THE IMPACTS OF GESTATIONAL DIABETES AND OBESITY OF MOTHERS ON INSULIN RESISTANCE AND ADIPOKINES LEVELS IN THE UMBILICAL BLOOD

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Key words: obesity, insulin resistance, adipokines, gestational diabetes, pregnancy

SUMMARY. Due to the stressful and sedentary lifestyle with the plenty of calorie-rich food obesity reaches pandemic proportions. It is increased interest of scientist to discovery previously unknown metabolic-endocrine effects on obesity. Aims. The aim of this study was to determine the influence of obesity of the mother and GDM as individual and combined factors on the levels of glucose, C-peptide, adiponectin and leptin in umbilical serum, and on insulin resistance in the child. Methods. This case control study included 160 pregnant women divided into four groups according to BMI and GDM. The concentration of C-peptide, adiponectin and leptin were determined using the ELISA method. Results. Obese women in both diabetic and healthy groups had higher concentrations of C-peptide. The concentration of adiponectin was positively correlated to newborn’s weight among women with normal BMI values in both diabetic and healthy groups and leptin concentrations and newborn’s weight only among obese diabetic women. Conclusion. This study has shown that the activities of increasingly common pathogenic factors in the mother – weight and increased insulin resistance – either independent or combined, alter the in utero metabolism of the child.

Introduction

The obesity of mothers prior to pregnancy is a significant risk factor for the disturbed development of the pregnancy and the child. Risks for the fetus include high birth weight and the risk of obesity later in life, and the development of metabolic syndrome (MS) or any of its components. It has been proven that the risk of birth of macrosomic babies is present in the groups of overweight women with the normal levels of the oral glucose tolerance test during pregnancy. These two groups of women have one thing in common: the components of MS. Some studies have indicated the connection between GDM and the risk of obesity in children and the development of MS later in life.

Adipocytes secrete numerous compounds having endocrine, paracrine and autocrine effects: leptin, adiponectin, resistin, visfatin, angiotensinogen, PAI-1, TNF-α, interleukin-6, ASP (an acylation stimulating protein), and likely many others that are yet to be discovered. Adipokines play an important role in the intake and the consumption of energy, the activity of insulin, the metabolism of fats and carbohydrates and in angiogenesis, the regulation of blood pressure and coagulation. Obesity leads to the increased secretion of all the said adipokines with the exception of adiponectin. The increased concentrations of TNF-α, IL-6 and resistin, and the reduced secretion of adiponectin and leptin, i.e. a resistance to leptin lead to the development of insulin resistance all contribute to the appearance of insulin resistance.

The aim of this study was to determine the influence of obesity of the mother and GDM as individual and combined factors on the levels of glucose, C-peptide, adiponectin and leptin in umbilical plasma, and on insulin resistance in the child.

Materials and methods

The study included 160 pregnant women, divided into four groups based on body mass index (BMI) and GDM. Pregnant women with normal body weight were those having a pre-pregnancy BMI of less than 25 kg/m², while overweight and obese patients were those with a pre-pregnancy BMI of greater than 25 kg/m² and 30 kg/m². Pregnant women who gained more than 20 kg during pregnancy were excluded from the study. The presence or absence of GDM was confirmed with the oral glucose tolerance test (oGTT) between the 20th and 28th week of pregnancy, with 75 grams of glucose, according to the criteria of the IADPSG. Only pregnant women with the diagnosis of GDM not receiving insulin treatment were included in the study. All preterm births, multiple pregnancies, pregnancies with other expressed pathology and pregnancies with chromosome anomalies or fetal deformations were excluded from the study. The gestational age was determined according to the first day of the last menstruation and confirmed by ultrasound examination in early pregnancy (6 to 10 weeks). After the delivery, a blood sample was taken from the umbilical vein in a standard test tube. After centrifuging, serum was kept at a temperature of –75°C. The concentrations of adipokines were determined in umbilical vein serum. Serum glucose levels were determined using the enzyme UV test (hexokinase method) on an Olympus AU2700 analyzer with reagents from the same manufacturer. We used the Mercodia C-peptide ELISA Immunoassay for the quantitative determination of human C-peptide concentrations. The ELISA
used to determine serum leptin and adiponectin levels were the Quantikine Human Leptin, Adiponectin/Acrp30, respectively. The HOMA 2 calculator was used to calculate the insulin resistance.

This study was approved by the Ethics Committee of School of Medicine University of Zagreb. All authors confirmed in writing that they have complied with the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving human subjects.

