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Oxidative stress biomarkers in diabetic mothers and their newborns

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ABSTRACT

The aim of the present study is to investigate oxidant / antioxidant status in type 1 and type 2 diabetic mothers and in their respective newborns to test the hypothesis that both diabetes increases maternal and fetal oxidative stress. 20 type 1, 25 type 2 diabetic and 40 healthy control mothers and their respective newborns were consecutively recruited from the Genecology and Obstetrics Department of Tlemcen hospital. The plasma total antioxidant capacity (TAC), vitamin C and glutathione, hydroperoxides, carbonyl proteins and erythrocyte antioxidant enzyme activities (catalase, superoxide dismutase) were measured in mothers and their newborns. Changes in plasma glucose and lipid levels were also determined. Type 1 and type 2 diabetic mothers had low TAC, vitamin C and glutathione values, catalase and superoxide dismutase activities, high plasma glucose, triglycerides, hydroperoxide and carbonyl protein levels as compared to control mothers. These abnormalities were worsened in type 2 diabetic mothers. Newborns of diabetic mothers also showed decreased TAC, glutathione, antioxidant enzyme activities and increased carbonyl proteins and hydroperoxides as compared to control newborns. In conclusion, diabetic mothers and their newborns are exposed to oxidative stress. Their oxidant and antioxidant status should be carefully considered and appropriate management should be organized during the pregnancy and the early postnatal period, including antioxidant supplementation.

Key words: diabetes; pregnancy; oxidative stress; mothers; newborns.

INTRODUCTION

Fetal growth is a complex process involving the interaction of mother, placenta and fetus. The growth and development of the fetus depend upon nutrients such as glucose, lipids and amino acids as well as the genetic makeup and maternal and fetal endocrine status. The fetus is dependent on the mother to supply its nutrients. It is also dependent on the placenta, an essential organ in pregnancy, to transfer these nutrients from the maternal circulation. Thus the fetal nutrition is a reflection of that of the mother's. This interaction exists in a sensitive equilibrium. Disturbances in this relation lead to fetal developmental consequences.

Diabetes has been considered as an important factor altering maternal metabolism and complicating fetal development, regardless of diabetic type (1,2). Changes in lipid metabolism during normal pregnancy are reflected by increased serum concentrations of nonesterified fatty acids, triglycerides, cholesterol, phospholipids and apolipoproteins (3). Diabetes mellitus is also associated with alterations in lipid levels and with changes in serum lipoproteins (4). It may be hypothesized, therefore, that diabetes during pregnancy may alter lipoprotein metabolism further. The lipid profile in gestational diabetes is related to the level of insulin resistance, and the insulin sensitivity

index is correlated negatively to lipids specially triglycerides (5). Decreased maternal insulin sensitivity in gestational diabetes may increase nutrient availability to the fetus, accounting for an increased risk of fetal overgrowth and adiposity (6). Infants of diabetic mothers also showed lipid changes (7).

On the other hand, pregnancy, mostly because of the increased oxygen requirement and mitochondria-rich placenta, is a condition exhibiting increased susceptibility to oxidative stress. Evidence for this concept includes studies demonstrating elevated levels of oxidative stress markers in normal pregnancy (8). In normal pregnancy, the rate of production of ROS is offset by their elimination by antioxidant defenses (8). However, in complicated pregnancies such as gestational diabetes mellitus, excessive ROS production overpowers antioxidant defenses, leading to an overall greater degree of oxidative stress (9). A significant correlation was found between some maternal and cord blood oxidative stress markers (10).

Evidence suggests that prenatal exposure to a diabetic intrauterine environment is associated with increased risk for impaired glucose tolerance, obesity and type 2 diabetes mellitus among offspring (11). The involvement of oxidative stress is one of the earliest abnormalities observed in diabetic subjects (12). Moreover, fetuses from mothers with gestational diabetes are at increased risk of developing oxidative stress, which subsequently induces the production of highly reactive oxygen radicals, which could be an important predisposing factor for adult diseases (13). In fact, Oxidative stress has been implicated in several diseases such as atherosclerosis, diabetes, obesity and metabolic syndrome (14). However, the implication of oxidative status in newborns of diabetic mothers is not well known. In addition, few data provided a global estimation of oxidative stress in diabetic mothers and their newborns, according to the type of diabetes. The question of the possible link between fetal redox status and the development of long term metabolic abnormalities is of a great interest.

