D-Glucaric Acid and Gamma-Glutamyltransferase as Indices of Hepatic Enzyme Induction in Pregnancy

M. Herzberg, E. Tenenbaum, B. Fishel, and M. H. Wiener

Increased activity of hepatic microsomal enzymes can be evaluated by measuring D-glucaric acid excretion in urine and γ-glutamyltransferase (EC 2.3.2.2) activity in serum. Aside from diverse foreign compounds, endogenous steroid hormones have been shown to be normal substrates of the microsomal enzyme system. Because there is an increase in steroid production in pregnancy, we sought to determine whether these indices of induction increase during pregnancy. In 90 women in various stages of pregnancy, all with normal kidney function, we measured glucaric acid excretion in urine and activity of the transferase in serum and in urine. Glucaric acid increased markedly during pregnancy, from 14.4 ± 2.1 in the first trimester to 23.5 ± 2.8 μmol of D-glucaro-1,4-lactone per gram of creatinine in the third trimester. We saw no correlation between glucaric acid excretion and the transferase activity in serum or urine. Activity of γ-glutamyltransferase remains within normal limits throughout pregnancy, which leaves doubt as to the value of this measurement in evaluating enzyme induction owing to endogenous steroids.

Additional Keyphrases: hepatic microsomal function • glucaic acid in urine during pregnancy • enzyme induction

Drug-metabolizing enzymes in liver microsomes can transform various foreign compounds into more polar metabolites, which are readily excreted in the urine (1). Endogenous steroids are also normal substrates of this enzyme system (2), being transformed predominantly into (polar) glucuronides and excreted in this form in urine and probably also into the bile.

Increased activity of hepatic microsomal enzymes (i.e., enzyme induction) can be evaluated by measuring the increase in excretion of glucaric acid (GA) in urine (3). In addition, γ-glutamyltransferase (EC 2.3.2.2; GGT) activity is increased in the serum of alcoholics and of epileptic patients who are receiving phenobarbital (4). This enzyme is localized in the microsomal fraction of the liver.

Martin et al. (5) suggest that increased GGT activity may reflect hepatic enzyme induction in hyperlipidemic subjects. They (6) also found an association between serum GGT activity and triglyceride concentration after treatment with contraceptives, which they suggest may reflect hepatic microsomal enzyme induction. Goldberg and Martin (7) claim that GGT is a more sensitive indicator of enzyme induction than are glucaric acid and 6-β-hydroxy-cortisol. Whitfield et al. (8) showed an increase in serum GGT when patients treated with warfarin were given amobarbital, secobarbital, or antipyrine—all of which are known to be good enzyme inducers.

We used both these indicators of enzyme induction to study induction due to endogenous metabolites formed in increasing amounts during normal human pregnancy.

Materials and Methods

Patients. Ninety pregnant women who were attending the prenatal clinic were investigated. Sixty-five women in the second and third trimester were studied for urinary glucaric acid excretion, creatinine clearance, and GGT activity in blood and in some cases in urine. All had normal creatinine clearance values. Twenty-five women in the first trimester, from whom a single morning urine specimen was obtained, were studied only for GA and creatinine. As controls, a group of men and women was used. These controls were in apparent good health, did not receive drugs, and did not smoke. As there were no differences between results by sex, they are considered as a single group.

Methods. In all subjects, we measured urinary D-glucaric acid (as glucuronidase inhibitor, D-glucaro-1,4-lactone) according to the method of Marsh (9), with the modifications of Latham (10). In all cases the urine specimen was obtained from a 24-h collection, except for 25 women in the first trimester, from whom the specimen was obtained in the morning. The inhibitory effect of glucarolactone was measured on β-glucuronidase extracted from Helix pomatia (Industrie Biologique Francaise). The substrate used was phenolphthalein mono-β-glucuronic acid (Sigma Chemical Co.). A standard curve was constructed for glucarolactone (Sigma) and the concentration of the glucaric acid in urine was calculated as micromoles of glucaro-1,4-lactone per gram of creatinine. The percent conversion of D-glucaric acid to D-glucarolactone was 23% according to Latham (10), 29% according to March et al. (11), and 30% according to Marsh (9). Therefore, to obtain results in terms of glucaric acid, the values for glucaro-1,4-lactone should be multiplied by 3.3. Serum GGT activity was measured by the kinetic method of Szasz (12). The substrate was L-γ-glutamyl-nitroanilide (4.4 mmol/liter) and tris(hydroxymethyl)aminomethane buffer (0.1 mol/liter, pH 8.6) was used. The reaction was done at 25 °C. Creatinine in serum and urine were estimated by the Jaffé reaction, all tests being run in duplicate.

