notes on methodology

The measurement of apolipoprotein A-I in human plasma by electroimmunoassay

J. Paul Miller, Simon J. T. Mao, Josef R. Patsch, and Antonio M. Gotto, Jr.

Department of Medicine, Baylor College of Medicine and The Methodist Hospital, Houston, TX 77030

Summary A variable proportion of the total apolipoprotein A-I (apoA-I) present in plasma or high density lipoproteins (HDL) is normally detectable by immunochemical methods. This has been attributed to masking of some of the immunoreactive sites of apo A-I by lipid in the intact HDL particle. This difficulty has been circumvented by heating or delipidation. We find that exposure of plasma to concentrations of urea greater than about 7.0 M in the barbital buffer used to dilute plasma samples for estimation by electroimmunoassay enables the complete detection of ApoA-I, as judged by comparison with samples delipidated with tetramethylurea. The need for time-consuming heating or delipidation is avoided.—**Miller, J. P., S. J. T. Mao, J. R. Patsch, and A. M. Gotto, Jr.** The measurement of apolipoprotein A-I in human plasma by electroimmunoassay. *J. Lipid Res.* 1980. **21:** 775–780.

Supplementary key words high density lipoproteins · rocket electrophoresis

Resurgence of interest in high density lipoproteins (HDL) as possible protective agents against ischaemic heart disease (1, 2) has been accompanied by the development of a variety of immunochemical methods (3) for the measurement of their major protein constitutent apolipoprotein A-I (apoA-I). Frequently only a small proportion of the total apoA-I believed to be present in plasma or HDL has been detectable by such methods (4–6) and this has been attributed to the masking of some of the immunoreactive sites of apoA-I by lipid in intact HDL (7). This difficulty in detecting the full complement of apoA-I in plasma or HDL has been circumvented by a variety of physical or chemical approaches including delipidation or heating (4–6). An alternative approach for the complete detection of apoA-I in plasma by electroimmunoassay (EIA), which avoids time-consuming heating or delipidation, is described here. It involves exposing the plasma to concentrations of urea in excess of 7.0 M in the barbital buffer used to dilute samples before assay.

Abbreviations: apoA-I, apolipoprotein A-I; EIA, electroimmunoassay; HDL, high density lipoproteins; RIA, radioimmunoassay; TMU, 1,1′,3,3′-tetramethylurea; RID, radioimmunodiffusion.

MATERIALS AND METHODS

Blood was obtained from healthy laboratory staff and from hospital patients who had normal plasma concentrations of cholesterol and triglycerides for their age and sex. It was anticoagulated with EDTA (1 mg/ml) and the plasma was separated by low-speed centrifugation.

Protein concentrations were determined by the method of Lowry et al. (8). Plasma cholesterol, HDL-cholesterol and triglycerides were measured as described in the Lipid Research Clinics Manual of Laboratory Operations (9).

Purification of apoA-I

HDL (d 1.063–1.21 g/ml) was prepared from the plasma of a normal human subject by sequential preparative ultracentrifugation (10) and delipidated by three extractions at 4°C with ether–ethanol 3:1 (v/v) over a 24-hr period. After a final extraction with ether alone, the sample was dried under nitrogen, the residue was dissolved in 0.1 M Tris HCl, 6.0 M urea, pH 8.6, and the apoproteins were separated on a 200 × 1.6cm Sephadex G-150 column (Pharmacia Fine Chemicals, Piscataway, NJ) using the same buffer. Fractions corresponding to apoA-I were collected and the apoprotein was shown to give a single band on SDS-polyacrylamide gel electrophoresis (11) which had the same R_f as known apoA-I. Amino acid analysis showed that the material contained no isoleucine or half-cystine. The apoA-I thus prepared was stored in aliquots (≤0.4 mg protein/ml) at both 4°C and −70°C. Samples kept at these two temperatures gave identical rocket heights after 3 months' storage.

Electroimmunoassay

Antisera to human apoA-I were raised in goats. Ten mg of apoA-I in 1 ml of 0.15 M NaCl was mixed with an equal volume of complete Freund's adjuvant (Difco Laboratories, Detroit, MI) and the emulsion was injected intradermally into multiple sites on the backs of the goats. After 6 weeks, a second injection of 5 mg of apoA-I in complete Freund's adjuvant was administered. The goats were bled weekly for 3 weeks after the booster injection and the antisera pooled. The EIA technique was based on that of Laurell (12).

