Abnormal Carbohydrate Metabolism During Pregnancy

Association with endothelial dysfunction

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OBJECTIVE — To evaluate whether abnormal endothelial function, a common finding in premenopausal women with type 2 diabetes, is present in early states of diabetes during pregnancy, such as impaired glucose tolerance (IGT) and gestational diabetes mellitus (GDM).

RESEARCH DESIGN AND METHODS — Brachial artery flow-mediated dilatation (FMD) (endothelium-dependent) and nitrate-induced dilatation (NID) (endothelium-independent) were measured in 23 pregnant subjects with carbohydrate abnormalities (10 IGT, 13 GDM) and in 15 pregnant control subjects during the third trimester of gestation. High-resolution vascular ultrasonography was used to perform these investigations. A fasting lipid panel was obtained, and glucose and insulin values in response to a 100-g oral glucose load were also measured.

RESULTS — FMD was significantly reduced in both groups of women with abnormal carbohydrate metabolism compared with control subjects (7.6 ± 1.1% in the IGT group and 4.1 ± 0.9% in the GDM group vs. 10.9 ± 1.1% in control subjects, P < 0.04 and P < 0.0001, respectively). Significant difference in FMD was also observed between IGT and GDM groups (P < 0.04). NID was comparable in the three groups. Among all subjects, FMD showed a strong independent negative correlation with glycemic area (r = −0.60, P < 0.0001).

CONCLUSIONS — Endothelial dysfunction, an early marker of macrovascular disease, is present in pregnancies complicated by IGT and GDM. This alteration, which seems to be directly related to glycemic levels, could explain, at least in part, the increased risk for concurrent hypertensive disorders during pregnancy in these women.

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Abnormal carbohydrate metabolism during pregnancy, such as impaired glucose tolerance (IGT) and gestational diabetes (GDM), is a relatively frequent disease affecting ~2–3% of all pregnancies (1). In common with other maternal disorders associated with macrovascular dysfunction, abnormal carbohydrate metabolism is associated with increased maternal and fetal-neonatal morbidity (1,2). It has been shown that women with GDM have an increased incidence of preeclampsia (3). Furthermore, these patients are more prone to development of type 2 diabetes, a known condition that leads to an increased risk for cardiovascular disease (1), later in life. Recently, several lines of evidence seem to indicate that vascular endothelial dysfunction has a role in the etiopathogenesis of vascular abnormality associated with preeclampsia and with type 2 diabetes (4–6). In addition, impaired endothelium-dependent vasodilation has been shown in euglycemic women with a history of GDM, thus supporting the assumption that glucose metabolism derangement is closely related to vascular dysfunction (7). On the basis of these findings, it could be argued that impaired endothelial function may be a factor occurring in pregnant women with abnormal carbohydrate metabolism. To verify this hypothesis, we evaluated endothelial function, along with carbohydrate and lipid metabolism, in women with impaired glucose tolerance and with GDM during the third trimester of gestation.

RESEARCH DESIGN AND METHODS — This study was conducted at the Department of Perinatal Medicine at Catholic University in Rome and was approved by the Institutional Review Board. Informed consent was obtained from each subject before the study. During the study, ~400 women were seen at our outpatient center for routine examination during the third trimester. A total of 30 women underwent oral glucose tolerance tests (OGTTs) diagnostic for IGT and GDM according to National Diabetes Data Group criteria (8), and 23 of the 30 women tested were enrolled in our study. A total of 20 glucose-tolerant pregnant women were approached at the comparable week of gestation to participate as control subjects; 15 of these women were enrolled in the study. All three groups of women were matched for age, parity, and gestational week of testing. All subjects were white. No subjects had hypertension during pregnancy, according to the National High Blood Pressure Education Program criteria (9). None of the subjects took medications known to affect endothelial function or glycemic and lipid metabolism. No subjects smoked before or during pregnancy. Demographic information from each subject included maternal age, parity, prepregnancy BMI (calculated

