Metabolic parameters and fetal development in offspring of mothers with gestational diabetes

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DISSERTATION

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Referral Centre for Diabetes in Pregnancy - "Department of Gynecology", Zagreb

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To whom it may concern

It is my hope that this Thesis will contribute to the future multidisciplinary research on the foetal development, metabolic parameters and its regulation in offspring of gestational Diabetes, and might serve as specific impact and importance in understanding a cause of enhanced foetal growth in well controlled gestational diabetic pregnancies, that predispose offspring to a significant risk of obesity, diabetes mellitus type 2, hypertension and metabolic syndrome in adulthood.

Table of Contents

Abbreviations

Gestational Diabetes mellitus (GDM)

Glycated haemoglobin (HbA_{1c})

Body mass index (BMI)

Ponderal Index (PI)

Small for Gestational Age (SGA)

Appropriate for gestational age (AGA)

Large for Gestational Age (LGA)

Normal Glucose Tolerance (NGT)

Impaired glucose tolerance (IGT)

Fasting plasma glucose (FPG)

Impaired fasting glucose (IFG)

Oral Glucose Tolerance test (OGTT)

Insulin Receptor (IR)

World Health Organization (WHO)

Free fatty-acid (FFA)

Very- low- density lipoprotein (VLDL)

Low- density lipoprotein (LDL)

High- density lipoprotein (HDL)

Homeostasis model assessment for insulin resistance (HOMA-IR)

Peroxisome proliferator-activated receptor (PPAR)-γ

Human Placental Lactogen   (hPL)

 Human placental growth hormone   (hPGH)

Tumor Necrosis Factor- α   (TNF α)

Human Chorionic Gonadotropin   (hCG)

White Adipose Tissue   (WAT)

Brown Adipose Tissue   (BAT)

Type 1 Diabetes Mellitus   (T1DM)

Type 2 Diabetes Mellitus   (T2DM)

International Association of Diabetes and Pregnancy Study Groups   (IADPSG)

1   INTRODUCTION AND OBJECTIVE OF THE STUDY

1.1   Insulin resistance and human placenta in Gestational diabetes

Gestational diabetes mellitus (GDM) is a carbohydrate intolerance of varying degrees of severity with onset or first recognition during pregnancy. It is one of the most frequent metabolic disorders of pregnancy affecting 1-10% of all pregnancies. Usually it disappears when pregnancy is over. Women who had GDM are at higher risk of developing diabetes, mainly type 2 Diabetes at a later stage in their life[1]. In parallel with obesity epidemic the incidence of gestational diabetes mellitus has doubled in last year's.

There is certain confusion in terms and criteria. Currently, WHO suggests partial adoption of International Association of Diabetes and Pregnancy Study groups (IADPSG) criteria[48]. Thus, if a nonsymptomatic pregnant women meets diagnostic criteria of diabetes during pregnancy it is diabetes in pregnancy (IAPDSG uses term "overt diabetes"). Gestational diabetes is diagnosed at any time in pregnancy according to IADPSG criteria:

Fasting plasma glucose: 5.1-6.9 mmol/l (92-125 mg/dl)

1 h post 75g oral glucose load: $\geq$ 10.0mmol/l (180 mg/dl)

2-h post 75g oral glucose load: 8.5-11.0mmol/l (153-199 mg/dl)

After previous acceptance of IADPSG approach to screening and diagnosis of gestational diabetes, current ADA guidelines suggest alternative(either/or) two-step approach with 50g glucose load in nonfasting individual with consequent measurement of BG after 60 minutes: if that is >7.8mmol/l (140mg/dl), a 100g OGTT should be performed and interpreted either et Carpenter-Coustan or National Diabetes Data Group. The two step approach has not been widely used in Europe.

Screening for GDM is generally undertaken in 24-28 week.

Women with risk factors such as glycosuria, age more than 30 year, family history of diabetes, previous GDM or glucose intolerance, previous adverse pregnancy outcomes, or high risk ethnicity, may be tested for GDM earlier by oral glucose tolerance test (OGTT) and if negative, re-tested at 26-28 weeks of gestation.

After the pregnancy ends, the woman should be re-classified as having either diabetes mellitus, or IGT, or normal glucose tolerance based on the results of a 75 g OGTT six weeks or more after delivery. It should be emphasized that such women, regardless of the 6-week post-pregnancy result, are at increased risk of subsequently developing diabetes. The significance of IFG in pregnancy remains to be established. Any woman with IFG, however, should have a 75 g OGTT.

Recent evidence indicates a worrisome rise in the prevalence of gestational diabetes that is largely explained by the increase in maternal obesity. The efforts to reverse this trend are critical and great evidence support the use of screening and treatment for women at risk.

Diabetes in pregnancy is associated with a number of adverse outcomes including birth trauma, neonatal hypoglycaemia, macrosomia and pre-eclampsia.

There are well-recognized associations between gestational diabetes and increased risks of fetalmacrosomia, birth trauma, and cesarean delivery.

The ***management of GDM*** still attracts some controversy regarding the exact modality of treatment to be used: lifestyle changes, diet and adjusted doses of insulin, or the emerging trend of the use of oral hypoglycemic drugs. There is agreement on the benefits of treating GDM, as untreated gestational diabetes has been associated with significant risks of perinatal morbidity in all levels of disease severity, and treatment has been associated with reduced perinatal complications and maternal morbidity.

The overall goal of diabetes management is to achieve as near normal physiological or ideal values as possible, without detriment to quality of life and, for glucose control in particular, without causing significant hypoglycemia.

Nutritional management in diabetes aims to assist in optimising metabolic control and reducing risk factors for chronic complications, this includes the achievement of blood glucose and glycosilated hemoglobin (HbA1c) levels as close to normal as is safety possible and serum lipid concentration as well as blood pressure values that may be expected to decrease the risk for macrovascular diseases. Individual therapeutic needs and the quality of life of the person with diabetes have to be considered when nutritional objectives are defined.

All women with GDM should receive nutritional counselling and be instructed in blood glucose self-monitoring. If blood glucose levels cannot be maintained in the normal range (fasting <95mg/dl and 1h after meals<140mg/dl) insulin therapy should be initiated.

Monitoring diabetes

Glycemic control should be monitored regularly for all patients with diabetes. The optimal method of determining risk of long-term complications is through HbA1c measurement and series of blood glucose measurements. Women with GDM should monitor the level of glycemia every day two hours after breakfast and at least one time per week before lunch.

When medical nutrition therapy is not successful in maintaining target glucose values during pregnancy complicated by GDM, medication is required. Insulin has been the

traditional treatment under such circumstances. The use of oral antidiabetic medications in the management of gestational diabetes has increased over the past several years.

Maternal and fetal monitoring is required in order to minimise maternal and fetal/neonatal morbidity and perinatal mortality.

It is of great importance understanding the relationships between glucose intolerance and pregnancy risk. For that reason even women with fasting hyperglycemia in pregnancy are at increased risk for fetal death. For that reason changes in recommendation criteria for GDM have resulted in the diagnoses of gestational diabetes in an increasing number of women who were previously normal.

Gestational diabetes results from insulin resistance and inadequate compensatory insulin secretion. Even in normal pregnancy, in the middle of the second trimester progressing to the third trimester, it is present physiological insulin resistance that result from increased maternal adiposity and hormones made by the placenta[2]. The human placenta expresses all known cytokines which are also produced in adipose cells, like TNF α, leptin and resistin[3].

Whether placenta at term is able to synthesize adiponectin is still in debate. These placenta cytokines contributes to low grade inflammation, developing during the third trimester of pregnancy[3]. Inflammatory cytokine TNF α, which is produced from adypocites, placenta, T cells, monocites, macrophagy, neutrophils and fibroblasts together with adiponectin from adypocites are active mediator in inducing insulin resistance during pregnancy[2]. First, it is described that TNF α impairs insulin signalling by diminishing insulin receptor (IR) tyrosine kinase activity[2]. Studies in pregnancy have reported that changes in insulin sensitivity from early (22-24 weeks) to late (34-36 weeks) gestation correlate with plasma TNF- α [4]. Second, TNF α suppress transcription factor peroxisome proliferator-activated receptor (PPAR)-γ and inhibit lipid cell differentiation, and lipid storage favouring increased lypolisis. This transition to insulin resistance contributes to greater postprandial increases in FFAs and increased hepatic glucose output that result in greater fuel availability to the fetus[2,4]. And, third TNFα and other proinflamatory mediators suppress the transcription of adiponectin in adypocite[5,6] contributing to insulin resistance.

In the other site, placental hormones like Human Placental Lactogen (hPL) and human placental growth hormone (hPGH), which are increased up to 10 fold during pregnancy, prevent insulin signalling downstream in skeletal muscle enhancing insulin resistance[2]. It is also shown that placenta growth hormone in the same way like TNFα; accelerate transition from lipogenesis to lypolisis by suppressing PPAR- γ[2]. All these metabolic changes are required to direct maternal nutritients toward fetoplacental unit allowing adequate fetal growth.

Leptin is cytokine secreted from the synciciotrophoblast of the placenta so during the first trimester of pregnancy its level rise and decline back to normal after delivery[7]. Increased level of leptin in maternal blood during pregnancy is caused by placental production and as well from adipose tissue[2,7]. Stimulation of leptin production is favorised by human Chorionic Gonadotropin

(hCG) and vice versa[8]. Insulin is another hormone that is involved in regulation of secretion of placenta leptin. There are number of insulin receptors found in the placenta with primary localisation during the beginning of gestation in syncytiothrophoblast and by the term of gestation in the endothelium[2]. In report from author Lappas it is indicated that leptin activate the release of proinflammatory cytokines and prostaglandins from human placenta, reflecting inflammatory, immunological and vascular response[7,9]. All these changes are in line with modifications of placental morphology with increased parenchyma tissue cellullarity and enhanced fetoplacental angiogenesis[10].

