papers and notes on methodology

Comparison of two commercial nephelometric methods for apoprotein A-I and apoprotein B with standardized apoprotein A-I and B radioimmunoassays

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Abstract Normotriglyceridemic and hypertriglyceridemic samples were analyzed for apoproteins A-I and B using the Beckman Array System and the Behring Nephelometer, and the nephelometric values were compared to values obtained by highly standardized radioimmunoassays developed at the Northwest Lipid Research Center. Although the means of the apoA-I values obtained by each method were similar, comparison of sample values by least-squares regression analysis revealed large differences (Sy = 20 mg/dl for Beckman, Sy = 18 mg/dl for Behring) (Sy = standard error of the estimate) regardless of whether the comparison included hypertriglyceridemic samples. For normotriglyceridemic samples, there was good agreement between apoB values obtained by the Behring Nephelometer and those obtained by RIA (r = 0.91, m = 1.03, Sy = 12 mg/dl). However, significantly higher apoB values were obtained on hypertriglyceridemic samples by the Behring Nephelometer. ApoB values for normotriglyceridemic samples obtained by the Beckman System and RIA showed fairly good correlation (r = 0.86, m = 0.71, Sy = 14 mg/dl). However, the nephelometric values for normotriglyceridemic samples averaged 29% lower than those obtained by RIA. This difference could largely be accounted for by the low apoprotein B value assigned to the Beckman calibrator. Significantly lower apoprotein B values were obtained on hypertriglyceridemic samples by the Beckman Nephelometer even after correction for calibration differences. Apoprotein values obtained by nephelometric methods may be inaccurate, particularly if the samples are hypertriglyceridemic.—**Adolphson, J.L., and J. J. Albers.** Comparison of two commercial nephelometric methods for apoprotein A-I and apoprotein B with standardized apoprotein A-I and B radioimmunoassays. *J. Lipid Res.* 1989. **30:** 597–606.

Supplementary key words nephelometry • hypertriglyceridemic plasma

Methodologies used for quantification of apolipoproteins (apo) include radial immunodiffusion (RID), radioimmunoassay (RIA), electroimmunoassay (EIA), enzyme-linked immunosorbent assay (ELISA), immunoturbidimetric assay (ITA), and immunonephelometric assay (INA). Commercial methods utilizing all but ELISA methodologies are currently available for apoA-I and apoB analysis. Each methodology takes advantage of different chemical and immunological properites of antigens and antigen-antibody complexes (1). As a result, conditions that prohibit measurement in some assays may not be a factor in other assays. For example, assays that are sensitive to particle size, such as RID methods, have difficulty accurately measuring apoB in lipemic or lyophilized plasma. Thus, even when common calibrators were used in all methods, different results were obtained on some samples. If the apoprotein values obtained by these different assays are to be used as predictors of coronary risk, it is important to be able to relate the values to other published apoprotein data, and to know the limitations of the apoprotein methods.

We have previously compared several commercial apoA-I and apoB RID kits and an apoA-I RIA kit to highly standardized apoA-I and apoB RIAs developed at the Northwest Lipid Research Center (2). This study highlighted the need for common calibrators whose value

Abbreviations: RID, radial immunodiffusion; RIA, radioimmunoassay; INA, immunonephelometric assay; PEG, polyethylene glycol; LDL, low density lipoproteins.

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has been established by a reference method. The differences observed in those assays could mainly be attributed to differences in the assigned value of the reference materials but method-dependent differences were also observed (2). The problems associated with measuring apoB in lipemic samples using RID methods have been well documented (3). Immunonephelometric methods were first applied to apoprotein measurement in the 1970s (4,5). Earlier INAs developed for other proteins often recommended the removal of lipoproteins from lipemic sera in order to avoid interfering light scatter from these larger particles. Improved optics and the use of kinetic methods (6) have helped to minimize these problems and have renewed interest in nephelometric methods for apoprotein analysis.

Beckman Instruments (Fullerton, CA) and Behring Diagnostics, Inc. (Somerville, NJ) manufacture nephelometers and reagent kits for the nephelometric analysis of many serum proteins. Both also produce kits for apoA-I and apoB determination. We were able to obtain, on separate occasions, the use of these nephelometers and their respective apoprotein reagents for the purpose of evaluating these nephelometric methods.

Several reviews have described the principles and kinetics of immunonephelometric and immunoturbidimetric assays (6–9). Whicher, Price, and Spencer (6) also reviewed in detail the specific operation of several nephelometers, including the Beckman ICS (the earlier manual version of the Beckman Array System).

Several hours of incubation time may be required to reach the maximum turbidity of a reaction. At longer incubation times turbidity may actually decrease as antigen-antibody complexes, particularly those formed with larger particles such as lipoproteins, precipitate from the solution. Rather than using an "endpoint" measurement, the Beckman and Behring methods take advantage of the linear relationship between the antigen concentration and the rate of complex formation.

The Beckman Array System uses a rate kinetic method for nephelometric determination of apoproteins. Samples for apoA-I and apoB analysis are diluted in a special apoprotein diluent buffer containing a nonionic detergent, and an aliquot is added to a cuvette containing a polyethylene glycol (PEG) reactant buffer. PEG enhances nephelometric reactions by increasing the rate of the reaction and by decreasing the solubility of the complexes (6). The specific antibody is added and the rate of complex formation is continuously monitored. When a maximum rate is obtained, usually within the first 60 sec, that rate is used to calculate the antigen concentration from the standard curve. The standard curve is factory-generated for each antibody and is read into the analyzer by a card-reading system. The analyzer is then calibrated using a lyophilized serum calibrator provided by the manufacturer.