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Abbreviations: AUC, area under the curve; FMD, flow-mediated dilatation; GDM, gestational diabetes mellitus; IGT, impaired glucose tolerance; MAP, mean arterial pressure; NEFA, nonesterified fatty acid; NID, nitrate-induced dilatation; OGTT, oral glucose tolerance test.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.
using habitual weight), and weight gain during gestation. Neonatal data included week of delivery, birth weight, and birth weight percentile. The study protocol, performed between gestational weeks 28 and 39, was divided into day 1 (OGTT and lipid evaluations) and day 2 (hemodynamic assessment). The OGTT (100-g oral glucose load) was performed after 2 days of a standard diet containing at least 250 g of carbohydrate per day. Samples were collected at 8:00 A.M. (after an overnight fast) and at 60, 120, and 180 min after glucose ingestion and were centrifuged immediately. An aliquot of plasma was stored at −20°C pending insulin assay. Plasma glucose levels were measured using the glucose oxidase method (Beckman, Fullerton, CA); plasma insulin concentrations were analyzed with a commercially available enzymatic assay (Wako Nefa C; Wako, Neuss, Germany).

The ultrasound investigation for measuring endothelium-dependent and endothelium-independent arterial dilatation was performed as described previously (10). Briefly, diameter of the brachial artery was measured using B-mode ultrasound images, with the use of a 7.5-MHz linear-array transducer and a standard ESAOTE AU 570 A system (Ansaldo, Milan, Italy). In all studies, scans were obtained with the subject at rest, during reactive hyperemia, again with the subject at rest, and after sublingual administration of nitroglycerin. The velocity of arterial flow was measured with a pulsed Doppler signal. Increased flow was induced by the inflation of a pneumatic tourniquet placed around the forearm (distal to the scanned part of the artery) to a pressure of 250 mmHg for 4.5 min, followed by release. A scan was performed continuously for 30 s before and 90 s after deflation of the cuff, including a repeated recording of flow velocity for the first 15 s after the cuff was released. Thereafter, 10–15 min was allowed for recovery of the vessel, after which an additional resting scan was performed. Sublingual nitroglycerin spray (400 μg) was then administered, and 3–4 min later, the last scan was performed. For the reactive-hyperemia scan, measurements of diameter were taken 50–60 s after deflation of the cuff. The vessel diameter in scans obtained after reactive hyperemia (flow-mediated dilatation [FMD]) and the administration of nitroglycerin (nitrate-induced dilatation [NID]) was expressed as a percentage of the average diameter of the artery in the two resting (or control) scans (considered as 100%). Reactive hyperemia was calculated as the maximal flow recorded in the first 15 s after cuff deflation divided by the flow during the first resting (baseline) scan. Each subject was studied in the morning after abstaining from alcohol, caffeine, and food for 8 h.

**Statistical analysis**
ANOVA with Fisher’s protected least significant differences test was used to detect the significance of the differences between means in the different groups. Repeated-measures ANOVA was used to identify differences in the insulin and glucose values in response to OGTT. Comparisons between frequencies were assessed by χ² analysis. Linear regression analysis was used for relationships between FMD and the metabolic characteristics studied. Subsequently, variables in which correlation with FMD achieved near statistical significance (P < 0.1) were entered into a stepwise regression model to assess the magnitude of their individual effects on FMD. The sample size for FMD was calculated for power 0.80, assuming a difference in means of ~5 and SD ~3 from our preliminary findings. Data are given as mean ± SD. Statistical significance was accepted at a level of P < 0.05.

