Lipemia is a potential cause of analytical interference (1, 2). Determinations of the lipemic index (L-index) or triglyceride concentrations are used to quantify lipemia (3, 4). The soy-based lipid emulsion, Intralipid, has been used to simulate lipemia in interference studies (2, 5), but without evidence of how well it simulates naturally occurring lipemia.

Many serum proteins can be quantified by immunoturbidimetric assays (6). Lipemia interferes by altering light scattering (1). Manufacturer often provide guidelines for the maximum acceptable lipemia that have been established by interference experiments using Intralipid-supplemented samples. For the Modular Analytics P analyzer (Roche Diagnostics), the maximum allowable triglyceride values range from 4000 mg/L for the prealbumin assay to 20 000 mg/L for the haptoglobin assay. Sample turbidity is only weakly correlated with triglyceride concentration in patient samples (4). Thus, to test the validity of the lipemic thresholds, we directly compared interference from lipemic patient samples with interference induced by supplementation with Intralipid.

We prepared pooled samples by mixing excess serum from two to five individual patient samples. Samples were assayed immediately or were stored at 4 °C for up to 2 weeks before testing. Samples were mixed by multiple manual inversions before analysis. The 16 pooled samples were simultaneously assayed for L-index, triglycerides, α1-antitrypsin, ceruloplasmin, haptoglobin, prealbumin, and transferrin on a Modular Analytics P 800 analyzer. All results are the means of duplicate measurements.

The protein assays are non-particle-enhanced immunoturbidimetry assays. The primary assay wavelength is 700 nm and the secondary wavelength 340 nm, except for transferrin (secondary wavelength, 505 nm). All absorbances measured at these wavelengths were within the linear range of the photometer. Triglycerides were measured by a colorimetric enzymatic assay with a primary wavelength of 700 nm and a secondary wavelength of 505 nm. The L-index was determined by the difference between the absorbance of the sample diluted in isotonic saline at 660 nm and 700 nm.

The protein assays used similar reagent and sample volumes. For the ceruloplasmin and prealbumin reactions, 20 μL of sample was mixed with the first reagent (250 μL), and then the second reagent (83 μL) was added. The α1-antitrypsin and haptoglobin reactions were similar except that the sample volumes were 10 μL. In the transferrin assay, the initial sample volume is 35 μL, the first reagent volume is 270 μL, and the second reagent volume is 100 μL.

After analysis, the samples were subjected to ultracentrifugation in an Airfuge (Beckman Coulter) at 100 000 g for 5 min. This markedly reduced the visual turbidity of the sample infranate. The infranate was then reassayed in duplicate for the same set of analytes. Intralipid (200 mL/L; Baxter Healthcare Corp.) was then added to each sample to produce L-index values comparable to or exceeding those of the original serum pool. The addition of Intralipid had a very small impact on the total volumes of the samples. In general, the addition of 10 μL of 200 mL/L Intralipid to a 1-mL nonlipemic serum sample generated a L-index increase of ~225. For each pool, the concentration of triglycerides in the Intralipid-supplemented infranate exceeded that in the original pool. The Intralipid-supplemented samples were then assayed in duplicate. The ratio of the measured analyte concentrations in each sample after ultracentrifugation to that in the original patient sample was calculated. Sample dilution was performed with sterile normal saline. All studies using samples obtained from humans were approved by the Institutional Review Board of the University of Utah.

The relationship between L-indices and triglycerides in patient samples and Intralipid-supplemented patient samples were examined (Fig. 1, A and B). The correlation coefficients for triglyceride concentrations and L-indices in Intralipid-supplemented serum pools (r = 0.84) and lipemic patient samples (r = 0.88) were comparable.

The effects of lipemia on test results were examined (Fig. 1C). Increasing triglyceride concentrations in the original pool were associated with substantial depressions of reported results for both ceruloplasmin and prealbumin. The degree of interference varied linearly with the triglyceride concentrations and correlated less well with L-index values (data not shown). Linear best fits of the data indicated that at a triglyceride concentration of 10 000 mg/L, the measured ceruloplasmin and prealbumin concentrations were depressed 52% and 36% compared with the observed values in infranates (Fig. 1C). Measured transferrin concentrations in the pooled sera were slightly depressed in lipemic samples (14% depression at a triglyceride concentration of 10 000 mg/L). These triglyceride concentrations (10 000 mg/L) roughly correspond to a L-index of 200 in patient samples (Fig. 1A). Lipemia in patient samples had little effect on measured concentrations of α1-antitrypsin and haptoglobin. In contrast to native lipemic samples, supplementation of pooled sera with Intralipid to a triglyceride concentration of 16 000 mg/L (L-index of ~400) had minimal effect on all analytes tested (Fig. 1D), although the measured ceruloplasmin concentration was observed increased slightly. As indicated by a linear fit of the data, at a triglyceride concentration of 10 000 mg/L, the measured ceruloplasmin concentration increased 7%.

