Fetal proinsulin and insulin and placental weight in pregnancies complicated by gestational diabetes and obesity

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Abstract

Objective. *We evaluated the role of fetal proinsulin and insulin on placental weight in patients with gestational diabetes.* ***Method.*** *We investigated a group of 15 patients diagnosed with gestational diabetes and 24 patients without diabetes. All cases of gestational diabetes during pregnancy found did not require insulin therapy. Were analyzed patient's age, parity, gestational age, sex and birthweight, Apgar score, placental weight immediately after birth, fetoplacentar index (weight divided by placental weight of the child), maternal weight and body mass index before pregnancy, blood pressure, and presence of metabolic syndrome. In addition were analyzed maternal glucose, total cholesterol, and triglycerides in the first trimester and proinsulin and insulin in cord blood at birth.* ***Results.*** *Fetal insulin and proinsulin showed significantly higher values in the group with diabetes compared to controls - Insulin 40.5 pmol /l (17.9-64.5) vs 81.3 pmol/l (53-121), p <0.001 and 7.73 pmol/l proinsulin (5-13) vs 13.3 pmol/l (8-23), p <0.001). The proinsulin/ insulin ratio was similar in both groups. Placenta is heavier in diabetic patients (686 g ± 77 vs 565 g ± 83 p <0.001), but fetoplacentar index showed no statistically differences in patients with gestational diabetes. Statistical analysis revealed the linear relationship of glucose with the fetal weight, placental weight and with the fetal proinsulin and insulin. Finally, fetal proinsulin and insulin are both increased in the same time with placental weight. The proinsulin/placental and insulin/placental ratios did not differ between studied groups, these hormones being in direct relationship with placental weight.* ***Conclusions.*** *Umbilical cord proinsulin and insulin levels are elaveted at birth in neonates from diabetic mothers. The fetal pronsulin/insulin ration could be a better marker of pancreatic beta cell function. Placental weight is increased in diabetic patients in relationship with hyperglycemia, and the present study showed a direct relationship of placental weight and fetal insulinemia and proinsulinemia.*

Keywords: *insulin, proinsulin, fetal pancreatic beta cells, placenta, umbilical cord, obesity, diabetes, apoptosis*

Introduction

Diabetes during pregnancy is associated with several adverse effects for both mother and newborn, with an increased perinatal morbidity and mortality, particularly associated to fetal macrosomia.

Maternal diabetes is associated with concentration changes of various hormones, and metabolites in the maternal as well as fetal circulation.

According to Pedersen's hypothesis[(1)], maternal hyperglycaemia increases transplacental transfer of glucose, inducing fetal hyperinsulinemia and fetal growth induction.

Obviously compensatory fetal hyperinsulinism can have independent influence on fetal weight, placental weight in diabetic patients[(2,3)].

So fetal hyperinsulinemia is associated with accelerated fetal growth and increased birth weight[(4,5)].

Proinsulin is a precursor of insulin prohormone synthesized in pancreatic beta cells of islets of Langerhans. The human insulin gene is encoded by the INS gene[(6,7)].

Molecular mechanisms of insulin synthesis and secretion are largely independent. Increased umbilical cord proinsulin was identified as a better indicator of pancreatic beta cell function. In studies using radioimmunometric techniques, proinsulin was independently correlated with fetal growth[(8)].

At placental level there are minimal structural differences, insulin higher levels was associated independently with increased capillary surface, thus providing a greater exchange surface adapted to increased fetal metabolic needs of pregnancy with diabetes[(9)].

Placental development starts early during pregnancy with the implantation of the blastocyst into the endometrium, and the placental morphology undergoes a continuous development by differentiation and proliferation processes.

Thus, the trophoblast differentiates into the placental villi expressing various degrees of maturation[(10)].

Frequently pregnancy pathologies related to placental dysfunction i.e.spontaneous abortion, IUGR or preeclampsia, occur in diabetic mothers[(11,12)]. This suggest that there is an influence of diabetic environment on trophoblast invasion.

Some biochemical agents like leptin, isoprostanes are involved in such diabetes-associated invasion defects.