**RESULTS** — Demographic characteristics of the pregnant control subjects and the two groups with abnormal carbohydrate metabolism are shown in Table 1. Women with GDM had a significantly higher BMI than control subjects. Similarly, their prepregnancy BMI was significantly higher than those of IGT and control subjects. Women with IGT showed a significant increase in weight gain compared with control subjects. Both groups of pregnant women with ab-

### Table 1—Demographic characteristics

<table>
<thead>
<tr>
<th></th>
<th>Control subjects</th>
<th>IGT</th>
<th>GDM</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>31.9 ± 0.5</td>
<td>30.4 ± 1.4</td>
<td>33.0 ± 1.2</td>
<td>1.903</td>
<td>0.236</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.65 ± 0.8</td>
<td>1.64 ± 2.3</td>
<td>1.63 ± 1.5</td>
<td>0.275</td>
<td>0.761</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>72.2 ± 1.2</td>
<td>75.7 ± 3.9</td>
<td>79.6 ± 6.6</td>
<td>1.444</td>
<td>0.276</td>
</tr>
<tr>
<td>Pregravid weight (kg)</td>
<td>64.4 ± 1.8</td>
<td>63.3 ± 3.2</td>
<td>71.0 ± 6.6</td>
<td>1.516</td>
<td>0.233</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.6 ± 0.4*</td>
<td>27.9 ± 1.1</td>
<td>29.7 ± 1.3</td>
<td>2.651</td>
<td>0.084</td>
</tr>
<tr>
<td>Prepregnancy BMI (kg/m²)</td>
<td>23.7 ± 0.5</td>
<td>23.4 ± 0.9</td>
<td>26.5 ± 1.4†</td>
<td>2.903</td>
<td>0.068</td>
</tr>
<tr>
<td>Weight gain (kg)</td>
<td>7.8 ± 1.3†</td>
<td>12.4 ± 1.1</td>
<td>8.6 ± 1.5</td>
<td>2.933</td>
<td>0.066</td>
</tr>
<tr>
<td>Parity (nulliparous/parous)</td>
<td>10/5</td>
<td>7/3</td>
<td>9/4</td>
<td>0.982</td>
<td></td>
</tr>
<tr>
<td>Week of delivery</td>
<td>39.3 ± 0.3§</td>
<td>38.4 ± 0.3</td>
<td>38.5 ± 0.3</td>
<td>2.861</td>
<td>0.070</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>3298 ± 86*</td>
<td>3444 ± 90</td>
<td>3696 ± 93</td>
<td>5.344</td>
<td>0.009</td>
</tr>
<tr>
<td>Birth weight percentile</td>
<td>58 ± 4∥</td>
<td>76 ± 4</td>
<td>86 ± 2</td>
<td>18.005</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are means ± SEM. *P < 0.04 control subjects versus GDM, †P < 0.05 GDM versus other groups, ‡P < 0.03 control subjects versus IGT, §P < 0.05 control subjects versus other groups, || P < 0.001 control subjects versus other groups.
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![Figure 1](image1.png)  
**Figure 1**—Mean plasma glucose (A) and insulin (B) levels at fasting and after OGTT in control (CTR) pregnancies (n = 15), in pregnancies with GDM (n = 13), and in pregnancies with IGT (n = 10). *P < 0.001 CTR versus other groups; †P < 0.002 GDM versus IGT, repeated-measures ANOVA.

![Figure 2](image2.png)  
**Figure 2**—Linear regression analysis of percentage increase in FMD and glucose area.

Newborns of GDM pregnancies had an 11% higher birth weight than that of control pregnancies (P < 0.04). Birth weight percentile, calculated according to a national standard curve of the general population (11), was significantly higher in both groups with carbohydrate metabolism abnormality than in control subjects.

