Simplified Turbidimetric Assay for Lipase Activity

Zakariya K. Shihabi and Charles Bishop

Serum lipase is determined by following turbidity changes during two 1-min intervals after adding serum to an olive oil emulsion containing desoxycholate. The olive oil emulsion is simply prepared and is stable for a month under refrigeration. Our observations confirm the findings of other investigators that increases in serum lipase activity are more accentuated than increases in serum amylase activity during pancreatitis. With the present fast and convenient method, serum lipase appears to be a better test for pancreatitis than is serum amylase.

Additional Keyphrases amylase • pancreatitis • normal values

While both amylase and lipase activities in serum are increased in pancreatitis, lipase has fallen from favor because it cannot be assayed quickly (e.g., in an emergency) and because of the notion that serum amylase activity will increase sooner in pancreatitis. The traditional titrimetric lipase assay of Cherry and Crandall (1), which requires a 24-h incubation, was shortened to 1–3 h (2, 3). By using a turbidimetric method, Vogel and Zieve (4) were able to complete the lipase assay in 20 min and show reasonable correlation with 3-h titrimetric values. There is general agreement that an olive oil emulsion is the best lipase substrate for detecting pancreatitis (5), but such emulsions are often tricky to prepare and may not remain stable very long (4, 6, 7).

The present paper describes a turbidimetric assay for serum lipase in which: (a) turbidity changes are followed during two 1-min intervals of incubation; (b) the kinetics are zero order; (c) a stable, easily-prepared, olive oil emulsion is used; and (d) turbidity changes are expressed in a manner independent of the spectrophotometer used. Results of this study, taken with the work of other authors (6, 8, 9), indicate that lipase and amylase rise simultaneously during pancreatitis, and that the increase in lipase activity may be greater. The present quick method for serum lipase thus offers a very convenient indicator for pancreatitis and an alternative to measurement of amylase, for which the substrate and measurement are not always well controlled.

Materials and Method

Reagents

1. Purified olive oil: Mix 50 ml of olive oil (Fisher Scientific Co.) with 10 g of aluminum oxide for 60 min, allow to settle, and filter.
2. Olive oil alcoholic solution, 1 ml/100 ml: Shake 2.000 g of purified olive oil in 200 ml of absolute ethanol until the olive oil dissolves.
3. Tris buffer: Dissolve tris(hydroxymethyl)-aminomethane (3.0 g) and sodium desoxycholic acid (6.0 g) in 1000 ml of water. Adjust the pH to 8.8 ± 0.1 with about 12 drops of concd. HCl. The pH is critical.
4. Olive oil emulsion: Add 7.5 ml of olive oil solution slowly to 500 ml of Tris buffer from a pipet. Keep the tip of the pipet under the surface of the emulsion while stirring. Refrigerated, this reagent is stable for a month.

Instrument

We used "Calbiometer" (Calbiochem, La Jolla, Calif. 92037) and Models 2400 and 222 spectrophotometers (Gilford Instrument Laboratories Inc., Oberlin, Ohio, 44074) with the temperature maintained at 37°C ± 1°C.

Procedure

1. Pipet 3.0 ml of olive oil emulsion into a cuvet. Warm to 37°C.
2. Add 100 μl of serum. Mix by inverting the cuvet.
3. Record the change in absorbance for two 1-min intervals (or until consistent).
Calculation

\[ \Delta A/\text{min} (F) = \mu \text{mol triglyceride bonds broken/}
\text{min per liter of serum} \] (For the Gilford Models 222 and 2400 at 340 nm, our value for \( F \) was 5300).

To derive \( F \) (the conversion factor from absorbance units to \( \mu \)mol/liter), add 50 ml of reagent 3 to each of three 100-ml beakers. Deliver 0.3, 0.6, and 0.9 ml of reagent 1 in the same manner as for the preparation of reagent 4. Measure absorbance at 340 nm (or the wavelength selected for the spectrophotometer to be used). Plot absorbance vs. \( \mu \)mol of olive oil per liter of solution. The top curve in Figure 5 is such a calibration, based on olive oil being pure glycercyl triolete (mol wt, 880). If one assumes that the turbidity of this olive oil suspension disappears when one triglyceride bond is broken, then the final calculation for our assay on the Gilford Model 2400 at 340 nm is

\[ \Delta A/\text{min} \times 5400 = \mu \text{mol triglyceride bonds hydrolyzed/min per liter of serum} \]

It is not easy to prove (or disprove) the assumptions in the foregoing calculation and, if preferred, the assay results could be expressed in turbidity units (i.e., \( \Delta A/\text{min} \)). In this case, each spectrophotometer and each wavelength will give a different value for a given serum; we therefore prefer the calibration as described here, even though the assumptions have not been fully validated.

Results and Discussion

Stable Olive Oil Emulsion

Olive oil emulsions are notoriously unstable and difficult to prepare. Using a method similar to Borgstrom (7), we made a solution of olive oil in absolute ethanol (1 ml plus 99 ml) instead of acetone, and stirred this into Tris buffer containing sodium desoxycholate.