The aim of the present study is to investigate oxidant / antioxidant status in type 1 and type 2 diabetic mothers and in their respective newborns, to test the hypothesis that both diabetes increases maternal and fetal oxidative stress. This status was evaluated by assaying both plasma total antioxidant capacity (TAC), markers of lipid and protein oxidation and blood antioxidant defenses, namely erythrocyte catalase and superoxide dismutase activities, reduced glutathione and plasma vitamin C levels.

MATERIALS AND METHODS

The study population included 85 women who gave birth at the Maternity department of Tlemcen Hospital, Tlemcen, Algeria. They were recruited successively from among the women who were admitted at the hospital. A written consent was obtained from all the subjects and the study was approved by the Tlemcen Hospital Committee for Research on Human Subjects. Forty control women claimed to have no history of chronic diseases, eclampsia, infections or fetal anomalies, and all had normal weight and normal glucose tolerance test during the third trimester and within 48 hrs of delivery. Twenty pregnant women with type 1 diabetes and twenty five women with type 2 diabetes were selected. All mothers with diabetes were treated with insulin during pregnancy (multiple injection self administration of short or long acting doses of insulin) or with oral hypoglycemic drugs. Care was taken to ensure that all the subjects were of similar age, weight, height, gestational age and parity. All these women had uncomplicated term singleton pregnancies. None showed any abnormalities during labor and delivered vaginally at term. Gestational age was estimated by the last menstrual period and confirmed by a first-trimester ultrasound scan. Newborn weight was recorded immediately after delivery. Maternal and neonatal characteristics are shown inTable1.

Blood samples

Maternal fasting blood samples were obtained from the arm veins of the mothers. Cord blood samples were obtained from the umbilical vein immediately following delivery and after the cutting of the umbilical cord. Blood samples were collected in heparinized tubes, centrifuged and plasma was separated for glucose, lipids, vitamins, total antioxidant capacity, hydroperoxides and carbonyl proteins determinations. The remaining erythrocytes were washed three times in isotonic saline, hemolysed by the addition of cold distilled water (1/4), stored in refrigerator at 4 °C for 15 min and the cell debris was removed by centrifugation (2000 g for 15 min). The hemolysates were appraised for antioxidant enzyme activities and reduced glutathione assay.

Chemical analysis

Glucose and lipid determination

Plasma glucose, triglycerides and total cholesterol contents were determined by enzymatic methods (Kits Sigma Chemical Company, St Louis, MO, USA).

Total antioxidant capacity
Plasma total antioxidant capacity (TAC) was assayed by a colorimetric method based on the suppression of the oxidation of the ABTS (2,2¢-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) by the whole of plasma antioxidants according a Sigma Aldrich Kit (Saint Louis, MO, USA). Trolox was used as a reference antioxidant for calculating the TAC values.

GSH determination
Erythrocyte reduced glutathione (GSH) levels were assayed by a colorimetric method based on the reduction of 5,5′-dithiobis-(2-nitrobenzoic) acid (DTNB) by GSH to generate 2-nitro-5-thiobenzoic acid which has yellow color, according a Sigma Aldrich Kit (Saint Louis, MO, USA).

Vitamin C assay
Vitamin C levels were determined in plasma by using the method of Roe and Kuether (15).

Determination of plasma and erythrocyte hydroperoxides
Hydroperoxides(marker of lipid peroxidation) were measured by the ferrous ion oxidation-xylenol orange assay in conjunction with a specific ROOH reductant, triphenyl phosphine, according to Pierce Kits (Thermo Fisher Scientific, USA).

Determination of plasma and erythrocyte carbonyl proteins
Plasma carbonyl proteins (marker of protein oxidation) were assayed by 2,4-dinitro phenyl hydrazine reaction, according a Sigma Aldrich Kit (Saint Louis, MO, USA).

Determinations of erythrocyte antioxidant enzyme activities
Catalase (CAT EC 1.11.1.6) activity was measured by spectrophotometric analysis of the rate of hydrogen peroxide decomposition at 240 nm (16). Enzyme activity was expressed as U/g Hb.