The Behring Nephelometer dilutes samples for apoprotein analysis in a standard buffer used for all Behring nephelometric tests. Aliquots of diluted sample and antibody are added to a cuvette containing reaction buffer, and a background (zero time) reading is taken. For apoA-I analysis an aliquot of supplemental buffer, containing a dissociating reagent, is added with the sample. After 6 min a second reading is taken and the net increase in scattered light is calculated. This type of nephelometric analysis is known as fixed time kinetic analysis because all readings are taken at a fixed time after addition of the antibody. The net increase in scattered light is compared to a standard curve and the concentration is calculated. Similar to the Beckman system, factory-generated standard curves provided with each antibody can be entered using a barcode reader, and the analyzer is calibrated using a lyophilized calibrator. A multipoint calibration option can be selected in which the analyzer serially dilutes the calibrator, takes readings, and generates the standard curve.

A Beckman Array Protein System was provided by Beckman Instruments, Inc., (Palo Alto, CA). All buffers, calibrators, and antibody reagents necessary for the measurement of apoproteins A-I and B were supplied by the manufacturer. Behring Diagnostics also provided a Behring Nephelometer and all reagents necessary for apoA-I and apoB measurement. The Behring Nephelometer was also made available to us at a later time for additional analyses. All apoA-I and apoB nephelometric determinations were performed according to the procedures provided by the manufacturers. Results for both nephelometric methods were calculated using the software provided with each instrument. Both companies provided technical advisors under whose supervision the calibration of the instruments was performed.

METHODS

Beckman states assayable ranges of 25 to 225 mg/dl for apoA-I and 30 to 200 mg/dl for apoB, using whole plasmas at a 1:36 dilution. The stated ranges for the Behring Nephelometer are somewhat wider, 18 to 580 mg/dl for both apoA-I and apoB, using plasma dilutions of 1:20. Whole plasma, apoA-I, and LDL were diluted to concentrations that exceeded both the maximum and the minimum of the ranges stated by each manfacturer for their respective procedures. Dilutions of purified standards for the Beckman Array System ranged from 0.4 to 8 mg/dl for apoA-I and from 0.2 to 6 mg/dl for apoB, and whole plasma dilutions ranged from 1:20 to 1:400. Purified standards analyzed on the Behring Nephelometer ranged from 0.6 to 28 mg/dl for apoA-I and 0.8 to 30 mg/dl for apoB, and whole plasmas from 1:5 to 1:200.

ApoA-I and apoB radioimmunoassays were performed as previously described (2, 10). ApoB RIA values are based on the LDL Lowry protein value with no correction factor. Bovine serum albumin obtained from the National Bureau of Standards was used as standard in the Lowry protein assay. Results for both radioimmunoassays were calculated using log/logit regression as described (2,10).

Four freshly thawed plasma pools were analyzed daily on both instruments. Each pool was made by pooling plasma from several donors who were selected for their plasma apoprotein levels. Protease inhibitors and antibiotics were added to the plasma, and aliquots were frozen at − 70°C. These pools have been used as quality control pools in the Northwest Lipid Research Center (NWLRC) assays and their mean values are based on 154 separate analyses performed during a 2-year period. Six fresh plasmas (five normolipidemic and one hypertriglyceridemic) were each analyzed 14 times on the same day using the Behring Nephelometer. Calibrators used for both nephelometric methods were analyzed for apoprotein A-I and B by the NWLRC radioimmunoassays. Normolipidemic samples that had been stored at − 20° for less than 30 days were thawed, allowed to reach room temperature, and analyzed by the nephelometric and the radioimmunoassay methods on the same day. Lipemic plasma with triglycerides levels greater than 300 mg/dl, cholesterol levels greater than 300 mg/dl, or both lipids greater than 300 mg/dl were also analyzed by both methods. These latter samples included plasma from lipoprotein lipase-deficient subjects with triglycerides greater than 1000 mg/dl, and also from two subjects whose apoB levels were greater than 300 mg/dl. Fresh plasma and serum were obtained from five normal and two hyperlipidemic subjects who had fasted for at least 10 hr. Aliquots of both plasma and serum were stored either frozen at − 20°C or at 4°C for 4 days, and were then analyzed twice each for apoA-I and apoB using the Behring Nephelometer. The fresh plasma samples from these subjects were analyzed for apoA-I and apoB 14 times to determine within-assay coefficient of variation for the Behring assays.

Serial dilutions of five hypertriglyceridemic plasmas were analyzed for apoB using the Behring Nephelometer and compared to results obtained for a normotriglyceridemic plasma.

RESULTS

Range and linearity

The Beckman Array System was able to measure apoA-I in whole plasma or as purified apoA-I in the range of 1 to 6 mg/dl. ApoB was measurable in plasma or purified LDL over the range of 1 to 6 mg/dl. Because the Beckman system dilutes plasmas 1:36 for analysis, these ranges are equivalent to plasma concentrations of 32–230 mg/dl for apoA-I and 32–220 mg/dl for apoB.