All mean analyte concentrations after ultracentrifugation were near the middle of their respective reference intervals. The mean (SD) for the analytes after ultracentrifugation were 300 (100) mg/L for ceruloplasmin (refer-
ence interval, 200–600 mg/L) for haptoglobin (reference interval, 300–2000 mg/L), 280 (60 mg/L for prealbumin (reference interval of 200–400 mg/L), 1400 (300) mg/L for α1-antitrypsin (reference interval, 1000–2000 mg/L), and 2800 (700) mg/L for transferrin (reference interval, 2000–4000 mg/L). The L-index in the samples after ultracentrifugation was dramatically reduced relative to the original samples and had a mean

Fig. 1. Results for the various analytes (A–D) and reaction monitor data (E and F).

(A–D), individual results (error bars, 1 SD) and linear regression lines are shown. For points with no apparent error bars, the SD is less than the size of the data point. (A), relationship between L-index values and triglyceride concentrations for 16 patient samples. The slope is 0.165; r = 0.876. (B), L-index values vs triglyceride concentrations for the same 16 samples after ultracentrifugation and supplementation with Intralipid. The slope is 0.256; r = 0.842. (C and D), results for α1-antitrypsin ( ), ceruloplasmin ( ), haptoglobin ( ), and prealbumin ( ). (C), the ratio of the analyte concentration measured in patient samples to the analyte concentration measured after removal of lipemia by ultracentrifugation is plotted on the y axis. (D), the ratio of analyte measured in Intralipid-supplemented samples to analyte measured after removal of lipemia by ultracentrifugation is plotted on the y axis. (E and F), reaction monitor data for ceruloplasmin and haptoglobin. Data were obtained from a single patient sample ( ); L-index, 121; triglycerides, 3570 mg/L), after ultracentrifugation ( ); L-index, 12; triglycerides, 1920 mg/L), and after Intralipid supplementation ( ); L-index, 195; triglycerides, 4560 mg/L). The mean net absorbances of time points 15 and 16 are subtracted from the net absorbance readings at time points 33 and 34 to obtain the absorbance change for each sample. Arrows indicate these time points. (E), volume-corrected data for the ceruloplasmin assay. The sample (20 μL) is mixed with the first reagent (250 μL) at time point 0. At time point 16, the second reagent (83 μL) is added (represented by the thick vertical line). The net absorbance after time point 16 is corrected for sample dilution by dividing by 0.765. The ceruloplasmin concentrations were 149 mg/L for the original sample, 216 mg/L for the sample after ultracentrifugation, and 225 mg/L for the Intralipid-supplemented sample. (F), volume-corrected data for the haptoglobin assay. All conditions are as described for the ceruloplasmin assay except for an initial sample volume of 10 μL and a dilution correction factor of 0.758. The measured haptoglobin concentrations were 1246 mg/L for the original sample, 1336 mg/L for the sample after ultracentrifugation, and 1308 mg/L for the Intralipid-supplemented sample.
The residual triglyceride concentrations in the samples after ultracentrifugation were reduced less uniformly, yielding a mean (SD) concentration of 3900 (2900) mg/L. Finally, ultracentrifugation of five Intralipid-supplemented samples was, in some cases, less effective in reducing L-index [mean (SD) L-index, 37 (28)] than ultracentrifugation of native patient samples.

Refrigerated storage of serum pools did not appear to significantly affect the lipemic interference. A freshly pooled sample with a triglyceride concentration of 6450 mg/L and a L-index of 160 exhibited lipemic interference comparable to the lipemic interference effects observed in stored serum pools as described above. For example, the original patient sample had a measured ceruloplasmin concentration of 174 mg/L and a prealbumin concentration of 220 mg/L, which increased after ultracentrifugation to 319 and 319 mg/L, respectively. After storage at 4 °C for 6, 9, and 15 days, the measured L-index and all analyte concentrations were identical or increased only slightly. After 15 days of storage, the measured L-index was 177, the measured triglyceride concentration was 6900 mg/L, and the ceruloplasmin and prealbumin concentrations were 208 and 230 mg/L, respectively.