Adiponectin is one of the adipokines released from adipose tissue which is exclusively synthesized in adipocytes and has insulin sensitising, anti-inflammatory and antiatherogenic properties[11]. With progression of obesity and insulin resistance, expression of adiponectin in fat and in plasma decrease[12]. It is shown that adiponectin secretion in white adipose tissue decline with advancing gestation, even in lean women[13], suggesting that there are pregnancy related factors that reduce adiponectin levels.

In diabetic environment, cytokines and inflammation related genes in adipose tissue and in placenta are over expressed[10]. In the study of Tatjana Radaelli[10] it is shown that one third of the placental genes are modified during GDM reflecting a state of chronic inflammation with signs of vascular dysfunction. Over expression of TNFα in GDM is associated with increased fetal adiposity[2,10]. There are other mediators that are upregullated in GDM like interleukin (IL)-6 and leptin[14]. All together they enhance insulin resistance. In addition adiponectin may be implicated in the loss of insulin sensitivity in pregnancy with GDM through a decrease in maternal concentration.

In women with GDM insulin resistance is more severe and leads to increase concentration of glucose and other nutrients such as lipids and amino acids in the maternal blood, increasing their transfer to the fetus[15]. Therefore different metabolic perturbations in the maternal environment are transmitted across the placenta which plays a central role in fetal programming regulating fetalnutritients supply and fetal growth [16].

With the growing prevalence of obesity, scientific interest in the biology of adipose tissue has been extended to the secretory products of adypocites, since they are increasingly shown to affect several aspects in the pathogenesis of obesity related diseases. In the last decade adipose tissue has been added to the panel of endocrine organs[20.]

In recent years, a number of additional signalling molecules secreted by adipose tissue have been discovered, commonly referred to as "adypocitokines". Among these, adiponectin is perhaps the most interesting and promising compound for the clinician since it has profound protective actions in the pathogenesis of diabetes and cardiovascular diseases. Adiponectin was found to be low in obese subjects and particular in insulin resistant patients[20]

It is well acknowledged that the sequel of obesity, particularly diabetes and cardiovascular disease, are influenced to a great extent by the actions of these adipocytokines[49]. Thereby, adipose tissue directly contributes to the pathogenesis of obesity related disorders.

1.2   Fetal development in inflammatory milieu

Altered metabolic environment in GDM mother expose the fetus to high level of glucose during critical windows of development that may result in changes of the biology of adypocites and result in increased fat mass in infants[17]. It is suggested that adverse influences during fetal life can alter the structure and function of distinct cells and organ systems thereby „programming" the individual for an increased risk of developing disease in adult life[16]. Increased glucose transfer from mother with GDM to the fetus results in β cell hyperplasia, increased insulin secretion and greater fetal adiposity[18].

Leptin plays an important role in fetal growth and metabolism, in thermo genesis, haematopoesis, regulate puberty onset and reproduction[19,20]. It is mainly produced in white adipose tissue (WAT) but it is evident in other tissue like in placenta, brown adipose tissue (BAT) skeletal muscle, pituitary, ovaries, liver and gastric epithelium[21].Fetal plasma leptin is derived from placenta in early gestation, in week 7-14 up to term and from adipose tissue which start to develop from 14 weeks of gestation[22]. During development of fetus in utero the level of leptin increases and it is shown that it correlate positively with birth weight and neonatal

adiposity[23]. This fact is also explained in the study of Linnemann[22] where in growth retarded fetus concentration of leptin was lower. There are a number of factors in feto/maternal/placenta unit which are involved in leptin production like insulin, cortisol and hCG[22]. Insulin plays a chronic role in the regulation of leptine gene expression and production by WAT. In cases of hyperinsulinemia plasma concentration and gene expression in WAT is increased[21]. This role of insulin in leptin production is seen in diabetic pregnancies and also in large for gestational age (LGA) infants where leptin levels are over expressed. Steroids also cause threefold increase in level of leptin and that was evident in preterm infants when the mother have taken corticosteroids and additionally this fact explain involvement of leptin in pulmonary development in utero[24]. During pregnancy almost all hCG is released in maternal circulation and cause increased release of leptin from adipose tissue[22]. Leptin have important role in osteogenesis and that is explained with high level of receptors and high level of its expression in cartilage and bone development[22]. It is described gender differences in leptin level, with lower concentration in males than in females and this explain negative correlation that exist between leptin and testosterone[25]. Finally, the process of transition from fetus to neonate represents one of the major physiological, hormonal and environmental challenges. To survive the fetus must be prepared to termorregullate and feed independently shortly after birth. In this process Leptin together with cortisol, thyroid hormones and sympathetic nervous system plays important role in initiating breathing and thermoregulation at birth[19].

Another important role of leptin is that it acts as an afferent satiety signal in the hypothalamus, suppressing food intake and stimulating energy expenditure[26]. After birth in healthy neonate's leptin level decline dramatically and this mechanism may be important for the stimulation of feeding behaviour. But, it is suggested that elevated level of insulin and leptin during "critical periods" of fetal development in different animal models leads to a perinatally acquired "malorgansiation" of orexigenic and anorexigenic neurons in hypothalamic arcuate nucleus that might contribute to the occurrence of hyperphagia, overweight and hyperinsulinemia throughout later life[27].

A number of studies have provided evidence that leptin is involved in the pathogenesis of atherosclerotic vascular diseases, proliferation of cancer cells, and stimulate bone formation.

Adiponectin is another adypocitokine, which influence insulin sensitivity and increase fatty acid oxidation in muscle. The most coherent and important effects to date have been shown for adiponectin, which renders it the most promising adypocitokine with the potential impact for the development of therapeutic strategies.

Contrary to leptin, adiponectin, the protective factor, is decreased in obesity and has protective actions by ameliorating insulin resistance, and inhibiting atherosclerosis and possible even cancer growth.

Hormones like insulin and glucocorticoids and, proinflammatory cytokines like TNF and IL-6 downregullate adiponectin expression in adypocite. And in the other site PPAR γ increases its expression[28]. In GDM mother the level of adponectin is decreased compared with normal pregnancies and this fact can be an early event in the natural history of type 2 diabetes[11]. In the study of Pardo Ines et al. adiponectin concentration in cord blood have shown positive correlation with birth weight, birth length, gestational age and leptin, indicating that adiponectin is synthesised in fetal tissue[29]. In some other studies it is shown that high adiponectin levels did not correlate with birth weight, but have only positive correlation with gestational age[30,31,32].

Compared with adults, level of adiponectin in umbilical samples from newborns are higher, and this suggest its multiple role in tissue differentiation and growth during fetal intrauterine development[30,29]. In fetuses of GDM mother's level of adiponectin are significantly lower compared to normal fetuses off the same gestational age[30]. Low adiponectin serum concentration at baseline independently may predict the future risk for developing obesity and obesity related disorder[12]. In the recent study of Jennifer Dyer[33] it is shown that LGA neonates born to mothers with GDM have decreased insulin sensitivity at birth, and this fact may reflect alterations in metabolic programming that could contribute to later development of metabolic syndrome or T2DM in childhood or adulthood. There are two studies, one in PIMA Indians[34] and another recent analyses of American youth[35] that have linked LGA non IDM and LGA IDM neonates with later development of type 2 DM and metabolic syndrome.

Taken together we can say that infant of a GDM mother may have a variable phenotype based on the interaction of genes and the in utero environment[36]. GDM increases the risk of macrosomia,

infant with birth weight greater then 90th percentiles for gestational age and increase risk for perinatal complications and long-term risk for obesity and type 2 diabetes. While, normalising maternal glucose levels have reduced neonatal morbidity in GDM, the macrosomia rate still has remained elevated compared with the normal obstetrical population[37].

All women have to be instructed about their (sevenfold increased relative) risk of type 2 diabetes at follow-up and possibilities for diabetes prevention, in particular weight management and maintenance/increase of physical activity. Monitoring of the development of the offspring and recommendation of healthy lifestyle of the children and family is recommended.

Recent data show that prevalence of gestational diabetes has increased in several race/ethnicity groups during the past 20 years[38]. Increasing GDM contribute to increasing diabetes and obesity in their offspring. This circulus vicious, as a public health concern require more inside in understanding metabolic changes in fetal environment that will help to address these problems in the next future.

2 HYPOTHESIS

We expect that:

- The concentration of leptin and insulin in offspring of GDM mothers will be increased proportionally with increased birth weight, but the level of adiponectin in LGA newborns from GDM mothers will be decreased compared with LGA newborns from normal healthy pregnancies.
- The HDL cholesterol in LGA newborns from GDM mothers will be lower than in LGA newborns from normal pregnancies and will have positive correlation with adiponectin.

3 AIMS OF THE STUDY

3.1 Main Aim of the Study

1. The main aim of this study will be to determine a cause of enhanced fetal growth (>90 percentile) in well-controlled (daily glucose profile in regular values and optimal HbA1c values) gestational diabetic pregnancies.

3.2 Specific Aims

1. To evaluate the concentration of adiponectin, leptin and C peptide in umbilical vein blood of newborns born from mother with gestational diabetes at different gestational age and to investigate their associations with fetal development.

2. To measure adiponectin, leptin and insulin levels in maternal serum and umbilical cord serum at delivery and to examine whether or not there are correlations between those hormonal levels and neonatal birth weights, maternal body weights and body mass indexes.