The Behring Nephelometer measured apoA-I in plasma or as purified apoA-I in the range of 2 to 28 mg/dl (plasma equivalent range 36 to 568 mg/dl for apoA-I), and measured apoB in plasma or LDL between 1 to 32 mg/dl for apoB (plasma equivalent range 18 to 650 mg/dl for apoB). For both nephelometric systems dilutions of purified A-I and LDL were parallel to the dilutions of whole plasmas within their respective ranges.

Serial dilutions of five hypertriglyceridemic plasmas were parallel to serial dilutions of a normotriglyceridemic plasma when analyzed for apoB by the Behring Nephelometer. The slope for the normolipidemic plasma was 1.00, compared to hypertriglyceridemic plasma slopes of 1.05 (TG = 252 mg/dl), 1.03 (TG = 332 mg/dl), 1.13 (TG = 713 mg/dl), 1.15 (TG = 1034 mg/dl), and 1.05 (TG = 1262 mg/dl). All correlation coefficients were greater than 0.995.

Reproducibility

Because of the limited time that the nephelometers were available to us, the between-assay variability was based on a relatively small number of observations. The values obtained for these are shown in **Table 1**. The coefficient of variation for the four frozen plasma pools run in the Beckman Array System ranged from 6.2 to 10.0% for apoA-I and from 5.5 to 8.4% for apoB (n = 7). The same plasma pools run in the Behring Nephelometer ranged from 3.2 to 8.6% for apoA-I and from 3.2 to 4.3% for apoB (n = 4). The between-assay coefficient of variation observed in the NWLRC RIAs during the same time period ranged from 2.0 to 3.9% for apoA-I and from 3.4 to 5.9% for apoB (n = 6). Later additional studies using the Behring Nephelometer and the same four plasma samples were conducted over a period of 3 weeks (in contrast to 4 days for the earlier study). Between-assay coefficients of variation ranged from 10.2 to 16.3% for apoA-I and from 4.1 to 10.4% for apoB (n = 11).

When five normotriglyceridemic plasmas and two hypertriglyceridemic plasmas were analyzed for apoA-I on the Behring nephelometer, the within-assay coefficients of variation ranged from 2.1 to 4.0% (n = 14). The same samples showed within-assay coefficients of variation for apoB ranging from 1.5 to 3.0% (n = 14). The coefficients of variation for the hypertriglyceridemic plasmas were not significantly different from those observed for the normotriglyceridemic plasmas.

Comparison of plasma and serum

No significant differences were observed between plasma and serum, either fresh or frozen for 3 days at

TABLE 1. Bias of commercial nephelometric methods on Northwest Lipid Research Center quality control pools

NWLRC RIA (n = 154)		Beckman (n = 7)				Behring (n = 4)			
NWLRC QC Pool	Mean	Mean	SD	CV	% Bias[a]	Mean	SD	CV	% Bias[a]
Apoprotein A-I values (mg/dl)									
QC 1	119	118	8.6	7.2	−1	129	8.6	6.6	+8
QC 2	134	137	8.6	6.2	+2	145	11.1	7.7	+8
QC 3	158	173	17.3	10.0	+9	179	9.8	5.4	+13
QC 4	137	141	13.0	9.2	+3	152	4.9	3.2	+11
Average			11.9	8.2	+3		8.6	5.7	+10
Apoprotein B values (mg/dl)									
QC 1	95	65	3.9	6.0	−32	100	4.0	4.1	+4
QC 2	88	66	5.5	8.4	−25	94	4.0	4.3	+6
QC 3	97	69	3.8	5.5	−29	100	3.9	3.9	+3
QC 4	148	115	7.9	6.9	−22	165	5.3	3.2	+10
Average			5.3	6.7	−27		4.3	3.9	+6

[a] % Bias = 100 × (assay value − NWLRC value)/NWLRC value.

−20°C, when analyses for apoA-I or B were performed using the Behring Nephelometer (n = 14).

Comparison to the NWLRC RIA methods

ApoA-I methods. The means obtained for each of the four quality control plasmas were compared to the mean values obtained by the NWLRC RIA methods. The percent bias was calculated as follows: 100 × (nephelometric mean − NWLRC RIA mean)/NWLRC RIA mean. The bias between methods was calculated as the mean of the percent biases for each pool (Table 1). The biases between the means of normotriglyceridemic samples analyzed by both methods were also calculated and are shown in **Table 2.** Also included in this table are the biases between the value assigned to the nephelometric reference materials by their respective manufacturers and the value obtained for them in the NWLRC RIA methods. Results obtained by both methods were compared using least-squares regression analysis and by Spearman's rank correlation (**Table 3**). All correlation coefficients obtained by least-squares regression (r) were within 0.04 of their respective Spearman's rank correlation coefficient (r_S).

The Beckman Array apoA-I quality control pool bias (+3%) and the bias between the sample means (−5%) showed little difference in the mean of the values obtained by both methods (Tables 1 and 2). However, the assigned value of the Beckman apoA-I calibrator was 16 mg/dl lower (−14%) than the value obtained when the calibrator was analyzed in the NWLRC apoA-I RIA.

