A Simple Method for the Intravenous Fat-Tolerance Test

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A simple method for measuring intravenous fat tolerance with an artificial fat emulsion (Intralipid®) was studied and compared with the currently used method of Carlson and Rossner [J. Lab. Invest. 29, 271–280 (1972)]. The proposed method uses as little as 3 mL of whole blood to follow, by nephelometry, the elimination of intravenously administered fat emulsions. There is a high correlation between the $K_2$ values obtained by the current method for plasma and those obtained by the proposed method ($r = 0.903$). The latter is simpler, quicker, and more acceptable to patients.

Additional Keyphrases: triglycerides • nephelometry

The intravenous fat-tolerance test (IVFTT), which involves an artificial fat emulsion (Intralipid®), is a convenient tool for the study of triglyceride dynamics in normal and abnormal metabolic states. Intralipid has been found to be a useful tracer for the study of the fractional turnover rate of circulating triglycerides (1). The rate of elimination ($K_2$) of Intralipid from the bloodstream for any person is reproducible when the same batch of the emulsion is used, even when repeated after an interval of six months (2). Recently, the IVFTT has also been found useful for determining the appropriate dose of Intralipid emulsion to use therapeutically in newborns of various gestational ages (3).

Carlson and Rossner (4) described a method for an IVFTT in which plasma Intralipid triglycerides is analyzed by nephelometry. However, this method requires 40 to 70 mL of blood and takes 1 to 2 h. We propose a simple method that uses as little as 3 mL of whole blood, takes much less time to complete, and yields results that correlate well with those of the method suggested by Carlson and Rossner.

Materials and Methods

Subjects

Twenty apparently healthy volunteers, nine men and 11 women between 23 and 58 years of age, participated in the study. Their informed written consent was obtained before the study.

Reagent

Intralipid (batch no. 195271) was supplied by Cutter Laboratories, Inc., Berkeley, CA 94710. Intralipid 10% intravenous fat emulsion contains per liter, fractionated soybean oil, 100 g; fractionated lecithin from egg yolk, 12 g; glycerol, 25 g; and sterile water to 1 L.

Procedure

After overnight fasting, collect "zero-time" samples of venous blood from subjects for nephelometric blank value and hematocrit. Then administer Intralipid 10% emulsion, 1 mL/kg of body weight (0.1 g/kg body weight) intravenously. Every 5 min for 1 h, collect 0.5 mL of venous blood into small heparinized conical tubes. Pipette 200 μL of this blood into duplicate tubes containing 10 mL of cooled (4 ºC), normal saline; mix immediately by inverting. Centrifuge samples in an I.E.C. refrigerated Model B20 centrifuge at 1500 rpm for 15 min. Transfer the supernate to cuvets and read the light-scattering index with a Coleman Model 2 Neph-Colorimeter (Coleman Instruments Division, Hinadale, IL 60521.)

Standards

Standards for the proposed method contained 10 mL of saline, 200 μL of "zero-time" whole blood, and various amounts of the emulsion to give final Intralipid-triglycerides concentrations of 0.25, 0.50, 1.0, 1.5, and 2.0 g/L. After these mixtures were centrifuged at 1500 rpm, the light-scattering index of the supernates was determined. To study the effect of initial turbidity on Intralipid-triglycerides values, we also prepared standards that contained 100, 300, and 400 μL of "zero-time" whole blood.

Storage of Samples

To study the effect of storage, we stored whole blood samples and the supernate at 4 ºC or at room temperature up to 96 h. We repeated the nephelometric readings every day.

Calculations

The mean of the duplicate nephelometric readings was converted to Intralipid-triglycerides by reference to a standard graph. The concentration of Intralipid-triglycerides in plasma was determined according to the following formula:

$$\text{Plasma Intralipid-triglycerides concentration} = \frac{\text{Intralipid-triglycerides concentration in whole blood supernate} \times 100}{100 - \text{hematocrit}}$$

The concentration of Intralipid-triglycerides in plasma was plotted on a logarithmic scale vs. time on a linear scale. The rate of elimination of the emulsion from the bloodstream, $K_2$, was calculated by the method of least squares (3). The linearity of the $K_2$ graphs was determined visually and by the $R^2$ value for the regression line. (The $R^2$ value is the proportion of total variability among the y-values that is accounted for by the regression line.)

Results

The graphs of the Intralipid standards were always linear.
and included the origin. This did not change by varying the amounts of “zero-time” blood, which suggests that the initial turbidity of the samples did not affect the Intralipid-triglycerides values.