3. To examine the influence of gestational diabetes on cord lipids at birth and relationship to body composition, cord insulin, leptin and adiponectin

4. To evaluate lipid metabolic profile in women with gestational diabetes and normal pregnancies.

4   MATERIALS AND METHODS

4.1   Subjects

This study was a cross sectional. Pregnant women admitted in Gynaecology Department „State Referral Centre for Diabetes in Pregnancy"in Zagreb have undergone routine physical examination and a 75 gr oral glucose tolerance test –OGTT. Blood samples were drawn at fasting and 120 min after glucose load. A diagnosis of GDM was defined by the WHO criteria of the following vein plasma glucose values: Fasting: $\geq$ 5.6 mmol/l   and 2 h: $\geq$ 7.8 mmol/l.

A total of 37 GDM mothers were randomly selected with their healthy normal babies and 46 healthy age-matched pregnant women were randomly selected with their newborn babies. Selected control women had no significant history of illness, no pregnancy related complications and no risk factor for gestational diabetes. They have normal glucose tolerance test in second and third trimester.

Inclusion criteria:

Signed informed consent

Pregnant women diagnosed for GDM for the first time.

Exclusion criteria:

 Personal history of GDM and history of previous abnormal glucose tolerance test

 Pregnancy related complications (hypertension, preeclampsia and eclampsia)

Acute or chronic infections, and

Current use of corticosteroids or other drugs

Maternal characteristics included in the study were: age, weight, height, BMI, parity, mode of delivery, and gestational age at delivery. Newborn characteristics included in the study were: gender, gestational age, head circumferences, weight, length and Ponderal index.

4.2   Methods

Baseline evaluation

On the day of the OGTT, demographic and historical information were collected by interviewer-administered questionnaire. Data collection included: 1) patient demographics; 2) information regarding current pregnancy including illnesses, infections, and medications; 3) personal medical, obstetrical, and smoking history; and 4) family history. Specific GDM risk factors were assessed including age, weight before pregnancy, weight gain during pregnancy, personal history of GDM, history of previous abnormal glucose tolerance test, previous delivery of large for gestational age infant, and family history of GDM or type 2 DM.

Analytical procedures - Biochemical measurements

The 75g OGTT was performed between 20 - 28 weeks of gestation, in the morning after an overnight fast of at least 8 h and venous blood samples were drawn at baseline and 120 min following ingestion of a standard 75-gr glucose load.

Blood samples were taken from mother before delivery and from neonates at the time of delivery from venous cord blood before separation of the placenta, and tested for concentrations of adiponectin, leptin, C-peptide, glucose, triglycerides (TG), total cholesterol (TCH), low density lipoprotein (LDL)-cholesterol and high density lipoprotein (HDL)-cholesterol. Analyses of the blood samples were blind. Serum samples obtained by centrifugation were immediately frozen and stored at −70°C until further analysis.

 Plasma triglycerides (TG) were measured by GPO-PAP method and total cholesterol by COD-PAP method; High density lipoprotein (HDL)-cholesterol and low density lipoprotein (LDL)-cholesterol were measured by cholesterol oxidase (CO) reaction method (DADE Behring, Dimension). Plasma glucose was determined by hexokinase glucose- 6-phosphate dehydrogenase method (Siemens DADE Behring, Dimension Xpand, clinical chemistry system).

Serum adiponectin, leptin and C-peptid levels were measured by the ELISA method using the kit of Bio Vendor Laboratory Medicine - Hamburg. The low limit of adiponectin detection will be 0.2μg/ml. and the intra and interassay coefficient of variation (CV) was 7.0% and 8.2%

respectively. The sensitivity of the leptin assay was 1.0 ng/ml and the intra and interassay CV was 5.95% and 11.55% respectively. The range of the C-peptide assay was between 0-16 ng/ml and the intra and interassay CV values 6.54% and 9.33% respectively.

Anthropometry

Anthropometric measurements of newborns were done immediately after delivery by paediatrician and included measurements of weight, height and head circumferences. Measurements of height and weight were obtained using a medical scale and gestational age was determined by paediatrician.

Calculation

Gestational age at delivery was calculated according to the last menstrual period and confirmed by ultrasound examination during the first trimester or early second trimester. *Gestational diabetes* was ruled out or confirmed by oral glucose tolerance test (OGTT) performed from 20-28 week of gestation. *Body mass index* (BMI) was calculated as weight (kg) per height (m^2). *Ponderal Index* at birth was calculated with the standard formula of weight (kg) divided by the cube of the length at birth (m^3). Birth weight percentiles were calculated according to published standards.

4.3 Statistical analysis

Data were analyzed with descriptive statistics, and differences between groups were assessed with t-test for normally distributed numerical variables, or nonparametric test for two independent groups (Mann-Whitney test) in case of not normally distributed numerical variables. Normality was tested with Kolmogorov-Smirnov test. Qualitative variables were tested with Chi-square statistics were possible, otherwise with two-way Fisher exact test. Spearman correlation test was used to compare not normally distributed variables and Pearson Correlation for normally distributed variables.

The level of statistical significance is chosen to be 0.05.

The SPSS version 17.0 was used to perform statistical analysis.

Power calculation was made upon published results (30, 31, 40 and 41). Estimation based on mean value for diabetic group was 23.1 µg/l with standard deviation 3.5µg/l, and control mean

value 29.7 µg/l with standard deviation 4.4 µg/l. With the expectation of equal sample sizes per group, alpha error probability of 0.05 for power of 0.90, total sample size was estimated to 18. Sample size was increased with the expectation of possible exclusion from the study, and sample stratifications for further data analysis, to 40.

4.4   Ethics

The study was conducted according to all currently valid and applied guidelines whose purpose is to assure proper conduction and protection of persons included in this research as examinees, including the Basics of Good Clinical Practice and Helsinki Declaration, Health Protection Law of the Republic of Croatia (NN 121/03), and Patient's Rights Law of the Republic of Croatia (NN 169/04).

Identity of healthy examinees and patients remained confidential and protected.

Consent form and subject information

Prior to the beginning of the trial, the investigator presented oral and written information about the investigation. The investigator had to be ensured that subject were fully informed about the aim of the study, procedures, potential risks, discomforts, and expected benefits which could come out from these investigations. Also, subjects were informed and agreed that the Health Authority personnel would require the access to data. It was emphasized that participation would be voluntary and that subjects would have the right to withdraw from the trial at any time without prejudice. A freely given, written Informed Consent was obtained from all subject prior their allocation for the treatment in accordance with the study protocol.

Ethics committee

Prior to the start of the trial, all three documents-research design of the thesis project proposal, informed consent, and agreement letter for participation of subjects, were submitted to the Research Ethic Board at Gynaecology Department Hospital in Zagreb and of the school of medicine, University of Zagreb. A written approval for these documents was obtained.

5   Results

A total of 37 pregnant women diagnosed for the first time with Gestational diabetes and their newborn babies participated in the study. As control subjects 46 healthy pregnant women and their newborn babies were selected.

Baseline characteristics and metabolic parameters of pregnant women are presented in table 1-2. The differences between groups are assessed with t-test for normally distributed numerical variables, and nonparametric test for two independent groups (Mann-Whitney test) in case of not normally distributed numerical variables.

Table 1. Patient characteristics

characteristics	GDM group	Control group	*P*-value
Patients (n)	37	46	
Maternal age(yr)	32.6 ± 6.1	30.2 ± 4.8	P=0.042
First trimester BMI (kg/m2)	29.6 ± 6.0	23.3 ± 4.3	P<0.0001
Weight gain in pregnancy (kg)	13.2 ± 6.3	15.9 ± 5.0	P=0.033
Fasting value: OGTT	5.6 ± 1.0	4.2 ± 0.4	P<0.0001
1-Hour value: OGTT	10.5 ± 1.4	7.6 ± 1.4	P=0.017
2-Hour value: OGTT	9.5 ± 1.4	5.7 ± 0.9	P<0.0001
HbA1c(%)	5.9 ± 0.4	4.0 ± 0.2	P=0.045

Values are given as mean ± SD; *T-test or U'- Mann Whitneytest.

Gestational diabetic mothers had significantly higher BMI (first trimester) than a control group (29.6 ±6.0 vs. 23.3 ± 4.3, P<0.0001) and had a higher Haemoglobin A1c value at the time of the OGTT (5.9 % vs. 4.0%, P=0.045), but the weight gain in well controlled gestational diabetic pregnancy was lower compared with normal healthy pregnancies (13.2 ±6.3 vs.15.9 ±5.0, P<0.05).

Table 2

Parameter	GDM group	Control group	*P*-value
Serum c - peptide (pmol/L)	1038.8 ± 628.1	976.9 ± 1031.3	P=0.08
Serum adiponectin (ng/mL)	10871.3 ± 5184.2	13418.9 ± 5148.6	P=0.021
Serum Leptin (ng/mL)	209.5 ± 194.4	242.6 ± 231.5	P=0.604
Serum Insulin (mU/L)	24.9 ± 23.7	22.7 ± 25.7	P=0.279
Plasma glucose (mmol/L)	5.3 ± 1.4	4.7 ± 0.6	P=0.019
HOMA - IR	5.5 ± 2.9	3.9 ± 1.8	P=0.002
Cholesterol (mmol/L)	6.11 ± 1.24	5.00 ± 0.52	P<0.0001
Tryglicerides (mmol/L)	3.80 ± 0.74	2.99 ± 0.58	P<0.0001
HDL (mmol/L)	1.80 ± 0.31	1.91 ± 0.33	P=0.866
LDL (mmol/L)	3.98 ± 0.66	2.93 ± 1.07	P<0.0001

Values are given as mean ± SD.

