Biological Variation and the Effect of Fasting and Halothane Anesthesia on Plasma Glutathione S-Transferase Concentrations

David C. Ray,1,4 Louise M. Aldridge,2 Heather J. Spens,1 Gordon B. Drummond,1 A. Forbes Howie,3 and Geoff J. Beckett3

Using a specific RIA, we have investigated in patients and volunteers whether fasting, diminished hepatic clearance, hemoconcentration, or within-day biological variation might be responsible for the transient increases in plasma glutathione S-transferase (GST) concentration observed after anesthesia. GST concentration was measured in 44 healthy volunteers after an overnight fast and at 3, 6, and 24 h after the fasting sample. The concentration was significantly lower at 3 and 6 h after than in the fasting sample ($P = 0.0019$ and $P = 0.015$, respectively). The change in GST concentration caused by fasting was examined in 30 subjects by comparing pre- and postfasting values. Fasting had no significant effect on GST concentration overall ($P = 0.4721$), but two individuals showed a marked increase in GST concentration after fasting overnight. In a separate study of 10 patients, plasma amylase activity and plasma concentrations of GST and albumin were measured immediately before and 3 h after induction of halothane anesthesia. Although GST concentration was increased at 3 h in each of the 10 patients, plasma amylase activity and plasma albumin concentration were significantly decreased in all patients ($P = 0.002$). Apparently, increases in GST concentration after anesthesia do not result from incidental factors.

**Indexing Terms:** variation, source of/enzyme activity/sex-related differences/liver function

The concentration of glutathione S-transferase (GST) B1 subunits in plasma or serum measured by RIA is an extremely sensitive index of acute cellular damage to hepatocytes in zones 1, 2, and 3 of the liver lobule ($J$). Measurement of plasma GST concentration, considered to offer several advantages over the measurement of aminotransferase activities, has received increasing interest in the detection of mild, drug-induced hepatocellular damage (2–9). Measurements of GST provide a highly specific test of hepatocellular damage, and GST results correlate better with histological changes than do the aminotransferases ($10$). In contrast to the aminotransferases, measurement of GST allows detection of both periportal and centrilobular damage ($J$). GST is readily released in quantity into the circulation after tissue damage has occurred, and its short plasma half-life (<90 min) allows early detection of hepatocellular necrosis and of its resolution (2).

Plasma concentrations of GST increase transiently in humans after anesthesia with halothane and enflurane, but not after isoflurane ($11$, $12$). These changes are considered to indicate a minor degree of hepatocellular damage, but could be caused by incidental factors such as fasting, hemoconcentration, within-day biological variation in plasma GST concentration, or a diminution in clearance of the enzyme. To elucidate the situation, we have now investigated these possibilities in two separate studies.

In the first study we examined both the influence of fasting and the within-day variation in plasma GST concentration by analyzing sequential blood samples from healthy, nonanesthetized volunteers after an overnight fast. In the second study, we investigated the possibility that GST concentrations might be increased as a result of hemoconcentration or of a general diminution in clearance of low-molecular-mass plasma proteins by measuring plasma albumin concentration and plasma amylase activity in samples obtained from patients in whom plasma GST concentration increased 3 h after halothane anesthesia. The plasma half-lives and molecular masses of GST and amylase are similar ($t_{1/2} < 90$ min and 180 min, and $M_r = 51 800$ and $50 000$ kDa, respectively), and both enzymes are thought to be removed from plasma via renal clearance ($13$, $14$). If hemoconcentration or a generalized reduction in clearance of low-molecular-mass enzymes was responsible for the observed increases in GST concentration after anesthesia, we would expect GST concentration, albumin concentration, and amylase activity to increase in parallel.

**Materials and Methods**

The studies were approved by the Area Ethics Committee.

**Within-day variation in plasma GST concentration.** We studied 45 healthy volunteers (30 men, 15 women), ages 21–49 years (median 31 years). Volunteers were excluded if they had recently undergone general anesthesia or blood transfusion, if they took regular medication, or if their average daily intake of alcohol exceeded 3 units (as defined by Health Education Councils in the UK). All volunteers fasted overnight. Blood was sampled for GST concentration and conventional liver biochemical tests at about 0900 the next day (time 0), after which the volunteers could eat and drink as usual. Further samples for measurement of

---

Departments of 1 Anaesthetics and 3 Clinical Biochemistry, Royal Infirmary of Edinburgh, NHS Trust, Lauriston Place, Edinburgh EH3 9YW, UK.