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Table 1. Mean Values of GA and GGT in Pregnancy and Controls

<table>
<thead>
<tr>
<th>D-Glucaric acid (as μmol D-glucaro-1,4-lactone/g creatinine)</th>
<th>γ-Glutamyltransferase U/liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>Mean ± SE</td>
</tr>
<tr>
<td>Controls</td>
<td>17</td>
</tr>
<tr>
<td>Trimester I</td>
<td>25</td>
</tr>
<tr>
<td>Trimester II</td>
<td>14</td>
</tr>
<tr>
<td>Trimester III</td>
<td>50</td>
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</tbody>
</table>

Statistical evaluation was done by Student's t-test.
The difference of means of GA excretion between controls and trimesters II and III \( P < 0.01 \)
The difference of means of GA excretion between controls and Trimester I n.s.
The difference of means of GA excretion between trimester I and trimesters II and III \( P < 0.05 \)
The difference of means of GGT excretion between trimesters I and II n.s.

We found no significant differences in serum GGT between controls and trimester II and III and between trimesters II and III.

Results

Table 1 contains the results for GGT in serum and GA excretion, presented as mean ± standard error, by trimester. The data for urinary GA excretion are presented as micromoles per gram of creatinine. When correlation studies between GA excretion per gram of creatinine and GA excretion per 24 h were done, a highly significant correlation was found \( r = 0.984, P < 0.001 \). From the data, it can be seen that there is an increase in urinary excretion of GA in the second and third trimester. The difference between trimester one and two, and between trimester one and three were significant \( P < 0.05 \) by Student's t-test.

In comparison with the control group, there is no significant difference in GA excretion between trimester 1 and control. On the other hand, there is a significant \( P < 0.01 \) difference between controls and trimesters two and three. GGT activity in serum did not change between trimester two and three (no data for GGT are presented for trimester one), and were not significantly different from controls.

Among the 35 patients studied urinary GGT ranged from 12.3 to 36.5 U/liter, with no significant correlation between the serum and urine \( r = 0.088 \). There also was no significant correlation between GA and GGT in urine \( r = 0.074 \), or between GGT in serum and GA in urine \( r = 0.19 \).

Figures 1 and 2 show the distribution of serum GGT and urinary GA in patients according to trimester of pregnancy and controls. From Table 2 and Figure 2 we can see that in the first trimester only eight of 25 women (32%) showed an excretion of GA higher than the upper normal limit, 15 μmol/g of creatinine. In the second trimester, 10 of 15 women (67%) and 32 of 50 (64%) in the third trimester show excretion of GA above upper normal limit. In contrast, (Figure 1, Table 2) no serum GGT activity exceeded 25 U/liter (upper normal limit).

Discussion

In 1963, Marsh (9) noted that the excretion of GA is especially high in pregnant women. In 1965, he found increased activity of the microsomal enzyme, β-glucuronidase, in pregnancy (13). Conney (2) and Conney (1), studying the influence of drugs on microsomal enzymes, found that normal body constituents such as hormones (cortisol, progesterone, estrogen) are also substrates of this same enzyme system. Davis (14) found an increased GA excretion that paralleled the course of pregnancy, and attributed this to a progressive increase in placental steroid production. These steroids undergo conjugation, mostly with glucuronic acid. Levy and Conchie (15) suggested that this may be a physi-
Table 2. Percentage of Elevated GA, by Trimester

<table>
<thead>
<tr>
<th>Trimester</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>8/25</td>
<td>32</td>
</tr>
<tr>
<td>II</td>
<td>10/14</td>
<td>67</td>
</tr>
<tr>
<td>III</td>
<td>32/50</td>
<td>64</td>
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