Effect of Gestational Diabetes on Circulating Levels of Maternal and Neonatal Carnitine

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Abstract

Objectives: Free carnitine is reported to decrease in normal pregnancies whereas data on the effect of gestational diabetes mellitus (GDM) on maternal and offspring carnitine status are lacking. This study aimed to assess changes in carnitine status in mothers with GDM and the offspring.

Subjects/methods: Prospective case-control study of 54 pairs of mothers - neonates (27 with GDM and 27 with normal pregnancies) and 26 non-pregnant controls. Serum glucose, lipids, and total fatty acids, blood β-hydroxybutyric acid, and whole blood carnitine were assessed in mothers before labor and neonates at birth. Carnitine was assessed in dried blood-spots on Guthrie paper using Tandem Mass Spectrometry.

Results: Compared to the controls, both groups of mothers had lower free carnitine and acylcarnitine and higher triglyceride and fatty acid levels. Compared to their respective maternal group, both groups of neonates had significantly higher free carnitine and acylcarnitine and lower triglyceride and fatty acid levels. Free carnitine and acylcarnitine, were comparable between the two maternal groups, whereas they were significantly higher in neonates of GDM mothers versus healthy neonates.

Conclusions: Well controlled GDM does not exacerbate changes in free carnitine, acylcarnitine, and fatty acid levels in pregnant women, albeit it is associated with increased carnitine in the newborn indicating enhanced fatty acid oxidation. The potential association of carnitine changes with macrosomia and long-term consequences in the offspring of GDM needs further investigation.

Keywords: Acylcarnitine; Dried blood spots; Fatty acids; Lipids; Pregnancy; Tandem mass spectrometry; Triglycerides

Abbreviations: AC: Acylcarnitine; FA: Fatty Acid; FC: Free Carnitine; GDM: Gestational Diabetes Mellitus; IDM: Infants of Diabetic Mothers

Introduction

Changes in intermediary metabolism occurring during pregnancy aim to meet the energy requirements of the pregnant women and ensure adequate nutrient supply to the fetus [1,2]. Carnitine (β-hydroxy-γ-trimethylaminobutyric acid) is an essential cofactor that plays a central role in fatty acid (FA) metabolism by transferring the long-chain FAs across the inner mitochondrial membrane into the mitochondrial matrix, where they are catabolized via β-oxidation [3,4]. Impairment of the carnitine shuttle may adversely affect FA oxidation leading to serious complications, especially during metabolic derangements, including liver dysfunction, skeletal myopathy, cardiomyopathy, and hypoketotic hypoglycemia [5]. Several studies in normal pregnancies have documented low plasma free carnitine (FC) levels approaching hypoketotic hypoglycemia [6-8].

Data on carnitine levels in women with gestational diabetes mellitus (GDM) are sparse. So far, there is only one published study on carnitine levels in pregnancies complicated with GDM, which reported increased plasma FC [9]. This finding is in disagreement with the decreased FC found in children and adolescents with type I diabetes [10]. Regarding the fetus/neonate, carnitine has been reported to be increased in neonates born to mothers with uncomplicated pregnancies [6,11], whereas there are no published data on the effect of GDM on the neonatal carnitine levels. The primary aim of this study was to assess the effect of GDM on maternal and offspring’s carnitine levels. In addition, we investigated the possible association between circulating carnitine levels and FA changes in the mothers and their neonates.

Subjects and Methods

This is a prospective case-control study. Participants were recruited among pregnant women who attended the antenatal clinic of the 2nd Department of Obstetrics and Gynecology of the Aristotle University of Thessaloniki, at the Hippokration General Hospital, during a two-year period. Eligible for the study were all pregnant women with single pregnancies who had an abnormal glucose tolerance test at 26-28 weeks of gestation and no history of pre-gestational diabetes. An
equal number of pregnant women with a normal glucose tolerance test and uneventful single pregnancies comprised the group of non-diabetic pregnant women. Exclusion criteria were hypertension or pre-eclampsia during the current pregnancy, chronic maternal health conditions, and perinatal problems that could possibly affect the neonatal carnitine levels, including delivery prior to the 36th week of gestation, perinatal asphyxia, congenital anomalies, evidence of intrauterine or perinatal infections, and metabolic diseases of the newborn. The study was approved by the Scientific Committee of the Hospital and the Ethics Committee of the Medical Faculty, Aristotle University of Thessaloniki, and written informed consent was obtained from all mothers prior to recruitment.

