Decreased fetal pre-adipocyte factor-1 in pregnancies complicated by gestational diabetes mellitus

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1. Introduction

Gestational diabetes mellitus (GDM) describes any degree of glucose intolerance recognized during pregnancy and affects 2–12% of all pregnancies [1,2]. Women with GDM are at increased risk of metabolic diseases including obesity, hypertension, dyslipidemia, insulin-resistance, cardiovascular disease and type-2 diabetes [12]. Transiitional hyperglycemia and macrosomia which increase delivery injury are short-term consequences of GDM. More importantly, maternal GDM confers lifetime risks of disease on the fetus [1,2].

Intrauterine hyperglycemia is one factor driving the development of overweight, obesity, hypertension, diabetes, and GDM in offspring. Fetuses exposed to GDM are at an increased risk of central obesity and being overweight during adolescence [3,4], a trend that continues into early adulthood [5]. Offspring of women with GDM have an 8-fold increased risk of diabetes/pre-diabetes (impaired glucose tolerance or impaired fasting glucose) in adulthood [6]. Young adults born to women with impaired glucose tolerance, GDM or type-1 diabetes have increased risks of developing type-2 diabetes [6–8]. At age 17 y, body mass index (BMI), systolic and diastolic blood pressure are significantly higher in GDM offspring compared to controls, and maternal GDM was positively associated with offspring BMI values independent of birth weight [4]. Infants born to GDM mothers, especially macrosomic infants, show increased concentrations of serum triglycerides and total and free cholesterol compared to control infants [7,8]. Investigations in a spontaneous, GDM animal model demonstrate the predisposition of offspring to develop metabolic and cardiovascular dysfunction, obesity and insulin resistance [9–11]. These findings confirm the association of GDM with an increased risk of metabolic diseases in GDM offspring; however, the precise mechanisms remain largely unknown.

Altered epigenetic modification is one potential mechanism, while hormones regulating the maternal-fetal adipo-insulin axis may also play a role. Soluble preadipocyte factor-1 (pref-1) is a factor secreted by preadipocytes that inhibits adipocyte differentiation and is significantly increased in small for gestational age (SGA) fetuses [12]. The authors proposed that increased pref-1 concentrations in early life determine adipocyte numbers, variations in fat tissue and vulnerability to metabolic diseases in later life. Our group has recently shown that fetuses born to women with severe preeclampsia had markedly increased serum pref-1 [13]. We hypothesized that pref-1 concentration was altered in fetuses born to women with GDM. To verify our hypothesis, we measured serum pref-1 concentrations of fetuses born to mothers with GDM compared to controls.

2. Materials and methods

2.1. Subjects

In this cross-sectional study, 37 women with GDM and 45 normal pregnant women were recruited with the approval of the Institutional Review Board, Women's Hospital, School of Medicine, Zhejiang University, Hangzhou, China. All participants gave informed consent. Pregnancy was diagnosed by a positive human chorionic gonadotropin (hCG) test
after missed menstruation. Gestational age was calculated by menstrual dating. Ultrasound was performed to confirm pregnancy and gestational age before 20 weeks gestation. GDM was diagnosed according to the criteria recommended by the International Association of Diabetes and Pregnancy Study Groups (IADPSG) [14]. All GDM women controlled their glycemia with diet control.

Exclusion criteria included multiple gestation, diabetes mellitus, chronic hypertension, infectious diseases recognized in pregnancy, premature rupture of membrane, active labor, polyhydramnios and signs of other, concurrent, medical complications. The women in the control group had no gestational complications and gave birth to healthy neonates of appropriate size for gestational age. To exclude the possible effect of labor on fetal pref-1 concentration, only women who received elective Cesarean section were included. The indications for elective Cesarean section were maternal request, breech presentation, cephalopelvic disproportion or GDM.

2.2. Sample collection and assay

Umbilical blood samples were taken immediately after the delivery of the baby. Blood samples were centrifuged after standing at room temperature for at least 30 min. Serum was separated and stored at −80 °C. Pref-1 concentrations were measured with a commercially available enzyme-linked immunosorbant assay (ELISA) kits (R&D Systems).

2.3. Statistical analysis

The Kolmogorov–Smirnov test determined data distribution. Normally-distributed data were presented as mean ± standard deviation (SD) and compared by Student’s t test. Categorical data were compared using the χ² test. Multiple variant linear regression was used to analyze the association of fetal pref-1 with the presence of GDM, maternal age, gestational age at delivery, fetal gender and birth weight. SPSS (Statistical Analysis System) was used for data analysis. A P < 0.05 was considered to be statistically significant.

3. Results

Table 1 showed the clinical data. GDM women were significantly older than those of normal pregnancy (P = 0.009). There were no significant differences in gestational age at delivery between normal pregnancy and GDM (P = NS). Neonatal birth weight was significantly heavier in GDM than normal pregnancy (P = 0.003 for birth weight and P < 0.001 for Z score). There was no significant difference in fetal gender between GDM and normal pregnancy (P = NS).

There were no significant differences in serum pref-1 concentrations between male and female fetuses in either the normal pregnancy group (22.32 ± 8.19 vs. 21.90 ± 10.00 µg/l; t = 0.154, P = 0.879) or the GDM group (15.83 ± 7.59 vs. 16.58 ± 4.14 µg/l; t = 0.336, P = 0.739).