Least-squares regression of the Beckman apoA-I data shows little difference between the correlation coefficient obtained for normotriglyceridemics (r = 0.74, n = 117) and that obtained when hypertriglyceridemics are in-

TABLE 2. Bias of commercial nephelometric apoprotein methods[a]

	ApoA-I Standards				Sample Means			
Company	Assigned Value	RIA Value	n	% Bias[b]	INA Value	RIA Value	n	% Bias[c]
Beckman	92	107	2	−14	138	146	117	−5
Behring	150	164	6	−9	151	157	109	−4
	ApoB Standards				Sample Means			
Beckman	110	195	2	−44	74	104	117	−29
Behring	123	131	6	−6	109	103	108	+6

[a] Results expressed in mg/dl.
[b] % Bias = 100 × (assigned value − NWLRC value)/NWLRC value.
[c] % Bias = 100 × (assay value − NWLRC value)/NWLRC value.

TABLE 3. Comparison of Northwest Lipid Research Center methods and commercial nephelometric methods by least-squares regression

Company	Samples	n	r	r_s	m	b	Sy
ApoA-I comparison							
Beckman	Normotriglyceridemic	117	0.74	0.76	0.86	12.7	20.8
	All samples	139	0.81	0.80	0.92	1.8	20.2
Behring	Normotriglyceridemic	109	0.69	0.73	0.57	62.2	17.2
	All samples	144	0.74	0.75	0.63	49.9	18.0
ApoB comparison							
Beckman	Normotriglyceridemic	117	0.86	0.86	0.71	0.2	12.4
	All samples	136	0.90	0.90	0.67	3.8	14.8
Behring	Normotriglyceridemic	108	0.91	0.93	1.03	3.3	13.5
	All samples	133	0.88	0.88	1.16	-4.9	20.2

n, Number of samples; m, slope; b, intercept; r, correlation coefficient; r_s, Spearman's rank correlation; Sy, standard error of y.

cluded (r = 0.81, n = 139). The higher correlation coefficient when hypertriglyceridemic samples are included may be because a higher proportion of those samples have either high or low apoA-I levels. For samples that have apoA-I levels above 150 mg/dl, the standard error of estimate (Sy) is higher than in those with lower apoA-I levels (**Fig. 1**). Sy is the standard deviation of the differences of the actual Y value from the Y value calculated from the least-squares equation (Y = mx + b) (11). Sy values were high whether or not hypertriglyceridemics were included (21 mg/dl and 20 mg/dl, respectively). Although the apoA-I means for normotriglyceridemic samples measured by both the Beckman and NWLRC methods are very similar, all five hypertriglyceridemic samples with apoA-I levels less than 100 mg/dl by RIA and triglyceride levels greater than 1300 mg/dl (1321, 1323, 1619, 2410, and 2850 mg/dl) appear to measure significantly less by the Beckman system. Three other samples with RIA apoA-I levels less than 100 mg/dl that did not measure significantly less by the Beckman system had triglyceride levels of 80, 1413, and 1611 mg/dl. In addition, the Beckman system was unable to detect any apoA-I in a sample whose triglyceride level was 4104 mg/dl, and which gave a value of 166 mg/dl when measured by the NWLRC apoA-I RIA. The HDL cholesterol of this subject was 226 mg/dl and the RIA apoA-I on the d > 1.063 g/ml fraction was 154 mg/dl. The average bias on the quality control pools analyzed by the Behring Nephelometer was +10% but the sample mean and the reference material biases were both negative (−4% and −9%, respectively). The apoA-I correlation coefficient for normotriglyceridemic plasmas measured by the Behring Nephelometer was similar to that observed when hypertriglyceridemic samples were included (r = 0.69, n = 109, vs. r = 0.74, n = 144). Samples with apoA-I levels greater than 160 mg/dl had a higher standard error of y (Sy) than samples with apoA-I levels less than 160 mg/dl (**Fig. 2**). The Sy was high regardless of whether or not hypertriglyceridemics were included (17 mg/dl vs. 18 mg/dl) (Table 3).

ApoB methods. The Beckman Array apoB quality control bias and the sample mean bias (−27% and −29%) showed that values obtained by the Beckman assay were much lower than those obtained in the NWLRC apoB RIA. When the Beckman reference material was analyzed in the NWLRC RIA, the value obtained also showed a significantly negative bias (−44%) when compared to the Beckman assigned value. The correlation coefficient of Beckman nephelometric apoB values and NWLRC apoB values for normotriglyceridemic samples

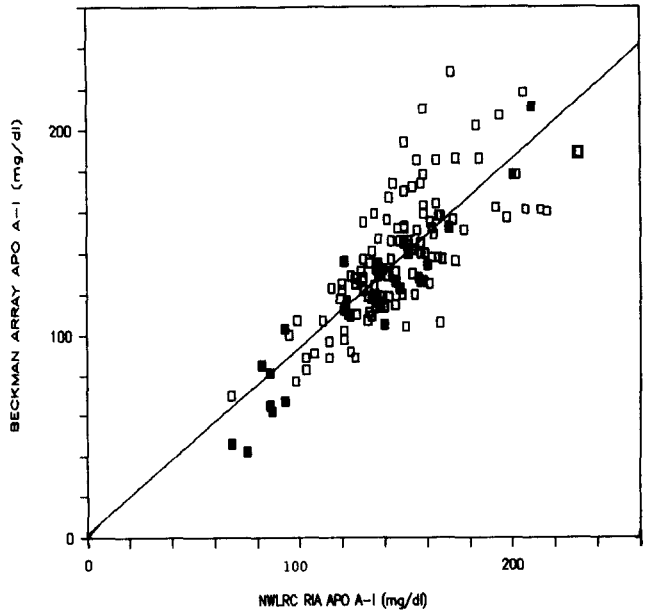

Fig. 1. Comparison of Northwest LRC apoA-I RIA with Behring Nephelometer. Regression line was calculated using data from all samples analyzed. Solid squares are hypertriglyceridemic samples.

