breathe 100% oxygen. Under these conditions, oxygen is delivered to tissues from the erythrocytes, the Fluosol, and the plasma.

Evaluation of the efficacy of the Fluosol-DA necessitates determination of the O₂ content of each of the compartments. Currently, an O₂-specific fuel cell (Lex-O₂-Con; Lexington Instruments, Waltham, MA 02154) is used to measure the total O₂ content of a whole-blood sample containing Fluosol. The O₂ content of the water compartment is calculated as the product of the measured DO₂, the volume concentration of water (1 - fluorocrit), and the solubility coefficient for O₂ in water. The O₂ content of the Fluosol compartment is the product of the DO₂, the volume concentration of the Fluosol (fluorocrit), and the solubility coefficient for O₂ in Fluosol. The O₂ content of the erythrocytes is obtained indirectly from the difference between the total O₂ content measured by the Lex-O₂-Con and the sum of the calculated O₂ contents for the Fluosol and plasma compartments.

Although the IL 282 CO-Oximeter (Instrumentation Laboratory, Lexington, MA 02173) provides a direct means of determining the O₂ content of whole blood, it is sensitive to light scattering and involves the use of a detergent to lyse the cells (3). Fluorocarbon emulsions cause significant light scattering in the CO-Oximeter. Our experience with lipid-encapsulated hemoglobin ("hemosomes") that similarly is not lysed by the CO-Oximeter, suggested that the determination of O₂ content is unaffected by light scattering. We tested to see if the same was true with whole blood in the face of the light-scattering effect of the fluorocarbon emulsions.

We diluted whole blood with Fluosol-DA, 20%, to cover an approximate fluorocrit range of 2 to 26% and a fluorocrit range of 4 to 13%. Identical dilutions were made with a clear, balanced electrolyte and colloid solution that did not contain the fluorocarbons. The hemocrits at each dilution point were matched between the two groups and were within 1% of each other. All samples were equilibrated with room air and the erythrocyte O₂ contents determined with the CO-Oximeter. The measured fluorocrits in the whole-blood-Fuoslol mixtures ranged from 4.3 to 13.8%, and the O₂ contents in both groups ranged from 9.0 to 116 cm³/L. A plot of the erythrocyte O₂ contents obtained for 25 whole-blood-control mixtures (true values, y) vs. those obtained for the corresponding whole-blood-Fluosol mixtures (x) was linear by the method of least squares. The linear correlation coefficient was 0.999, the slope of the line was 0.903, and the intercept was 0.22. The standard error of estimate was 0.6 cm³/L.

Our data suggest that over a range of fluorocrits (4 to 13%) that exceeds the maximum permissible clinical concentration, the IL 282 CO-Oximeter can be used to determine directly the erythrocyte O₂ content in the case of a patient who is receiving Fluosol-DA, 20%.

References

L. R. Sehgal
H. L. Sehgal
A. L. Rosen
S. A. Gould
L. M. Dalton
C. L. Rice
G. S. Moss
Div. of Biochem. Res.
Dept. of Surgery
Michael Reese Hosp. & Medical Center and The University of Chicago
Chicago, IL 60616

Fasting and Gender (and Altitude?) Influence Reference Intervals for Serum Bilirubin in Healthy Adults

To the Editor:

The influence of fasting and gender on serum bilirubin concentration has been previously evaluated in rather ill-defined populations (1-5). The most frequently quoted reference interval for total serum bilirubin concentrations in adults is 0 to 12 mg/L. It still is a problem for the clinician to evaluate marginal increases in serum bilirubin in otherwise healthy patients. Serum bilirubin concentrations below 10-12 mg/L are correctly considered to be normal (6-9), at least for 95% of the population. Concentrations as high as 15 mg/L are, however, often considered abnormal and may prompt a more extensive (and expensive) workup of the patient, even in the absence of other clinical evidence of disease.