Metabolic characteristics of the groups studied are shown in Fig. 1 and Table 2. As expected, glucose AUC and values at fasting and in response to OGTT were significantly lower in control subjects than the other groups. Similarly, lower glycemic values were also found in IGT than GDM subjects (Fig. 1A). Insulin levels, at fasting and in response to glucose challenge, did not differ among the three groups studied (Fig. 1B). Total cholesterol, LDL cholesterol, HDL cholesterol, and triglyceride levels were similar among all groups. NEFAs were significantly higher in both groups with abnormal glucose metabolism than in control subjects. Blood pressure, although in normal range in all subjects, was higher in women with GDM, in whom mean arterial pressure (MAP) levels were significantly higher than in the other groups. Baseline brachial artery diameter (vessel size) was similar in the three groups (Table 2). Baseline velocity and the percentage increase in blood velocity after ischemic stimulus (reactive hyperemia) were comparable in all groups. Interestingly, FMD (i.e., endothelium-dependent dilatation) of women with IGT was 70% of that in pregnant control subjects (P < 0.04), whereas women with GDM exhibited values of FMD at ~38% of that of control subjects (P < 0.0001). FMD of subjects with GDM was roughly half that of subjects with IGT (P < 0.04). NID (endothelium-independent) was comparable in the three groups.

To better investigate the relation between endothelial function and carbohydrate metabolism, we performed linear regression analysis between FMD and the various parameters examined. Linear regression analysis showed a strong negative relation between FMD and glucose AUC, which was highly significant (r = −0.60, P < 0.0001; Fig. 2). Similarly, even if less strong, correlation was found between FMD and fasting glucose (r = −0.58, P = 0.0001), as well as plasma glucose levels at 60, 120, and 180 min after OGTT (r = −0.57, P = 0.0001; r = −0.56, P = 0.0002; r = −0.58, P = 0.0001; respectively). In contrast, FMD did not show any significant relation to plasma insulin levels determined at fast-

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**Table 2—Comparison of metabolic and vascular characteristics between control pregnancies and women with GDM and IGT**

<table>
<thead>
<tr>
<th></th>
<th>Control subjects</th>
<th>IGT</th>
<th>GDM</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week of study</td>
<td>35.7 ± 0.3</td>
<td>33.8 ± 1.3</td>
<td>34.0 ± 0.5</td>
<td>4.666</td>
<td>0.460</td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>67.1 ± 0.8*</td>
<td>73.2 ± 9.7</td>
<td>82.9 ± 2.3†</td>
<td>27.303</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>AUC glucose 0–180</td>
<td>16,979 ± 2177#</td>
<td>20,107 ± 132</td>
<td>24,844 ± 7888</td>
<td>51.476</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fasting insulin (µU/ml)</td>
<td>10.5 ± 1.2</td>
<td>10.4 ± 0.7</td>
<td>11.2 ± 2.0</td>
<td>0.097</td>
<td>0.907</td>
</tr>
<tr>
<td>AUC insulin 0–180</td>
<td>13,622 ± 1,095</td>
<td>15,756 ± 623</td>
<td>15,938 ± 2,154</td>
<td>0.790</td>
<td>0.461</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>219.1 ± 12.4</td>
<td>229.6 ± 9.3</td>
<td>199.4 ± 9.9</td>
<td>1.757</td>
<td>0.187</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>244.5 ± 9.6</td>
<td>248.4 ± 8.9</td>
<td>240.2 ± 4.7</td>
<td>0.276</td>
<td>0.760</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>58.8 ± 2.5</td>
<td>64.7 ± 2.1</td>
<td>65.0 ± 2.4</td>
<td>2.197</td>
<td>0.126</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>148.1 ± 4.9</td>
<td>154.3 ± 4.7</td>
<td>140.9 ± 3.7</td>
<td>1.945</td>
<td>0.158</td>
</tr>
<tr>
<td>NEFA (mg/dl)</td>
<td>0.317 ± 0.017</td>
<td>‖</td>
<td>0.520 ± 0.058</td>
<td>0.495 ± 0.023</td>
<td>11.043</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>79.7 ± 1.3</td>
<td>78.7 ± 1.3</td>
<td>87.0 ± 2.6</td>
<td>6.314</td>
<td>0.0046</td>
</tr>
<tr>
<td>Vessel size (mm)</td>
<td>3.4 ± 0.06</td>
<td>3.3 ± 0.13</td>
<td>3.5 ± 0.10</td>
<td>1.612</td>
<td>0.214</td>
</tr>
<tr>
<td>FMD (%)</td>
<td>10.9 ± 1.1#</td>
<td>7.6 ± 1.1**</td>
<td>4.1 ± 0.9</td>
<td>11.63</td>
<td>0.0001</td>
</tr>
<tr>
<td>NID (%)</td>
<td>15.2 ± 1.6</td>
<td>14.6 ± 2.1</td>
<td>11.3 ± 0.9</td>
<td>1.933</td>
<td>0.1599</td>
</tr>
<tr>
<td>Baseline velocity (m/s)</td>
<td>0.15 ± 0.004</td>
<td>0.15 ± 0.008</td>
<td>0.15 ± 0.008</td>
<td>0.135</td>
<td>0.873</td>
</tr>
<tr>
<td>Reactive hyperemia (%)</td>
<td>325 ± 38</td>
<td>350 ± 68</td>
<td>335 ± 30</td>
<td>0.073</td>
<td>0.929</td>
</tr>
</tbody>
</table>