2 Current address: Department of Anaesthetics, Royal Hospital for Sick Children, Sciennes Rd., Edinburgh EH9, UK.

4 Author for correspondence. Fax 44-131-536 3672.

Received October 31, 1994; accepted February 13, 1995.

688 CLINICAL CHEMISTRY, Vol. 41, No. 5, 1995
GST concentration were taken 3, 6, and 24 h after this initial sample, time intervals that correspond to those of previous studies of GST concentrations in patients undergoing anesthesia.

Effect of an overnight fast on plasma GST concentration. In 30 of the above subjects (21 men, 9 women), GST concentration was also measured at about 1600 on the day before commencing their overnight fast to assess the change caused by fasting.

Effect of halothane anesthesia on the circulating concentrations of GST, amylase, and albumin. We measured retrospectively plasma amylase activity and albumin concentration in samples obtained from 10 patients who received halothane and in whom GST concentration increased 3 h after induction of anesthesia. Serum amylase activity and concentrations of GST and albumin were measured in the samples obtained immediately before induction of anesthesia (time 0) and 3 h later.

Plasma measurements and statistical analysis. Plasma samples for GST and amylase measurements were stored at −20°C before analysis. Measurements of standard liver-function tests were made on fresh plasma stored at 4°C. All samples from the same subject were measured in the same assay run. The plasma concentration of GST B₁ subunits was measured by a specific RIA (15), for which the reference interval was 0.5–4.5 µg/L. The inter- and intraassay CVs were <10% and <5%, respectively, over the range 0.2–40 µg/L. Activities of alanine aminotransferase and γ-glutamyltransferase and concentrations of bilirubin and albumin were measured with a Hitachi (Osaka, Japan) 747 analyzer with reagents provided by BCL Clinical Diagnostics, Lewes, UK. Plasma amylase activity was measured by using a BCL α-amylase kit with p-nitrophenyl phosphate substrate.

The Wilcoxon signed-rank test was used to examine changes in GST concentration from time 0 to 3, 6, and 24 h and also to examine differences between the changes in GST concentration and amylase activity. The sample size of 45 subjects provides the study with a power >80% for a change in GST concentration of >0.5 µg/L.

Results and Discussion

Within-Day Variation and Effects of Overnight Fasting

In one volunteer the initial (prefasting) GST concentration measured was outside the reference interval (Table 1); this subject was excluded from statistical analysis. The individual changes in GST from the value at time 0 are shown in Fig. 1; the median and interquartile ranges of GST concentrations for all subjects, and for men and women separately, are shown in Table 2. GST concentration was slightly but significantly less at 3 and 6 h than at time 0, but by 24 h was not significantly different from time 0. Although the men had higher concentrations of GST than the women did at all sample times, none of these differences was statistically significant. Two volunteers with a normal GST concentration at time 0 exceeded the upper reference limit later—at 3 h in one subject and at 24 h in the other (Table 1). No subject had any abnormality in the results of conventional liver-function tests, i.e., bilirubin, alanine aminotransferase, γ-glutamyltransferase, and albumin.

<p>| Table 1. Glutathione S-transferase profiles (µg/L) in volunteers whose plasma GST concentration exceeded the upper reference limit during the study. |
|---|---|---|---|---|</p>
<table>
<thead>
<tr>
<th>Time of sampling</th>
<th>Prefast</th>
<th>0</th>
<th>3 h</th>
<th>6 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.4</td>
<td>4.0</td>
<td>8.1*</td>
<td>3.7</td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td>3.8</td>
<td>7.1*</td>
<td>4.5</td>
<td>4.3</td>
<td>6.6*</td>
<td></td>
</tr>
<tr>
<td>3.9</td>
<td>7.3*</td>
<td>6.0*</td>
<td>7.6*</td>
<td>6.9*</td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>1.6</td>
<td>1.2</td>
<td>2.1</td>
<td>6.6*</td>
<td></td>
</tr>
<tr>
<td>(---)²</td>
<td>(5.7)*</td>
<td>(3.8)</td>
<td>(3.1)</td>
<td>(3.3)</td>
<td></td>
</tr>
</tbody>
</table>

* Results outside the reference interval.
² Results for the subject who was excluded from analysis are shown in parentheses.