*T-test or U'- Mann Whitnney test .

Serum adiponectin concentration were significantly lower in gestational diabetic mothers when compared with healthy control subjects (10871.3 ± 5184.2 vs. 13418.9 ± 5148.6, P=0.021). The secretions of adiponectin in normal and complicated pregnancy with gestational diabetes suggest an involvement of adiponectin in insulin resistance during gestation. Concentration of glucose was significantly higher in GDM (5.3 ± 1.4 vs. 4.7±0.6, P<0.05) and HOMA –IR (5.5 ± 2.9 vs. 3.9 ±1.8, P=0.002) than in control subjects.

To assess the role of adiponectin in lipid metabolism it was tested lipid profile in GDM and control group. Cholesterol concentration was significantly higher in GDM (6.11 ± 1.24 vs. 5.00 ± 0.52, P<0.0001), triglycerides (3.80 ± 0.74 vs. 2.99 ± 0.58, P<0.0001) and LDL (3.98 ± 0.66 vs. 2.93 ± 1.07, P<0.0001) compared with control subjects.

Spearman correlation test was used to assess how adiponectin level correlate with BMI and as aspected, adiponectin in maternal blood correlated inversely with BMI(r= -0.428, P=0.008).

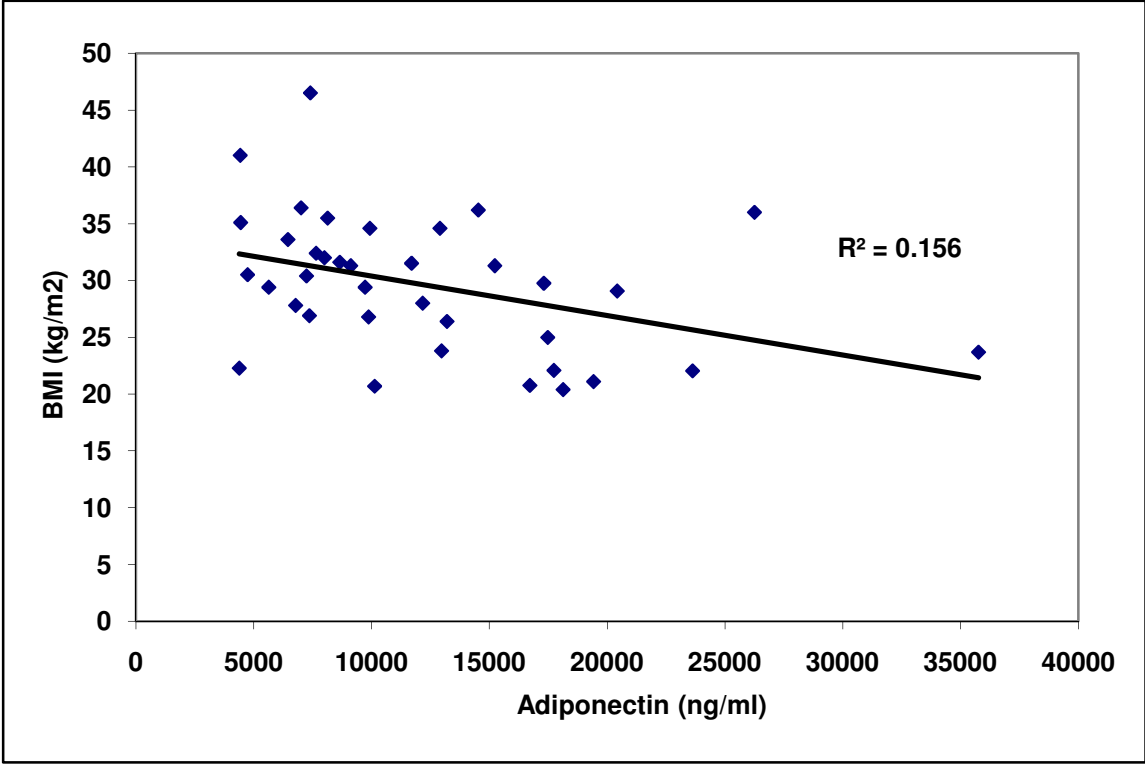Correlation: r= -0.428, 95% Cl-0.666 to -0.111, P=0.008

Figure 1.Correlation between adiponectin concentration in maternal blood and maternal body mass index-BMI

Baseline characteristics and metabolic parameters in newborns of gestational diabetic mothers and newborns of normal healthy pregnancy are shown in table 3-4.

Table 3.

Characteristics	GDM group	Control group	*P*-value
n	37	46	
Gestational age (weeks)	39.4 ± 1.6	39.4 ± 1.0	P=0.719
Body weight (g)	3644.1 ± 765.1	3513.0 ± 597.4	P<0.0001
Body length (cm)	50.8 ± 2.7	50.2 ± 2.8	P=0.921
Apgar score 1'	9.2 ± 1.6	9.5 ± 1.1	P=0.584
Apgar score 5'	9.7 ± 0.6	10.1 ± 1.5	P=0.403
HOMA- fetal IR	1.4 ± 0.9	0.6 ± 0.4	P<0.0001
Ph_umbilical	7.2 ± 0.1	7.2 ± 0.1	P=0.853

Values are given as mean ± SD.

*T-test or U'- Mann whitnney test .

Offspring's of GDM were born almost in the same gestational weeks as a control group but the weight was significantly higher in GDM (3644.1 ± 765.1 vs. 35130 ± 597.4, P<0.0001) and Insulin resistance HOMA-IR was significantly higher in offspring of GDM (1.4 ±0.9 vs. 0.6 ±0.4, P<0.0001).

The Apgar scores of the study and control patients were comparable and satisfactory.

Table 4.

Parameters	GDM group	Control group	P-value
n	37	46	
Cord c-peptid (pmol/L)	478.0 ± 285.3	282.3 ± 108.9	P=0.0002
Cord Adiponectin (ng/ml)	32136.6 ± 11103.5	39842.3 ± 8105.2	P=0.0016
Cord Leptin (ng/ml)	104.0 ± 106.3	71.8 ± 98.8	P=0.034
Cord Insulin (mU/L)	11.5 ± 12.4	4.4 ± 3.1	P<0.0001
Cord glycose (mmol/L)	3.8 ± 0.7	3.0 ± 0.7	P<0.0001
Cord cholesterol (mmol/L)	1.86 ± 0.37	1.72 ± 0.29	P=0.042
Cordtriglycerides (mmol/L)	0.37 ± 0.22	0.28 ± 0.09	P=0.026
Cord HDL (mmol/L)	0.59 ± 0.15	0.70 ± 0.17	P=0.0026
Cord LDL (mmol/L)	1.01 ± 0.28	0.74 ± 0.15	P<0.0001

Values are given as mean ± SD.

*T-test or U'- Mann whitnney test .

As shown in table 4, concentration of c- peptide (478.0 ± 285.3 vs. 282.3 ± 108.9, P=0.0002), insulin (11.5 ± 12.4 vs. 4.4 ± 3.1, P<0.0001) and leptin (104.0±106.3 vs. 71.8 ± 98.8,P=0.034) in umbilical cord blood of offspring of GDM, were significantly higher compared with normal subjects and only adiponectin concentration was significantly lower(32136.6 ±11103.5 vs. 39842.3 ±8105.2, P=0.0016).

Lipid profile also shows significantly disturbances in newborns of GDM with higher concentration of cholesterol (1.86 ±0.37 vs. 1.72 ±0.29, P=0.042), triglycerides (0.37±0.22 vs. 0.28 ±0.09, P=0.026) and LDL (1.01 ± 0.28 vs. 0.74 ±0.15, P=<0.0001) and lower HDL (0.59±0.15 vs. 0.70 ±0.17, P=0.0026) compared with newborns of normal healthy pregnancies.

On Spearman correlation analysis, maternal adiponectin correlated inversely with neonatal birth weight and maternal leptin correlated positively with neonatal birth weight but not statistically significant.
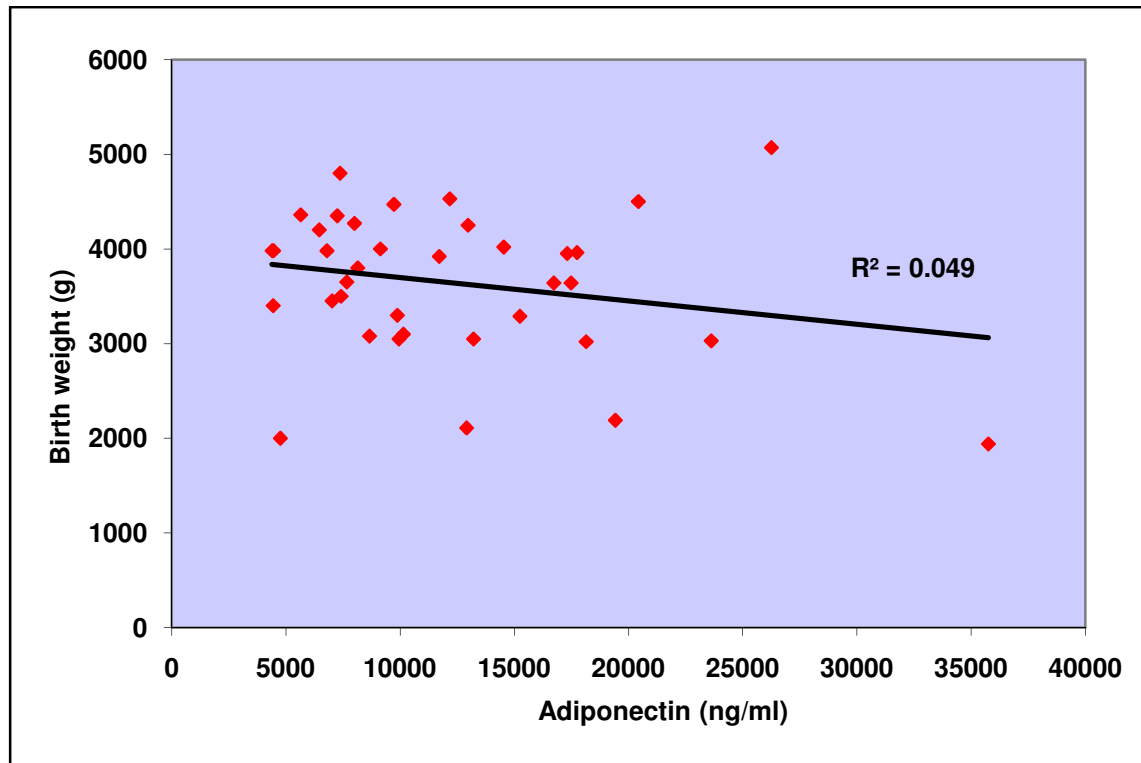
Correlation: r= -0.203, 95% Cl 0.502-0.139, P=0.228