The gel consisted of 1.0 or 1.5% agarose (Bio-Rad Laboratories, Richmond, CA), 5% dextran T10 (Pharmacia Fine Chemicals, Piscataway, NJ) and 1% anti-apoA-I in 0.05 M barbital buffer, pH 8.6. The agarose and dextran were dissolved in the buffer by heating in a boiling water bath and the antiserum was added subsequently when the temperature had cooled to less than 50°C. Gel dimensions were either 8.0 × 9.0 × 0.1 cm or 18.5 × 9.0 × 0.1 cm. These were achieved by pouring the gel between two glass plates separated by a 1 mm thick U-frame (Bio-Rad Laboratories, Richmond, CA). Electrophoresis was performed in a water-cooled (15–17°C) IEP cell (Behring Diagnostics, Somerville, NJ) containing the same barbital buffer that was used to make the gel. Gel and buffer were connected by wicks made of two thicknesses of chromatography paper. The wick to wick distance across the gel was 5–6 cm.

Samples of plasma for assay without physical or chemical modification were diluted 1:5 to 1:20 in barbital buffer. When the effects of urea (Ultra Pure, Bethesda Research Laboratories, Inc., Rockville, MD) were investigated, this was incorporated into the diluent buffer at the concentration stated. Plasma was diluted 1:20 to 1:100. Urea was never dissolved in the buffer used for making the gels or for filling the electrophoresis tank.

The effects of delipidation were studied by incubating plasma with an equal volume of TMU at 37°C for 30 min (13). The precipitate was removed by centrifugation and the TMU-soluble fractions used for EIA after appropriate dilution in barbital buffer (final plasma dilution 1:20 to 1:100).

The effect of heat on the detection of apoA-I in plasma was studied by diluting plasma (1:5 to 1:20) in barbital buffer (without urea) and then heating to 52°C for 3 hr (5).

Samples were applied to the wells in volumes of 2.5 μl using a spring-loaded dispenser (#701, Hamilton Co., Reno, NV) while a low voltage was applied across the gel to minimize radial diffusion. The voltage from the power source was then increased to give a voltage as measured across the gel of 4–6 v/cm. Electrophoresis was performed overnight for periods of 16–18 hr. The following day the gel was soaked for at least 1 hr in 1 M NaCl to remove unprecipitated antiserum before staining in Coomassie Brilliant Blue (Bio-Rad Laboratories, Richmond, CA) and drying down on acetate sheets (500 WM, Bienfang Paper Co., Metuchen, NJ) for storage.

ApoA-I was quantitated from the measurement of peak heights of standard apoA-I preparations and unknowns. These values were compared initially with values obtained by measurement of rocket area. This was achieved by tracing the magnified rocket image using an overhead projector and measuring the area of the traced rockets by planimetry. For eight samples assessed by both methods, the largest discrepancy was 3.3% and the means for the two methods differed by less than 0.3%.

The intraassay coefficient of variation for a single sample measured in replicate (n = 22) on the same gel was 3.3%. The interassay coefficient (n = 10) was 7.7%.

RESULTS

Measurement of apoA-I in delipidated plasma

Plasma was obtained from 16 normolipemic subjects (eight males, eight females) whose ages and plasma lipid concentrations are given in **Table 1.** The apoA-I concentrations measured in plasma after delipidation with TMU were 112.9 ± 25.0 mg/dl for men and 131.5 ± 13.8 mg/dl for women. These values are very similar to the mean normal values reported by several other laboratories using a variety of methods (**Table 2**).

Measurement of apoA-I in untreated and heated plasma

When plasma samples from the same 16 individuals were diluted in urea-free barbital buffer without any other kind of physical or chemical treatment, the mean concentration of apoA-I detected by EIA was 23.6 ± 11.8 mg/dl, for males and females combined, which represents 19.3% of the amount detected in delipidated plasma (Table 1). Heating the plasma samples after dilution with the barbital buffer to 52°C for 3 hr increased the apparent apoA-I concentration to an average of 35.7 ± 9.6 mg/dl or 29.2% of the amount detected in delipidated plasma (Table 1). Heating did not influence the height of the rockets given by the pure apoA-I standards.