Statistical analysis

Data were analyzed using the Statistica 7.1 software package (StatSoft, Inc. Tulsa, USA). The Smirnov – Kolmogorov test was used to explore normality. The independent sample t-test was used to explore the difference between two groups. The Smirnov – Kolmogorov test was used to explore normality. The independent sample t-test was used to explore the difference between two groups. The ANOVA was used to determine the differences between more than two independent samples, followed by the Tukey test for post hoc analysis. Preliminary analyses were performed to ensure no violation of the assumptions of normality, linearity and homoscedasticity.

Results

Among mothers without GDM, there was a statistically significant difference between glucose levels in umbilical blood in mothers with increased BMI as compared to mothers with normal BMI values (Table 1). Among mothers with a diagnosis of GDM, there was no significant difference in the levels of umbilical glucose between overweight mothers and mothers with a normal BMI level (3.66±0.67 mmol/L vs. 3.92±0.84 mmol/L, P=0.304). Among mothers with normal BMI levels, there was a significant difference between the value of glucose in umbilical blood in the group of mothers with and without GDM. There was no statistical difference in the level of umbilical glucose among overweight mothers with and without GDM.

The C-peptide values in umbilical blood within the control group of women, i.e. normal BMI and without diabetes, was 262.94±99.48 pmol/L. A comparison of the C-peptide values among healthy women with normal BMI vs. those with increased BMI gave significantly higher levels of umbilical C-peptides in the overweight women. The same results were obtained when considering the groups of women with GDM: the overweight group had statistically higher levels of C-peptides in the umbilical blood. Mother’s weight was associated with the increased values of umbilical C-peptides in women with and without GDM.

There was a significant main effect for all variables when one-way ANOVA performed. Afterwards, post-hoc comparisons using the Tukey test was assessed. The difference between C-peptide concentrations among GDM-BMI>25 and CTRL-BMI≥25 groups did not reach statistical significance. The insulin resistance among two groups not having GDM did not differ significantly. As we analyzed differences in adiponectine

| Table 1. Mean scores and standard deviations for analyzed molecules in umbilical blood |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
|                                  | GDM-BMI≥25 (A) n=39 | GDM-BMI<25 (B) n=42 | CTRL-BMI≥25 (C) n=38 | CTRL-BMI<25 (D) n=41 | post hoc P<0.05 |
| GLUCOSE* (mmol/L)                | 3.66±0.67            | 3.92±0.84            | 3.3±0.89             | 2.89±0.6             | a:d, b:c, b:d       |
| C-PEPTIDE* (pmol/L)              | 510.1±276.5          | 355.2±331.2          | 345.12±126.4         | 262.9±99.5           | a:b, a:c, a:d       |
| INS. RES.* (HOMA 2)             | 1.19±0.35            | 1.04±0.24            | 0.94±0.17            | 0.81±0.14            | a:c, a:d, b:c, b:d  |
| LEPTIN* (ng/mL)                 | 46.3±18.1            | 36.3±16.2            | 41.31±16.2           | 29.12±15.7           | a:b, a:d, c:d       |
| ADIPONECTINE* (µg/mL)           | 32.1±9.23            | 39.3±4.4             | 33.5±5.8             | 38.53±8              | a:b, a:d, b:c, c:d  |

| Table 2. Correlations between newborn’s weight and adipokines |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
|                                  | GDM-BMI≥25 (A) n=39 | GDM-BMI<25 (B) n=42 | CTRL-BMI≥25 (C) n=38 | CTRL-BMI<25 (D) n=41 | Newborn’s weight (g) |
| LEPTIN (ng/mL)                  | r=0.502 P=0.001        |                                |                                |                                |                                |
| ADIPONECTINE (µg/mL)            | r=0.418 P=0.007        | r=0.471 P=0.002                |                                |                                |
levels, there was no difference between GDM-BMI ≥ 25 group and CTRL-BMI ≥ 25 (two groups with BMI ≥ 25), and between GDM-BMI < 25 and CTRL-BMI < 25 (two groups with BMI < 25).

The relationship between newborn’s weight and adipokines was investigated using Pearson’s product moment correlation coefficient (Table 2). There was a strong, positive correlation between the concentration of leptin and newborn’s weight in GDM-BMI ≥ 25 group. There were also medium, positive correlations between adiponectin concentrations and newborn’s weight in GDM-BMI < 25 and CTRL-BMI < 25 group.