Dilution was examined as an additional method to reduce assay interference. After 15 days of storage, the same pooled sample was diluted 1:3 with normal saline, and the concentrations of the analytes were measured. The L-index was 168, the triglyceride concentration was 6690 mg/L, and the ceruloplasmin and prealbumin concentrations were 318 and 345 mg/L, respectively, comparable to concentrations observed after ultracentrifugation. Thus, dilution appears to markedly reduce lipemic interference in these assays.

Examination of the P 800 analyzer reaction monitors for the ceruloplasmin and haptoglobin assays revealed a possible explanation for the negative interference by native lipemia seen for ceruloplasmin. The original sample examined was moderately lipemic and had a L-index of 121 as is frequently observed in patient samples. When reagent was added after timepoint 16, the absorbance decreased slightly at timepoints 17 and 18 for the native sample but increased noticeably for both the centrifuged and Intralipid-supplemented samples (Fig. 1E). This decrease in absorbance after reagent addition to a lipemic sample likely plays a role in the 30% depression of the measured ceruloplasmin concentration because it decreases the difference between the initial sample absorbance measurements before second reagent addition and the final absorbance measurements. This effect was not observed for the measurement of haptoglobin, although a larger change in absorbance during the haptoglobin assay may render the effect relatively inconsequential (Fig. 1F).

Finally, a slight slowing of the rate of the reaction in the lipemic sample relative to that seen after ultracentrifugation cannot be entirely excluded.

The observed correlation between the triglyceride concentration and turbidity was relatively poor ($r < 0.90$) in both the Intralipid-supplemented and patient lipemic samples. The poor correlation between measured turbidity and triglycerides in patient samples has been noted previously, although supplementation with Intralipid led to a good correlation between triglycerides and patient sample turbidity when lipid emulsion was titrated into a single pool of patient samples (4, 7). The nonlinear relationship between triglyceride concentration and sample turbidity in lipemic patient samples likely results from nonhomogeneity in the organization of lipids in patient samples (4, 8–10). Triglycerides are likely to be present in various macromolecular forms, including both VLDL particles and chylomicrons. Chylomicrons can contribute substantially to sample turbidity, whereas VLDL particles make more minor contributions. The distribution of triglycerides in these macromolecular complexes is patient-specific.

Lipemia found in patient samples, patient samples after ultracentrifugation, and in samples supplemented with Intralipid elicited markedly different degrees of interference in some clinical assays. Turbidimetric assays may be particularly sensitive to selective perturbation by lipemic samples. Differences in particle size in lipemic samples can dramatically affect light scattering and alter turbidity (11). Ultracentrifugation preferentially removes larger buoyant macromolecular lipid complexes such as chylomicrons from patient samples and thus alters the lipid profile. The lipid composition in the soy-based emulsion Intralipid consists predominantly of small, relatively dense, phospholipid-rich liposomes and triglyceride-rich artificial chylomicrons (12), whereas patient samples contain a complex mixture of macromolecular lipid-and-protein structures (8).

The assay-specific nature of the interference in lipemic patient samples appears to be predominantly attributable to disruptions of complex macromolecular structures on the introduction of assay-specific reagents. This may be caused by differences in the composition of these reagents or by the presence of trace amounts of detergents. Polyethylene glycol, which is a component of all of the reaction reagents, may contain trace amounts of contaminating detergents. Patient samples, after either ultracentrifugation or Intralipid supplementation, exhibit only minor assay interference, presumably because of the lack of higher-order lipid-lipoprotein complexes. However, assay-specific interference in lipemic samples arising through phase-partitioning of the analyte and increases in the volume of the nonpolar phase that slow the reaction rate cannot be completely ruled out (13).

These results have important implications for the evaluation of lipemic interference in the clinical laboratory. In some assays, the lipemic interference in patient samples was not observed in Intralipid-supplemented samples with comparable or higher L-index and triglycerides values. This may not be limited to turbidimetric assays, as it has been noted that for a rate-blanked compensation assay for creatinine, lipemic interference can occur in patient samples but not in samples containing synthetic lipid emulsions (7). Thus, the use of samples supplemented with Intralipid does not provide a universally applicable method to estimate endogenous assay interfer-
ference in lipemic patient samples. This suggests that interference studies should be performed using lipemic patient samples rather than Intralipid-supplemented samples.

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References

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