Fig. 1. Individual changes in plasma glutathione S-transferase concentration from the initial sample (n = 44).

| Table 2. Median and interquartile ranges for plasma glutathione S-transferase concentrations (µg/L) in healthy volunteers. |
|---|---|---|---|---|
| Time, h | 0 | 3 | 6 | 24 |
| All subjects (n = 44) | 2.0 | 1.8* | 1.9* | 2.0 |
| Median | 1.5–2.7 | 1.4–2.1 | 1.5–2.2 | 1.6–2.6 |
| Interquartile range | 2.2 | 1.9 | 2.0 | 2.3 |
| Men (n = 29) | 1.8–2.9 | 1.5–2.2 | 1.7–2.3 | 1.6–2.8 |
| Median | 1.7 | 1.6 | 1.7 | 1.8 |
| Interquartile range | 1.4–2.3 | 1.3–2.1 | 1.3–2.2 | 1.6–2.2 |
| Women (n = 15) | 0.1097 | 0.2642 | 0.2974 | 0.0847 |
| P (difference between sexes) | * | 0.0019 | 0.015 |

* Significantly different from the time 0. (Wilcoxon signed-rank test.)
The median GST concentration measured in 30 subjects before fasting commenced was 2.2 μg/L (interquartile range: 1.6, 3.0). This value was not significantly different from the value at time 0 for these patients (2.1 μg/L; interquartile ranges 1.5, 2.7; \( P = 0.4721 \)). The individual changes in GST concentration after fasting (Fig. 2) show that two subjects had a GST concentration exceeding the upper reference limit at time 0; in both cases, the prefasting value was within the normal range (Table 1).

Numerous studies have reported changes in plasma GST concentration after anesthesia with various agents (3-9, 11, 12), but none of these studies had addressed the possibility that the measured changes in GST concentration may simply reflect within-day variation in GST concentration over the sampling period of the study. Similarly, the influence of an overnight fast on plasma GST concentration has not been reported. In our studies, we have shown that, as a group, healthy volunteers had no significant increase in plasma GST concentration over a 24-h period or after an overnight fast. In contrast, halothane anesthesia caused significant and consistent increases in GST concentrations, with peak concentrations occurring 3 h after anesthesia. Although we were unable to demonstrate any significant effect of an overnight fast on GST concentration in our volunteers overall, two individuals exhibited a marked increase in GST concentration after an overnight fast.

The marked and rapid changes we observed in GST concentrations in some of our volunteers during the 24 h of the study and the possible effects of an overnight fast is relevant to the use of GST measurements in clinical practice because the degree of the abnormality in GST concentration we observed in some subjects is similar to that found in patients with chronic liver disease such as chronic active hepatitis and alcoholic cirrhosis (16, 17). An increased plasma GST concentration should therefore be confirmed on further occasions before a provisional diagnosis of chronic liver dysfunction is made. We cannot be sure that the changes in GST concentration in some volunteers after an overnight fast resulted from the fast, or merely reflected biological variation: Much larger studies are needed to clarify this point. Fasting could influence plasma GST concentration by depleting hepatic energy reserves, which may be required for the maintenance of hepatocellular integrity. The increase in GST concentration observed in response to insulin-induced hypoglycemia in healthy volunteers tends to support this hypothesis (18).

We found also that men had higher concentrations of GST than women at all times during the study period, although these differences were not statistically significant. Recent work with a time-resolved immunofluorometric assay has suggested that the reference interval for plasma GST concentration is different for males and females (19). Our findings would seem to support the possibility of using different reference intervals for GST concentration in evaluating males and females.

Effects of Halothane Anesthesia on GST, Amylase, and Albumin

The changes in plasma GST concentration, plasma amylase activity, and plasma albumin concentration in each of the 10 patients after halothane anesthesia are shown in Fig. 3. In each, GST concentration was increased at 3 h, but plasma amylase activity and
plasma albumin concentration were lower at 3 h than at time 0 ($P = 0.0002$). This suggests that the increase in plasma GST concentration seen 3 h after halothane anesthesia does not result from hemococoncentration or from diminished clearance of GST, for, if this had been the case, albumin concentration and amylase activity would also have been increased. The parallel decrease in amylase activity and albumin concentration 3 h after halothane anesthesia is probably due to hemoconcentration rather than altered clearance. We have observed similar changes in albumin concentrations in previous studies (11).

In conclusion, our findings suggest that incidental factors do not result in changes in plasma GST concentration after anesthesia. Because changes in GST concentration may occur in healthy individuals within the day or after an overnight fast, single measurements of GST may be of limited value in the diagnosis of chronic liver disease.

References