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The insulin may contribute to stimulate the invasion by complex transcriptional up-regulation and activation of specific matrix metalloproteinase MT1-MMP type 14[13].

Invasion regulation is a complex process resulting from the balance between invasion inhibiting and promoting factors, and obviously the diabetic environment appears to shift this balance.

If the placentomegaly is a distinct feature of the diabetic pregnancies at the end of gestation, there could be postulated that there is a period of accelerated placental growth in these pregnancies, parallel to foetal growth in these fetuses.

Altered placental structure and morphology is in relationship with various maternal or fetal growth factors, acting either on maternal side or fetal side of syncyto-trophoblast membrane[14].

In addition, studying fetal proinsulin could be interesting because recent data showed that increased proinsulin aggregates was associated with a decrease in insulin conversion, with the accumulation of massive protein aggregates that trigger apoptotic cellular mechanisms[15-17].

These data are supported by recent research showing that overproduction of nonnative proinsulin in adults may predispose to cellular toxicity and premature loss of pancreatic beta cells[18-22].

Impaired placental growth, development and function as well as relation to fetal insulin are major causes of impaired fetal growth[23].

The mechanism of growth disorders is complex, most likely there are other factors that may contribute - endogenous oxidative-stress, placental disorders, pregnancy-induced hypertension frequently associated, as exogenous food, environmental, toxic, smoking, drugs, alcohol etc.

Methods

The purpose of this study was to investigate the role of fetal proinsulin and insulin on placental weight in patients with gestational diabetes.

Exploratory observational study included a 15 patients group diagnosed with gestational diabetes during pregnancy and a control group of 24 patients without diabetes, with negative screening test for gestational diabetes. All patients were followed during pregnancy and gave birth in hospital "Dr I. Cantacuzino" in 2009.

Gestational diabetes diagnostic criteria are those accepted by the ADA (American Diabetes Association) and WHO (World Health Organization): fasting plasma glucose >126 mg/dl (7 mmol/L), plasma glucose above 200 mg/dl (11.1 mmol/l) at any time of day.

It was used glucose tolerance test to 100 g according to ADA from 1 h values over 180 mg/dL (10 mmol/l), 2 h - over 155 mg/dL (8.6 mmol/l) and 3 h over 140 mg/dL (7.8 mmol/l). Obesity was calculated for body mass index (BMI) before pregnancy: normal weight - between 18.5 - 24.9 kg/sqm, overweight between 25 and 29.9 kg/sqm, obesity over 30 kg/sqm.

Inclusion and exclusion criteria

Inclusion criteria: 18-40 years old pregnant, regardless of parity, with term singleton pregnancy, obtained spontaneous, with or without gestational diabetes. All cases of gestational diabetes were found during pregnancy and did not required insulin therapy.

Exclusion criteria: other types of dyabetes, pre-existing pathology: inflammatory disease, *lupus erythematosus*, anemia with hemoglobin less than 7 mg/dL, known pregnancy diabetes, congenital and acquired thrombophilia diagnosed during pregnancy, benign pathology, malignant fetal malformations suggestive of trisomy diagnosed in current pregnancy.

Informed consent was obtained for all patients and internal ethical board approved the study. The study complies with international recommendations on human studies and meet ethical standards for human experimentation as specified in the Declaration of Helsinki[21].

Investigations and procedures

It was assessed patients age, rank, parity, gestational age, maternal weight and body mass index before pregnancy, weight gain, blood pressure. We measured maternal hemoglobin, glucose, triglycerides and cholesterol in the first trimester, and for newborn was noted the sex, weight and Apgar score.

A morphological placental evaluation was performed by measuring the placental weight at birth and fetoplacental index - child weight (grams) divided by placental weight (grams).

Recruitment and sample collection

Serological detection methods included enzymatic immunoassay for insulin, proinsulin at birth from cord blood.

