Circulating and Placental Growth-Differentiation Factor 15 in Preeclampsia and in Pregnancy Complicated by Diabetes Mellitus

Meryam Sugulle, Ralf Dechend, Florian Herse, M. Susanne Weedon-Fekjaer, Guro M. Johnsen, K. Bridget Brosnihan, Lauren Anton, Friedrich C. Luft, Kai C. Wollert, Tibor Kempf, Anne Cathrine Staff

Abstract—Growth-differentiation factor 15 (GDF-15), a stress-responsive transforming growth factor-β–related cytokine, is emerging as a new risk marker in patients with cardiovascular disease. We explored GDF-15 in preeclampsia and in diabetic pregnancies, because these conditions are associated with augmented risk for cardiovascular disease, both in mother and in offspring. Plasma from pregnant women (n=267; controls: n=59, preeclampsia: n=85, diabetes mellitus: n=112, and superimposed preeclampsia in diabetes mellitus: n=11), fetal plasma (n=72), and amniotic fluid (n=99) were analyzed by immunoassay for GDF-15. Placental GDF-15 mRNA and protein expression levels were analyzed by quantitative real-time PCR and immunoblots in 78 and 18 pregnancies, respectively. Conditioned media from preeclamptic (n=6) and control (n=6) villous placenta explants were analyzed by immunoassay for GDF-15. Median maternal GDF-15 concentration was elevated in those with diabetes mellitus, as compared with controls (91 549 versus 79 875 ng/L; P=0.02). Median GDF-15 concentration was higher in patients with preeclampsia than in controls in term maternal blood samples (127 061 versus 80 319 ng/L; P<0.001). In the fetal circulation and amniotic fluid, GDF-15 was elevated in preeclampsia and superimposed preeclampsia in diabetes mellitus, as compared with controls. GDF-15 placental mRNA expression was elevated in preeclampsia, as compared with controls (P=0.002). Placenta immunoblots confirmed a single GDF-15 protein band, and a time-dependent increase in GDF-15 protein was detected in the conditioned media. Our study is the first to show that GDF-15 is dysregulated, both in preeclampsia and in diabetic pregnancies. The mechanisms and diagnostic implications of these findings remain to be explored. (Hypertension. 2009;54:00-00.)

Key Words: cardiovascular disease ■ growth differentiation factor 15 ■ preeclampsia ■ diabetes mellitus ■ pregnancy

Preeclampsia (PE) is a major complication affecting ∼3% to 4% of all pregnancies and is globally responsible for ∼50 000 maternal deaths annually. PE develops after 20 weeks of gestation and is characterized by hypertension and proteinuria. The pathophysiology of PE remains unknown; however, circulating factors produced by an oxidatively stressed placenta have been proposed to cause an excessive systemic inflammatory response and generalized maternal endothelial dysfunction, contributing to the maternal clinical features of PE. A shallow placentation, with abnormal invasion of cytotrophoblasts and incomplete remodeling of placenta-supplying maternal uterine spiral arteries, is proposed to cause the altered circulation and the ensuing oxidative stress in the placenta and associated release of endothelial deranging factors to the maternal circulation.

Women with PE have an augmented risk of cardiovascular morbidity and mortality later in life.6-8 PE, diabetes mellitus (DM), and cardiovascular disease share many risk factors: endothelial dysfunction, obesity, hyperglycemia, insulin resistance, hypertension, metabolic syndrome, and dyslipidemia.9,10 Women with pregestational DM or gestational DM have a 2- to 4-fold increased risk for PE.12 Gestational diabetes is a strong risk factor for developing subsequent diabetes13 and for developing future cardiovascular disease.14

Growth-differentiation factor 15 (GDF-15) is a member of the transforming growth factor-β superfamily, also named macrophage-inhibitory cytokine 1.15 GDF-15 has been ascribed a cardioprotective role in vivo in the adult heart16 and has been identified as a prognostic biomarker in patients with acute coronary syndrome,17 myocardial infarction,18 and...
chronic heart failure. An association between increased circulating GDF-15 concentrations and an augmented risk of future cardiovascular disease has also been observed in apparently healthy elderly women.