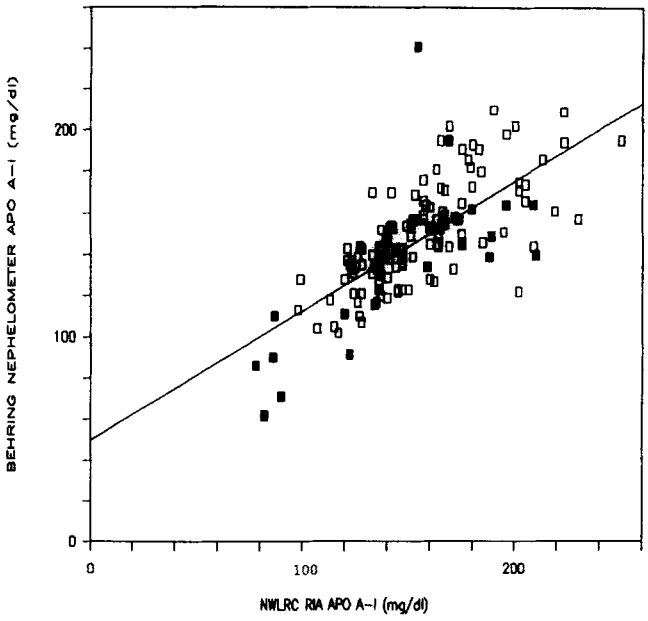

Fig. 2. Comparison of Northwest LRC apoA-I RIA with the Beckman Array System. Regression line was calculated using data from all samples analyzed. Solid squares are hypertriglyceridemic samples.

was similar to that obtained when hypertriglyceridemic samples were included in the calculation (r = 0.86, n = 117, and r = 0.90, n = 136). However, the Sy was greater when the hypertriglyceridemic samples were included (Sy = 15 mg/dl for all samples, Sy = 12 mg/dl for normotriglyceridemics). Samples with RIA apoB values greater than 176 mg/dl had nephelometric apoB values that averaged 16 mg/dl lower than expected by the regression line obtained with the normotriglyceridemic samples, and seven out of nine of these samples were hypertriglyceridemic (**Fig. 3**). For 30 samples that had Beckman apoB values greater than 100 mg/dl, the correlation with NWLRC apoB values was only 0.11 with a slope of 0.14 and an intercept of 93 mg/dl. **Table 4** lists all hypertriglyceridemic samples analyzed by both the Beckman Array System and the NWLRC RIA, and the apoB values obtained in each method. The Beckman values were adjusted upwards by a factor of 1.4 before the calculation of the percent difference because of the difference in the values of the reference materials used to calibrate the assays. The Beckman Array System was unable to obtain apoB values for four plasmas that had triglyceride levels greater than 2400 mg/dl. Nine of eleven samples analyzed with triglycerides greater than 500 mg/dl had significantly lower apoB values than those obtained by RIA even after adjustment for differences in calibration (Table 4).

Only a slightly positive bias (+6%) was observed for the quality control and sample mean biases for the Behring Nephelometric apoB assay. However, when Behring reference material was analyzed in the NWLRC apoB RIA, the assigned value showed a slightly negative bias (−6%) compared to the RIA value. The Behring apoB assay showed good correlation with the NWLRC method for normotriglyceridemic samples (r = 0.91, n = 108). The Sy for normotriglyceridemic samples was 14 mg/dl, but increased to 20 mg/dl when the hypertriglyceridemic samples were included (Table 3). Lipid data for all hypertriglyceridemic samples analyzed by both the Behring Nephelometer and the NWLRC RIA, and the apoB values obtained by each method are shown in **Table 5**. When compared to the NWLRC RIA apoB values, Behring nephelometric apoB values for hypertriglyceridemic samples showed a significant positive bias (**Fig. 4**,Table 5).

Table 6 lists all hypertriglyceridemic samples analyzed for apoA-I and apoB by all three methods. The apoB values obtained by the Beckman Array System are significantly lower than apoB values obtained by the Behring System even after adjustment for differences in calibration. The apoB values obtained by the RIA method are intermediate between the values obtained by the two nephelometric methods. No significant biases are seen among the apoA-I data of the three methods other than those also observed for sample mean biases.