Superoxide dismutase (EC 1.15.1.1) activity was measured by the NADPH oxidation procedure (17) and was expressed as units of SOD per g Hb.

Statistical analysis
Values are means ±SD. Statistical analysis of the data was carried out using STATISTICA (version 4.1, Statsoft, Tulsa, OK). The significance of the differences between two groups was determined by Student's t-test. Multiple comparisons were performed using ANOVA followed by the least significant difference (LSD) test. A value of $P < 0.05$ was considered to be statistically significant.

RESULTS

Biochemical parameters
Plasma glucose and triglyceride concentrations were significantly increased in type 1 and type 2 diabetic mothers as compared to control mothers; the highest values were obtained with type 2 diabetes mellitus. Plasma cholesterol levels were increased only in type 2 diabetic mothers as compared to controls.

No statistically significant differences between plasma glucose, cholesterol and triglyceride levels in newborns of type 1 diabetic mothers and control newborns were confirmed. However, newborns of type 2 diabetic mothers had significantly higher glucose and triglyceride levels than other newborn groups.

Antioxidant status markers
Plasma total antioxidant status (TAC) and erythrocyte GSH levels were lower in diabetic mothers than in control mothers whatever the type of diabetes; the lowest values were obtained with type 2 diabetes mellitus. Plasma vitamin C levels were significantly lower in type 2 diabetic mothers as compared to their controls. Erythrocyte superoxide dismutase (SOD) and catalase activities were found to be significantly decreased in diabetic mothers versus controls with an important fall in type 2 diabetic mothers.

Variations in the biomarkers of oxidative stress which were observed in the newborns of diabetic mothers were parallel to those seen in their mothers. In fact, TAC, GSH levels and SOD and catalase activities were decreased in newborns of diabetic mothers as compared to control newborns, regardless of the type of maternal diabetes. However, Vitamin C levels did not differ between newborns of diabetic mothers and control newborns.

Oxidant status markers

Plasma and erythrocyte hydroperoxide and carbonyl protein levels were higher in type 1 and type 2 diabetic mothers than in controls. The highest values were observed in type 2 diabetes mellitus. Oxidant status markers were also increased in newborns of diabetic mothers as compared to newborns of control mothers.

Table 1. Maternal and neonate characteristics

Characteristics	Control mothers	Type 1 diabetic mothers	Type 2 diabetic mothers
Number	40	20	25
Age (years)	28 ± 3.25	27 ± 4.50	30 ± 4
BMI (Kg/m^2)	22.61 ± 2.13	24.35 ± 3.12	34 ± 3.50 **$^{++}$
Duration of diabetes (years)	-	5 ± 2	7 ± 2
Parity	3 ± 1	2 ± 1	3 ± 1
Blood pressure (cmHg) systolic (SBP)	11.56 ± 1.03	12 ± 2	11.94±1.10
Blood pressure (cmHg) diastolic (SBP)	7.62 ± 1.02	7.88 ± 1.50	7.69 ± 1.40
Gestational age (weeks)	38.50 ± 1.30	38 ± 2	39 ± 1
Birth weight (g)	3270 ± 131.50	3478 ± 240	4650±188*
Sex ratio (M/F)	18/22	10/10	11/14

Values are means ± SD. BMI, Body mass index (weight / height^2); M / F, males / females.
*Significant differences between diabetic and control groups are indicated as : * P<0.01.*
Significant differences between type 2 and type 1 diabetic groups are indicated as: + P<0.01.