GDF-15 is highly expressed in the placenta, but its function in pregnancy remains unknown. We recently found augmented GDF-15 mRNA expression levels in preeclamptic placentas while screening for dysregulated genes in pooled placenta samples. GDF-15 is also known to be produced by adipose cells in response to oxidative stress. Because PE is associated with placental oxidative stress and because pregnancies complicated by DM and PE are associated with an augmented risk for later cardiovascular disease in the mother and offspring, we hypothesized that the circulating concentrations of GDF-15 are elevated in both conditions. We, therefore, explored whether circulating, fat tissue, and placental GDF-15 were dysregulated in pregnancies complicated by PE or DM.

Methods

Patients and Sample Collection

We included 267 patients from an ongoing biobank collection at Ulleval: 208 women with complicated pregnancies (85 with PE, 112 with DM, and 11 with superimposed PE in DM [diabetic PE; DPE]) and 59 with uncomplicated pregnancies (controls). Of the 112 patients with DM, 46 had pre-existing DM type 1, 11 had DM type 2, and 55 had gestational DM (including 36 diet treated and 19 treated with both diet and insulin). Only women with singleton pregnancies were included, and none had pre-existing hypertension, rupture of membranes, clinical signs of infection, or were in labor at the time of blood sampling. PE was defined as blood pressure augmentation after 20 weeks' gestation to >140/90 mm Hg on ≥2 occasions 6 hours apart in a previously normotensive woman, combined with proteinuria. Proteinuria was defined as protein dip stick ≥1+ on ≥2 midstream urine samples 6 hours apart or a 24-hour urine excretion of ≥0.3 g of protein, in the absence of urinary infection. The diabetes groups were defined according to the World Health Organization criteria. Of the 267 women, 139 fasting women (60 PE, 56 control, 14 DM, and 9 DPE) were included directly before cesarean section, and 128 women (3 control, 25 PE, 98 DM, and 2 DPE, most nonfasting) were recruited during pregnancy (regardless of later mode of delivery), the latter group, therefore, with only maternal citrate blood available. At cesarean section, amniotic fluid was sampled from 99 of these deliveries (36 control, 46 PE, 10 DM, and 7 DPE). After delivery of the placenta, umbilical vein EDTA blood samples were collected in 72 cases (34 control, 23 PE, 8 DM, and 7 DPE). Both maternal and fetal blood, as well as amniotic fluid, samples were obtained and stored, as described previously, the processing of citrate plasma being identical with that of EDTA plasma. Decidual tissue from the placental bed was collected using a vacuum suction method developed and evaluated by our group. Decidual represents the maternal-fetal interface and includes the maternal endometrium of pregnancy invaded by fetal extravillous trophoblasts. Placental biopsies were excised from a macroscopically normal looking, centrally located cotyledon, omitting the decidual layer. In addition, a maternal abdominal wall subcutaneous fat tissue biopsy was collected. All of the tissue samples were snap frozen in liquid nitrogen directly after collection and stored at −80°C until further processing.

The newborn birth weight percentiles were calculated according to national birth registry data, as well as according to ultrasound based percentiles. The Regional Committee of Medical Research Ethics in Eastern Norway approved the study, and informed written consent was obtained from each woman.

Conditioned media of placenta villous explants were obtained from a study of cesarean delivered placentas in 6 women with PE and 6 women with uncomplicated pregnancies (controls). The explants were prepared as described previously, and the conditioned media were collected at different time points (0, 2, 4, and 8 hours).

GDF-15 Immunoassay

GDF-15 in plasma and conditioned media of placental villous explants was measured by an immunoradiometric sandwich assay using a polyclonal, affinity chromatography-purified goat antihuman GDF-15 IgG antibody (R&D Systems). All of the analyses were performed in duplicate at the laboratory where the assay was developed. GDF-15 concentration is independent of the added anticoagulant matrix, and we could, therefore, compare maternal citrate plasma concentrations with fetal EDTA plasma concentrations.

