Low Bias in Assayed Values of Lipoprotein Antigens—Lipoprotein(a) and Apolipoproteins A-I and B—in Midday Postprandial Blood Specimens Compared with Morning Fasting Specimens

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Two-hour postprandial specimens have a -14% proportional bias for lipoprotein(a) (Lp(a)), a -0.035 g/L systematic bias for apolipoprotein (apo) A-I, and a -9% proportional bias for apo B, compared with values in 12-h fasting specimens. Although a physiological hemodilution appears to account for a portion of these biases, other major factors must be implicated for Lp(a) and apo B. Even after dilutional effects are controlled for, assayed values of Lp(a) are 11-13% lower, and assayed values of apo B are 8-9% lower, in postprandial specimens than in fasting specimens. Therefore, the time of collection of a blood sample relative to the last meal can significantly affect assayed values of lipoprotein antigens. Further studies are needed to determine whether these observations result from a physiological sequestration of lipoproteins in the postprandial state or from negative interference affecting the assays of lipoprotein antigens.

Additional Keyphrases: sample collection • variation, source of

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References
ments, Brea, CA 92621) and reagents from Beckman; Lp(a) was quantified with the Macra Lp(a) kit (Terumo Corp., Elkton, MD 21921). To determine whether observed differences between postprandial and fasting specimens were the result of hemodilution, I also analyzed for albumin and total protein aliquots of sera frozen at -80 °C; for these assays, I used the Ektachem® (Clinical Products Division, Eastman Kodak Co., Rochester, NY 14650).

To determine whether the observed differences between postprandial and fasting concentrations of lipoprotein antigens were significant, I used both paired sample t-tests and linear-regression analyses. For Lp(a), an additional statistical analysis was performed: Subjects were divided into two subgroups based on fasting Lp(a), and the mean differences were compared by using Satterthwaite's approximation of the t' distribution (3).

I used a multivariable regression analysis to determine whether any portion of the observed difference (y) could be attributed to dilutional effects. In this multivariable-regression analysis, either albumin or total protein was used to control for dilutional effects by using (as a second independent variable) the derived quantity

$$x_2 = x_A \frac{(x_B - x_B)/x_B}{x_B}$$

where $x_A$ and $z_A$ (see equations 2 and 3) are the fasting and postprandial concentrations, respectively, of either Lp(a), apo A-I, or apo B, and $x_B$ and $z_B$ are the fasting and postprandial concentrations, respectively, of either albumin or total protein.

The rationale for the definition of $x_2$ as a derived variable to measure the amount of change that can be attributed to dilutional effects is as follows. If the difference between the postprandial and fasting concentrations of all analytes is exclusively the result of hemodilution, then, for any given pair of specimens,

$$z_A/x_A = z_B/x_B$$

which would imply that

$$y = z_A - x_A = x_A \frac{(x_B - x_B)/x_B}{x_B}$$

Results

The mean fasting values were 208 mg/L for Lp(a) (n = 51), 1.463 g/L for apo A-I (n = 54), and 0.865 g/L for apo B (n = 56). For all three analytes, assayed values were lower for the postprandial specimen than for the fasting specimen. The differences (postprandial minus fasting) were -23 mg/L for Lp(a) (P = 0.0001), -0.035 g/L for apo A-I (P = 0.0008), and -0.036 g/L for apo B (P = 0.0001). For Lp(a), the mean difference of -62 mg/L for the 14 subjects having a fasting value >300 mg/L was significantly lower (P < 0.001) than the mean difference of -8 mg/L for the 37 subjects having a fasting value <300 mg/L.

Linear-regression data and plots are shown in Figure 1. These data suggest that postprandial specimens have a -14% proportional bias for Lp(a), a -0.035 g/L systematic bias for apo A-I, and a -9% proportional bias for apo B, all of which are statistically significant. Multivariable-regression data are shown in Table 1. The regression coefficients for $x_i$ in Table 1 suggest that, even after controlling for dilutional effects, there is still a -11% to -13% proportional bias for Lp(a), and a -8% to -9% proportional bias for apo B, in postprandial specimens. On the other hand, for apo A-I, whether total protein or albumin is used to control for dilution effects,
the partial correlation coefficient for \( x_2 \) is much greater than that for \( x_1 \); furthermore, the corresponding partial F-tests are significant. These data suggest that dilutional effects account for at least some of the systematic bias for apo A-I.

### Discussion

The results of this study suggest that clinically significant biases occur when postprandial specimens are analyzed for lipoprotein antigens. Therefore, reference ranges established for these analytes, based on fasting samples, must not be used to interpret results for specimens obtained at other times. Furthermore, when these analytes are measured as a part of research protocols, preparation of the patient for the collection of the sample must be carefully standardized.

It is somewhat surprising that assayed values of lipoprotein antigens are lower in the postprandial state. Although lower serum concentrations appear to result, at least in part, from increased intravascular volume in the postprandial state, this dilutional effect accounts for a relatively small fraction of the proportional biases for Lp(a) and apo B. Because the Lp(a) lipoprotein (also known as the "low-density lipoprotein variant") contains both the Lp(a) and apo B antigens, a single mechanism exerted only on this particular lipoprotein may account for the proportional biases of both analytes.

This study suggests that there may be a sequestering of lipoproteins in the postprandial state; however, there is also the possibility that the immunoassays used to detect lipoprotein antigens are subject to negative interferences from postprandial serum. In this and other laboratories (4), the Array has been shown to be free of interferences from moderate amounts of lipemia. Therefore, it is unlikely that the lower postprandial values of apo A-I and apo B are the result of sample turbidity. The Lp(a) assay is a "sandwich" enzyme immunoassay; after incubation with the solid-phase antibody, the sample is washed away. Therefore, turbidity cannot affect the results of the Lp(a) assay. Of greater concern is the possibility that, in the postprandial state, increased serum lipids reduce the immunoreactivity of lipoprotein antigens (primarily Lp(a) and (or) apo B), either by masking them or by inducing a conformational change in them.

To my knowledge, the decrease of Lp(a) in the postprandial state has not been reported previously. Further studies are needed to determine whether this observation is an artifact of the Lp(a) assay. Because triglycerides tend to associate with Lp(a) lipoproteins in the postprandial state (5), this redistribution of triglycerides may account for altered immunoreactivity of the Lp(a). In another study from this laboratory, involving a different commercial Lp(a) assay, a small (but statistically significant) inverse correlation between Lp(a) and triglycerides was observed in fasting sera from 853 healthy donors (6). If triglycerides do reduce the immunoreactivity of Lp(a), then this may be a fundamental limitation of the assay, because more than one commercial test kit seems to be affected.

### References


