Serum Homocysteine Levels Are Increased in Women With Gestational Diabetes Mellitus

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Serum homocysteine (sHcy) has been found to be elevated in patients with type 2 diabetes mellitus, as well as in other clinical conditions associated with insulin resistance and/or vascular diseases. The aims of this study were to measure the relationship between sHcy with biohumoral markers of insulin resistance in pregnant women affected with gestational diabetes mellitus (GDM). We studied 2 groups of pregnant women categorized, after a 100-g, 3-hour oral glucose tolerance test (OGTT) as nondiabetic (n = 78) or affected with GDM (n = 15), by measuring sHcy, serum folate, albumin, vitamin B_{12}, uric acid, and lipids. In both groups, peripheral insulin sensitivity was measured by using the OGTT-derived index of Matsuda and DeFronzo (ISI_{OGTT}). Serum homocysteine was significantly higher in the group with GDM compared with nondiabetic women (5.88 ± 2.26 μmol/L *v* 4.45 ± 1.52 μmol/L; *P* = .003); was inversely related to serum folate (*r* = -.48; *P* = .0001), and was significantly related to serum albumin (*r* = .27; *P* = .009), 2-hour plasma glucose (*r* = .25; *P* = .01), as well as to serum uric acid (*r* = .23; *P* = .03). No relationship was observed between sHcy and serum vitamin B_{12}, serum triglycerides, total, or high-density lipoprotein (HDL) cholesterol, mean blood pressure and ISI_{OGTT}. Vitamin B_{12} was correlated with ISI_{OGTT} (*r* = .36; *P* = .0005) and inversely with mean blood pressure (*r* = -.24; *P* = .02). GDM remained significantly associated with higher sHcy concentrations also after adjusting for age, serum folate, albumin, uric acid, ISI_{OGTT}, and vitamin B_{12} (*P* = .006). In conclusion, we found that sHcy is significantly increased in women with GDM, independently of other confounding variables, is significantly related to 2-hour OGTT plasma glucose, and seems unrelated to insulin resistance in these subjects.

SERUM HOMOCYSTEINE (sHcy) has previously been found to be reduced during pregnancy, and associated with adverse pregnancy outcomes or early pregnancy losses.[1-4] High levels of sHcy have been found to be associated with clinical conditions of insulin resistance, including type 2 diabetes mellitus or preeclampsia,[5-7] and there is evidence that sHcy is a major independent risk factor for vascular disease both in diabetic and nondiabetic subjects.[5,8]

Based on these premises, we studied the relationship between sHcy and glucose or insulin levels in pregnant women with normal glucose tolerance or gestational diabetes mellitus (GDM): conditions that are both physiologically or pathologically related to increased peripheral insulin resistance.

SUBJECTS AND METHODS

This study included 93 pregnant women who consecutively performed during the last semester of the year 2001 a 100-g, 3-hour oral glucose tolerance test (OGTT) between the 24th and the 28th gestational week at the Outpatient Clinic of the Diabetes Unit of the Hospital of Pistoia according to a standardized program of the healthy authorities of the Region of Tuscany and in agreement with the recommendations of the American Diabetes Association.[9] According to this protocol a full 100-g, 3-hour OGTT should be performed in all women who are glucose intolerant to a previous 1-hour 50-g oral glucose challenge or have other risk factors for GDM (history of glucose intolerance or of macrosomia during the previous pregnancies or history of diabetes in first-degree relatives). Both plasma glucose (Glucose GOD-PAP; Roche Diagnostic, Mannheim, Germany) and insulin measurements (Immunometric assay; DPC, Los Angeles, CA) were performed in all women at baseline and after 100 g glucose oral load at 60, 120, and 180 minutes. Homocysteine was measured at the OGTT baseline (Fluorescence Polarization ImmunoAssay; Abbott, Wiesbaden, Germany; coefficient of variation [CV] intra-assay, 2.2%; interassay, 3.8%) in serum samples after centrifugation for 10 minutes at 3,000 × *g* at 4°C. Blood samples were also obtained in all women to measure serum albumin (by nephelometric assay; Beckman, Fullerton, CA), folic acid and vitamin B12 (Chemiluminescent ImmunoAssay; Bayer, Leverkusen, Germany; CV intra-assay, 3.1%; interassay, 4.2%), total and high-density lipoprotein (HDL)-cholesterol, uric acid, and triglycerides, which were assayed by standardized automated methods using common commercial kits.