RNA Isolation

The placenta, decidua, and fat tissues were pulverized in liquid nitrogen using a mortar and pestle, followed by homogenization of 10 to 15 mg of tissue in 800 μL of RNA lysis buffer with an Ultra Turrax homogenizer (IKA Labortechnik) for 30 seconds. Total RNA was isolated from placenta and decidual tissue using an ABI6100 (Applied Biosystems) and from fat tissue with an RNaseasy Lipid Tissue Mini kit (Qiagen). The RNA quality and quantity were determined using spectrophotometry (NanoDrop 1000, NanoDrop Technologies) and capillary electrophoresis (Agilent 2100 Bioanalyzer, Agilent Technologies) according to the manufacturer’s protocol and were found to be sufficient for quantitative real-time PCR analysis with 260:280 and 260:230 ratios >2 and an RNA integrity number >7.

mRNA Expression by Quantitative Real-Time PCR

Total RNA (400 ng per tissue sample) was reverse transcribed to single-stranded cDNA using the High-Capacity cDNA Reverse Transcription kit (Applied Biosystems), according to the manufacturer’s instructions. Quantitative real-time PCR was performed using TaqMan gene expression assays (Applied Biosystems) for GDF-15 (Hs00171132_m1). After testing 4 endogenous controls, we used tyrosine 3-monooxygenase/trypophan 5-monooxygenase activation protein, α-polypeptide (YWHAZ; Hs00237047_m1), for placental and decidual tissue and TATA binding protein (Hs99999910_m1) for fat tissue. The assays were run on 96-well clear plates using TaqMan Gene Expression Master Mix on the ABI Prism 7900HT Sequence Detection System (Applied Biosystems). Gene expression values were then calculated on the basis of the ΔΔCt method.

Immunoblotting

GDF-15 protein levels in homogenized placenta tissue from 10 patients with PE and 8 control pregnancies were quantified by immunoblotting, as described previously. The affinity-purified polyclonal goat antihuman GDF-15 IgG antibody was used to determine GDF-15 expression levels in the human placenta. An antibody against β-actin was obtained from Cell Signaling Technology.

Statistical Analysis

The GDF-15 results and other continuous variables are presented as median values and ranges. Statistical analyses were performed using SPSS 15 (SPSS Inc). Differences in continuous variables between groups were tested by nonparametric Mann–Whitney tests. Student’s t test was applied for the tissue gene expression and immunoblot calculations, because these were normally distributed. Spearman’s correlation was used to calculate correlation coefficients. A P<0.05 was considered statistically significant.

Results

Clinical characteristics of the 267 pregnant women included in the GDF-15 maternal citrate plasma analyses are shown in Table 1. Fewer patients were included in the fetal plasma
Median birth weight percentiles did not differ among the gestational age groups. In PE, nearly all the women delivered after week 34. In the uncomplicated group results. In PE, median GDF-15 concentration in maternal plasma was higher than in controls (99 124 versus 79 875 ng/L), although the difference was not statistically significant (\(P=0.1\)). However, maternal GDF-15 concentration was significantly elevated in the DM group as compared with the control group (93 129 versus 79 875; \(P=0.001\)), whereas the expression in the DM (n=85; Spearman’s correlation: 0.5; \(P<0.001\)) and the DM group (n=112; Spearman’s correlation: 0.3; \(P<0.001\)), when analyzed separately.

**GDF-15 in Fetal Circulation**

As shown in Table 2, median GDF-15 concentration in EDTA plasma from the umbilical vein (transporting blood from placenta to the fetus) was elevated both in PE and DPE compared with controls (\(P<0.001\) and \(P=0.001\), respectively). Fetal GDF-15 concentrations were much lower than maternal concentrations, with a median relative concentration for fetal versus maternal samples of 1:21 (72 paired samples). There was a significant positive correlation between maternal and fetal plasma GDF-15 concentrations for the whole patient group (n=72; Spearman’s correlation: 0.5; \(P<0.001\)).

**GDF-15 in Amniotic Fluid**

As shown in Table 2, amniotic fluid GDF-15 was elevated in PE and DPE as compared with controls (\(P<0.001\) and \(P=0.001\), respectively). Amniotic fluid GDF-15 concentrations were intermediate between maternal and fetal concentrations.