**Discussion**

Among mothers with normal BMI levels, there was a significant difference between the value of glucose in umbilical serum in the group of mothers with and without GDM. There was no significant difference in the level of umbilical glucose among overweight mothers with and without GDM. Therefore, we can conclude that there is a link between GDM and an increase in the values of umbilical glucose when the mother has a normal BMI. These results correspond to those obtained by Taricco et al.,7 but their study did not separate mothers with GDM by weight.

There is a link between mother’s weight and increased values of umbilical glucose, when the mother does not suffer from GDM. The human fetus is virtually entirely dependent on the mother’s glucose, with only a very small amount of own glucose production. Glucose passes via the mechanism of facilitated diffusion, by the existence of protein carriers in the cell membrane that bind glucose.8

In women with a normal BMI, there was a significant difference in values between the groups with and without GDM, i.e. GDM was associated with higher levels of umbilical C-peptides. The same results were also obtained in overweight women, i.e. GDM was associated with increase values of umbilical C-peptides. It can then be concluded that both weight and GDM in the mother are associated with increased levels of C-peptides in umbilical blood, and that this effect is further increased by their combined effect.

In the group of women without GDM, no difference was observed in insulin resistance in the children of mothers with normal BMI and mothers with high BMI, though there was a significant difference in the results of mothers with GDM in comparison with mothers with a high BMI, i.e. the children of mothers with GDM had higher insulin resistance than children of mothers with high BMI. Therefore, we can conclude that the mother’s weight is connected to increased insulin resistance in children only when the mother also has GDM.

In 2009, Catalano et al.9 published a study in which they examined a group of overweight mothers and a group of mothers with a normal BMI and determined that the children of overweight mothers had increased insulin resistance. The difference between that study and this one is that Catalano et al. did not separate the pregnant women into those with increased insulin resistance during pregnancy. Given the connection between weight and GDM, it is logical that there would be more mothers with GDM in the overweight group.

In women with a normal BMI, there was significantly higher insulin resistance in the children of mothers having GDM than in the control group. The same results were obtained in overweight mothers, i.e. the presence of GDM was associated with higher values of insulin resistance in children.

Leptin levels in serum are usually in low concentrations, measured in ng/mL,10,11 and are somewhat higher in pregnant women than in non-gravid women.12 The increase of leptin in mother’s serum that appears in early pregnancy is explained by the synthesis of leptin within the placenta or the increasing share of free leptin. Highman et al. conducted a large study in which they determined the referential values in the general population within pregnancy.13

The question is then posed as to the values of leptin in umbilical blood, as fetal fatty tissue, like the placenta, also produces leptin.14 In this study a significant difference was found between the control groups and the group of overweight mothers without increased insulin resistance. There was also a significant difference in the levels of umbilical leptin in women with GDM having a normal BMI and those who were overweight. There are few comparable studies in the literature, though the 2006 study by Hauguel-De Mouzon et al.15 arrived at a similar conclusion that there is an association between the mother’s weight and increased values of umbilical leptin. Simmons et al.16 reported higher levels of leptin in mothers with GDM and Catalano et al. obtained similar results.9 However, Maffei et al.17 did not find increased levels of umbilical leptin with GDM.

The present study covered a significantly larger sample and showed a significant increase in the level of umbilical leptin in mothers with GDM and the control group of mothers with normal BMI, even though the differences were substantially less than when considering the effect of weight.

In the present study, the increased level of leptin in both umbilical and mother’s blood proved to be positively correlated with baby birth weight, even though the relevant data published to date is very contradictory.17

Adiponectin plays an important role in lipid metabolism, glucose homeostasis and energy balance, and that it possesses an antiatherogenic and antiinflammatory role.

In the serum of non-gravid women, most authors have reported the levels of about 13.5 μg/mL.18 Mazaki-Tovi et al. noted that adiponectin levels decrease as pregnancy advances, and are somewhat lower than the above values during the third trimester.19

The levels of adiponectin in the umbilical serum of newborns are significantly higher than in adults.20 The exact mechanism of how such high levels of adiponec-
tin are present in fetal serum has not yet been fully explained. The development of umbilical adiponectin in the control group of mothers with normal insulin resistance and normal BMI was 38.5±3.8 µg/mL. In some studies, the level of adiponectin in umbilical blood was lower in the fetuses of mothers with GDM in comparison to levels in fetuses of healthy mothers. However, our findings in the comparison of women with GDM and those without did not yield a significant difference.