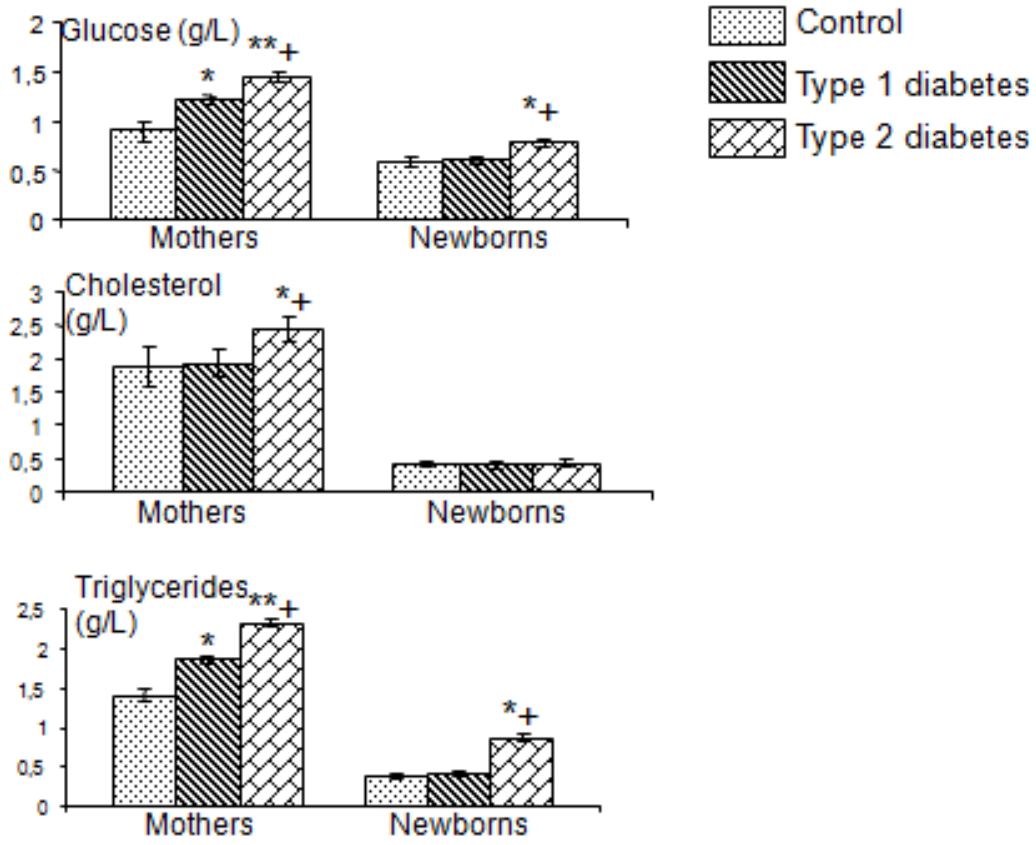

Figure 1. Plasma glucose and lipid levels in mothers and in their newborns
Values are means ± SD.
*Significant differences between diabetic and control groups are indicated as: * P<0.01; ** P<0.001.*
Significant differences between type 2 and type 1 diabetic groups are indicated as: + P<0.01.