**Tissue GDF-15 mRNA and Placental Protein Expressions**

Mean GDF-15 mRNA expression in placental tissue was elevated in PE (n=29) as compared with controls (n=33; \(P=0.002\)), whereas the expression in the DM (n=10) and DPE (n=6) groups did not differ significantly from controls (Figure 3). The GDF-15 expression in fat tissue was elevated in the small DM group as compared with controls (Figure 3), although it was not statistically significant (\(P=0.2\)) and was unaltered in PE. GDF-15 expression in the decidual samples was similar for all of the study groups. The expression of

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>C (n=59), Median (Range)</th>
<th>PE (n=85), Median (Range)</th>
<th>DM (n=112), Median (Range)</th>
<th>DPE (n=11), Median (Range)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient age at delivery, y</td>
<td>33 (21 to 40)</td>
<td>31 (18 to 42)</td>
<td>30 (20 to 42)</td>
<td>32 (21 to 36)</td>
<td>0.4/0.5/0.8</td>
</tr>
<tr>
<td>BMI before pregnancy, kg/m²</td>
<td>22.4 (17.4 to 39.7)</td>
<td>23.4 (18.9 to 41.1)</td>
<td>25.4 (17.0 to 45.4)</td>
<td>27.7 (21.5 to 34.5)</td>
<td>0.02/(&lt;0.001*/0.001*)</td>
</tr>
<tr>
<td>BMI at blood sampling, kg/m²</td>
<td>28.1 (20.3 to 38.2)</td>
<td>30.5 (21.6 to 49.6)</td>
<td>31.1 (23.0 to 51.4)</td>
<td>35.5 (24.2 to 44.6)</td>
<td>0.005*/(&lt;0.001*/0.001*)</td>
</tr>
<tr>
<td>Parity</td>
<td>0 (0 to 6)</td>
<td>0 (0 to 3)</td>
<td>0 (0 to 5)</td>
<td>0 (0 to 4)</td>
<td>0.3/0.5/0.4</td>
</tr>
<tr>
<td>Gestational age at test day, wk</td>
<td>38.7 (24.3 to 41.7)</td>
<td>33.9 (24.9 to 41.0)</td>
<td>37.4 (32.7 to 42.0)</td>
<td>35.9 (29.1 to 38.4)</td>
<td>(&lt;0.001*/0.001*/0.001*)</td>
</tr>
<tr>
<td>Gestational age at delivery, wk</td>
<td>38.7 (25.3 to 41.9)</td>
<td>33.9 (24.9 to 41.4)</td>
<td>39.0 (32.7 to 42.0)</td>
<td>35.9 (29.1 to 38.4)</td>
<td>(&lt;0.001*/0.003*/0.001*)</td>
</tr>
<tr>
<td>Neonatal weight, g</td>
<td>472 (856 to 4325)</td>
<td>2163 (540 to 5036)</td>
<td>3814 (1534 to 5857)</td>
<td>2582 (985 to 4592)</td>
<td>(&lt;0.001*/0.001*/0.001/0.004)</td>
</tr>
<tr>
<td>Neonatal weight percentile</td>
<td>68 (6 to 99.5)</td>
<td>12 (0.5 to 99.5)</td>
<td>83 (1.5 to 99.9)</td>
<td>83 (0.5 to 99.9)</td>
<td>(&lt;0.001*/0.007*/0.3)</td>
</tr>
<tr>
<td>Systolic BP &lt;20 wk, mm Hg</td>
<td>112 (80 to 135)</td>
<td>115 (90 to 140)</td>
<td>111 (85 to 155)</td>
<td>124 (95 to 160)</td>
<td>0.03/0.5/0.003*</td>
</tr>
<tr>
<td>Diastolic BP &lt;20 wk, mm Hg</td>
<td>65 (50 to 93)</td>
<td>70 (50 to 89)</td>
<td>70 (50 to 90)</td>
<td>76 (50 to 90)</td>
<td>0.002*/0.2/0.001*</td>
</tr>
<tr>
<td>Systolic BP at test day, mm Hg</td>
<td>120 (90 to 153)</td>
<td>160 (140 to 220)</td>
<td>120 (90 to 189)</td>
<td>160 (140 to 183)</td>
<td>(&lt;0.001*/0.7*/0.001*)</td>
</tr>
<tr>
<td>Diastolic BP at test day, mm Hg</td>
<td>70 (57 to 92)</td>
<td>100 (90 to 119)</td>
<td>78 (50 to 100)</td>
<td>100 (90 to 110)</td>
<td>(&lt;0.001*/0.008*/0.001*)</td>
</tr>
</tbody>
</table>

C indicates controls; BMI, body mass index. All \(P\) values are as compared with control group, by Mann–Whitney test.