In total there were 15 samples from diabetic patients and 24 patients without diabetes at birth. Sampling protocol was available in the delivery-rooms, collection being made immediately after delivery from the umbilical venous blood after clamping. To minimize the influence of hemolysis on detection Insulin samples were sent immediately to the laboratory, within maximum 20 minutes to centrifugation and plasma was frozen in 60 minutes. Antenatal corticosteroids were not administered in the last 24 hours, deliveries were at term - over 37 weeks. The placenta was examined and weighed immediately after birth.

Statistical processing of student t test and used multiple regression analysis using SPSS17.0 program trial version. Statistical significance was considered for p value <0.05.

Results

Maternal and fetal general characteristics were included in Table 1 and 2.

As shown in the table, both groups had similar age and gestational age at birth.

We focused on the patient's background, the main outcomes at birth and the biochemical results.

Table 1 Maternal and fetal characteristics

	Control	Diabetes	p*
N	24	15	
Age (years)	27.92 ± 5.9	26.8 ± 2.7	0.43
Parity			
1	12 (50)	8 (53.3)	
2	10 (41.7)	5 (33.3)	
>2	2 (8.3)	2 (13.3)	0.91
Gestational age (weeks)	39.21 ± 1.53	39.27 ± 1.28	0.90
BMI (kg/sqm)	22.9±4.3	30.9±2.8*	<0.01
AP systolic	120±10	139±5.7*	<0.01
Sex (M/F)	13 / 11	8 / 7	0.96
Birthweight (grams)‡	3425.5 ± 458	4150 ± 469.4*	<0.01
masculin	3511±308	4425±486*	<0.01
feminin	3322 ± 590	3835 ± 149*	0.005
Placenta weight (g)	571.28 ± 96	693 ± 79*	<0.01
Feto-placentar index	6.03 ± 0.41	5.99 ± 0.32	0.78
Umbilical cord			
N	24	15	
Insulin (pmol/l)	42.29 (15.25–64.58)	81.3 (53–121)*	<0.01
Proinsulin (pmol/l)	8.71 (4–18)	13.4 (8-23)*	<0.01
Proinsulina/Insulina ratio	0.114	0.164	NS
Proinsulin/placental ratio	0.015	0.019	NS
Insulin/placental ratio	0.074	0.117	NS
Maternal blood samples			
Haemoglobin (%)	11.54±1.25	12.19 ±0.90	0.08
Glycemia (mg/dl)	81.98± 12	124,73±44*	<0.01
Total cholesterol (mg/dl)	192±30	240±17.11*	<0.01
Triglycerides (mg/dl)	170.5±30	224.5±28*	<0.01
Newborn *n*	24	15	
Apgar score	8.5±0.65	8.33±0.90	0.50

** Statistically significant (p <0.01)*

Table 2 Statistical analysis on personal history and family history in batches analyzed

	Control n=24	Diabetes n=15	*P**
Diabetes heredity	4 (16%)	9 (60%)	<0.05
Obesity heredity	5 (20%)	7 (46%)	<0.05
Macrosomia personal history	0 (0%)	3 (20%)	<0.05
Metabolic syndrome history	4 (16%)	12 (80%)	<0.05

** Hi square test non-parametric variables.*

As shown in the table when analyzing heredity for diabetes and obesity data, we found a significantly higher prevalence in patients for the heredity of diabetes, obesity, macrosomia and metabolic syndrome in the family in study group.

When analyzing the body mass index before pregnancy our data showed in patients with diabetes a body mass index significantly higher and the difference is statistically significant compared to those in control group (30.9 kg/sqm vs 22.9 kg/sqm, $p<0.01$).

Considering the lipid profile, we found significantly elevated concentrations of the maternal cholesterol (240 vs 192 mg/dl, $p<0.01$) and triglycerides (224 vs 170 mg/dl, $p<0.01$).

In addition, arterial pressure was significantly higher in diabetic patients (139 mmHg vs 120 mmHg).

All these findings: the presence of the higher values of arterial pressure, altered lipid profile in patients with glycemic intolerance, and obesity reflected the presence of the metabolic syndrome in diabetic patients.

