suggests that different targets should be equally effective for quantification of the total DNA in the plasma of pregnant women. Diagnostically, this would simplify the choice of maternal targets for circulating DNA analysis in pregnant women. This finding is particularly relevant to pregnancy-associated disorders in which quantitative aberrations of the total plasma DNA have been found [e.g., in preeclampsia (12, 16–18)]. It is important to emphasize, however, that the CGH analysis provided data only for the predominant DNA species in maternal plasma, which are of maternal origin. Methods for analyzing the genomic representation of fetal DNA in maternal plasma will be interesting but much more difficult to achieve. It would also be interesting to analyze the genomic representation of plasma DNA in other conditions, including cancer (19, 20) and trauma (21).

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References

Gestational diabetes occurs with variable severity in 3%–5% of all pregnancies and may be related to oxidative stress and impaired antioxidant defenses (1). Antioxidant enzymes include superoxide dismutase, which produces hydrogen peroxide, and catalase, which consumes hydrogen peroxide. Catalase is the main regulator of hydrogen peroxide metabolism (2), which is associated with diabetes mechanisms such as Glut 4 expression, insulin secretion, insulin signaling, protein tyrosine phosphatase regulation, and glucose transport stimulation (3). Hydrogen peroxide has novel insulin-like effects, e.g., inhibition of lipolysis and reactivation of phosphoenolpyruvate carboxy kinase (4, 5), and insulin moderates hydrogen peroxide generation (6, 7) and catalase synthesis (8). High concentrations of hydrogen peroxide may damage heme proteins, cause cell death, and together with redox active metal ions, produce highly toxic hydroxyl radicals.

High catalase activity in erythrocytes seems to provide antioxidant defense for tissues with low catalase activity, particularly pancreatic beta cells. Catalase is important in antioxidant defense against hydrogen peroxide (9, 10), but there are conflicting reports of decreases (11, 12),

Blood Catalase Activity in Gestational Diabetes Is Decreased but Not Associated with Pregnancy Complications, Laszlo Geth,1,2 Zoltan Toth,2 Ildiko Tarnai,1 Maria Berczes,3 Peter Toerok,2 and William N. Bigler4 (1 Department of Clinical Biochemistry, Molecular Pathology, and Clinical Analytical Chemistry, 2 Department of Obstetrics and Gynecology, and 3 Neonatal Intensive Care Unit, Medical and Health Science Center, University of Debrecen, Debrecen, Hungary; 4 Center for Biomedical Laboratory Science, San Francisco State University, San Francisco, CA; 5 Department of Laboratory, Municipal Hospital, Simeg, Hungary; * address correspondence to this author at: Department of Clinical Biochemistry Molecular Pathology, Medical and Health Science Center, University of Debrecen, PO Box 70, Debrecen H-4012, Hungary; fax 36-52-417-631, e-mail goth@jaguar.dote.hu)
increases (13), and no change (14) in catalase activity in diabetes. A high incidence (14%) of diabetes mellitus observed in 63 Hungarian patients with inherited catalase deficiency (1 with type 1 and 7 with type 2 diabetes) could be associated with damage to oxidation-sensitive pancreatic beta cells by exposure to long-term increased hydrogen peroxide concentrations (15), but there have been conflicting reports from small studies of maternal and embryonic catalase in rat (16) and human (17) gestational diabetes.

We compared blood catalase activity in patients with gestational diabetes, pregnant patients without diabetes, and nonpregnant nondiabetic individuals. We examined the catalase gene mutations associated with decreased catalase activities and evaluated the effects of decreased maternal blood catalase activity on complications in newborns.

Study participants included 60 pregnant women with gestational diabetes but no family history of diabetes mellitus who were undergoing treatment at the Department of Obstetrics and Gynecology of the Medical and Health Science Center (Debrecen, Hungary). All received insulin therapy. Blood samples were taken from 32 women in the second trimester and 28 in the third trimester. For comparison we used 235 age- and sex-matched nonpregnant, nondiabetic women (nonpregnant group) from the outpatient clinics of the Municipal Hospital (Sümeg, Hungary). From this group we evaluated the catalase activity of 129 pregnant, nondiabetic women from the second trimester and 136 from the third trimester. We received consent from the participants, and the samples were deidentified. We determined blood catalase activity with a simple spectrophotometric assay (18) [reference mean (SD), 108.6 (13.1) MU/L (n = 235) for women 20–40 years of age]. Blood hemoglobin A1c (Hb A1c) was measured with a Diamat system (Bio-Rad; [reference interval, 4.2%–6.1%]), and blood hemoglobin with a blood cell counter (SF; Sysmex). Genomic DNA was extracted with a QIAamp Blood Kit (Qiagen) from leukocytes of 38 women from the nonpregnant group, 30 from the pregnant diabetic group with blood catalase activity within the reference interval, and 9 from the gestational diabetes group with blood catalase activity <50% of the mean activity in the reference population. The C-to-T regulatory mutation was examined in 22 patients with gestational diabetes with blood catalase activity below the lower limit of the reference interval (80.3 MU/L) and in the 38 nonpregnant controls. Exons 2 and 7 and the catalase promoter regions were PCR-amplified and the PCR products analyzed for known catalase gene mutations (19, 20).