Effect of urea on detection of apoA-I in plasma

To study the effect of urea concentration on the detection of apoA-I in whole plasma, we diluted plasma samples in a series of 0.05 M barbital buffers, pH 8.6, containing concentrations of urea up to 9.0 M. Urea had no effect on the peak height of apoA-I standards. As described in the methods section, the apoA-I in the standard stock solution was isolated in a urea-containing buffer. To test the effect of urea, it was removed by dialysis and two series of dilutions were then made in the barbital buffer used to dilute the plasma samples, one with and the other without

TABLE 1. Plasma lipid and apoA-I concentrations in 16 normolipemic subjects

Subject	Sex	Age	Cholesterol	Triglycerides	HDL-cholesterol	ApoA-I			
						Untreated[a]	Heated[b]	Urea[c]	TMU[d]
			mg/dl	*mg/dl*	*mg/dl*	*mg/dl*			
1	M	34	178	113	25	12	25	93	89
2	M	59	145	151	29	11	21	98	90
3	M	65	265	82	42	18	30	127	109
4	M	62	191	146	47	53	45	143	124
5	M	70	216	77	77	49	58	175	166
6	M	59	178	189	31	24	40	125	121
7	M	35	170	67	45	17	34	109	103
8	M	60	189	190	35	15	24	106	101
Mean		55.5	191.5	126.9	41.4	24.9	34.6	122.0	112.9
± S.D.			± 35.9	± 49.4	± 16.4	± 16.6	± 12.5	± 27.1	± 25.0
9	F	57	253	123	40	25	39	135	144
10	F	55	201	93	41	19	30	119	119
11	F	68	208	120	59	27	44	179	144
12	F	52	192	107	52	17	30	165	145
13	F	58	253	83	72	28	45	166	148
14	F	67	198	161	43	21	32	139	119
15	F	60	167	57	60	19	34	137	124
16	F	54	215	132	59	22	41	154	139
Mean		58.9	210.9	109.5	53.3	22.3	36.9	149.3	131.5
± S.D.			± 29.6	± 32.0	± 11.3	± 4.0	± 6.2	± 20.0	± 13.8
All subjects Mean ± S.D.			210.2 ± 33.3	118.2 ± 41.2	47.3 ± 14.9	23.6 ± 11.8	35.7 ± 9.6	135.6 ± 27.0	122.2 ± 21.7

[a] Whole plasma diluted in 0.05 M barbital buffer, pH 8.6. No urea.
[b] Whole plasma diluted in 0.05 M barbital buffer, pH 8.6 without urea and then heated to 52°C for 3 hr.
[c] Whole plasma diluted in 0.05 M barbital buffer, containing 9.0 M urea, pH 8.6.
[d] Plasma delipidated with TMU and then diluted with 0.05 M barbital buffer, pH 8.6.

9.0 M urea. The rocket peak heights given by the two series of dilutions were indistinguishable.

The effect of constant dilution of a given plasma sample with buffer containing progressively increasing urea concentrations was profound (**Fig. 1**). At a concentration of about 7.0 M urea, the rocket height reached a maximum. It should be noted that these are the actual concentrations of urea in the sample wells, as diluted by the plasma sample, and not the concentrations of urea in the diluent buffer. The effect of the urea seemed to occur rapidly. Plasma samples diluted in buffer containing 9.0 M urea at room temperature immediately before assay gave rockets as high as those which had been exposed to urea for 48–72 hr. In order to ensure that rocket height was maximized and that plasma apoA-I was measured under conditions in which urea concentration was not limiting, all subsequent measurements using urea-containing buffer were made using 9.0 M urea in the buffer.

Not only does the presence of urea increase the height of the rockets generated by the plasma dilu-

TABLE 2. Concentrations of apoA-I in normolipemic subjects

Authors	Ref.	Method	ApoA-I, mg/dl ±S.D.	
			Males	Females
Schonfeld et al.	4	RIA	100 ± 35	104 ± 34
Karlin et al.	3, 5	RIA	130 ± 20	149 ± 30
Albers et al.	15	RID	120 ± 20	129 ± 25
Reman et al.	25	RID	137 ± 23	137 ± 23
Curry et al.	24	EIA	143 ± 24	146 ± 78
Shepherd et al.	21	EIA	127 ± 7	136 ± 16
Caslake et al.	22	EIA	105 ± 19	111 ± 14
Avogaro et al.	16	EIA	128 ± 22	134 ± 20