All pregnant women were screened for gestational diabetes between the 26th and 28th week of gestation. The 2-hour oral glucose tolerance test using a loading dose of 75 g of glucose was performed. The plasma glucose threshold values for the diagnosis of gestational diabetes were 95 mg/dL, 180 mg/dL, and 155 mg/dL for the fasting, 1-hour, and 2-hour glucose values, respectively. In the case of abnormal or ambiguous oral glucose tolerance test results the test was repeated. The level of metabolic control was evaluated with regular blood glucose checks and blood HbA1c assessment according to the department policy. During the study period 27 pregnant women with GDM (GDM mothers) and 27 non-diabetic pregnant women (non-GDM mothers) matched for age, gestational age and the mode of delivery were included in the study. In 15 GDM mothers, glucose control was managed with diet whereas 12 of them required insulin treatment. Twenty-six healthy non-pregnant female volunteers, matched for age, served as controls.

Recorded data included maternal history of health problems and obstetric history, current pregnancy complications and perinatal history. Anthropometric measurements included maternal weight, height, maternal triceps skinfold thickness, birth weight (BW), length, and neonatal triceps and quadriceps skinfold thickness. The maternal body mass index (BMI = weight [kg]/height [m]²) a few days before delivery and neonatal ponderal index (PI = [BW [g]×100]/length [cm]³) at birth were also calculated.

Laboratory measurements

Fasting blood samples were obtained from all pregnant women about 1 week prior to the expected delivery date and from the non-pregnant controls. Venous blood samples from the neonates were obtained within 1 hour after birth, before the first feeding. All measurements were performed in venous blood obtained by a single bleeding. Biochemical tests included measurement of blood β-hydroxybutyrate and serum glucose, triglycerides, total cholesterol, HDL- and LDL- cholesterol, and FAs. The serum triglycerides, total cholesterol, HDL- and LDL- cholesterol were assayed using the OLYMPUS AU2700 biochemical analyzer (Olympus, Hamburg, Germany). Measurement of β-hydroxybutyrate was performed in venous blood using the MEDISENS® OPTIMUM BLOOD GLUCOSE AND KETONE SENSOR (Abbott Laboratories, Hellas, S.A., Athens, Greece). The assay range is 0.0–6.0 mmol/L. A previous study reported that the intra-assay coefficients of variation were tested on three different levels of β-hydroxybutyrate (low at the mean of 0.43, moderate at 1.08, and high at 3.55 mmol/L) were 10.5, 5.5, and 3.2%, respectively [12].

Serum samples were stored at -80°C until measurement of serum FAs. Serum FAs were measured according to the method described by Lagerstedt et al. [13] by using Gas Chromatography/Mass Spectrometry. The analysis used in our study was the AGILENT 7890A GAS CHROMATOGRAPHY (Agilent Technologies, Inc., Agilent


J Diabetes Metab
ISSN:2155-6156 JDM, an open access journal
Volume 4 • Issue 3 • 1000250
Biochemical parameters

Comparison between the non-GDM and GDM mothers showed comparable levels of serum glucose, β-hydroxybutyric acid, and lipids (Table 1). In comparison with the controls, both groups of pregnant women had significantly lower blood glucose and higher serum triglycerides and cholesterol (Table 1). Comparison between the two groups of neonates showed that the IDM had significantly lower blood glucose, although only 2 of them developed hypoglycemia (blood glucose lower than 2 mmol/L). In addition, the IDM had significantly higher serum triglyceride levels (Table 2).

Blood carnitine

Results regarding the levels of total carnitine, FC, and AC fractions in blood and comparisons among the groups of mothers and neonates are summarized in table 3 (Supplementary file-Figure 1). Values of AC are presented as sum of total AC, as well as major AC fractions, namely short-chain AC (C2- C5), medium-chain AC (C6 – C12), and long-chain AC (C14 – C18). The two groups of mothers had comparable FC, AC, and AC fraction levels. In comparison to the controls, both maternal groups had significantly lower FC, whereas the non-GDM but not the GDM mother had significantly lower AC. In all neonates carnitine fraction levels were significantly higher than in their respective mothers, except for the medium-chain AC. Comparison between healthy neonates and IDM showed significantly higher FC, AC, and short-chain AC in the IDM, whereas the difference in long-chain AC was of borderline significance (Table 3).

Serum fatty acid levels

The two groups of mothers had total FA and FA fractions significantly higher than the control levels. The neonates had total FA, long-chain (C13-C18) FA, and very long-chain (C20-C22) FA lower than the respective maternal levels. The FAs did not differ either between the two maternal groups or between the two neonatal groups (Table 4).