Data are means ± SEM. *P < 0.03 control subjects versus IGT, †P < 0.0005 GDM versus other groups, ‡P < 0.0002 GDM versus IGT, †P < 0.01 control subjects versus other groups, ‡P < 0.005 GDM versus other groups, §P < 0.0001 control subjects versus GDM, **P < 0.04 IGT versus other groups.
ing and at 60, 120, and 180 min after OGTt (r = –0.03, P = 0.83; r = –0.04, P = 0.79; r = –0.23, P = 0.17; r = –0.21, P = 0.21; respectively). A relatively more robust correlation was found to insulin AUC (r = –0.26, P = 0.10).

FMD exhibited significant correlation with NEFA (r = –0.41, P < 0.01) and with BMI (r = –0.41, P < 0.01). It has been shown that hyperglycemia, hyperinsulinemia, adiposity, and increased levels of NEFA all may affect endothelial function. Therefore, to evaluate their independent contribution to predict FMD, we performed stepwise regression analysis. The analysis showed that glucose AUC accounted for 35% of the variance of FMD (P < 0.0001), whereas NEFA contributed an additional 5% (P < 0.0001). BMI and insulin AUC did not contribute to the regression model.

**CONCLUSIONS** — The results of this study show the following new findings: 1) compared with control pregnancies, women with abnormal carbohydrate metabolism show an impaired FMD, more remarkable in subjects with GDM than those with IGT; and 2) FMD is inversely and strongly related to glucose area.

FMD was examined, at resting condition, by measuring the percentage increase in vessel diameter in response to an increased blood flow during postocclusion hyperemia. It is recognized that FMD depends on the ability of the endothelium to release nitric oxide in response to shear stress and is used as a reliable method to assess endothelial function in various clinical conditions (12). This technique was the method of choice for pregnant women in view of its noninvasiveness and the brief duration for the study.

Although, to our knowledge, no previous study has assessed in vivo maternal endothelial function in GDM, our results are consistent with evidence that women with pregnancies complicated by GDM have abnormal endothelial function. Knock et al. (13) reported impaired endothelial response to acetylcholine in small artery rings of pregnant women with gestational diabetes. Other authors found defective endothelium-dependent vasodilation in euglycemic women with previous GDM (7).

Endothelial dysfunction in diabetes has been attributed mainly to hyperglycemia (14,15). In keeping with this assumption, we found a greater degree of abnormal endothelial function in women with GDM than in women with IGT. Furthermore, it is interesting to note that, among all subjects, the percentage increase in FMD was strongly and negatively related to the magnitude of glucose level, at both fasting and after oral glucose challenge.