Figure 2.Correlation between maternal adiponectin concentration and birth weight in newborns
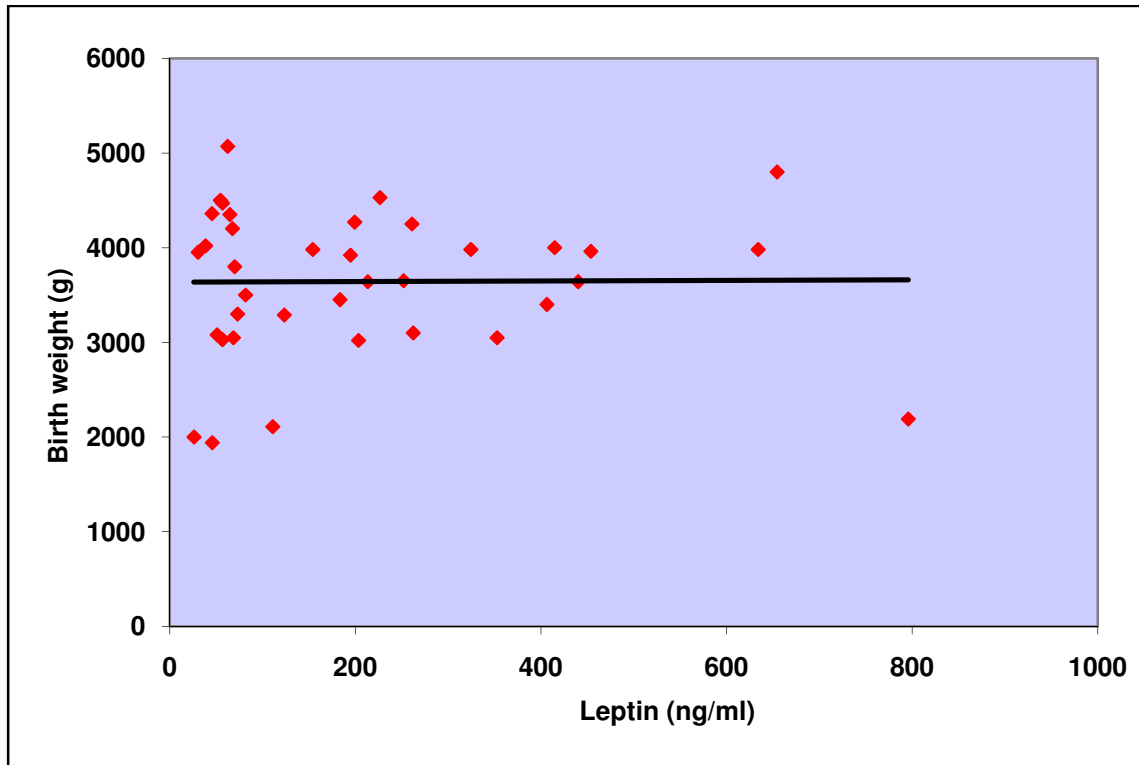
Correlation: r=0.0169, P=0.907

Figure 3.Correlation between maternal leptin concentration and birth weight in newborns

T-test was performed to assess lipid profile in offspring of GDM and control group based on neonatal growth percentiles, table 5-6.

Table 5.HDL (High density lipoprotein) cholesterol in umbilical cord blood in SGA, AGA and LGA newborns

Study group	Data	GDM group	Control group	P-value
SGA ($<10^{th}$ percentiles)	n	1	2	
	Mean ± SD	0.93 ± 0.0	0.68 ± 0.17	
AGA (10-90th percentiles)	n	26	39	0.0002
	Mean ± SD	0.58 ± 0.14	0.73 ± 0.16	
LGA ($>90^{th}$ percentiles)	n	10	5	
	Mean ± SD	0.49 ± 0.14	0.68 ± 0.19	0.043

SGA-small for gestational age, AGA-appropriate for gestational age, LGA-large for gestational age

Table 6.LDL (low density lipoprotein) cholesterol in umbilical cord blood in SGA, AGA and LGA newborns

Study group	Data	GDM group	Control group	P-value
SGA (<10th percentiles	n	1	2	
	Mean ± SD	0.78 ± 0.0	0.85 ± 0.09	
AGA (10-90th percentiles)	n	26	39	<0.0001
	Mean ± SD	0.97 ± 0.26	0.71 ± 0.13	
LGA (>90th percentiles)	n	10	5	0.023
	Mean ± SD	1.32 ± 0.36	0.85 ± 0.26	

SGA-small for gestational age, AGA-apropriate for gestational age, LGA-large for gestational age

Not only LGA neonates but also AGA neonates of GDM have significantly lower HDL (0.58 ± 0.14 vs. 0.73 ± 0.16, P=0.0002)) and higher LDL (0.97 ± 0.26 vs. 0.71 ± 0.13, P= <0.0001) compared with neonates of healthy control group.

Cord blood adiponectin concentration in LGA newborns of GDM shows significantly lower concentration compared with AGA newborns (24469.0± 10478.8 vs. 34871.4 ± 10269.7, P=0.01).Cord blood leptin and insulin concentration did not show any statistical significance between two groups.

Table 7. Concentration of cord blood adiponectin, leptin and insulin in AGA and LGA newborns of GDM

	AGA (10-90th percentiles)	LGA (>90th percentiles)	
n	**26**	**10**	***P*-value**
Cord Adiponectin(ng/ml)	34871.4 ± 10269.7	24469.0 ± 10478.8	P=0.01
Cord Leptin (ng/ml)	102.6 ± 111.8	113.6 ± 100.1	P=0.787
Cord Insulin(mU/L)	11.3 ± 14.1	12.9 ± 6.4	P=0.787

*T-test.

As aspected, concentration of insulin and C –peptide in umbilical cord blood correlated positively with neonatal birth weight. Concentration of leptin in umbilical cord blood also correlated positively with birth weight but not significantly (r=0.257, 95% CI,- 0.0824 to 0.544, P=0.124) and concentration of adiponectin correlated inversely with birth weight but not significantly(r= - 0.213, 95% CI,-0.51 to 0.128, P=0.203).
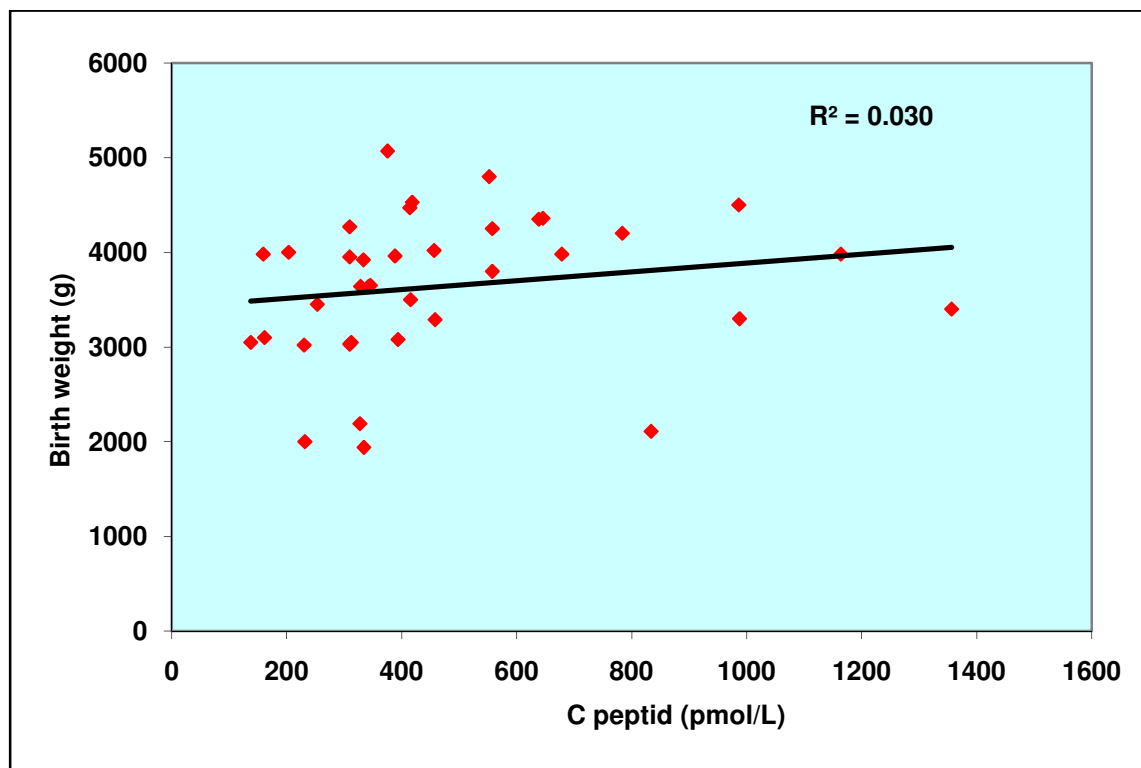
Correlation: r=0.351, 95% CI (0.021-0.612), P=0.033