We used the Student t-test to evaluate the statistical significance of differences between means. Values were considered significant at P <0.05.

Blood catalase and hemoglobin data are presented in Table 1. The mean (SD) blood catalase activity for pregnant women [89 (18) MU/L; n = 169] was decreased 18% (P <0.001) compared with the control group [109 (13) MU/L; n = 235]. The decrease in mean catalase activity was even greater (32%) for the gestational diabetes group [74 (14) MU/L; n = 60; P <0.001]. The mean blood hemoglobin concentrations for the pregnant nondiabetic group [120 (10) g/L] and for the gestational diabetes group [117 (10) g/L] were decreased 7% (P <0.001) and 9%, respectively, compared with the controls [129 (10) g/L]. The mean ratio of blood catalase to hemoglobin was significantly lower (P <0.001) than in the controls [0.84 (0.10) MU/g; set as 100%] for both the nondiabetic pregnant group [0.75 (0.10) MU/g; 90% of control value] and the gestational diabetes group [0.63 (0.11) MU/g; 75% of control value].

In the pregnant nondiabetic group, there was no difference in mean hemoglobin or catalase between the second and third trimesters, whereas in the gestational diabetes group, there was significant (P <0.05) variation of mean

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**Table 1. Mean (SD) blood hemoglobin concentrations, blood catalase activities, and catalase-to-hemoglobin ratios in nonpregnant controls, nondiabetic pregnant women, and in women with gestational diabetes.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Nonpregnant controls</th>
<th>Pregnant, nondiabetic</th>
<th>Gestational diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. in group</td>
<td>235</td>
<td>169 for all; 29 for 2nd trimester; 136 for 3rd trimester</td>
<td>60 for all; 32 for 2nd trimester; 28 for 3rd trimester</td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>129 (10)</td>
<td>120 (10)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>118 (10)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2nd vs 3rd trimester</td>
<td>118 (13) vs 121 (14)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>117 (10) vs 119 (9)&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Catalase, MU/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>109 (13)</td>
<td>89 (18)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>74 (14)&lt;sup&gt;a,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>2nd vs 3rd trimester</td>
<td>89 (17) vs 90 (19)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>67 (18) vs 80 (15)&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Catalase/hemoglobin, MU/g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>0.84 (0.10)</td>
<td>0.75 (0.10)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.63 (0.11)&lt;sup&gt;a,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>2nd vs 3rd trimester</td>
<td>0.76 (0.11) vs 0.74 (0.11)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.57 (0.10) vs 0.62 (0.02)&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Significantly different from nonpregnant controls (P <0.05).
<sup>b</sup> Not significantly different (P >0.05).
<sup>c</sup> Significantly different from pregnant controls (P <0.05).
<sup>d</sup> Significantly different from second trimester (P <0.05).
blood catalase [second trimester, 67 (18) MU/L (62% of control value); third trimester, 80 (15) MU/L (74% of control value)] but the mean blood hemoglobin did not vary.

We observed significant differences in the decreases in mean blood hemoglobin concentrations (6%–9%) and catalase activities (18%–38%) in the nondiabetic pregnant and gestational diabetes groups compared with the control group (P <0.05). The mechanisms that decreased hemoglobin may have decreased the catalase activity, but catalase-specific mechanisms probably caused the larger decreases in catalase activity in the women with gestational diabetes. To detect a catalase-specific mechanism, we searched for mutations in the catalase gene in samples from the women with gestational diabetes who had <50% of the normal blood catalase activity.

Mutations (19) that are known to cause catalase deficiencies in some Hungarians (exon 2, Hungarian type A and B; intron 7, type C) were not detected in any of the 38 nonpregnant individuals, 30 pregnant women without diabetes, or 9 women with gestational diabetes and catalase activity <50% of the mean activity of the reference population.

We investigated 22 patients with gestational diabetes who had decreased blood catalase activities (below the lower limit of 80.3 MU/L of the reference interval) for the catalase gene regulatory mutation (C-to-T substitution in the 5′ promoter region, which may influence catalase activity (20). Although this mutation should yield the highest activities for the TT types and lowest for the CC types (20), our results showed nearly the same (P >0.7) catalase activities for the CC types [63.6 (9.7) MU/L; n = 7], CT types [62.2 (11.6) MU/L; n = 10], and TT types [64.5 (8.1) MU/L; n = 5]. Furthermore, the 38 nonpregnant individuals also showed a different trend because the highest, (nonsignificant) activity values were found for patients with the CC types [96.4 (21.1) MU/L; n = 19], lower for the CT types [93.3 (24.1) MU/L; n = 12], and lowest for the TT types [89.4 (12.8) MU/L; n = 7]. Thus, the presence or absence of these catalase gene mutations did not appear to influence catalase activity.