DISCUSSION

Nephelometric and turbidimetric methods are more susceptible than other immunoassay methods to factors

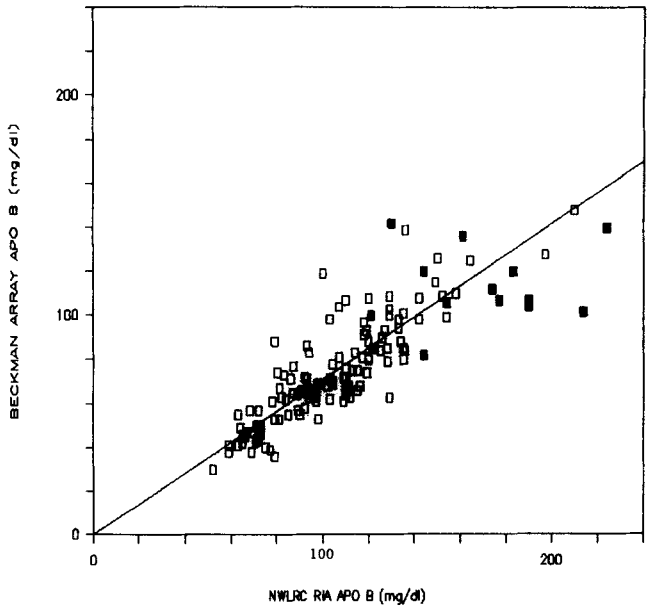

Fig. 3. Comparison of Northwest LRC apoB RIA with the Beckman Array System. Three samples with apoB levels greater than 250 mg/dl are not included. Regression line was calculated for normotriglyceridemic samples only. Solid squares are hypertriglyceridemic samples.

TABLE 4. ApoB in hypertriglyceridemic samples analyzed by the Beckman Array System

CHOL	TG	NWLRC RIA	Beckman Array	× 1.4	Percent Difference[a]
mg/dl					
581	4104	1216	<30		
814	3180	155	[b]		
310	2850	552	[b]		
288	2410	47	[b]		
509	2005	270	147	206	−24
415	1619	292	217	304	4
345	1611	214	102	143	−33
321	1554	190	107	150	−21
371	1413	190	104	146	−23
451	1323	345	278	389	13
209	1321	71	43	60	−15
227	878	177	107	150	−15
335	640	224	140	196	−13
243	570	174	112	157	−10
253	512	144	82	115	−20
248	496	121	100	140	16
252	471	154	106	148	−4
251	422	183	120	168	−8
230	394	144	120	168	17
312	354	161	136	190	18
180	301	102	68	95	−7
201	288	122	85	119	−2
351	268	130	142	199	53

[a]Percent difference = 100 × (adjusted value − RIA value)/RIA value.
[b]ApoB could not be determined.

that interfere with light transmission such as dust, lipoproteins, fibrin, and other plasma proteins. Although plasma and serum have been shown to give similar results for apoA-I and apoB using the Beckman Array System (12), serum is usually recommended for nephelometric assays because many plasma proteins, including fibrinogen, are removed in the clotting process. Although most of our comparison data was obtained on plasma samples, we showed that identical results were obtained for plasma and serum and no differences were observed between fresh and frozen samples when analyzed by the Behring Nephelometer.

The application of nephelometry to quantitation of other apoproteins may be limited by the ability of nephelometric systems to detect low levels of plasma proteins, especially when these proteins are associated with large particles such as lipoproteins. Some nephelometric assays on serum proteins have recommended the removal of lipoproteins from lipemic plasma before measurement in order to minimize light scatter due to large lipoprotein particles. Behring Diagnostics has developed methods using latex beads coupled to antibodies to enhance the sensitivity of some of their assays. Theoretically, this technology could be applied to other analytes, including apoproteins.

Current apoprotein nephelometric procedures use polyclonal antibodies, since monoclonal anitbodies do not form cross-linking precipitates. Future assays could, however, use a combination of monoclonals reactive with different epitopes on a specific protein.

Thus far, nephelometric systems that perform apoprotein analysis only offer methods for apoA-I and apoB quantification. The measurement of other analytes represents a further challenge to the sensitivity and versatility of nephelometric methods. Other apoproteins such as apoprotein[a] of the Lp[a] lipoprotein (13), have been implicated as important predictors of coronary heart disease. The ability to develop and add new assays to an existing system is an important feature of that system. The Beckman Array System necessitates the manual dilution of all standards and samples, and requires that data be transferred to another system for analysis, as no inter-

TABLE 5. ApoB in hypertriglyceridemic samples analyzed by the Behring Nephelometer

CHOL	TG	NWLRC RIA	Behring INA	Percent Difference[a]
mg/dl				
581	4104	1216	632	−48
814	3180	155	557	259
288	2410	47	161	243
326	2030	202	358	77
420	1772	190	346	82
345	1611	214	379	77
371	1413	190	328	73
209	1321	71	138	94
394	1242	91	386	324
238	1074	184	256	40
435	1056	249	348	40
232	868	127	204	61
272	754	146	210	44
345	700	242	264	9
254	670	143	180	26
335	640	224	200	−11
243	570	174	214	23
249	564	131	188	43
253	512	128	166	30
252	471	154	196	27
213	470	123	181	47
330	460	159	218	37
181	450	85	127	49
210	417	116	173	49
230	394	144	199	38
292	362	171	230	35
312	354	161	226	40
194	338	122	150	23
277	336	167	207	24
162	317	95	117	23
207	310	103	117	13
180	301	102	124	22
238	295	139	163	17
201	288	122	139	14
177	276	106	135	28
287	266	187	193	3

[a]Percent difference = 100 × (adjusted value − RIA value)/RIA value.