Figure 4.Correlation between concentrations of cord c- peptide and birth weight in newborns

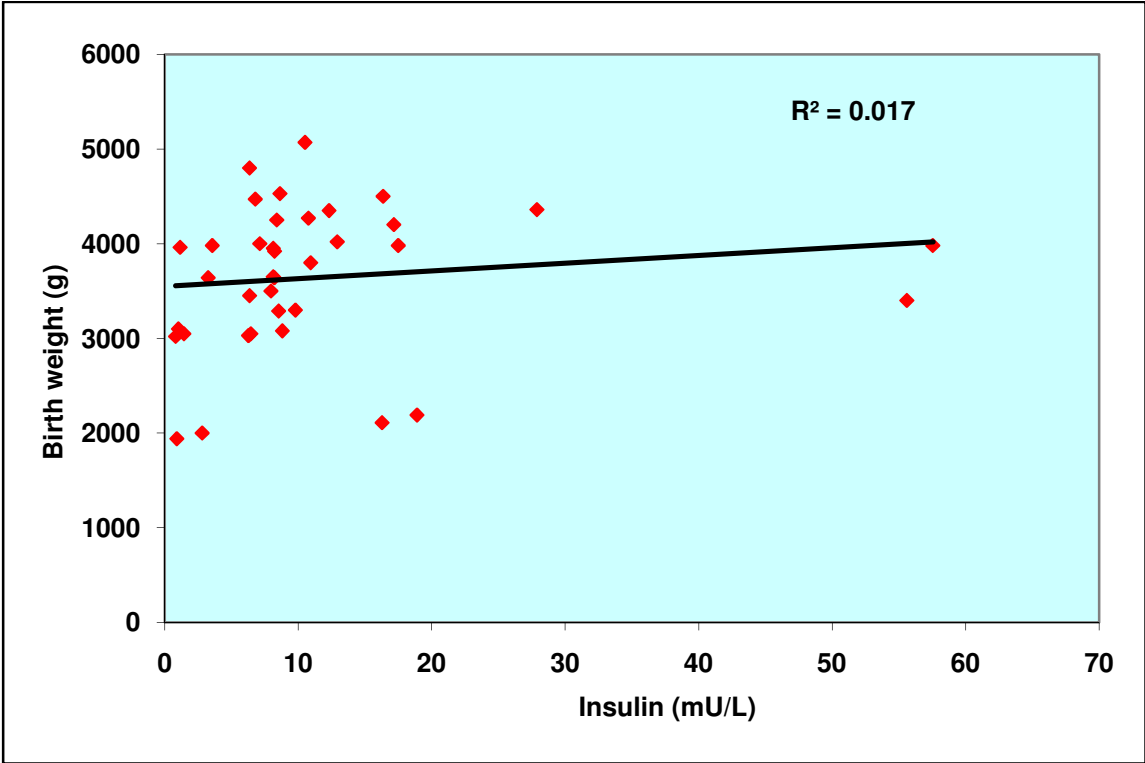Correlation: r=0.363, 95% CI (0.0351-0.621), P=0.0269

Figure 5. Correlation between concentrations of cord insulin and birth weight in newborns

Adiponectin concentration in umbilical cord blood of newborns of GDM was significantly inversely correlated with neonatal percentiles, respectively z-score (r= - 0.383, P=0.0192).

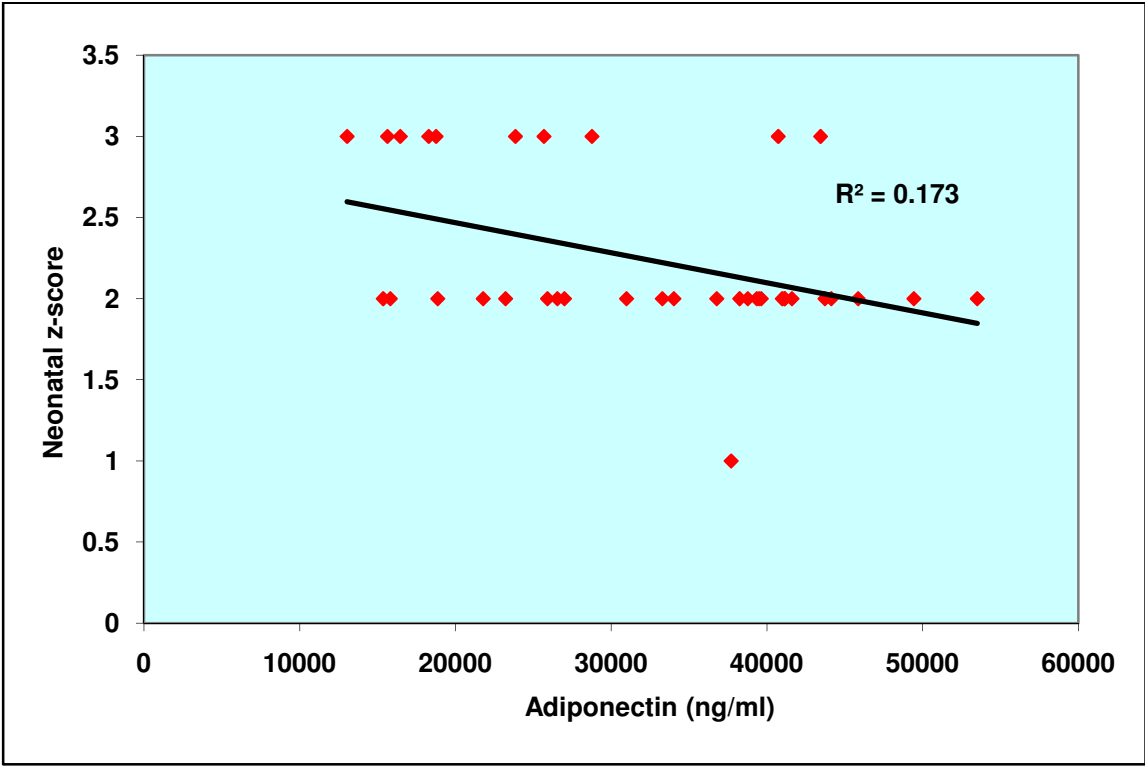Correlation: r= - 0.383, 95% CI (- 0.635 to -0.057), P=0.0192

Figure 6. Correlation between concentrations of cord adiponectin and neonatal z-score

Insulin concentration in umbilical cord blood of newborns of GDM was significantly positively correlated with neonatal percentiles, respectively z-score (r= 0.384, P=0.0187)

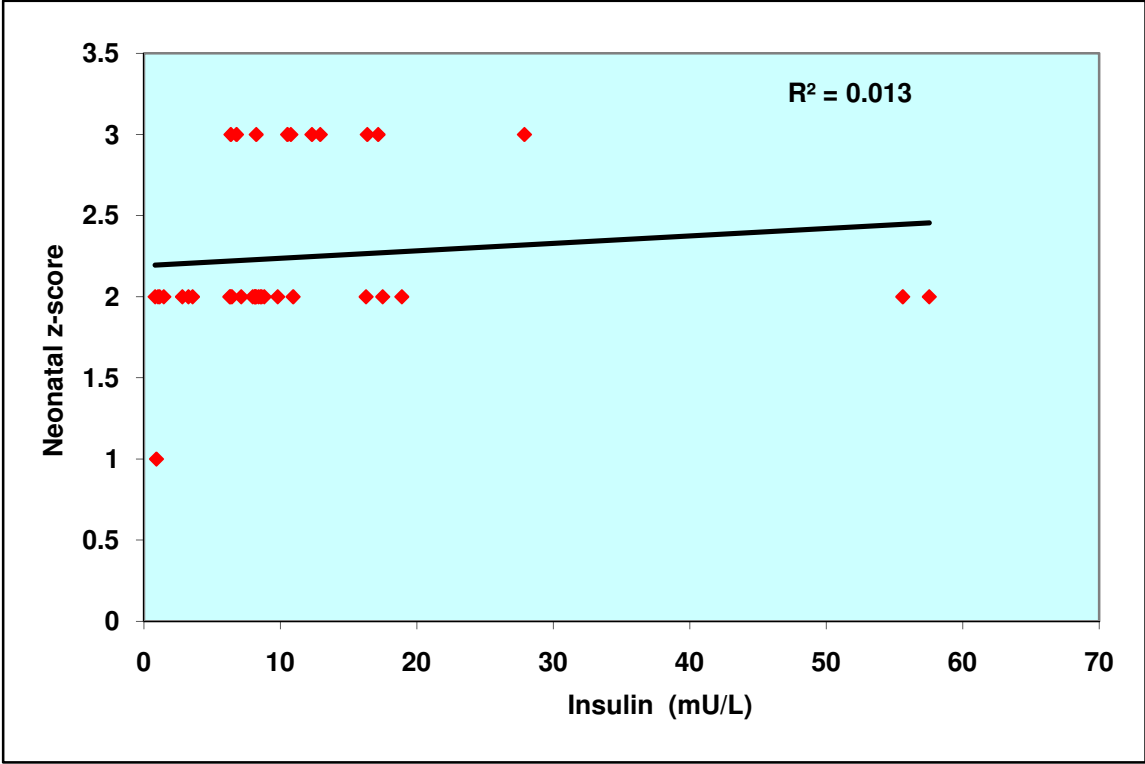Correlation: r= 0.384, 95% CI (0.059 to 0.636),P=0.0187

Figure 7. Correlation between cord insulin and neonatal z-score

Spearman rank Correlation was used to assess correlation of adiponectin with HDL, and as aspected umbilical cord adiponectin was positively correlated with HDL (r=0.325,P=0.0068).