The presence of gestational diabetes was significantly correlated with higher birth weight, and was noticed an increased incidence of fetal macrosomia (weight above 4,000 g), and this difference is maintained regardless of newborn sex.

However, the Apgar score was not significantly different in cases of diabetes.

This study showed no statistical differences on maternal hemoglobin in studied groups (12.19 vs. control 11.54 g/dl).

When analyzing the placental weight, the results showed significant higher values in cases of diabetes, but the calculated fetoplacental index was similar (5.99 vs 6.03). Finally we analyzed the insulin and proinsulin concentrations in umbilical cord and our results showed significantly higher values in case of diabetic pregnancies.

However the calculated proinsulin/insulin ratio was similar in studied groups (0.11 vs. 0.16).

We observed a direct relationship of placental weight with fetal weight, and the same direct relationship of proinsulin and insulin with fetal weight.

The proinsulin/placental ratio and insulin/placental ratios did were similar between studied groups, these hormones being in direct relationship with placental weight.

Discussion

This paper analyzed the correlation of maternal diabetes and fetal pancreatic beta cell function measured by insulin and proinsulin values.

In addition were depicted correlations of these hormones to placental weight and birth weight.

We observed significant differences maintained for maternal blood glucose, lipid profile and hormonal profile of cord blood. In our study group was observed that diabetic mothers met already all criteria for metabolic syndrome characterized by the association of obesity, impaired glucose tolerance, elevated blood pressure and serum lipids. According to Pedersen's hypothesis macrosomia observed in maternal gestational diabetes is fetal hyperinsulinism consequence of maternal hyperglycemia.

Even if we know the results on fetal development, there is no consensus on the mechanisms that regulates in placental compartment.

HAPO prospective study data revealed the direct connection between increasing maternal blood glucose, neonatal body composition and neonatal hyperinsulinism[24].

Of course, other risk factors for macrosomia and gestational diabetes were frequently associated as confounding factors: pregestational obesity - observed in our study and other studies, as well as the pregnancy excessive weight gain, maternal age >40 years and multiparity >4[25]. It is interesting that fetal insulin and proinsulin are nearly two times higher in cases with diabetes.

The same results were observed in placental weight - statistically significantly higher in diabetic pregnancies, but it is interesting to note that no significant difference was observed for fetoplacentar index, placental weight was directly related with fetal weight.

This suggests that there is an adaptive development of the placenta in direct proportion to fetal weight, in close relationship to increased metabolic needs and increased fetal hemodynamics.

In a recent study, Nelson analyzed the placentas in a cohort of mothers with type 1 diabetes, and he found an increased placental weight but villous, nonparenchymal, trophoblast, and capillary volumes did not differ. Villous surface area, capillary surface area, membrane thickness, and calculated morphometric diffusing capacity were also similar in type 1 diabetic and control subjects. In multivariate analysis of cord parameters in OT1DM, fetal IGF-I emerged as a significant correlate of most components (intervillous space, villous, trophoblast, and capillary volumes). By contrast, fetal insulin was only independently associated with capillary surface area, and IGF I emerged as key correlate of placental substructural volumes[26].

In our study the placentae in GDM were heavier, and there was a direct relationship of placental weight with glycemia and fetal proinsulin and insulin.

Furthermore, the placental vascular development is regulated by various growth factors like and specific cytokines. Thus, vascular endothelial growth factor (VEGF), placental growth factor (PlGF), insulin-like growth factors 1 and 2 (IGF1, IGF2), fibroblast growth factor (FGF-2), tumor necrosis factor (TNFA), interleukin 8 (IL-8) have been identified. The TNFA can act as well in an angiostatic manner[27]. In diabetic pregnancies, there is evidence of alteration of these factors.

The precise action of each of these factors in early placental angiogenesis is still unclear and further investigation is necessary[28-31].

So there was noted a reduced IGF1, in the diabetic context of hyperglycemia and hyperinsulinemia[32,33].

However, other growth factors and cytokines may be altered and further studies should demonstrate the role of each factor.