We investigated serum bilirubin concentrations in a cross-sectional study of 50 men and 50 women between the ages of 21 and 44 who had no prior history of disease, had taken no medication (including alcohol) for at least one week before blood sampling, had passed a brief physical examination, and had all other laboratory results within the reference intervals (chloride, carbon dioxide, potassium, sodium, serum urea nitrogen, glucose, total protein, albumin, calcium, inorganic phosphorus, cholesterol, uric acid, creatinine, alkaline phosphatase, lactate dehydrogenase, aspartate aminotransferase, alanine aminotransferase, iron, direct bilirubin, and total bilirubin determined with a Technicon SMA® analyzer; and blood-cell counts performed with a Coulter S Plus®). All subjects were within ±10% of the Metropolitan Life Insurance Co. averages for height and weight (10). The age range, mean, median, and mode for the male and female population samples were as similar as possible (without taxing the study's financial and time constraints). For the men the age range was 21 to 42 years, with a mean of 28, median of 29, and mode of 29. In comparison, the age range for the women was 21 to 44 years, with a mean of 29, median of 27, and mode of 24. Finally, subjects were residents of the geographic area served by our hospital (average elevation, 500 ft. above sea level). We obtained blood specimens from each subject, non-fasting (2 ± 0.5 h post-prandial) and after a 12-h fast. Total bilirubin was determined with a SMA® analyzer by the automated diazo method of Gambino and Schreiber (11), which is based on the Jendrassik/ Grof procedure as modified by Nosslin (12).

Method precision was assessed from the controls, which were included as every eighth specimen; normal and abnormal controls were alternated in this repeating pattern. During the study the normal control mean was 5.9 (SD 0.7) mg/L (CV 11.9%) and the abnormal control mean was 48.3 (SD 1.7) mg/L (CV 3.5%). Six proficiency specimens...
Table 1. Influence of Gender and Fasting on Total Bilirubin Concentration (mg/L) in Serum

<table>
<thead>
<tr>
<th></th>
<th>12-h fasting</th>
<th>Non-fasting</th>
<th>Fasting vs non-fasting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (and SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>10 (5)</td>
<td>8 (4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Women</td>
<td>7 (4)</td>
<td>6 (3)</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Men vs women, p</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Ninety-five Percentile Reference Intervals for Serum Bilirubin (mg/L)

<table>
<thead>
<tr>
<th></th>
<th>±2 SD</th>
<th>±2 SD</th>
<th>5–95 percentiles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men, fasting</td>
<td>0 to 20</td>
<td>4 to 20</td>
<td>5 to 24</td>
</tr>
<tr>
<td>Men, non-fasting</td>
<td>0 to 15</td>
<td>2 to 16</td>
<td>3 to 14</td>
</tr>
<tr>
<td>Women, fasting</td>
<td>0 to 12</td>
<td>2 to 13</td>
<td></td>
</tr>
<tr>
<td>Women, non-fasting</td>
<td>0 to 12</td>
<td>2 to 13</td>
<td></td>
</tr>
</tbody>
</table>

were analyzed during the study; their standard deviation index averaged ~0.2 and ranged from ~0.4 to 0. In these proficiency challenges our results were compared with those of as many as 270 laboratories using the same methodology.

The non-fasting, fasting results within the men’s and women’s population samples were analyzed by the paired t-test. An unpaired t-test was used to compare results both for non-fasting and fasting from the men’s and women’s groups. The significance level was set at p <0.05 for all statistical evaluations.

In both sexes the bilirubin concentrations during fasting and non-fasting differed significantly (Figure 1), bilirubin concentrations increasing in the fasting specimens. In men, the average bilirubin increased 2 mg/L after fasting for 12 h (Table 1). In women, this increase resulting from fasting was less pronounced (average 1 mg/L), but still statistically significant (p <0.05). The individual changes in serum bilirubin concentration due to fasting averaged 1.8 (SD 2.3) mg/L for women and 2.4 (SD 2.5) mg/L for men (fasting values minus the non-fasting values). In some individuals the changes were relatively large. One woman’s serum bilirubin changed from 13 to 14 mg/L, the latter value being outside most reference intervals in current use. Among the men, the greatest increase attributable to fasting was 14 mg/L (from 11 mg/L during non-fasting to 25 mg/L during fasting). Clearly, fasting has an important effect on bilirubin concentration. But in 21 women and 13 men the bilirubin concentrations were not increased during fasting at all; some even showed decreases.