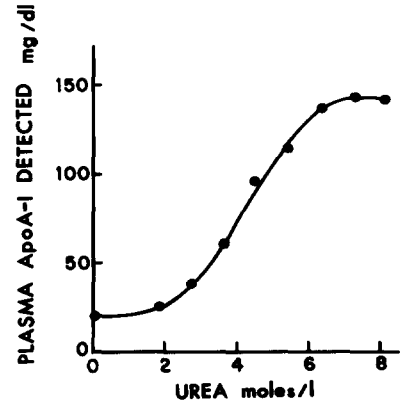

Fig. 1. Effect of urea concentration on detection of apoA-I in a single normal human plasma assayed at constant dilution (1:11) in 0.05 M barbital buffer, pH 8.6, containing increasing concentrations of urea. Abscissa is final urea concentration in sample well.

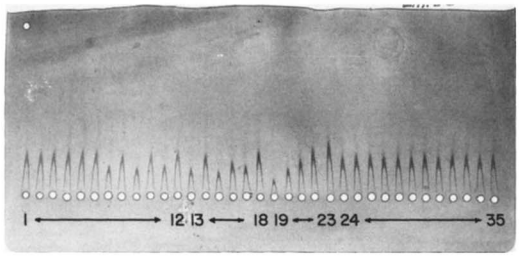

Fig. 2. Gel containing 1.5% agarose, 5% Dextran T10, 1% anti-apoA-I antiserum. Rockets developed by electrophoresis at 4 V/cm for 16 hr. Wells 1–12 contain plasma diluted in urea-containing buffer. Wells 13–18 contain plasma dilutions delipidated with ether–ethanol 3:1. Wells 19–23 contain apoA-I standard which give linear relationship between peak height and protein concentration. Wells 24–35 contain plasma dilutions delipidated with TMU. Plasma samples were derived from several sources and were applied in different dilutions, hence variation in rocket height. The form of all rockets is similar whether generated by pure apoA-I, delipidated plasma or plasma diluted in urea-containing buffer.

tion, it also increases their intensity, and their morphology resembles that of the apoA-I standards (**Fig. 2**) which is a criterion for antigenic identity between standards and unknowns (12). Heating had no effect on the detection of apoA-I in plasma samples which had been diluted in buffer containing 9.0 M urea.

The concentrations of apoA-I in plasma from the 16 normolipemic subjects already studied, when measured in the presence of 9.0 M urea, are shown in Table 1. These values with one exception were always at least as high as those found in delipidated plasma and on average the concentrations of apoA-I measured in the presence of urea were about 10% higher than those found in delipidated plasma. The concentrations measured in these two ways were highly correlated ($r = 0.954, P \ll 0.001$) (**Fig. 3**) The concentrations measured in the presence of urea were also significantly correlated ($r = 0.857$, $P < 0.001$) with the plasma α-cholesterol concentrations. Plasma apoA-I concentrations in the women were significantly higher than in the men ($P < 0.05$).

We have measured the apoA-I concentration in the same 16 plasma samples in the presence of urea using a second antiserum (goat 6434). The overall mean (±S.D.) was 137 ± 30.8 mg/dl and the individual values correlated highly ($r = 0.907$, $P < 0.001$) with those obtained using the original antiserum (goat 174). Thus, the total plasma apoA-I concentration detected in the presence of 9.0 M urea was largely independent of the antiserum used. It was notable, however, that in the absence of treatment with urea, the second antiserum detected only 4.5% on average (cf. 17% with the original antiserum) of apoA-I measured in the presence of urea.

DISCUSSION

Exposing plasma to urea concentrations in excess of 7.0 M appears to allow all the apoA-I present to be detected in our EIA since the values obtained were at least as high (10% higher on average) than those obtained after delipidation with TMU. Moreover, TMU-delipidation or heating to 52°C for 3 hr in addition to treatment with urea did not lead to a further increase in the amount of apoA-I detected. In our system, heating plasma (52°C for 3 hr) without treatment with TMU or urea did not allow the full complement of apoA-I to be detected. The reason for the slightly higher values of apoA-I detected in the presence of urea compared to delipidated samples is uncertain but may be due to small amounts of apoA-I being trapped in the heavy precipitate formed when whole plasma is treated with TMU. Support for this explanation is gained from experiments in which ^{125}I-apoA-I was incubated with whole plasma before TMU-delipidation. Recovery of radioactivity from the supernatant after TMU-precipitation was on average 12% less than that predicted on the basis of dilution of plasma with an equal volume of TMU. It is likely that the increased detection of apoA-I in the presence of high concentrations of denaturant is at least partly the result of dissociation of apoA-I from the parent HDL particle (14).[1]

The concentrations of apoA-I which we have observed in our 16 normolipemic individuals are very similar to those reported from other centers using a variety of methods (Table 2). Our data support those workers who have found higher plasma apoA-I concentrations in women than in men (5, 15) but our

[1] Miller, J. P. Unpublished observations.

