Stability of Insulin in Normal Whole Blood

To the Editor:

For estimating the concentration of insulin in clinical investigations of hypoglycemia, laboratories require plasma or serum to be separated without delay and stored at −20 °C. When there is a delay of several hours before separation, the insulin assay may either be refused or the result disregarded by the clinician or biochemist.

It is hard to find evidence that insulin is unstable in whole blood. Insulin could be degraded by proteolysis in the plasma, but Livesey et al. (1) have shown that, in separated plasma, insulin is stable at 20 °C for up to three days. After α1-antitrypsin binds to receptors on the blood cells, the binding of 125I-labeled insulin to erythrocytes (2) and granulocytes (3) is, in the absence of unlabeled insulin, <2 milli-int. units/L at concentrations of cells equivalent to those in blood. Granulocytes can degrade insulin (3), but only in small quantities. Erythrocytes degrade internalized insulin at 37 °C (4, 5), but at room temperature the binding to cells and degradation of insulin would be expected to have only a small effect on the plasma insulin concentration.

In our experience, delays of up to 5 h between venesection and arrival of the sample in the laboratory can occur; therefore, we conducted this study to determine the stability of insulin in whole blood.

Samples of heparinized blood were obtained from each of 10 subjects, four of whom had taken a 75-g glucose load 40 min before venesection. Each sample was split; one part was separated within 15 min, the other after standing at room temperature (18–20 °C) for 5 h. Immediately after separation the plasma was frozen and stored at −20 °C until the insulin concentrations were determined by RIA in a routine batch, subjects 1 to 6 by one RIA method, subjects 7 to 10 by another. With both assays the highest plasma insulin concentrations after a 75-g glucose load are commonly between 50 and 50 milli-int. units/L; for fasting normal subjects the value is usually between 4 and 10 milli-int. units/L. The results were as follows:

<table>
<thead>
<tr>
<th>Subject</th>
<th>Immediately</th>
<th>After 5 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>28</td>
<td>27</td>
</tr>
<tr>
<td>2</td>
<td>79</td>
<td>72</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
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<td>7</td>
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<td>8</td>
<td>103</td>
<td>97</td>
</tr>
<tr>
<td>9</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>10</td>
<td>19</td>
<td>17</td>
</tr>
</tbody>
</table>

*Received glucose load.

In four cases with high insulin concentrations the concentration in the plasma separated after 5 h was slightly lower than in the plasma separated immediately, but not significantly (p > 0.25, Student’s t-test). In none of the 10 samples allowed to stand at room temperature for 5 h before the plasma was separated was the change in the insulin concentration large enough to be of diagnostic importance. It follows that in such circumstances the request for insulin assay should be complied with, and the same significance attached to low values as when the plasma is separated immediately. Of course it would be prudent, as in all such patients, to confirm the findings with another specimen.

We thank Michele Amoroso and Swarna Simpson for technical assistance.

References

Table 1. Typical Course of Emergency Therapy of a Patient with Diabetic Ketoadiposis *

<table>
<thead>
<tr>
<th>Time interval, h</th>
<th>Venous blood pH</th>
<th>Glucose mmol/L</th>
<th>3-HBA mmol/L</th>
<th>Acetoacetate</th>
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<tr>
<td>0</td>
<td>7.307</td>
<td>43.3</td>
<td>13.1</td>
<td>+++</td>
</tr>
<tr>
<td>2.5</td>
<td>—</td>
<td>51.1</td>
<td>8.1</td>
<td>+++</td>
</tr>
<tr>
<td>3.5</td>
<td>7.333</td>
<td>45.3</td>
<td>5.5</td>
<td>+++</td>
</tr>
<tr>
<td>5</td>
<td>—</td>
<td>39.3</td>
<td>2.9</td>
<td>++</td>
</tr>
<tr>
<td>8</td>
<td>—</td>
<td>26.3</td>
<td>0.5</td>
<td>–</td>
</tr>
<tr>
<td>10</td>
<td>—</td>
<td>5.9</td>
<td>0.4</td>
<td>–</td>
</tr>
</tbody>
</table>

* At the first three time intervals shown here, intravenous therapy with insulin, 10 units/h; at 8 h this was discontinued; at 10 h the patient was returned to his usual subcutaneous injections.