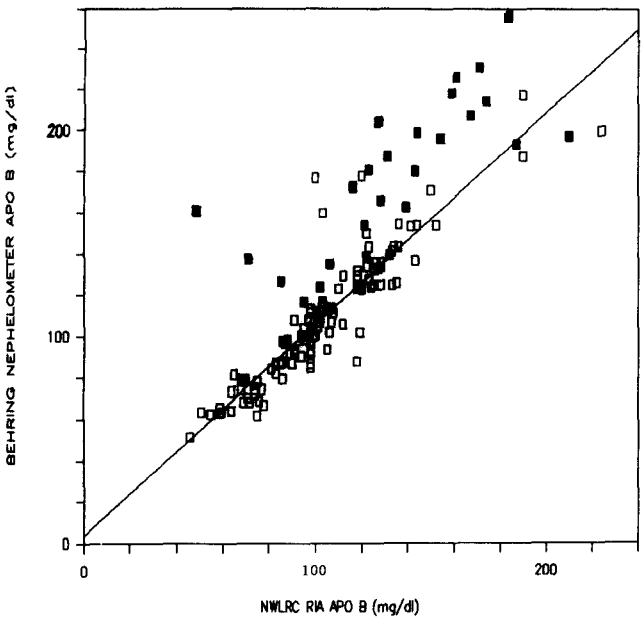

Fig. 4. Comparison of Northwest LRC and apoB RIA with the Behring Nephelometer. Samples with apoB levels greater than 250 mg/dl are not included. Regression line was calculated for normotriglyceridemic samples only. Solid squares are hypertriglyceridemic samples.

nal data reduction software is provided for user-developed methods. Thus, development of new methods or modification of existing methods is not easily accomplished on this instrument. On the other hand, the Behring Nephelometer system performs all dilutions and data reduction necessary for the user-developed assays that are performed for the manufacturer's methods.

Both nephelometric assays measured apoproteins A-I and B levels in purified A-I and LDL, respectively, as well as in plasma. The same values were obtained regardless of the dilution used for the measurement, and this linearity was observed over the ranges claimed by each manufacturer. Other materials, such as lyophilized serum or synthetic peptides, should also be tested for linearity before they are used as reference materials or standards.

Serial dilutions of hypertriglyceridemic plasmas analyzed for apoB by the Behring Nephelometer were parallel to those for a normotriglyceridemic plasma, indicating that the elevated apoB observed for hypertriglyceridemic samples is probably not due to interfering light scatter by large numbers of large particles or to the presence of high concentrations of lipids, as the concentration of both large apoB particles and the amount of lipid present during the determination would be diluted out. The fact that apoA-I measurement in hypertriglyceridemic plasma is not significantly affected (except in one sample with triglyceride > 4000 mg/dl) also indicates that the high apoB values associated with hypertriglyceridemic plasmas are due to the large apoB particle itself. Antigen-antibody lattices formed from larger apoB-containing particles (VLDL) would be larger than those formed from smaller apoB-containing particles (LDL) even if equal numbers of apoB antigenic sites were present.

The between-assay coefficients of variation observed for four normolipidemic quality control pools analyzed in the Beckman Array System averaged 8.2% for apoA-I and 6.7% for apoB (Table 1). Between-assay coefficients of variation for the NWLRC RIA during this period were 2.9% for apoA-I and 4.4% for apoB. The between-assay coefficients of variation for the same pools analyzed by the

TABLE 6. Comparison of apoA-I and apoB in hypertriglyceridemic samples analyzed by NWLRC RIA, Beckman Array, and Behring Nephelometer

		ApoA-I (mg/dl)			ApoB (mg/dl)		
CHOL	TG	NWLRC RIA	Beckman Array	Behring Nephelometer	NWLRC RIA	Beckman Array	Behring Nephelometer
mg/dl							
201	288	140	105	119	122	85	139
180	301	172	156	158	102	68	124
312	354	153	142	157	161	136	226
230	394	122	114	135	144	120	199
252	471	147	123	138	154	106	196
253	512	137	135	133	128	82	166
243	570	124	109	131	174	112	214
335	640	136	134	144	224	140	200
209	1321	86	65	90	71	43	138
371	1413	123	110	133	190	107	328
345	1611	82	85	62	214	102	379
288	2410	87	62	110	47	[a]	161
814	3180	154	120	241	155	[a]	557
581	4104	166	<25	195	1216	<30	632

[a]The Beckman Array System was unable to measure apoB.

Behring Nephelometer over a period of 4 days were 5.7% for apoA-I and 3.9% for apoB. A later analysis of between-assay variation was performed on the Behring Nephelometer over a period of 3 weeks and higher between-assay coefficients of variation were observed, particularly for apoA-I. Marked differences in the measured apoprotein levels of the four plasma control pools were noted at each change of calibration. Calibration was performed at 7-day intervals as per the manufacturer's recommendation. More consistent results could be obtained by following each calibration with several sets of quality control analysis. When the results of the control pools fall outside acceptable ranges, the instrument should be recalibrated and the control pools again analyzed. While variation in the value of a single plasma pool in an assay may not indicate a general shift in an assay, the observation of similar changes in several plasma pools is more conclusive. Assay variations that change the slope of the standard may affect only the higher or lower values of an assay. Thus, a quality control system that includes several plasma pools, each at a different apoprotein level, can more accurately guard against instrument drift.

