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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 sequestering of lipoproteins in the postprandial state or from negative interferences affecting the assays of lipoprotein antigens.

Additional Keyphrases: *sample collection · variation, source of*

One previous report (*1*) stated that there is no significant difference between fasting and postprandial serum values for apolipoprotein (apo) A-I and apo B, whereas another report (*2*) stated that both of these analytes are lower during the postprandial period. Both studies involved only a few subjects (10 and 8, respectively), and statistical analysis was limited to paired sample *t*-tests. Here, I report the relationship between fasting and postprandial apo A-I, apo B, and lipoprotein(a) [Lp(a)] measured in a larger number of subjects.

Materials and Methods

Fasting and postprandial blood samples were obtained from healthy human donors, all of whom were laboratory employees. Donors were instructed to fast overnight for at least 12 h. After the fasting specimen was obtained, the subjects consumed a lipid-rich breakfast of their choice from the Clinical Center cafeteria. Each subject consumed at least (*a*) two eggs, (*b*) one egg and three strips of bacon, (*c*) one egg and three breakfast sausages, or (*d*) one egg and an order (~100–200 g) of fried potatoes. Individuals were allowed to consume larger meals if this was their habit. The postprandial specimen was obtained 2 h after completion of the meal.

Blood samples were drawn into 7-mL Vacutainer® Tubes (Becton Dickinson, Rutherford, NJ 07070) containing no additive ("red top"). Samples were allowed to clot for 30–60 min and then were centrifuged at 2000 × *g* for 15 min. On the same day that the samples were collected, sera were assayed for apo A-I and apo B (both apo B-100 and apo B-48 are recognized by the latter assay) with the Array® nephelometer (Beckman Instru-

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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

$$x_2 = x_A\,[(z_B - x_B)/x_B] \qquad (7)$$

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,

$$z_A/x_A = z_B/x_B \qquad (8)$$

which would imply that

$$y = z_A - x_A = x_A\,[(z_B - x_B)/x_B] \qquad (9)$$

Results

The mean fasting values were 208 mg/L for Lp(a) (n = 51), 1.453 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_1 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,

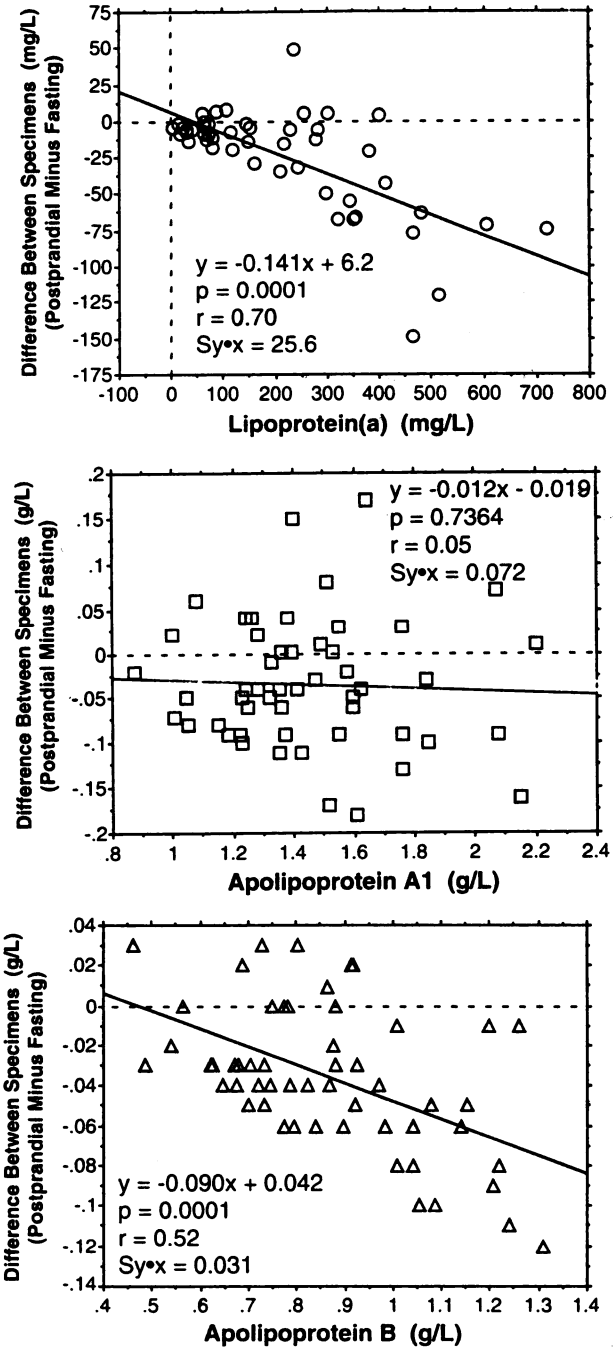

Fig. 1. Changes in assayed values of lipoprotein antigens in 2-h postprandial specimens vs 12-h fasting specimens

Table 1. Changes in Assayed Values of Lipoproteins Controlled for Hemodilutional Effects

	Regression coefficients	Correlation coefficient[a]	Probability[a]
Lp(a) (total protein)			
Regression		0.723	0.0001
x_1	−0.111	0.530	0.0001
x_2	−2.177	0.277	0.0556
Intercept	2.740		
Apo A-I (total protein)			
Regression		0.376	0.0256
x_1	−0.016	0.066	0.6302
x_2	−3.061	0.373	0.0071
Intercept	−0.009		
Apo B (total protein)			
Regression		0.589	0.0001
x_1	−0.082	0.503	0.0001
x_2	−1.793	0.320	0.0278
Intercept	0.039		
Lp(a) (albumin)			
Regression		0.704	0.0001
x_1	−0.125	0.564	0.0001
x_2	−1.188	0.156	0.2792
Intercept	4.300		
Apo A-I (albumin)			
Regression		0.287	0.1280
x_1	−0.015	0.066	0.6583
x_2	−2.369	0.283	0.0440
Intercept	−0.001		
Apo B (albumin)			
Regression		0.561	0.0001
x_1	−0.086	0.518	0.0001
x_2	−1.159	0.260	0.1137
Intercept	0.040		

Multivariable-regression models demonstrate the relative importance of fasting lipoprotein antigen concentration (x_1) vs dilutional effects (x_2) in determining the difference (y) between postprandial and fasting lipoprotein antigen concentrations. The variable x_2, which controls for hemodilutional effects, was calculated (see text) by using either albumin or total protein as the control analyte (listed in parentheses).

[a] Correlation coefficients and F-test probabilities for the complete model are shown in rows labeled Regression. Partial correlation coefficients and partial F-test probabilities are shown in the rows labeled x_1 and x_2.

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.

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