Optimal Conditions

Extract of human (cadaver) pancreas had a pH optimum for lipase of 9.0 as shown in Figure 1; the lipase in pooled serum has an optimum of 8.3. These results were similar to those of Vogel and Zieve (4), who chose pH 9.1 in order to optimize conditions for pancreatic lipase in pancreatitis. For our final assay, we chose pH 8.8 in order to use Dade Control Serum (American Hospital Supply Corp., Miami, Fla, 33152) in daily quality control and to assay the normals with a certain degree of reproducibility. The type of buffer (phosphate, glycine, or triethanolamine) has no effect on lipase activity.

Vogel and Zieve (4) found that sodium desoxycholate enhances serum lipase activity and that 3.5 g/liter is an optimal concentration. We chose 3 g/liter (2.95 g/liter as final concentration) on the basis of our results, as shown in Figure 2.

Lipase activity increased with olive oil concentration, as shown in Figure 3, the optimal concentration being about 270 \( \mu \)mol/liter for normal serum and about 170 \( \mu \)mol/liter for pancreatic lipase (based on olive oil being pure glycercyl.
trioleate). We chose the concentration of 170 μmol/liter of olive oil.

The kinetics of lipase action on an olive oil emulsion do not remain zero order for very long (2); only for the first few minutes of incubation (Figure 4). Sera with low activity have a lag of 30 s.

Calibration of Turbidimetric Measurements, Units

The absorbance of an olive oil emulsion is greater at shorter wavelengths (Figures 5 and 6). Furthermore, turbidity measurements vary from one spectrophotometer to another. Thus, absorbance units alone are unsuitable as a common measure for lipase activity (Table 1). Instead we preferred to use the unit μmol/min per liter of serum, as described under Method.

Vogel and Zieve (4) correlated turbidimetric units (20-min incubation) with titration units (3-h incubation). Because their titration curves were not linear with time (their Figure 2), but constantly decreasing, it must be apparent that their factor relating change in turbidity to liberation of fatty acid is an accident of their conditions. The practical problem in obtaining a factor is that the lipase reaction loses zero-order kinetics before there is enough fatty acid for a reliable titration.

---

**Table 1. Lipase Activity for a Serum Sample, as Determined by Use of the "Calbiometer" and the Gilford Spectrophotometer**

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Wavelength, nm</th>
<th>ΔA/min</th>
<th>Factor</th>
<th>Activity, μmol/min/liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calbiometer</td>
<td>Fixed&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.017</td>
<td>7000</td>
<td>119</td>
</tr>
<tr>
<td>Gilford, Mod. 222</td>
<td>340</td>
<td>0.021</td>
<td>7000</td>
<td>111</td>
</tr>
<tr>
<td>Gilford, Mod. 222</td>
<td>450</td>
<td>0.010</td>
<td>7000</td>
<td>114</td>
</tr>
</tbody>
</table>

<sup>a</sup> Standardized with NADH at 340 nm.

---

**Fig. 5.** The absorbance of emulsions containing different concentrations of olive oil, as determined on the "Calbiometer" (G—O), and the Gilford (Model 222) at 340 nm (Δ—Δ) and 450 nm (●—●)

**Fig. 6.** The absorption spectrum of the olive oil emulsion

---

**Normal Range**

The normal range (95% confidence limits) for 80 healthy individuals (males and females) is 7–120 μmol/min per liter of serum at 37°C. We observed no significant differences between the results for males and females.

Figure 7 shows the correlation between amylase as measured by the Caraway method (10) and lipase by the present method. While both show similar trends for amylase values of about 300 Somogyi units (about twice normal) or less, the rise in lipase thereafter is faster than for amylase. This reinforces the conclusion of others (5) that lipase activity is a better indicator of pancreatitis than is amylase activity.

Ticktin et al. (11) noticed that amylase and lipase activities were sometimes (not always) increased in the presence of increased blood urea concentrations. As shown in Table 2, we observed five patients with blood urea N of 80–180 mg/100 ml who had lipases from 190–900 μmol/min per liter and amylases from 150–400 units. Another group,
of five patients had urea nitrogen values of 54–120 mg/100 ml, but lipase activities of 40–100 μmol/min per liter.

Interferences

We noticed that in about 5% of sera of normal individuals there is a slight nonlinear increase, rather than a linear decrease, in turbidity by the present method (probably owing to slight precipitation of some proteins). Because this increase in turbidity is nonlinear, it can be avoided by many simple approaches, such as waiting till two consistent readings are obtained or by using a smaller serum volume, or more preferably by diluting the serum twofold or fivefold with the Tris buffer (containing desoxycholate) and warming this diluted serum at 37°C for 5 min before assay.

Reproducibility

The coefficient of variance for this method is ±7.2%, when the Gilford 2400 spectrophotometer (equipped with a recorder) is used for reference sera ("Moni-Trol II X," Dade Division, American Hospital Supply Corp., Miami, Fla. 33152). This figure is based on 30 determinations (mean, 115 μmol/min per liter), performed by different technicians and with different batches of reagent, during 33 days.

We are happy to acknowledge the technical assistance of Mr. Bruce Kovach. The graphs were drawn by Mr. Alexander Molnar.

References