In both nephelometric apoA-I methods and the Behring apoB method, the mean values for normotriglyceridemic samples were similar to those obtained by RIA, but manufacturer-assigned values for the reference materials were significantly less than the values obtained when the materials were run in the NWLRC apoA-I RIA. The assigned value of the Beckman Array apoB reference material also was significantly less than expected on the basis of the bias on the plasma samples. Beckman provides a lyophilized serum pool for calibration of both their apoA-I and apoB assays, while Behring uses lyophilized purified standards for calibration of their apoA-I and apoB methods. The reconstituted Beckman calibrator is visually turbid, even when diluted 1:3 for use as the apoA-I calibrator, while the Behring reference material appears clear. Lyophilized materials that have particulate matter upon reconstitution could have interfering light scatter. Although lyophilized materials may give reproducible results, the turbidity associated with many reconstituted preparations may result in inaccurate measurement of the analyte. If the analyte itself is associated with the particulate matter, further inaccuracies could result from the inclusion of the particulate matter in the antigen-antibody lattice. A closer examination of the use of lyophilized materials in nephelometric assays is needed.

The Beckman Array System is standardized to a lyophilized pool produced and distributed by Centers for Disease Control (CDC). The value for this pool is based on values obtained by several laboratories using several different assay methods including radial immunodiffusion (RID). Our laboratory has observed that when analyzed by an apoB RID method (10), lyophilized pools from various sources resulted in values 15 to 40% lower than their corresponding value by the RIA method, even though plasma samples gave similar values in both methods (Adolphson, J. L., and J. J. Albers, unpublished observations). Since the methods used to establish the consensus value for the CDC lyophilized pool included a number of RID procedures, and because the consensus values do not represent accuracy-based target values, we feel that setting the calibration of a commercial assay to the CDC pool is inappropriate. Calibration materials must be optimized for the method in which they are to be used in order to produce accurate results. Materials optimized for one method but used in another may produce unexpected results (matrix effects). When Tago RID standards were analyzed in the NWLRC RIAs (2), reagents added to the RID standards (possibly detergents) resulted in inaccurate measurement of the apoprotein levels.

The CDC pool, which has an assigned apoB value of 59 mg/dl (14), was analyzed 14 times in the NWLRC apoB RIA and an average value of 84 mg/dl was obtained. Thus, the absolute difference between the two methods can be largely attributed to the value assignment of this reference material. Although the Beckman Array System results in apoB values that are 29% lower than the NWLRC apoB values, correlation with the NWLRC apoB RIA is good (r = 0.86). The use of a different calibrator or increasing the value of the Beckman calibrator by a factor of 1.4, would result in equal apoB sample means for normotriglyceridemic samples (NWLRC mean = 104 mg/dl, Beckman mean = 104 mg/dl), and a slope (m) of 1.00. However, this adjustment also results in an increase in Sy from 12 mg/dl to 18 mg/dl. Comparison of scatter graphs of the two nephelometric apoB methods compared to the NWLRC RIA does suggest somewhat greater differences with the Beckman method.

In conclusion, nephelometric apoA-I methods show considerable differences from the NWLRC apoA-I RIA which are method-dependent. These differences are observed with both normotriglyceridemic and hypertriglyceridemic samples. Both apoB nephelometric methods show fairly good correlation with the NWLRC apoB RIA for normotriglyceridemic samples. The Beckman Array System gives apoB values 29% lower than the RIA due to the low apoprotein B value assigned the calibration material. Both nephelometric methods show significant differences from the apoB RIA in the measurement of apoB in hypertriglyceridemic samples. Table 6 compares apoA-I and apoB values obtained for hypertriglyceridemic plasmas that were analyzed by all three methods. ApoA-I values show no significant biases. The apoB values for hypertriglyceridemic samples obtained by the Beckman Array System are significantly different from those obtained by the Behring Nephelometer, even after correction for differences in calibration. The correlation

coefficient between apoB values of hypertriglyceridemic samples measurable by both nephelometric methods was only 0.43 (n = 11). The correlation coefficient increased to 0.88 when two samples with triglyceride levels of 1413 and 1611 mg/dl, respectively, were excluded from the calibration. The NWLRC apoB RIA values are intermediate between the nephelometric apoB values. Given the finding that apoB values greater than 100 mg/dl by the Beckman Array System had very poor correlation, a very low slope, and a high intercept when compared to the NWLRC RIA apoB values, the values obtained by the Beckman System are not likely to be useful for predicting atherosclerotic risk.

The NWLRC apoA-I RIA has been shown to give good agreement with an apoA-I RID procedure developed at the NWLRC (2) and with three commercial apoA-I RID kits (2). The standard error of y was less than 12 mg/dl for all four comparisons. Further evaluation is needed to determine the reasons for the large differences between the NWLRC apoA-I and the nephelometric apoA-I methods. Because of potential inaccuracies, A-I values obtained by nephelometric methods should be interpreted with caution. Hypertriglyceridemic samples still pose significant problems for the accurate measurement of apoB by nephelometric methods. However, the fairly good agreement in apoB measurement for normotriglyceridemic samples suggests that nephelometric apoB methods may be useful for the initial screening of large numbers of predominately normotriglyceridemic samples. ApoB values that were elevated as measured by nephelometric methods, or that were obtained from hypertriglyceridemic samples could be reanalyzed by an independent method to confirm that the apoB values are elevated. Because values for hypertriglyceridemic samples are lower by the Beckman Array System, hypertriglyceridemic plasmas with elevated apoB levels may appear to have normal apoB levels. Thus, greater care must be taken to identify hypertriglyceridemic samples when using the Beckman System.

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