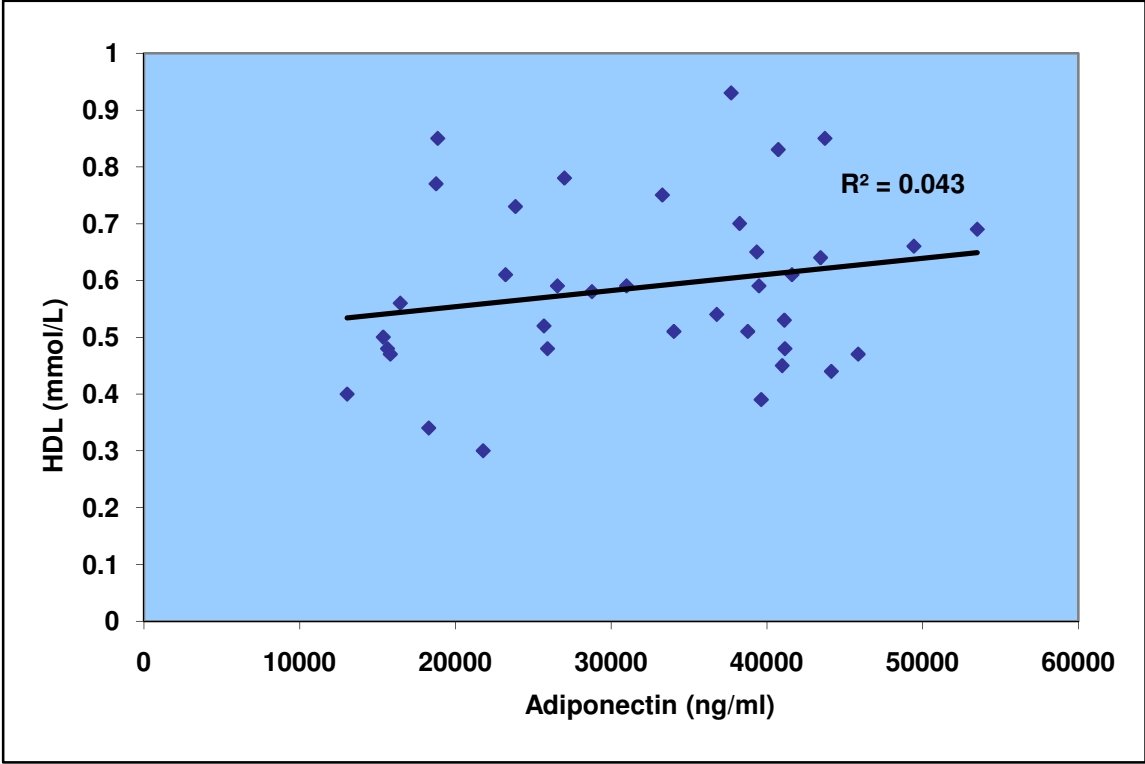Correlations: r=0.325, 95% CI (0.087 to 0.528, P=0.0068)

Figure 8. Correlation between concentrations of cord adiponectin and HDL

Concentration of adiponectin (24469.0 ± 10478.8 vs. 45359.6 ± 7592, P=0.0017) is significantly lower in LGA newborns of GDM mothers compared with LGA newborns of healthy control.

Table 8.

adiponectin	n	GDM group Mean ± SD	n	Control group Mean ± SD	P - value
AGA	26	34871.4 ± 10269.7	39	38791.3 ± 7992.6	P=0.089
LGA	10	24469.0 ± 10478.8	5	45359.6 ± 7592.0	P=0.0017

T -test

Concentration of Leptin (102.6 ±111.8 vs. 62.0 ±94.4, P=0.039) in umbilical cord is significantly higher in AGA newborns of GDM mothers compared with AGA newborns of healthy control.

Table 9.

leptin	n	GDM group Mean ± SD	n	Control group Mean ± SD	P - value
AGA	26	102.6 ± 111.8	39.0	62.0 ± 94.4	P=0.039
LGA	10	113.6 ± 100.1	5.0	127.2 ± 110.5	P=0.813

Man Whitney test , T-test

Concentration of Insulin is significantly higher in AGA (11.3±14.1 vs.4.3 ±3.2, P<0.0001) and LGA (12.9 ±6.4 vs. 5.8 ±2.9, P=0.035) newborn of GDM mothers compared with AGA and LGA newborns of healthy control.

Table 10.

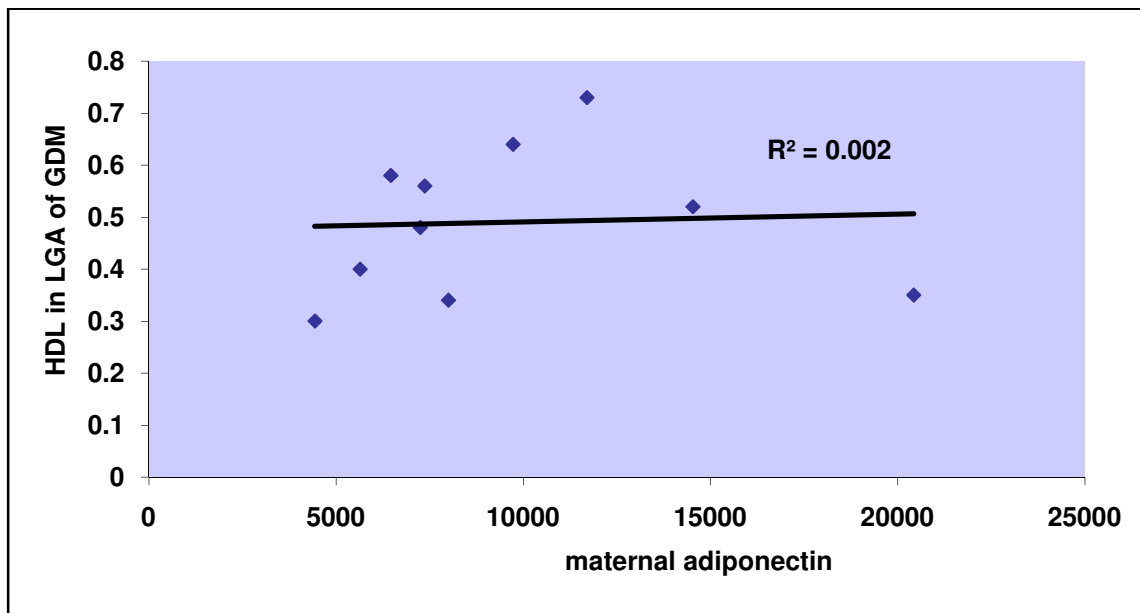| insulin	GDM group		Control group		P - value
	n	Mean ± SD	n	Mean ± SD	
AGA	26	11.3 ± 14.1	39.0	4.3 ± 3.2	P<0.0001
LGA	10	12.9 ± 6.4	5.0	5.8 ± 2.9	P=0.035

T-test

Pearson Correlation test was used to compare correlation between maternal Adiponectin and HDL cholesterol in LGA newborns of GDM mothers(r= 0.05, 95% CI -0.57 to 0.65, P=0.88)but we did not find any correlation.

Figure: 11

33

6   Discussions:

To our knowledge, this study is the first cross sectional study planned to determine a cause of enhanced fetal growth (>90 percentile) in well controlled gestational diabetic pregnancies (ondiet only) and to evaluate the level of adiponectin and its role in lipid profile in LGA newborns from GDM.

We achieved significant changes in metabolic parameters between newborns of GDM mothers and normal healthy pregnancies.

First, weight gain in offspring of GDM was significantly higher compared with offspring of normal healthy pregnancies, increased maternal BMI and increased level of serum lipids and triglycerides were the strongest predictor of fetal macrosomia in our study.

Women with GDM diagnosed for the first time during pregnancy have significantly higher BMI. Our study have shown that even in well controlled GDM mothers we find significant metabolic changes in lipid profile, increased concentration of cholesterol, triglycerides and LDL cholesterol. These changes in lipid profile can be attributed to the level of adiponectin which is significantly lower in GDM mothers compared with normal healthy control, upholding the importance of adiponectin in pregnancy induced insulin resistance.

Adiponectin as an adipokine is recognised in many studies as a key regulator of insulin sensitivity and inflammation. In our study adiponectin concentration in GDM mothers inversely correlated with BMI.In addition, significant increased of glucose and Insulin resistance in maternal blood of GDM mothers predispose the fetus to develop in inflammatory environment.

Diet control GDM mothers have significantly lower weight gain during pregnancy but this intervention seems to be not sufficient to prevent altered metabolic milieu during pregnancy.

So, there is no doubt that maternal glycaemia and BMI in women with GDM are involved in determining birth weight of newborns.