5.1 mmol/L gave CVs of 3.2% and 2.2%, respectively. We compared results with the kit and a modified Williamsen et al. (10) procedure, assaying 21 patients' samples by both methods. The resulting regression coefficient (r) was 0.998. Blood samples from 57 clinically fasted men and women were also assayed for 3-HBA; the mean value was 0.2 (SD 1.2) mmol/L.

Our pre-treatment range of 3-HBA concentrations for 11 ketoacidotic patients was 2.3 to 21.3 mmol/L. Table 1 gives results for a typical ketoacidotic patient on treatment with insulin. This case illustrates the time course of an episode of diabetic ketoacidosis caused by non-compliance with insulin regimen at home. Here, a significant decrease in 3-HBA preceded by several hours a decrease in the glucose and acetoacetate test values. Acetoacetate as determined by "Acetest" (Ames Co., Elkhart, IN 46514) did not respond to treatment with insulin until the 5-h sample, by which time 3-HBA had decreased to 78% of its pre-treatment concentration. Upon return of the 3-HBA concentration to within normal limits, the intravenous therapy was discontinued and the patient was returned to a regular insulin dose.

The above case is typical of the six other cases we have monitored. In all of them, earlier and better control of the insulin dose was achieved with 3-HBA monitoring. Thus, quantitative monitoring of 3-HBA in ketoacidotic patients is of greater clinical utility and should supersede the semiquantitative monitoring of acetoacetate. The availability of a convenient and inexpensive kit method for 3-HBA as described here should allow for more widespread use of 3-HBA measurements.

We thank Dr. Quincy Crider (Sigma) for supplying us with 3-HBA kits.

References

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Does Assay of Cholesterol in High-Density Lipoprotein Subclasses Give Clinically Useful Information?

To the Editor:

In a Letter, Joven et al. (1) point out the lack of evident diagnostic value of measuring cholesterol (Chol) in HDL subclasses. This is a much-debated question, most probably because of:

* the heterogeneity of analytical procedures for the separation of subclasses by zonal or preparative ultracentrifugation and polyamionic separation (2–4)
* the variety of methods used in determining cholesterol
* the lack of adequate reference values
* the poor interlaboratory reproducibility.

A review of published data (5) demonstrates the poor agreement of results in healthy subjects, with consistent discrepancies in the HDL Chol content as a percentage of total HDL Chol. For instance, HDL cholesl concentration usually is higher in females than in males (6, 7), but the opposite results were found in two studies (6, 9), and in others the distribution of HDL Chol in HDL varies remarkably. We consider unreliable those methods that produce results not reflecting a different sex-related (and perhaps also disease-related) distribution of HDL in HDL.

We report here our data for 507 consecutive randomized determinations of cholesterol in HDL subclasses, with respect to the percentage distribution of a precipitable fraction in total HDL.

We used a rapid procedure for total HDL separation, treating 0.10 mL of fresh serum with 1.0 mL of a 100 g/L unbuffered solution of polystyrene glycol (PEG-6000). The results correlate well with those by ultracentrifugation (10). HDL was subfractionated by precipitation of apoprotein B-containing lipoproteins and an HDL-insoluble fraction by this procedure, except we used a precipitation mixture containing PEG-6000 100 g/L, dextran sulfate 15,000 (Schib, Boulogne, France) 0.187 g/L, and MgCl2 13 mmol/L. After a 15-min incubation of the mixture at room temperature and 15-min centrifugation at 1500 × g, the cholesterol was determined in the supernate in a

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Fig. 1. Distribution of precipitable subtraction of HDL in men and women

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