The highest mean serum bilirubin concentrations were found in fasting men: 10 (SD 5) mg/L, as compared with 7 (SD 4) mg/L in fasting women. This difference is statistically significant (p <0.001). The average difference between results for men and women during non-fasting was only slightly less (2 mg/L), and had the same statistical significance (p <0.001). The results have been summarized in Table 1.

Separate reference intervals were established for each of the four test groups, i.e., fasting and non-fasting men, fasting and non-fasting women. The limits used to include 95% of the results in each group were determined by three different approaches for comparison: the parametric gaussian distribution function involving the ±2 SD arithmetic solution, a logarithmic transformation, and the nonparametric percentile method (13,14). The 95% reference intervals calculated by these three approaches are given in Table 2. With only 50 data points in each group, the error obtained by the percentile method would be quite large (14). The lower limits established by use of the logarithmic transformation approach were considered to be relatively unimportant, and the upper limits were quite similar to the ±2 SD determinations. We elected to use the reference intervals calculated by using the mean ±2 SD. When all results during non-fasting (both sexes) were combined, the ±2 SD limits were 0 to 13 mg/L, mean 6.7 (SD 3.4) mg/L, and when all fasting results were combined the limits were 0 to 17 mg/L, mean 8.2 (SD 4.4) mg/L. The limits when all data were combined (both sexes, fasting and non-fasting) were 0 to 15 mg/L. This upper limit is significantly higher than most published reference intervals.

In line with the method selected for calculating the reference intervals, namely, ±2 SD, 2.5% of the results exceed the reference ranges, i.e., 2% of non-fasting men, 4% of fasting men, 2% of non-fasting women, and 2% of fasting women. As expected, five of 200 (2.5%) of the results exceed the upper 2 SD limit. However, if we use for comparison the most frequently quoted reference interval for serum bilirubin—0 to 12 mg/L—14% of the non-fasting men, 20% of the fasting men, 2% of the non-fasting women, and 14% of the fasting women exceed the reference range; 25 of the 200 results (12.5%) would be considered “abnormal.” About 22 of the results would be falsely classified as “abnormal” and might prompt further workup.

Several investigators have reported that males tend to have higher concentrations of serum bilirubin than females, but these findings seem to have had little impact on published reference intervals (6–9). Our findings indicate that separate reference intervals for serum bilirubin should be established for each sex.

Living at a relatively high altitude, 5000 feet, appears to increase bilirubin concentrations in serum. When the data from our study are grouped without regard to gender or state of fasting, the ±2 SD intervals are significantly increased in comparison with most published reference intervals. It is well established that individuals living at an altitude of 5000 feet above sea level have significantly increased hematocrits, yet we found no significant correlation between individual bilirubin concentrations and either hemoglobins or hematocrits. The average SDI of ~0.2 achieved on the CAP proficiency challenges would seem to rule out analytical bias as being responsible for the increased bilirubin concentrations. To our knowledge, the effect of altitude on serum bilirubin concentration has not previously been described in the literature. Laboratories serving patients who live at unusually high elevations should establish reference information from a population sample living at a similar altitude.

This investigation was supported in part by a research contract with Sterling-Winthrop Research Institute; grant R01 CA5K04 (400016-05 from NICHS, NIH; and Associated University Pathologists.

References


George L. White, Jr.1
James A. Nelson2
Donald M. Pedersen1
K. Owen Ash3

Depts. of Family and Community Medicine,1
Radiology,2 and Pathology3
University of Utah School of Medicine
Salt Lake City, UT 84132

Address correspondence to K. O. A.

Easy Measurement of Viscosity of Semi-Micro Serum Samples

To the Editor:

The viscosity of serum is measured to ascertain hyperviscosity syndrome (HVS) and the need for therapeutic pheresis in patients with hyperglibulinemia (1). Usually the viscosity relative to water is determined with a capillary viscometer (2). A recent case review (3) reported the need to draw repeated samples and up to 40 mL of blood to get sufficient serum for the viscosity measurement. We have found an apparatus with which one can make conventional relative viscosity measurements on less than 1 mL of serum. Values for a group of healthy individuals matched the reported range (2).