Leptin is and adipokine secreted by several tissue into the circulatory system. There are contradictory results about the level of leptin in GDM mothers. Leptin levels have been reported either elevated [39,40] or reduced [42] or unaltered [32]. In our study leptin level was lower in GDM mothers compared with healthy controlled subjects, and this might be explained that plasma leptin are markedly lowered by dieting.

Adipocytokines secreted by adipose tissue are required for a number of physiological and metabolic processes[39]. Despite their potential importance as mediators in metabolic disorders, very little is known about their implications in GDM and in offspring of GDM mothers. There are some controversial thoughts about adipocytokines concentration in cord blood of offspring from GDM mothers. In recent study of Ategboet al[40], it is shown that the level of leptin and adiponectin in LGA infants of gestational diabetic mothers were lower than in their age matched control newborns. In opposite the study of Jennifer Shine Dyer[33] shows that leptin level was higher in LGA newborns and no changes in adiponectin, whereas study of Louise Pircet al[41], did not find any differences in cord blood serum leptin, but adiponectin level was lower between those two groups. In our study, the level of adiponectin was significantly lower in LGA newborns of GDM because of higher fat mass. In the other site, leptin was slightly lower in LGA of GDM compared with LGA of control subjects with no statistical significance that might be due to a small sample size of LGA babies. Insulin concentration was significantly higher in AGA and LGA newborns of GDM compared to newborns of normal healthy pregnancies.

The exact role of adiponectin in regulating intrauterine fetal growth has not been fully elucidated. Adiponectin hypersecretion observed in neonates may suggest a multiple role for adiponectin in tissue differentiation and growth during fetal development. Adiponectin has a great role in development of metabolic syndrome, which is further supportted by the close correlations of adiponectin with risk factors and components of the metabolic syndrome, such as insulin resistance, hyperinsulinemia, triglycerides and low density lipoprotein cholesterol, all uniformly reported in a variety of studies. Association between adiponectin and blood lipids have been demonstrated in adults and adolescents[11,12], but limited data have been published on blood lipids in offspring of gestational diabetic mothers and their relation to adiponectin[43,44]. It is

known that adiponectin may modulate the plasma lipid profile in an antiatherogenic manner, as associations with high cholesterol have been shown in adults[45]. In our study the mean level of serum adiponectin in offspring of GDM was significantly lower than in offspring of healthy control subjects. In addition, LGA newborns of GDM have significantly lower adiponectin levels than AGA newborns of GDM. One possible explanation for these findings can be that adipose tissue exerts its negative feedback mechanism and the correlation with birth weight is disrupted (46). So, when the quantity of adipose tissue is abnormally higher, like in LGA newborns, the negative feedbacks intensify to a degree that depresses the level of adiponectin[46]. Several lines of evidence indicate that an increase in fat mass leads to a down-rregullation of adiponectin.LGA newborns have not only excessive amount of adipose tissue, but also larger adypocites[47]. Therefore, both the large number and size of adypocites in LGA newborns may account for the lower adiponectin levels. All these finding place the LGA newborns in a risc group for metabolic syndrome, type 2 DM and cardiovascuar diseases.

Not only macrosomic babies, also AGA newborns of GDM mothers have disturbed lipid profile compared with newborns of healthy mothers, HDL cholesterol was significantly lower and LDL cholesterol significantly higher. These finding suggest that not only LGA newborns but also AGA newborns are at increased risk for obesity related disorders

In our knowledge, there is no cross sectional study that have examine correlation of maternal adiponectin with HDL cholesterol at birth in LGA newborns born form mother with and without gestational diabetes, but our study failed to show any difference.

7   CONCLUSIONS

The GDM is a condition that can impact on the short-term and long term health of both the mother and her baby. Adipokines secreted by adipose tissue are required for a number of physiological and metabolic processes. Despite the potential importance of these agents as mediators of metabolic disorders still it is little known about their implication in GDM and macrosomia.

Current temporal trends are showing increase incidence of gestational diabetes and increasing in LGA births. Hypoadiponectinemia in women with increased BMI is associated with the pathogenesis of GDM and macrosomia.

Hyperinsulinemia and hypoadiponectinemia in macrosomic infants are predisposing factors that can contribute in development of obesity related disorder.

The findings of this study describe an altered metabolic environment for the fetus even in well controlled GDM mothers. Decreased adiponectin levels in offspring of GDM regardless of body fat mass, confer a substantially increased risk for diabetes and cardiovascular diseases, suggesting that adiponectin may even contribute directly to the pathogenesis of these diseases.

Hyperlipidemia and increased insulin resistance found in offspring of GDM mothers increase the risk for both neonatal and childhood complication such as obesity.

It is of great importance that offspring of gestational diabetic mothers should be prospectively evaluated for obesity related diseases and get more understanding in the pathogenesis of metabolic syndrome and type 2 DM.

8 SAŽETAK (ABSTRACT IN CROATIAN)

*Uvod:*Povećanje učestalosti Diabetesa u trudnoci I makrosomne djece nosi veliki rizik za razvitak bolesti povezane za pretilost kao metabolički sindrom I tip 2 dijabetesa .

Ciljevi: Cilj istraživanja je ispitati uzrok pojačanog fetalnog razvitka(> 90 centile) kod trudnica sa dobrom kontrollom gestacijskog diabetesa.

Pacijenti i Metode: 37 trudne zene sa GDM i 46 zdravih trudnica kao kontrolna grupa I njihova novorođenčad su sudjelovali u istrazivanju. Uzorci krvi uzeti su od majke prije poroda i od novorođenih u vrijeme poroda I testirana je koncentracije adiponektina, leptina , insulin , c peptida, kolesterola, triglicerida, HDL i LDL kolesterol.

Nalazi: Majke sa diabetesom u trudnoči imaju značajno veći BMI (prvotromjesečje) od kontrolne skupine (29,6 ± 6,0 vs. 23,3 ± 4,3 , P < 0,0001) , a koncentracija adiponektina su značajno niže u Majke sa diabetesom u trudnoci u usporedbi sa zdravim kontrolnim ispitanicima (10.871,3 ± 5184,2 vs 13.418,9 ± 5148,6 , P = 0,021) .
U djece majki sa diabetesom koncentracije adiponektina (24.469,0 10.478,8 ± vs 45.359,6 ± 7.592 , P = 0.0017) su znatno niže kod LGA djece majki s dijabetesom u usporedbi sa LGA djece zdrave kontrolne grupe ; I koncentracija inzulina je značajno viša u AGA (11,3 ± 14,1 vs.4.3 ± 3,2 , P < 0,0001) i LGA (12,9 ± 6,4 vs 5,8 ± 2,9 , P = 0,035) djece majki s diabetesom u usporedbi sa AGA i LGA djece zdrave kontrolne grupe.

Zakljucak::Nalazi ovog istraživanja opisuju metabolicke promene koje okružuju plod I kod dobro kontroliranog diabetesa u trudnoci . Hiperinzulinemija I hypoadiponectinemia u makrosomne dojenčadi su predisponirajući čimbenici koji mogu pridonijeti u razvoju bolesti povezane za pretilosti kasnije u životu.

9 ABSTRACT

Introduction: Increasing incidence of GDM and LGA births predispose children for obesity related diseases, metabolic syndrome and type 2 Diabetes.

Aims: The aim of the study was to investigate the cause of enhanced fetal growth (>90 centiles)in well controlled gestational diabetic pregnancies.

Subjects and methods: 37 GDM mothers and 46 healthy control subjects and their newborns participated in the cross sectional study. Blood samples were taken from mother before delivery and from neonates at the time of delivery and tested for concentration of adiponectin, leptin, insulin, c peptide, cholesterol, triglycerides, HDL and LDL cholesterol.

Results: Gestational diabetic mothers had significantly higher BMI (first trimester) than a control group (29.6 ±6.0 vs. 23.3 ± 4.3, P<0.0001) and adiponectin concentration were significantly lower in gestational diabetic mothers compared with healthy control subjects (10871.3 ± 5184.2 vs. 13418.9 ± 5148.6, P=0.021).   In offspring of GDM concentration of adiponectin (24469.0 ± 10478.8 vs. 45359.6 ± 7592, P=0.0017) is significantly lower in LGA newborns of GDM mothers compared with LGA newborns of healthy control; and concentration of Insulin is significantly higher in AGA (11.3 ±14.1 vs.4.3 ±3.2, P<0.0001) and LGA (12.9 ±6.4 vs. 5.8 ±2.9, P=0.035) newborn of GDM mothers compared with AGA and LGA newborns of healthy control.

Conclusions: The findings of this study describe an altered metabolic environment for the fetus even in well controlled GDM mothers. Hyperinsulinemia and hypoadiponectinemia in macrosomic infants are predisposing factors that can contribute in development of obesity related disorder later in their life.

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11   CURRICULUM VITAE

Vjosa Mulliqi Kotori was born on October 21, 1966, in Prishtina, Kosovo, where she completed primary school and gymnasium.

Education and professional training (graduation year):

MD: School of Medicine, University of Prishtina, 1993

Specialisation in Pediatrics: Kosovo University Clinical Centre (KUCC), 2005

Postgraduate Training:

CME Postgraduate course (1st category) in the field of Diabetology at Univeristy Clinic "VukVrhovac" School of Medicine, University of Zagreb, Croatia, 2007.

CME postgraduate course on Pediatric Endocrinology and Nephrology in Salzburg , Austria, 2011

CME postgraduate course on Endocrinology, Dubrovnik, 2010

First ISPAD CME on Diabetes, Varna, Bulgaria, 2011, 2013, 2015.

Publications: 6 papers in peer reviewed journals, 1 paper in "DiabetologiaCroatica", abstracts and international conferences.

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