However, there is a significant difference between the values of umbilical adiponectin between the babies of mothers with normal and high BMI. It could be concluded that the association between GDM and reduced levels of umbilical adiponectin in some studies could be due to the small sample size, and correlation between GDM and the mother’s weight.

An association was established between the value of adiponectin in umbilical blood and in the plasma of mother’s with babies with high birth weights, which is in line with papers published to date.22

Conclusions
This study has shown that the activities of the increasingly common pathogenic factors in the mother, weight and increased insulin resistance, either independently or combined, alters the in utero metabolism of the child. The importance of adipokines and the influence of fetal metabolism can also been seen it the fact that weight in the mother has an impact on increasing birth weights in groups where mothers were not insulin resistant, and no difference was observed between umbilical glucose levels and insulin resistance of the child.

References

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UTJECAJ GESTACIJSKOG DIJABETESA I PRETILOSTI MAJKE NA INZULINSKU REZISTENCIJU I RAZINE ADIPOKINA U UMBILIKALNOJ KRVI

Izvorni članak

Ključne riječi: pretilost, inzulinska rezistencija, adipokini, gestacijski dijabetes, trudnoća

Sažetak. Zbog stresnog i sedentarnog načina života i uz obilje kalorijski bogate hrane došle su razmjere pandemije. Njenu povećanu incidenciju prati i povećani interes znanstvenika i otkrivanje dosad nepoznatih metaboličko-endokrinoloških učinaka debljine. Cilj. Željeli smo odrediti standardne vrijednosti inzulinske rezistencije djeteta, te koncentracije adiponektina i leptina u umbilikalnoj krvi zdravih trudnica za vrijeme porođaja, te odrediti njihov omjer. Isto tako, željeli smo odrediti utjecaj pretilosti majke i gestacijskog dijabetesa, kao zasebnih i združenih faktora na koncentracije navedenih adipokina u umbilikalnoj krvi, kao i na inzulinsku rezistenciju djeteta. Metode. U istraživanje je uključeno 160 trudnica, podijeljenih u četiri skupine; u skupinu trudnica s normalnom tjelesnom težinom (ITM<25) i isključenim gestacijskim dijabetesom (n=41), u skupinu trudnica s normalnom tjelesnom težinom i gestacijskim dijabetesom (n=39), u skupinu pretilih trudnica (ITM ≥25) s isključenim gestacijskim dijabetesom (n=42), u skupinu pretilih trudnica (ITM ≥25) s isključenim gestacijskim dijabetesom (n=38) i skupinu pretilih trudnica s gestacijskim dijabetesom (n=39). Uzorci krvi iz umbilikalne vene prikupljeni su za vrijeme porođaja, te su određene koncentracije glukoze, C-peptida, adiponektina i leptina. Naknadno su prema formuli HOMA-IR izračunate vrijednosti inzulinske rezistencije. Rezultati. Koncentracije unutar naše kontrolne skupine u žena bez gestacijskog dijabetesa i ITM<25 za umbilikalnu kriv iznose: glukoza 2.89±0.60 mmol/L, C-peptid, 262.94±99.48 pg/l, leptin 29.12±15.7 ng/mL, adiponektin 38.53±8.00 µg/mL, a inzulinska rezistencija djeteta iznosi 0.44. Pretile žene u obje skupine (sa i bez gestacijskog dijabetesa) su imale povećanu koncentraciju C-peptida. Debljina i gestacijski dijabetes povećali su koncentraciju umbilikalne glukoze zasebno, ali je izostao združeni utjecaj. Gestacijski je dijabetes majke povezan s povišenom inzulinskom rezistencijom djeteta, a debljina majke samo pojačava taj učinak kod već postojećeg GDM. Pretilost majke povećuje koncentraciju leptina u umbilikalnoj krvi. Gestacijski dijabetes povećuje koncentraciju leptina, ali samo u žena sa ITM<25. Moglo bi se zaključiti da je učinak debljine izraženiji na porast koncentracije leptina, te da doseže određeni limit na koji više gestacijski dijabetes nema učinak. Koncentracija adiponektina je pozitivno povezana sa porođajnom težinom novorođenčeta kod žena sa normalnim ITM u obje skupine (sa i bez gestacijskog dijabetesa). Debljina majke povezana je sa sniženom koncentracijom adiponektina u umbilikalnoj krvi, ali ne i gestacijski dijabetes. Zaključak. Našim smo istraživanjem dokazali da djelovanje sve učešćujih patogenih čimbenika majke; debljine i povišene inzulinske rezistencije, zasebno ili združeno, mijenja metabolizam djeteta in utero.