Before a diagnosis of gestational diabetes is made, usually during the second trimester, and before initiation of insulin therapy, oxidative stress and glucose autooxidation may increase hydrogen peroxide generation and diminish catalase synthesis (5–12). The observed higher mean catalase activity in the third than the second trimester in the gestational diabetes group may reflect the success of intensive insulin therapy and decreased blood glucose and oxidative stress. This is the first report of this change in catalase activity associated with control of hyperglycemia in human gestational diabetes; our observation is consistent with animal experiments in which insulin therapy normalized catalase activity and protein expression (12).

To evaluate the change in hyperglycemia control in the gestational diabetes group, we measured Hb A1c concentrations in second and third trimester samples. Samples from both trimesters had mean Hb A1c values within the reference interval, but the second trimester mean (5.7 (0.6); n = 32) was significantly higher (P <0.01) than the third trimester mean (5.1 (0.8); n = 28). This Hb A1c change correlated negatively with the change in mean catalase activities [second trimester, 67 (18) MU/L (n = 32) vs third trimester, 80 (15) MU/L (n = 28); P <0.001]. Similar effects have been observed for serum and blood catalase in patients with type 2 diabetes (21, 22).

The low catalase activities of the 60 insulin-treated patients with gestational diabetes were not associated with clinical disorders. During their pregnancies, 53 of these patients had no complications, 7 had hypertension, and 3 had preeclampsia.

We evaluated 53 newborns to assess the impact of decreased gestational maternal catalase activity during gestational diabetes. The infants were divided into 2 groups based on maternal catalase activities: group A with lower [52 (8) MU/L; n = 9] and group B with higher [78 (10) MU/L; n = 44] activity. The cutoff for groups A and B was 60 MU/L, the mean of the maternal blood catalase activities. There were no significant differences in mean birth weights [3271 (473) g for group A vs 3456 (566) g for group B] or mean gestation times [38.7 (1.2) weeks vs 38.9 (1.0) weeks]. The frequencies of cesarean section [3 of 9 (33%) vs 14 of 44 (32%)] and high (>3500 g) birth weights [4 of 9 (45%) vs 22 of 44 (50%)] were similar in these groups. Group B had a higher frequency of respiratory distress syndrome [10 of 44 (22%) vs 1 of 9 (11%)] and of other complications (icterus, 44%; hypoxia, 5%; anemia, 5%; acidosis, 5%; erythema, 5%; pylectasy, 2%; prematurity delivery, 2%). Similar complications were not detected in group A, possibly because of the low number of patients in this group. Although we could not perform a clear statistical analysis because of the small number of patients, these results suggest that low maternal catalase activity does not represent a significant risk for mothers or newborns.

Our data suggest that dysregulation of catalase synthesis could be responsible for decreased blood catalase in gestational diabetes and its change in the second and third trimester. We found no link between known mutations in the catalase gene and such dysregulation, but as yet unknown mutations could be responsible for the low catalase activities associated with gestational diabetes.

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References
Addition of a Homologous Internal Control to a Real-Time PCR Assay for Detection of Bordetella pertussis, Christoph Koidl, Michael Bozic, Gerhard Mühlbauer, Egon Martha, and Harald H. Kessler

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Pertussis, also called whooping cough, is caused by Bordetella pertussis. The disease may show an atypical course, particularly in neonates and elderly patients. A rapid and safe diagnostic method is thus essential for appropriate treatment and prophylaxis. Culture has been considered the gold standard for detection of B. pertussis, but this method often lacks sensitivity, and a minimum of 4 days may be required to obtain results (1, 2). PCR is a rapid, sensitive, and specific method for the diagnosis of pertussis (3–5).

In this study, a new molecular assay was established based on real-time PCR and including a homologous internal control (IC). We evaluated the performance of this assay with a commercially available genomics DNA kit and with clinical samples.

The new molecular assay consisted of a protocol for manual extraction of DNA followed by generation of the amplification product by real-time PCR. The assay was based on the amplification of a 181-bp fragment of the repetitive insertion sequence IS481, which has been described in B. pertussis and Bordetella holmesii and may be present in Bordetella bronchiseptica (6–10) (see Table 1 in the Data Supplement that accompanies the online version of this Technical Brief at http://www.clinchem.org/content/vol51/issue12). We determined assay linearity and detection limit by analyzing 10-fold dilutions of the ATCC genomic DNA isolate 9797D from B. pertussis. Interassay variation was determined with 7 dilutions of the genomic DNA isolate (5 determinations on 5 different days), whereas intraassay variation was determined with 3 samples (5 determinations within a single assay). All assays for determination of inter- and intraassay variation included negative controls.

A total of 219 nasopharyngeal swabs were tested in this study. All specimens were collected with the Copan Venturi Transystem® culture swab transport system (CO-PAN Italia Spa) according to the manufacturer’s instructions. Samples were obtained from patients (99 females, 120 males; mean age, 9.1 years; range, 0–86 years) with a diagnosis of pertussis. Samples were obtained from patients (99 females, 120 males; mean age, 9.1 years; range, 0–86 years) with a diagnosis of pertussis.