, maternal FGF21, neonatal sex, BMI gain.

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

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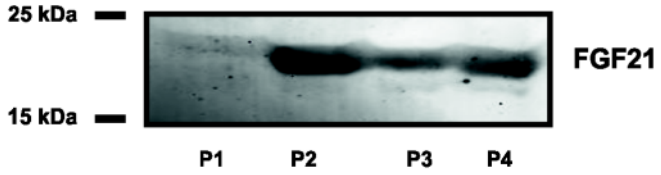
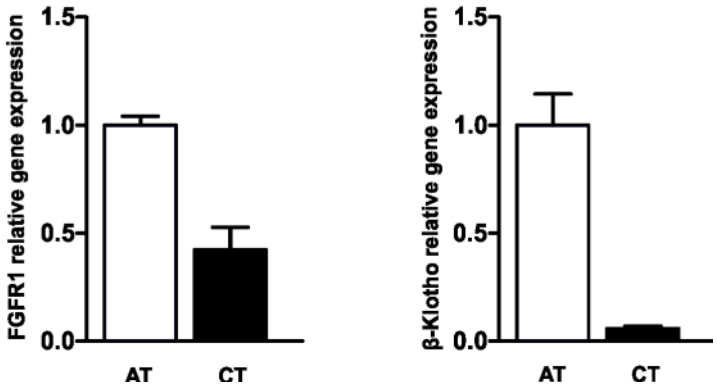
**Figure 1**

**A**




**B**

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