Correlations

The maternal FC, AC, and AC fractions were significantly positively correlated with the respective neonatal carnitine levels (Table 5). The maternal total FAs and FA fractions were not correlated with the respective neonatal FA levels (Table 5). Among the lipids, the triglycerides were significantly positively correlated the AC levels in the mothers (r=0.279, p=0.043) and the neonates (r=0.373, p=0.006).

Discussion

Our results showed that GDM was associated with increased FC, AC, and short-chain AC in the neonate whereas it did not further compromise the maternal carnitine concentrations. No mother or neonate had clinical manifestations of carnitine insufficiency. The low circulating FC levels in normal pregnancies documented
concluded that carnitine deficiency is not common in juvenile diabetes

on carnitine levels are controversial. Studies in juvenile type 1-diabetes
in GDM compared to normal pregnancy. Data on the impact of diabetes
long-chain polyunsaturated FAs for growth and development [20].

fetal development, which requires the availability of essential FAs and
utilized by the fetus [18,19]. This process is very important for normal

with lipoprotein lipase, phospholipase A2, and intracellular lipases in
the fetus is facilitated by the presence of lipoprotein receptors together
mother but it is rather transferred to the fetus. The transport of FAs to
findings support the view that the excess of lipids is not used by the
of triglycerides and FAs by inhibiting β-oxidation. Moreover, our
suggests attenuated lipid oxidation. Consequently, the low FC observed
occurring in pregnancy [17]. However, the low FC along with low AC
disputed. The increased serum triglycerides, cholesterol and FAs in late


Values are expressed as means and standard deviations; parametric ANOVA with Dunnett T3 test for multiple comparisons; significant differences: * mothers vs. controls; † IDM vs. controls; ‡ healthy neonates vs. IDM. The differences of all carnitine fractions (except for the MCAC) between the each maternal group and the respective neonatal group as well as between controls and healthy neonates were significant at p<0.001; no difference between the two maternal groups was significant; IDM: Infants of Diabetic Mothers; TC: Total Carnitine; FC: Free Carnitine; IAC: Total Acyl-Carnitine; SCAC: Short-Chain AC; MCAC: Medium-Chain AC; LCAC: Long-Chain AC

Table 3: Whole blood levels (μmol/L) of free carnitine, total acyl-carnitine, and acyl-carnitine fractions.

Values are expressed as means and standard deviations; parametric ANOVA with Dunnett T3 test for multiple comparisons; significant differences: * mothers vs. controls; † mothers vs. respective neonatal group; ‡ neonates vs. controls; no difference either between the two groups of mothers or between the two groups of neonates was significant; IDM: Infants of Diabetic Mothers; FAs: Fatty Acids; C8–C12: Medium-Chain FAs; C13–C18: Long-Chain FAs; C20–C22: Very Long-Chain FAs

Table 4: Serum levels (μmol/L) of fatty acids.

in the present study, as in several previous reports [6–8], have been
attributed to transplacental transfer of FC to the fetus, which is not
capable of producing carnitine. This is attributed to low activity of the
enzyme γ-butyrobetaine hydroxylase being about 12% of adult values at
birth [15,16]. Although no clinical evidence of carnitine insufficiency in
pregnancy has been observed, the possible involvement of the reduced
FC in the metabolic changes observed on normal pregnancy cannot be
disputed. The increased serum triglycerides, cholesterol and FAs in late
pregnancy have been attributed to the effect of the hormonal alterations
occurring in pregnancy [17]. However, the low FC along with low AC
suggests attenuated lipid oxidation. Consequently, the low FC observed
in normal pregnancy can potentially contribute to accumulation of
triglycerides and FAs by inhibiting β-oxidation. Moreover, our
findings support the view that the excess of lipids is not used by the
mother but it is rather transferred to the fetus. The transport of FAs to
the fetus is facilitated by the presence of lipoprotein receptors together
with lipoprotein lipase, phospholipase A2, and intracellular lipases in
the placenta, which allow the free FAs to be released from maternal
lipoproteins and triglycerides, transferred through the placenta and
utilized by the fetus [18,19]. This process is very important for normal
fetal development, which requires the availability of essential FAs and
long-chain polyunsaturated FAs for growth and development [20].