\(\ast P<0.05\).
GDF-15 in placenta and decidua compared with fat tissue in healthy controls was also investigated and indicated 1500- and 60-times higher expressions of GDF-15 in the placenta and decidua compared with fat tissue (data not shown). The GDF-15 immunoblots from the 18 placenta samples (10 PE and 8 control pregnancies) detected a single band at 50 kDa (data not shown). Densitometric analyses revealed elevated but not significantly different GDF-15 protein expression levels (adjusted for β-actin expression levels, mean±SD expression in percentage of control group) in the PE group (111±25%) as compared with the control group (100±18%; P=0.14).

GDF-15 Placental Expression and Correlation With Circulating GDF-15
Maternal and fetal blood samples were available in 68 and 42 of the 77 women with placental tissue GDF-15 mRNA analysis. We found a significant positive correlation between placental GDF-15 gene expression and maternal GDF-15, as well as between placental expression and fetal GDF-15 concentrations for the whole patient group (Spearman’s correlations: 0.3 and 0.4, respectively; P<0.02 for both). Also, placental GDF-15 expression and the GDF-15 concentration in the amniotic fluid samples (available in 55 women with placental tissue samples) were positively correlated (Spearman’s correlation: 0.4; P=0.01).

GDF-15 in Conditioned Media of Placental Villous Explants
There was a time-dependent increase in the GDF-15 concentration, as analyzed by immunoassay, in the conditioned media of placental villous explants (6 PE and 6 controls). Median 0-, 2-, 4-, and 8-hour GDF-15 concentrations in the control versus the PE group were as follows: 1745 versus 1892 ng/L, 9726 versus 15 309 ng/L, and 14 596 versus 17 927 ng/L. This increase in placental GDF-15 protein production in the PE group as compared with the control group, however, was not statistically significant (all P>0.1).

Discussion
Pregnant women, as demonstrated in our study, have much higher concentrations of GDF-15 than nonpregnant fertile women, as reported by Moore et al37 and as confirmed in blood samples that we analyzed from 11 healthy premenopausal women (aged 24 to 44 years), with a low median GDF-15 of 889 ng/L (data not shown). We found in a large pregnancy cohort an elevated median plasma GDF-15 concentration in PE at term. Also, we found an elevated median maternal GDF-15 concentration in DM. We are not aware of previous studies looking at circulating GDF-15 levels in pregnancy complicated by DM, but DM has been shown to be independently associated with elevated levels of GDF-15 in nonpregnant individuals with established cardiovascular disease.17 Interestingly, the infants from PE pregnancies had elevated circulating GDF-15 concentrations as compared with infants from uncomplicated pregnancies. It is remarkable that fetal GDF-15 concentrations in our study were ~10-fold higher than circulating GDF-15 in elderly persons or nonpregnant healthy, fertile women.37 Because both PE and pregnancy complicated by DM are associated with augmented future cardiovascular risks for both mother6–8 and offspring,26,27 longitudinal follow-up of our pregnancy cohort could possibly further clarify whether elevated circulating GDF-15 concentrations, in mother and/or offspring, are indicative of an increased future cardiovascular risk, similar to the situation in apparently healthy elderly women.20 As illustrated in Figure 1, there is a large heterogeneity within all pregnancy groups regarding GDF-15 concentrations, and whether differences within groups are associated with differ-
ences in future cardiovascular risk remains unknown. This heterogeneity could possibly explain the findings of a previous study, demonstrating no significant difference in maternal GDF-15 in PE as compared with uncomplicated pregnancy; however, less women were included as compared with our study.38

The main direct or indirect source of the high maternal and fetal circulating GDF-15 in pregnancy is most likely the placenta. Previous studies have identified the villous trophoblast cells as the main source of placental GDF-15 protein production.39 In general, we observed very high maternal GDF-15 concentrations, relatively lower fetal concentrations, and intermediate amniotic fluid concentrations (Table 2). These findings support the notion that the placenta is the primary source of GDF-15 in pregnancy and that GDF-15 is preferentially transported to the maternal side of the placenta. We cannot, however, exclude that other placental cell types, in addition to the villous trophoblasts, contribute to the increase in maternal and fetal GDF-15 seen in PE at term.