$K_2$ values obtained by the proposed method gave a high correlation ($r = 0.903$) with the $K_2$ values obtained by the current method of Carlson and Rossner. The mean $K_2$ values by their method was $6.34 \pm 0.68$ (SEM) and by the proposed method was $7.15 \pm 0.70$ (SEM) (Table 1). The graphs for elimination rates vs. time appeared nearly linear, which was confirmed by the high values for $R^2$. Graphs of results by both methods showed slight but similar patterns of deviation from linearity. As is seen from the relative magnitude of $R^2$ for the two methods, there is no difference between them with respect to linearity.

**Effect of Storage on Samples**

Heparinized whole blood samples stored at 4 °C and at room temperature developed fibrin clots overnight and showed various amounts of hemolysis. Repeating the analysis with these samples gave inconstant and unreliable elimination values.

$K_2$ values for the supernate from whole blood samples stored for 24, 48, or 96 h were the same as the original values (Table 2). Storing these samples at either 4 °C or at room temperature did not affect the readings.

### $K_2$ in Samples Obtained over a 30-Min Period

We calculated $K_2$ from the Intralipid-triglycerides concentrations of samples obtained during the first 30 min of monitoring and compared it with that obtained by the current method of sampling for 60 min. The correlation between the two values was high ($r = 0.937$), which suggests that the length of the sampling can be decreased to 30 min.

### Discussion

We found that the current method for the IVFTT poses two main problems:

(a) Because of the recentrifugation and the tedious pipetting involved in the preparation of the plasma samples, it takes about 1.5 to 2 h to complete the analysis.

(b) To obtain sufficient plasma so that the Intralipid does not float to the top, analysts must use large volumes of blood (a total of 40 to 70 mL). Removal of such large amounts of blood is undesirable in many subjects and impractical in children. An alternative suggestion of using capillary blood involves pricking the finger 13 times, which is not generally acceptable to patients.

The proposed method involves only one centrifugation, in which the blood cells form a firm deposit at the bottom of the tube. There is no problem with the Intralipid floating to the top or sticking to the sides of the tube. Because the supernate can be transferred directly into the cuvetts, the time-consuming pipetting can be avoided. The whole analysis takes only 30 to 45 min and requires a much smaller amount (6 to 7 mL for duplicate analysis) of venous blood. The amount of blood could be further reduced to as little as 3 mL by de-
creasing the duration of sampling to 30 min. Finally, results by this method correlate well (r = 0.903) with results by the currently used method.

We suggest that this method is simpler, quicker, and more acceptable to the patient, whilst at the same time just as accurate as the method of Carlson and Rosner. The proposed method can also be used to study children, even newborns.

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References


Assessment of Fetal Lung Maturity by Colorimetric Phospholipid Determination without Digestion

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A previously described method for the determination of phospholipid concentration in amniotic fluid without digestion has been modified to make it more suitable for use in a routine laboratory. Results compare well with those by the original procedure. Results from nearly 70 amniotic fluids, collected at delivery, were compared with lecithin/sphingomyelin (L/S) ratios determined on the same fluids. Statistical analysis of these data showed that for the prediction of lung immaturity, determination of total phospholipid concentration was at least as good as the L/S ratio.

The undoubted usefulness of measurement of the lecithin/sphingomyelin ratio (L/S ratio) in amniotic fluid in the assessment of fetal lung maturity is reflected by the large amount of literature that has followed the early work by Gluck and his colleagues (1). Disadvantages of L/S ratio measurement include the labor intensiveness of the procedure, its unsuitability for a routine laboratory, and its precision, which is only that of a semi-quantitative technique. These problems are overcome by measuring total phospholipid concentration colorimetrically by a method not involving a digestion step. We have previously described such a method in which phospholipids are complexed with molybdophosphate through their quaternary nitrogen and then reduced to molybdenum blue (2). This method has been modified by carrying out the initial reaction and the reduction in a single system instead of a biphasic one. Results on more than 100 specimens have been compared with those obtained with the L/S ratio or those obtained by using our previously described method (2). These comparisons enable us to comment on the suitability of this method as a routine technique for the assessment of fetal lung maturity.

Materials and Methods

Reagents

Dipalmitoyl phosphatidyl choline (synthetic, 98% pure), Sigma Chemical Co., Poole, Dorset, U.K.
Dodeca-molybdophosphoric acid, BDH Chemicals Ltd., Poole, Dorset, U.K.
Chloroform, analytical grade.
Methanol, analytical grade.
Stannous palmitate, Sigma Chemical Co.

Determination of Lecithin by Reduction of Its Molybdophosphate Complex

In the procedure for lecithin determination described previously, molybdophosphoric acid in aqueous solution was reacted with lecithin in a chloroform solution, resulting in the transfer of molybdophosphate into the chloroform phase to form a lecithin–molybdophosphate complex, which was then reduced to molybdenum blue with a reducing agent prepared