(a) Normal serum samples. Blood samples were drawn from employees at the annual physical examination. (Informed consent for an extra red-top tube of blood was obtained before venipuncture.) Serum was obtained by centrifugation. Samples not measured on the day of collection were stored covered, in a refrigerator. Electrophoresis was run independently on the same samples to establish normal values on a new densitometer.

(b) Viscometer. A no. 100 Cannon-Manning semi-micro viscometer, with a sample volume of 0.6 mL, was used in the procedure provided by the manufacturer (Cannon Instruments, State College, PA).

(c) Procedure. Temperature was maintained at 37.0 ± 0.01 °C in a Sargent Thermostor bath (Sargent-Welch Scientific Co., Skokie, IL). The requisite sample volume was drawn into the viscometer with a Clay-Adams pipette suction apparatus. Flow time was measured with a hand-held stopwatch accurate to 0.1 s.

The sample was drawn above the mark and allowed to drain for five measurements; the average flow time was calculated. Water was measured first, to assure that the flow time matched previous calibrations and to provide the baseline for calculation of relative viscosity. Between samples, the viscometer was cleaned with the aid of a vacuum line and trap, with detergent solution followed by a water rinse, and then dried with acetone.

(d) Results. The flow times for water in the two viscometers used in this study were 45.6 and 46.4 s. A CV of 0.13% was found for a single run with water; the day-to-day CV was 1.4% over 13 days. The mean relative viscosity of serum samples from 60 healthy subjects was 1.64 (SD 0.07), the range 1.52 to 1.90. A normal range of 1.4 to 1.8 has been reported (2). The total running time for a sample plus water calibration is about 30 min. Patients’ samples may require more time, in relation to their degree of hyperviscosity.

No change in relative viscosity was found after refrigerated sample storage for up to eight days in a Parafilm-sealed tube.

(e) Discussion. If a patient has hyperviscosity syndrome, it is difficult to separate the serum from the clot and cells. Thus it is a problem to procure sufficient sample (5 mL) for viscosity measurements as generally outlined in a textbook of laboratory procedures (1, 2). The semi-micro viscometer we used gave excellent reproducibility and required only 0.6 mL of sample. The procedure is really more accurate than necessary in that changes of three- to fourfold are usually necessary before symptoms are seen (1).

HVS causes multiple symptoms and is readily treated by therapeutic pheresis (1). Availability of this simple, rapid procedure, involving conventional venipuncture techniques, would encourage close monitoring of patients with HVS. Plasmapheresis may then be performed before clinical signs are obvious.

The availability of a simple, accurate, and semi-micro viscosity procedure may also permit other useful clinical measurements on biological fluids. For example, the viscosity of pleural fluid provides data for differential diagnosis of mesothelioma (4). The viscosity of any fluid free of cells, clots, and particles may be determined.

References


Harold Van Kley
Peggy E. Lathrop

Res. Labs.
St. Mary’s Health Center
6420 Clayton Rd.
St. Louis, MO 63117

Improved Method for Determination of Triglycerides In Plasma Lipoproteins by an Enzymic Kit Method

To the Editor:

Human plasma lipoproteins have been classified, on the basis of density, into chylomicrons, very-low-density lipoproteins (VLDL, relative density <1.006 kg/L), low-density lipoproteins (LDL, rel. dens. 1.019–1.063), and high-density lipoproteins (HDL, rel. dens. 1.063–1.21). A major function of these lipoproteins is to transfer the lipids and regulate the lipid metabolism. In patients with hypertriglyceridemia, their VLDL- and chylomicron-triglycerides are drastically increased and their HDL concentrations are usually lower than those of normal persons. The methods available for estimating triglycerides in chylomicrons and VLDL include thin-layer and gas–liquid chromatography, liquid scintillation counting procedures (1), and fluorometric techniques (2). Enzyme kits are cur-