Other studies have shown that small amounts of GDF-15 mRNA are expressed in the adult kidney, pancreas,39,40 and colon,41 but the effect of pregnancy on GDF-15 expression in these tissues has not been explored. Interestingly, GDF-15 is strongly induced by oxidative stress, nitrosative stress, interleukin 1β, and interferon γ, as well as by simulated ischemia/reperfusion injury in cultured cardiomyocytes.36,42 Increased placental oxidative and nitrosative stresses are also features of PE,24,25,43 as is ischemia/reperfusion.44 Expression of GDF-15 mRNA in monocyted cells is upregulated by interleukin 1β and tumor necrosis factor-α,15 circulating cytokines that are elevated in PE.45 On the basis of these considerations, elevated GDF-15 protein concentrations in the maternal and fetal circulations, as well as in the amniotic fluid, may well be related to an excessive placental production of GDF-15 in PE, and GDF-15 could represent a marker for placental stress.

In contrast to the PE group, we found no increased GDF-15 expression levels in the placentas from the DM group as compared with the controls (Figure 3). Because we observed increased placental oxidative and nitrosative stresses are also features of PE, as is ischemia/reperfusion.44 Expression of GDF-15 mRNA in monocyted cells is upregulated by interleukin 1β and tumor necrosis factor-α,15 circulating cytokines that are elevated in PE.45 On the basis of these considerations, elevated GDF-15 protein concentrations in the maternal and fetal circulations, as well as in the amniotic fluid, may well be related to an excessive placental production of GDF-15 in PE, and GDF-15 could represent a marker for placental stress.

In contrast to the PE group, we found no increased GDF-15 expression levels in the placentas from the DM group as compared with the controls (Figure 3). Because we observed increased GDF-15 expression levels in the fat tissue in DM

Table 2. GDF-15 Immunoassay Results for the Study Groups

<table>
<thead>
<tr>
<th>Sample Source</th>
<th>C</th>
<th>PE</th>
<th>DM</th>
<th>DPE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>GDF-15, maternal citrate plasma, ng/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.1/0.02/0.6</td>
</tr>
<tr>
<td>No.</td>
<td>59</td>
<td>85</td>
<td>112</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>79 875</td>
<td>99 124</td>
<td>91 549</td>
<td>81 160</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>12 854 to 203 176</td>
<td>28 082 to 373 203</td>
<td>36 952 to 264 179</td>
<td>37 829 to 287 638</td>
<td></td>
</tr>
<tr>
<td>GDF-15, fetal EDTA plasma, ng/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001*/0.1/0.001*</td>
</tr>
<tr>
<td>No.</td>
<td>34</td>
<td>23</td>
<td>8</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>37 110</td>
<td>59 778</td>
<td>46 699</td>
<td>60 202</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>(1860 to 6266)</td>
<td>(3822 to 15652)</td>
<td>(3256 to 5918)</td>
<td>(4230 to 11 830)</td>
<td></td>
</tr>
<tr>
<td>GDF-15, amniotic fluid, ng/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001*/0.7/0.001*</td>
</tr>
<tr>
<td>No.</td>
<td>36</td>
<td>46</td>
<td>10</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>29 565</td>
<td>52 775</td>
<td>34 140</td>
<td>59 595</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>(9105 to 65 455)</td>
<td>(18 870 to 15 7920)</td>
<td>(16 275 to 75 455)</td>
<td>(33 055 to 81 720)</td>
<td></td>
</tr>
</tbody>
</table>