The placental amount of MT1-MMP is elevated in the first trimester in type 1 diabetes mellitus, and in the normal placentae, the active MT1-MMP decreases in late first trimester, whereas in diabetic pregnancies remain high.

Insulin and also TNFA up-regulated the MT1-MMP expression *in vitro* and *in vivo*, and doses of insulin with which diabetic mothers were treated correlated directly with MT1-MMP expression in these placentae.

The higher expression of these proteases could be in correlation with dysregulation of invasion control systems early in diabetic pregnancies, and shows the sensitivity of placental development to expression of the specific factors like insulin, other growth factors or cytokines.

All these factors may have an impact on placenta in third trimester, acting on the microvillous syncytiotrophoblast membrane - for maternal factors, and on the endothelium or basal syncytiotrophoblast membrane.

Furthermore, recent data showed evidence that gestational diabetes mellitus in pregnancy is a pathological condition associated with placenta vascular dysfunction and with metabolic changes at the fetoplacental microvascular and macrovascular endothelium.

This pathological state could be considered as a metabolic marker that could predict occurrence of diseases in later in life, such as cardiovascular disease, diabetes mellitus (including gestational diabetes), obesity, and metabolic syndrome[34].

The originality of the present study was to try to investigate the correlations of umbilical proinsulin as well as the insulin levels at birth, with placental weight and other markers such as: pregestational weight, maternal blood sugar. This study showed increased values of fetal proinsulin and insulin in diabetic patients directly correlated with fetal and placental weight.

In the same time the proinsulin/insulin ratio could be a better marker in assessing the dysfunction of pancreatic beta cells early at delivery.

Morphological alterations were seen in few studies in diabetic placentas, but there was no evidence showing correlations of placental alterations with fetal proinsulinemia as precursor of insulin, and early marker of fetal pancreatic beta cell adaptative function.

We searched in addition the keyrole played by placenta by fetoplacental index, as well as proinsulin/placenta and insulin /placenta ratios in regulating fetal growth. The linear relationship placental and fetal weight in diabetic patients shows the adaptive mechanisms in placental structure and harmonious relationship between fetus and its placenta.

The mechanism of increased secretion of pancreatic beta cells is complex probably by hyperfunction but also by adaptive hyperplasia.

It was proven the persistence of fetal hyperinsulinism after birth increased the risk of neonatal hypoglycemia through direct and indirect mechanisms, inhibiting the main metabolic pathways of insulin glucose[35-37].

However, neonatal hypoglycemia risk is difficult to estimate, in literature the percentage of cases requiring intravenous treatment is low - about 5%, macrosomia and poor metabolic control are recognized as risk factors[38].

The importance of studying the fetal beta cell function at birth is that assessing the degree of secretory dysfunction may explain fetal growth disorders such as macrosomia and intrauterine growth restriction, as well as other diseases later in adulthood, and identifying their triggers and timing of action could guide the therapeutic intervention[39].

Elevated levels of proinsulin and insulin ratio in umbilical cord may reflect at delivery, the function of fetal pancreatic beta cells, according to recent studies, which is incriminated later in life of adult as pathogenic mechanisms of cardiovascular disease, diabetes, of obesity and metabolic syndrome[40,41].

However, further research in molecular changings in fetal pancreatic beta cell function is necessary to better understanding of adaptive changings at this level in relationship with metabolic alterations in diabetic mothers.

Conclusions

Umbilical cord proinsulin and insulin levels are elevated at birth in neonates from diabetic mothers.

Although placental weight is increased in diabetic patients in relationship with hyperglycemia, the present study showed no statistical role of placental weight for the fetal insulinemia and proinsulinemia. Other factors are involved in fetal hyperinsulinism, the most important is maternal hyperglycemia.

Hyperglycemia is independently correlated with increased fetal and placental weight.

Proinsulin level was significantly higher in cord blood, a parameter more specific than insulin in assessment of fetal pancreatic beta cell function.

Future studies on molecular mechanisms of placental and fetal growth could provide more information about intrauterine growth disorders and the role of prolonged exposure to high levels of glucose on fetal pancreatic beta cell with subsequent impact later in life. ■

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