Fetal serum pref-1 concentrations were significantly lower in GDM group compared to the normal pregnancy group (Fig. 1) (P = 0.001). Multiple variant linear regression analysis revealed that fetal serum pref-1 was significantly associated with exposure to GDM and gestational age (r² = 0.388, P < 0.001 after adjustment for maternal age, fetal gender and birth weight) but not maternal age, fetal gender and birth weight.

4. Discussion

De Zegher et al. [12] demonstrated that soluble pref-1 is abundantly present in the fetal circulation and that SGA fetuses have significantly higher serum concentrations of pref-1 than control fetuses at birth. These authors proposed that soluble pref-1 is one of the mediators determining adipocyte differentiation in the fetus and subsequently lipid storage capacity in adulthood. Our group has recently demonstrated that fetuses born to pregnancies complicated by severe preeclampsia had increased serum pref-1 concentrations while their birth weight was lower compared to normal pregnancy, gestational hypertension and mild preeclampsia at birth [13]. In the current investigation, we observed decreased serum concentrations of pref-1 in GDM fetuses. Regression analysis showed that fetal pref-1 concentrations were associated with gestational age, but not birthweight or maternal age. Our findings suggest that reduced pref-1 concentrations in fetal circulation may be among the mechanisms linking intrauterine exposure to GDM with high risk of metabolic diseases in later life.

Pref-1, cloned from a 3T3-L1 preadipocyte cDNA library, is highly expressed in preadipocytes but decreases during differentiation, and is absent in mature adipocytes [15,16]. Since pref-1 reflects the degree of adipocyte differentiation in vitro and in vivo, it is used as a preadipocyte marker [16–22]. Constitutive expression of pref-1 in 3T3-L1 cells by stable transfection inhibits the differentiation of adipocytes [15]. Soluble pref-1 prevents lipid accumulation and expression of adipocyte transcription factors such as PPAR-γ and C/EBP-α as well as late adipocyte markers (including fatty acid synthase (FAS), stearoyl-coenzyme A desaturase (SCD), and fatty acid binding protein 4 (FABP4/aP2)) by upregulating Sox9 in preadipocytes, suggesting that pref-1 inhibits adipocyte differentiation. Conversely, decreasing pref-1 expression by

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<th>Clinical data.</th>
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<td>Normal pregnancy</td>
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<tr>
<td>n</td>
<td>45</td>
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<tr>
<td>Maternal age (y)</td>
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<tr>
<td>Gestational age at delivery (w)</td>
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<td>Female: 25</td>
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<tr>
<td>Birth weight (g)</td>
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<td>Weight ranges (g)</td>
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<td>Weight Z score</td>
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![Fig. 1. Comparison of fetal pref-1 between normal pregnancy and gestational diabetes. There was a significant difference in fetal pref-1 concentrations (t = 3.339, P = 0.001).](image-url)
transfection of antisense sequence greatly enhances adipogenesis in vitro [23–26].

The body weight of pref-1-null mice at weaning, whether male or female, was markedly decreased compared with wild-type mice [27]. However, pref-1-null mice gained body weight more rapidly. The major fat depots (inguinal, retroperitoneal and gonadal) increased significantly in pref-1-null mice, indicating that the accelerated body weight gain in pref-1-null mice was due to the increase in adipose tissue mass [28]. Histological analysis revealed that adipocytes in fat depots from pref-1-null mice were significantly larger than those from wild-type controls [29]. Moreover, the expression of late markers of adipocyte differentiation (SCD and FAS) was significantly enhanced in adipose tissue from pref-1-null mice. pref-1-null mice showed some characteristics associated with obesity such as increased blood concentrations of triglycerides, cholesterol, and free fatty acids as well as an enlarged fatty liver. When fed a high-fat diet, pref-1-null mice were vulnerable to develop impaired insulin resistance and glucose intolerance compared to wild-type mice on the same diet [27–29]. Conversely, over-expression of pref-1 significantly inhibited adiposity in transgenic mice [30].

Relatively high birth weight is one of the characteristics of GDM-complicated pregnancy [31,32]. Data in the current and previous studies of ours confirmed the increased birth weight in GDM [33]. In contrast, GDM animal model induced by injection of streptozocin usually have over-expression of pref-1 significantly inhibited adiposity in transgenic mice [27].

To exclude the possible effect of labor on fetal pref-1 concentration, only infants delivered by elective Cesarean section was included. This was a limitation in study design and limited the extrapolation of our results to infants delivered via vagina. In a very primary observation comparing pref-1 concentrations in infants of normal pregnant women delivered by Cesarean section and vaginal delivery, we found that pref-1 concentrations were comparable (data not shown), implying that active labor did not affect fetal pref-1. However, further study is needed to address the change in pref-1 in infants delivered via vagina because the sample size was small and no GDM infants were included in our primary observation.

Data from in vivo studies with pref-1-null mice models are consistent with findings of in vitro studies. Published data have strongly demonstrated that pref-1 inhibits adipogenesis and subsequently leads to enhanced insulin sensitivity and improved glucose tolerance. It is accepted that impaired glucose tolerance, insulin resistance and dyslipidemia are high risk factors for metabolic diseases such as diabetes, hypertension and cardiac-vascular diseases. Based on these findings, pref-1 may be among the mediators linking intrauterine exposure to hyperglycemia with increased risks for metabolic diseases in late life. Further clinical observations and in vivo investigations will be necessary to support this assertion.

Acknowledgments

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References