Accumulating evidence seems to indicate that the effect of acute hyperglycemia on endothelial function is mediated through an attenuated secretion of nitric oxide and an increased production of oxygen-derived free radicals (16–18). Although the present study cannot give any information about the mechanism by which hyperglycemia leads to endothelial dysfunction, our data indicate that functional damage of endothelial cells occurs even during relatively moderate hyperglycemia, similar to that found in women with IGT. Furthermore, considering that the functional endothelial evaluation was performed under fasting condition, when all subjects were basically euglycemic, these data suggest that a chronic deleterious effect of hyperglycemia on endothelium could be hypothesized.

We and others have demonstrated that pregnant women with abnormal carbohydrate metabolism presented a marked decrease of sensitivity to insulin (19,20). Because it is known that resistance to insulin action is associated with endothelial dysfunction (21), it is likely that this association could explain the impairment in endothelial function in women with GDM and IGT. However, in this study, insulin resistance was not formally evaluated. Insulin secretion, at fasting and in response to glucose challenge, has been shown to be a good indicator of insulin sensitivity in large studies (22). On the contrary, in our hands, this variable failed to be a marker of insulin sensitivity and, although we found differences in insulin values among the groups studied and negative correlation between insulin area and FMD, these analyses did not achieve a statistical significance. Nevertheless, we must note that the lack of statistical significance might reflect a β error due to small sample size.

Several reports indicate that hypertension, obesity, and dyslipidemia all may affect endothelial function (21–23,24). Therefore, we analyzed all the variables to assess which of these play a major role in the endothelial dysfunction displayed by women with abnormal carbohydrate metabolism.

As described in RESEARCH DESIGN AND METHODS, all subjects studied were normotensive. Despite that, women with GDM presented a 10% higher MAP than that in IGT and control subjects. Therefore, one could hypothesize that this difference in blood pressure may account for the blunted endothelial function observed in patients with GDM. However, among all subjects, we did not find a significant correlation between FMD and MAP (r = 0.23, P = 0.16). Therefore, although we cannot exclude a contribution of blood pressure in endothelial function of patients with GDM, it is likely that this contribution could be minimal.

As in our previous study (25), BMI was shown to be directly related to endothelial dysfunction. Given that both women with GDM and those with IGT exhibited a somewhat higher BMI than the pregnant control subjects, it is possible that the association between endothelial function and glycemic area could be influenced by this variable. However, when we examined the value of all the variables studied in the prediction of endothelial function using a multivariate analysis, we found that glycemic area, but not BMI, persisted independently in the model.

In keeping with other reports, total cholesterol and LDL cholesterol levels were similar among the three groups (26). Moreover, there was no relationship between these lipids and the percentage increase in flow-mediated vasodilatation. Therefore, it is highly unlikely that these lipid parameters could account for the differences in endothelial function observed between our study groups. As previously reported, NEFA levels were higher in both groups of pregnancies complicated by carbohydrate abnormality than in control subjects (26). In addition, NEFA values did correlate with FMD. Interestingly, this relation was independently maintained, even after adjusting for parameters usually associated with NEFA levels (such as insulin and glucose values and adiposity), explaining 5% of the variance in flow-mediated vasodilatation. Therefore, NEFA levels seem to negatively affect endothelial function, not only at the high degree of elevation seen during experimental studies (27), but also with its relatively moderate ele-
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vascular dysfunction exhibited by pregnant subjects with abnormal carbohydrate metabolism.

In conclusion, this study provides evidence that women with abnormal glucose metabolism in pregnancy are characterized by endothelial dysfunction. This dysfunction seems to be strongly associated with elevated glycemic levels, independent of the degree of adiposity. Considering the vasodilator role of the endothelium, these findings could explain, at least in part, the increased risk for concurrent hypertensive disorders during pregnancy in women with GDM and IGT. Furthermore, these data support the clinical and epidemiological findings of augmented cardiovascular disease in subjects with previous pregnancy complicated by GDM and extend it to women with previous IGT during pregnancy.

References