C indicates controls. All of the P values are as compared with the control group, by Mann–Whitney test. *P<0.05.
(although not statistically significant), but not in PE (Figure 3), we speculate that extraplacental sources (ie, the fat tissue) contribute to the increased circulating maternal levels of GDF-15 in diabetic pregnancies as compared with controls and PE. Excess GDF-15 stemming from fat tissue, rather than from the placenta itself, could also explain the lack of increased GDF-15 levels in amniotic and fetal blood that we observed in DM, in contrast to the situation in PE, where GDF-15 is also elevated in these compartments (Table 2). In support of fat as a GDF-15 source, it could be argued that the fat tissue represents a much larger tissue mass in a pregnant woman as compared with the placenta (normally 0.5 kg at term). On the other hand, we found higher absolute GDF-15 expression levels in the placental tissue as compared with fat and decidual tissues (on the basis of the Ct values, with \(\approx 1500\)-fold and 60-fold higher relative placental expressions); therefore, the net contribution of fat tissue to the elevated circulating maternal GDF-15 levels in DM pregnancy remains unknown.

There was no evidence of more severe PE being associated with more elevated maternal GDF-15 concentrations. The PE pregnancies with higher infant weight percentiles (as in clinically less severe PE with less placenta dysfunction) had in fact a higher maternal GDF-15 concentration than pregnancies complicated by intrauterine growth restriction or low birth weight percentiles (data not shown). We found no indication for higher maternal GDF-15 concentrations in DM pregnancies with less tight blood glucose control, either evaluated by high fasting blood glucose or by HbA1c (data not shown).

A weakness of our study is the limited patient number in the DM subgroups, as well as the small DPE group, with probable type II errors for some of the statistical subanalyses. However, the advantage of our biobank is the unique opportunity to analyze GDF-15 levels in the maternal, fetal, and amniotic fluid compartments, as well as tissue expression of GDF-15 (in placental, decidual, and fat tissues) from the same pregnancies, which is not feasible unless the cesarean delivery mode is chosen. Another strength of our study is the fact that all of the patients were included before labor; therefore, differences in labor duration and delivery mode (vaginal versus cesarean) did not confound our analyses. Such differences may cause variations in placental oxidative stress, with unknown effects on circulating concentration and tissue expression of GDF-15. Women with DM mostly deliver vaginally; therefore, few tissue samples could be recruited in the subgroups of DM in our study. Although pregestational and gestational diabetes represent different phenotypes of DM, these patients share many physiological and clinical aspects, supporting the merging of the diabetic subgroups to 1 DM group in our article.

The lack of matching for gestational age of preeclampsia and control subjects may be viewed as a limitation of our study. However, women with PE often deliver prematurely to reduce maternal and fetal morbidity and mortality (because removal of the placenta and, thus, ending the pregnancy are the “treatment” of PE). Premature delivery (either vaginal or by cesarean section) does not occur in uncomplicated pregnancies, but is attributed to a pathological cause, most often inflammation/infection, thereby largely restricting the possibility of recruiting age-matched premature control pregnancies if tissue samples and circulating samples are warranted. Also, correction for gestational age in the PE group may be statistically feasible but may not necessarily be biologically correct, because prematurely delivered women with PE will have more severe disease than women with PE who are delivered at term. Nevertheless, because GDF-15 serum concentrations have been found previously to increase with gestational age in normal pregnancy, we propose that the lack of a statistically significant difference between maternal GDF-15 in the total PE group and the control group is attributed to differences in gestational age. Supporting this notion is our finding that women with PE who delivered at term had significantly elevated circulating GDF-15 concentrations as compared with the control group delivered at term.

**Perspectives**

Increased circulating levels of GDF-15 are associated with an augmented risk for subsequent cardiovascular events in non-pregnant subjects. Our data should encourage further research into the (patho)physiological role of GDF-15 during pregnancy. Follow-up studies of women and their offspring after pregnancy complications, eg, PE and DM, are warranted to explore whether circulating GDF-15 remains elevated also after pregnancy and birth and to address the question of whether elevated maternal and fetal GDF-15 levels could play a role in the excessive risk of cardiovascular diseases after these conditions.

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**Disclosures**

T.K. and K.C.W. have filed a patent and have a contract with Roche Diagnostics to develop a GDF-15 assay for cardiovascular applications. The remaining authors report no conflicts.

**References**


Circulating and Placental Growth-Differentiation Factor 15 in Preeclampsia and in Pregnancy Complicated by Diabetes Mellitus

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