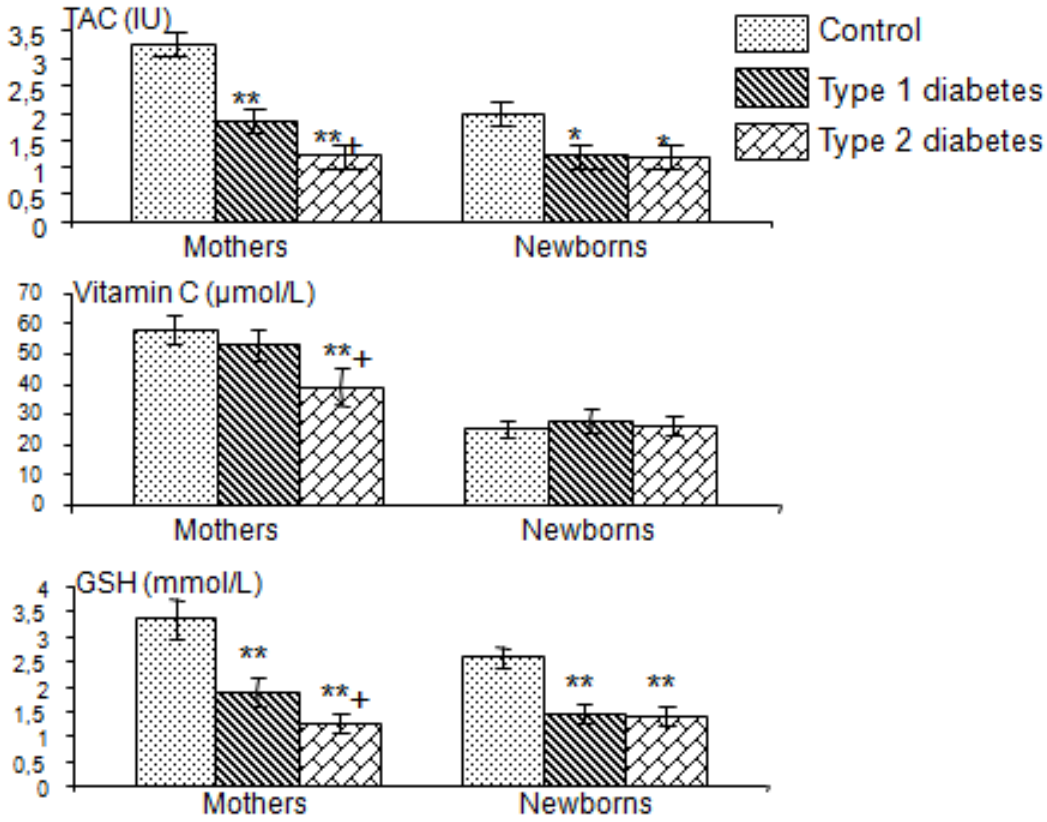

Figure 2. Antioxidant status in mothers and in their newborns
Values are means ± SD. GSH: reduced glutathione; TAC: total antioxidant capacity.
*Significant differences between diabetic and control groups are indicated as: * P<0.01; ** P<0.001.*
Significant differences between type 2 and type 1 diabetic groups are indicated as: + P<0.01.

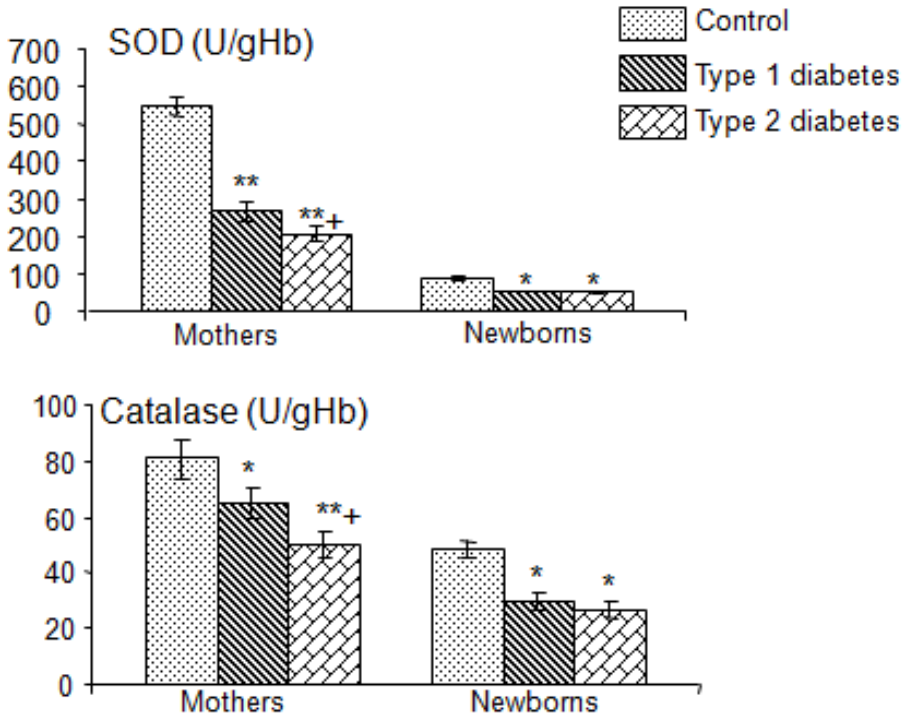

Figure 3. Erythrocyte antioxidant enzyme activities in mothers and in their newborns
Values are means ± SD. SOD: superoxide dismutase.
*Significant differences between diabetic and control groups are indicated as: * P<0.01; ** P<0.001.*
Significant differences between type 2 and type 1 diabetic groups are indicated as: + P<0.01.