Women who had taken oral folate supplements 2 weeks before the test were excluded. All women had hemoglobin values exceeding 110 g/L and were allowed to take iron supplementation as prescribed. GDM was diagnosed in 15 women using the criteria suggested by the American Diabetes Association.[9] Peripheral insulin sensitivity was graded by using the OGTT-derived insulin sensitivity index (ISI_{OGTT}) of Matsuda and DeFronzo[10] obtained as follows: ISI_{OGTT} = 10,000/square root (fasting plasma glucose [mg/dL]) × fasting plasma insulin [mU/L]) × (mean glucose value during the OGTT × mean basal insulin value during the OGTT). This index has recently been validated in pregnant women with or without GDM by comparing it with the euglycemic-hyperinsulinemic clamp[11] The study was approved by the ethical committee of our Hospital.

Statistical Analysis

Two-tailed unpaired Student's *t* test was used to calculate differences in mean values between diabetic and nondiabetic women. Univariate and multiple logistic regression analyses were performed to determine associative relationships between variables. Values are expressed as means ± SD, and $P < .05$ was considered significant. All statistical evaluations were performed using SAS package for personal computers (SAS Institute, Cary, NC).[12]

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Table 1. Main Characteristics of Pregnant Women

	Normal Glucose Tolerance (n = 78)	Gestational Diabetes Mellitus (n = 15)	*P*
Age (yr)	32.3 ± 3.7	34.6 ± 3.1	.03
Parity (no.)	1.6 ± 0.8	1.7 ± 0.9	NS
Pregestational BMI (kg/m^2)	23.2 ± 3.8	23.8 ± 2.6	NS
BMI at time of test (kg/m^2)	26.3 ± 3.7	26.7 ± 3.2	NS
Gestational week of the test (no.)	29.2 ± 2.8	28.7 ± 3.4	NS
Mean blood pressure (mm Hg)	85.5 ± 6.9	86.8 ± 5.6	NS
OGTT 2-h plasma glucose (mmol/L)	7.8 ± 1.3	10.7 ± 0.8	.0001
OGTT 2-h plasma insulin (pmol/L)	746.9 ± 425.6	1,208.2 ± 910.7	NS
Serum albumin (g/L)	35.4 ± 3.1	34.5 ± 3.9	NS
Serum triglycerides (mmol/L)	2.4 ± 0.8	2.8 ± 1.3	NS
Total cholesterol (mmol/L)	6.7 ± 1.1	7.3 ± 1.2	NS
HDL-cholesterol (mmol/L)	1.6 ± 0.3	1.6 ± 0.4	NS
Serum uric acid (μmol/L)	202.2 ± 47.6	226 ± 53.5	NS
ISI_{OGTT} (mg/dL · min)	9.1 ± 3.6	7.1 ± 2.8	.02
Serum folate (nmol/L)	31.3 ± 16.5	33.3 ± 17.9	NS
Serum vitamin B_{12} (pmol/L)	234.5 ± 295.9	160.4 ± 32.1	.005*
Serum homocysteine (μmol/L)	4.45 ± 1.52	5.88 ± 2.26	.003

Abbreviations: BMI, body mass index; HDL, high-density lipoprotein; OGTT, oral glucose tolerance test; NS, not significant.
*After log transformation.

RESULTS

Women with GDM were older, had significantly higher 2-hour OGTT glucose, lower ISI_{OGTT}, and serum vitamin B12 (Table 1).

sHcy was significantly higher in the group of women with GDM (Table 1). As expected sHcy was inversely related to serum folate and was significantly related to serum albumin; it was, moreover, related to 2-hour OGTT glucose and to serum uric acid, as reported in Table 2, concerning the whole group of women. No relationship was observed between sHcy and serum vitamin B_{12}, serum triglycerides, total or HDL-cholesterol, mean blood pressure, and ISI_{OGTT}. Vitamin B_{12} was significantly related to ISI_{OGTT} ($r = .36$; $P = .0005$) and inversely to mean blood pressure ($r = -.24$; $P = .02$).

GDM remained significantly associated with higher sHcy, as well as inversely to ISI_{OGTT} values, also after adjusting for age, serum concentration of folates, albumin, uric acid, and vitamin B_{12} by means of a multiple logistic model (Table 3). Similarly, after a multiple regression model, 2-hour OGTT glucose was significantly linearly related to sHcy, independently from other possible confounders, such as serum albumin, uric acid, and folates ($P = .0017$; $R^2 = .404$).