The regulation of metabolic pathways may be substantially different in
GDM compared to normal pregnancy. Data on the impact of diabetes
on carnitine levels are controversial. Studies in juvenile type 1-diabetes
showed decreased FC and increased AC [10] whereas other studies
concluded that carnitine deficiency is not common in juvenile diabetes
[21]. Data regarding the carnitine status in GDM are rare. In our study,
 neither the FC nor the total AC and AC fractions differed between
diabetic and normal pregnant women. This finding could be attributed
to the good glycemic control of maternal diabetes, which provided a
metabolic balance similar to that of normal pregnancy. This assumption
is supported by the lack of fasting ketosis in our GDM population and
it is in line with previous studies that reported comparable fasting
eketonemia in normal pregnancies and well-controlled GDM [22].
Similarly, studies in diabetic patients showed that the FC levels are not
decreased in the absence of diabetes-associated complications [23] or
ketosis [24]. In contrast to our results, the only previous study dealing
with carnitine levels in diabetic pregnancies reported increased FC
and decreased AC in pregnancies complicated with GDM compared to
normal pregnancies [9]. We can not find a plausible explanation for the
increased FC found in the previous study. The different assay
(radioisotopic) used by Pappa et al. [9] and measurements in plasma
instead of whole blood may have contributed to the different results.

Regarding the effect of GDM on maternal serum lipids, the
concentrations of triglycerides and FAs did not differ between the
normal and GDM pregnant women. These results contrast previous
reports of accentuated dyslipidemia in women with GDM [25]. The
only plausible explanation for this disparity could be the good glycemic
control of diabetes in our study population. In fact, previous studies in
well-controlled GDM reported that the whole body net carbohydrate
and FA utilization did not differ significantly between the GDM patients
and control subjects [26]. Other authors also reported comparable
levels of maternal FAs between normal and diabetic pregnancies [27].
These data suggest that well controlled GDM does not exacerbate the effect of pregnancy on FA metabolism.

Sufficient carnitine supply is very important for the fetus/neonate to stimulate FA oxidation and improve glucose homeostasis in order to meet the high energy requirements during delivery and early postnatal life [28]. In agreement with previous reports in cord blood, the levels of FC and AC were increased in the healthy neonates immediately after birth compared to the respective maternal values [6,7,11]. This finding is suggestive of an active, carrier-mediated transplacental transfer of FC to the fetus. The active transport of carnitine through the placenta is supported by experimental studies documenting the presence of the carnitine transporter organic cation transporter novel type II (OCTN2) in the placenta [29]. Levels of triglycerides and FAs were decreased in the neonates compared to the respective maternal levels. This finding could be due to either decreased transplacental transfer or increased utilization of FAs by the fetus/neonate. The notion of decreased transplacental transfer contrasts the high maternal FA levels as well as the findings of previous studies demonstrating the presence of lipoprotein receptors and enzymes in the placenta, which facilitate the free FAs transplacental transfer to the fetus [18,19]. On the other hand, the elevated FC and ACs found in the neonates support the notion of enhanced FA utilization by the fetus. This is in agreement with experimental studies showing high activity of the enzymes involved in FA oxidation in the human placenta [30] and the fetus [31].

So far there is no published data regarding the effect of GDM on the fetal/neonatal levels of carnitine. In our study, the IDM had significantly higher FC and AC levels than the healthy neonates. These findings are suggestive of increased β-oxidation in the IDM that is facilitated by the potentially increased FC supply via the placenta. The increased triglyceride levels in the IDM are also compatible with enhanced β-oxidation. It seems therefore that the GDM affects the carnitine-associated metabolic pathways of the fetus/neonate even in pregnancies with good glycemic control. Future studies may delineate the role of the carnitine changes and increased β-oxidation on the fetal metabolism and the short- and long-term consequences on the offspring of pregnancies complicated by GDM.

The strength of our study is that it is the first to report that the GDM affects the carnitine status in the offspring and that the changes are suggestive of increased β-oxidation. A weakness of our study is the fact that, due to limitations on the quantities of blood allowed to be withdrawn from neonates, we did not measure the neonatal serum insulin, which could add useful information regarding the glucose metabolism in the IDM.

In conclusion, our results indicate that gestational diabetes is associated with carnitine shuttle alterations in the fetus/neonate, which are compatible with enhanced FA oxidation, while it does not exacerbate the changes in carnitine-shuttle system occurring in the normal pregnant women. The potential association of the carnitine changes found in the offspring of GDM pregnancies with fetal programming during early pregnancy, i.e. before diagnosis of GDM and treatment implementation, and consequently, with the short- and long-term effects of GDM on the offspring needs further investigation.

References