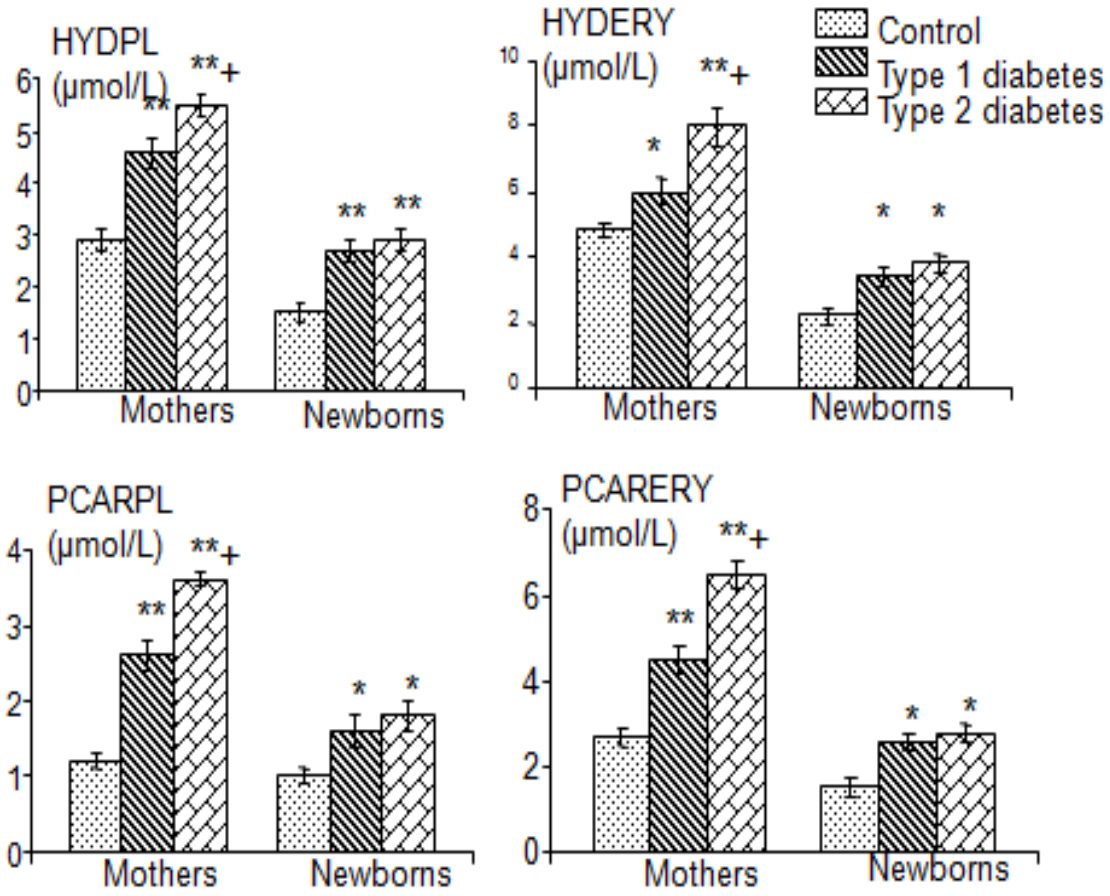

Figure 4. Oxidant status in mothers and in their newborns

Values are means ± SD. HYDPL: plasma hydroperoxides; HYDERY: erythrocyte hydroperoxides; PCARPL: plasma carbonyl proteins; PCARERY: erythrocyte carbonyl proteins.

*Significant differences between diabetic and control groups are indicated as: * P<0.01; ** P<0.001.*

Significant differences between type 2 and type 1 diabetic groups are indicated as: + P<0.01.

DISCUSSION

This study provides evidence that diabetes mellitus alters the oxidant / antioxidant status in mothers and their newborns, whatever the type of diabetes. Type 1 diabetic mothers had high serum glucose and triglyceride concentrations, while total cholesterol values were unchanged when compared with control values. However, type 2 diabetic mothers showed higher serum glucose, triglyceride and cholesterol levels than control mothers. The highest values were observed in type 2 diabetic mothers.

Hypertriglyceridaemia is well known in diabetic subjects (4) and can be accounted for by two mechanisms: enhanced hepatic VLDL and triglyceride production and reduced adipose tissue lipoprotein lipase activity which restrains VLDL removal from the circulation. In agreement with our findings, previous studies have shown that diabetic mothers had hypertriglyceridaemia (6,7). However, type 2 diabetic mothers with associated obesity had more altered glucose and lipid profiles as compared to type 1diabetic mothers and to control women (6,11). High cholesterol levels are seen in obesity which is associated with the insulin resistance state (18). Montelongo and colleagues hypothesized that the development of excess hyperlipidaemia in diabetic pregnancy depends on the balance between the degree of glycaemic control and the plasma sex hormone values during pregnancy (19).