Table 2. Correlation Coefficients (*r*) of Univariate Regressions Relating sHcy to Other Variables in the Whole Group of Women

	Correlation Coefficient *r*	*P*
Age	0.16	NS
Parity	0.0003	NS
Prepregnancy BMI	0.03	NS
BMI at time of test	−0.05	NS
OGTT 2-h plasma glucose	0.25	.01
OGTT 2-h plasma insulin	0.02	NS
Serum folates	−0.48	.0001
Serum vitamin B_{12}	−0.17	NS
Serum triglycerides	0.04	NS
Total cholesterol	0.17	NS
HDL-cholesterol	−0.06	NS
Serum uric acid	0.23	.03
Serum albumin	0.27	.009
Mean blood pressure	0.08	NS
ISI_{OGTT}	0.02	NS

DISCUSSION

The mean value of sHcy changes during normal gestation, and the sHcy concentrations in our population are, on average, very similar to the mean sHcy concentration reported at 20 to 28 weeks' gestation by Walker et al[1] in a Canadian population (4.3 ± 1 μmol/L). Nonetheless, according to our study, sHcy is significantly increased in women with GDM, and it is unlikely that this increase is due to possible differences in exogenous oral folate supplementation or in serum albumin, the 2 major mechanisms previously recognized to modulate sHcy during normal pregnancy.[1]

Table 3. Multiple Regression Logistic Model Indicating the Relationship Between GDM and sHcy Adjusted for Serum Folates, Albumin, and Prevailing Insulin Sensitivity

	Parameter Estimate	*P*
Intercept	5.415	.05
Age	0.074	NS
sHcy	0.359	.006
Serum folates	0.052	NS
Serum vitamin B_{12}	0.000	NS
Serum albumin	−0.462	NS
Serum uric acid	0.234	NS
ISI_{OGTT}	−0.131	.01

NOTE. GDM scored as present (1) or absent (0) is the dependent variable (y) in the model.

Abbreviations: GDM, gestational diabetes mellitus; sHcy, serum homocysteine.

The modest increase in sHcy in women with GDM (1.4 μmol/L, about 30% of the mean value of nondiabetic women) does not allow any conclusion about the biologic significance of such a modification. Neither is it possible to draw conclusions about any putative influence of this small sHcy increase in eventually worsening the pregnancy outcomes, as no previous study has related the risk of adverse pregnancy outcomes with sHcy levels measured during the gestation period when, as above mentioned, they are, on average, much lower than out of pregnancy. It should be noted, however, that similar increments in sHcy have been described in patients with type 2 diabetes or in pre-eclampsia when compared with unaffected control subjects.[13,14]

The question whether sHcy can be considered a simple marker of GDM or whether sHcy may cause deterioration of glucose tolerance remains unanswered by this study, similarly to other previously published studies, which have been unable to clarify whether increased levels of sHcy may somehow cause or, on the contrary, simply be a marker of hypertension, pre-eclampsia, or diabetes and its complications.[13-15] Only well-designed intervention trials with folic acid supplementation will be able to give more exhaustive explanations about this point.

Our data suggest that sHcy is related to after load glycemic levels as expressed by the 2-hour OGTT glucose concentrations. This seems to be in disagreement with a previous report by Kaplan et al[16] describing no relationship between plasma Hcy and metabolic control (expressed as glycated hemoglobin) in pregnant women affected with both type 1 and type 2 diabetes. Nevertheless, it is difficult to compare our findings with those of Kaplan et al due to major differences in the study design. The final impression, which arises from the results of these studies, however, is that sHcy in pregnant women seems to be more related to postload glucose excursions than to mean metabolic control as monitored by glycated hemoglobin values.

In this group of pregnant women, sHcy levels seem to be unrelated to insulin sensitivity similarly to what has previously been described in healthy or in nondiabetic hypertensive individuals.[17-19] It has, on the contrary, been emphasized by previous studies that in diabetes sHcy is directly related to insulin resistance, especially when associated with the presence of micro- or macrovascular complications.[5,13,20,21] Similarly, in women with pre-eclampsia and who present biohumoral signs of endothelial dysfunction, sHcy has been found to be augmented and inversely related to peripheral insulin sensitivity.[6,22] In this regard, it has previously been demonstrated that GDM is also associated with abnormal endothelial function,[23] thus somehow justifying the main finding of this study.

The association between vitamin B_{12} and GDM or insulin sensitivity during pregnancy, as testified by this study, remains unclear, because any link with vitamin B_{12} and GDM loses its significance after a multiple regression analysis in which vitamin B_{12} is a covariate. As we know, no sound results have been produced in previous studies about this issue. Some contradictory reports exist about serum vitamin B_{12} during pregnancy or pre-eclampsia[24,25]: some found this vitamin reduced in preeclamptic women,[6] while other investigators did not find any difference in vitamin B_{12} levels of pregnant women with preeclampsia compared with healthy pregnant women.[26,27]

In conclusion, our findings justify further studies to (1) investigate the biologic significance of the relationship between sHcy increase and GDM and (2) to clarify whether any intervention in pregnant women aimed at reducing the postoral load plasma glucose and increasing plasma folate lead to a synergistic action in lowering sHcy levels and, at the same time, in preserving mothers from GDM and their offspring from its detrimental effects.

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