Glucose and lipid profiles were not significantly altered in newborns of type 1 diabetic mothers, in agreement with previous studies (7). Significant positive correlation was found between maternal and foetal plasma glucose and triglyceride levels in type 2 diabetic pregnancy, supporting the hypothesis that increased plasma glucose and triglycerides may lead to an enhanced glucose and fatty acid transport through the placenta after placental lipoprotein lipase hydrolysis and may consequently increase supply to the fetus and enhance the fetal growth and

hepatic triglyceride synthesis. In our study, birth weight of newborns of type 2 diabetic mothers was significantly increased compared to control newborns. In addition, glucose and triglyceride levels in these newborns were higher than the control values. Clinical studies indicate that maternal type 2 diabetes mainly results in fetal macrosomia (1,5). Previous studies reported raised fetal hepatic VLDL secretion and hypertriglyceridemia (7). The hypercholesterolemia in the macrosomic pups at birth reflected an increase in lipoprotein particles, resulting probably from their enhanced synthesis.

Our data revealed that the total antioxidant activity (TAC) was decreased in the plasma of type 1 and type 2 diabetic mothers in favor of an oxidative stress in these women. The reduction of TAC was associated with increased oxidative stress biomarkers such as hydroperoxide and protein carbonyl levels in diabetic mothers. Elevated levels of oxidant markers in these diabetic women could result from their abnormal metabolism, hyperglycemia, or decreased antioxidant status. All forms of diabetes are associated with oxidative stress (20). Hydroperoxides and carbonyl proteins are commonly used as indicators of lipid peroxidation and protein oxidation. Protein carbonyl contents were found to be increased in diabetic subjects (21,22) and they reflect the amount of oxidative stress which the person has been exposed to, during a long time period.

Vitamin C levels were lower in type 2 diabetic mothers than in controls, in agreement with previous studies in diabetes (23). Low plasma levels of vitamin C could reflect its high utilization rate, thus, suggesting that this vitamin may be used to reduce oxidative stress in diabetic mothers.

It is well reported that oxidative stress is induced by both the increases in free radicals and by the disturbance of the free radical scavenging system in diabetes. Alternatively, it is also possible that reduced vitamin C concentrations reflect low intake (24), which resulted in a decreased antioxidant defence system in diabetic mothers.

The increase in the plasma lipid peroxidation and protein oxidation in maternal diabetes which is seen in the present study was associated with reduced erythrocyte antioxidant catalase and SOD activities and GSH levels in diabetic mothers. A reduction in SOD, the primary enzyme that inactivates the superoxide radical and in the catalase activity which is involved in the detoxification of H2O2, would lead to increased numbers of free radicals and this could thereafter be responsible for the increased levels of hydroperoxide and carbonyl proteins in diabetic mothers.

Antioxidant enzymes may also be consumed or inactivated in high oxidative conditions.

In our study, higher levels of hydroperoxides and lower levels of TAC, GSH, catalase and SOD activities in infants of diabetic mothers, when compared with control newborns suggested that these infants were exposed to greater oxidative stress. In diabetic pregnancy, neonatal oxidant/antioxidant status was closely related to the maternal status. In diabetes as well as in macrosomia, protein glycation and glucose auto-oxidation may generate free radicals, which, in turn, catalyze lipid peroxidation (25). Moreover, disturbances in the antioxidant defense system in diabetes and macrosomia have been reported as follows: alteration in antioxidant enzymes activities, impaired glutathione metabolism, and decreased ascorbic acid levels (1).

Persisting abnormalities in oxidant and antioxidant balance in newborns of diabetic mothers could be one of the processes that link fetal growth deviations with adult metabolic diseases, especially metabolic syndrome (26).

We therefore suggest follow up studies on oxidant and antioxidant status of these infants to investigate the long term consequences of oxidative markers impairment at birth.

Nevertheless, oxidant and antioxidant status in these neonates should be carefully considered, and appropriate management should be organized during the early postnatal period, including antioxidant supplementation.

Alternatively, diabetic mothers could be supplemented with additional antioxidant nutrients during pregnancy to enhance the endogenous ability of their newborns to resist oxidative stress.

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