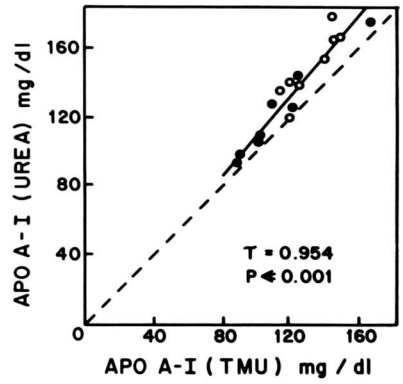

Fig. 3. Comparison of apoA-I concentrations detected in 16 normolipemic plasma samples measured either in the presence of urea or after delipidation with TMU. Dashed line is line of equality. (●) Males, (○) Females. Equation of regression line, y = 1.18x − 8.9.

sample size was small and since it may not be representative of the general population, this observation should be interpreted with caution. It was not our principal objective to investigate this question. In common with others (4, 15, 16), we have observed a significant relationship between plasma apoA-I concentration and HDL-cholesterol concentration.

The observation that, in contrast to apoA-II (17), only a small proportion of apoA-I is detectable by RIA in native plasma or HDL (4, 5) has been interpreted as implying that the majority of the apoA-I antigenic sites are masked by lipid or protein and it is possible to reduce the immunoreactivity of apoA-I or apoHDL by recombination with egg lecithin or HDL lipid (7). Experiments using "region-specific" antibodies (18, 19) suggest that in HDL_2 at least, the COOH-terminal region of apoA-I is more exposed than the NH_2-terminal.

The exact proportion of apoA-I immunochemically detectable in untreated plasma or HDL is very variable, whether RIA (4, 5, 20), EIA (16, 21–24), or radial immunodiffusion (RID) (15, 24, 25) is used. The effect of different assay methods, while using the same antiserum, has not been widely studied. It may be however that they make relatively little difference. Using the antiserum from goat 174 for EIA we found about 17% of the total plasma apoA-I was detectable in 16 normolipidemic subjects. This compares with 15% detected by RIA using the same antiserum on a pool of plasma drawn from the same subjects (26).

It is more likely that most of the variability lies in the antiserum used. Those authors (15, 16, 21, 22) who have used high concentrations of urea in the plasma diluent for EIA and RID would be expected, on the basis of our findings here, to be able to detect apoA-I completely, whether or not they delipidated their samples. Others, however have detected maximal amounts of apoA-I without the need for prior physical or chemical treatment (20, 24). In the present study the mean proportion of apoA-I detectable in the plasma of our 16 normolipidemic subjects was 17% and 4.5%, depending on the antiserum used.

Any antiserum is a heterogeneous mixture of antibodies, and the amount of apoA-I detected in unmodified plasma by a given antiserum is likely to depend on the titer of those antibodies directed against the antigenic sites of apoA-I that are exposed in native HDL ("surface antigens") (26). It is likely that much of the variability in the detection of apoA-I reported by different authors using different antisera is explicable on this basis.

In conclusion, it is frequently necessary to resort to physical or chemical treatments for the full detection of apoA-I in plasma by immunochemical methods whether RIA, RID, or EIA. An increase in the amount of apoA-I detected when such treatments are used should be sought when setting up a new assay. For EIA, at least, dilution of plasma in barbital buffer containing 9.0 M urea is a simple method of detecting all the apoA-I present that is less time-consuming than delipidation.

We are very grateful to Mr. Nauro Nava of the Lipid Research Clinic for determining the plasma lipid concentrations on the 16 normolipemic subjects studied here and to Ms. Debbie Mason and Mrs. Susan Davies for preparing the manuscript. This work was supported in part by the Atherosclerosis, Lipids and Lipoproteins Section of the National Heart and Blood Vessel Research and Demonstration Center, Baylor College of Medicine, a grant-supported research project of the National Heart, Lung, and Blood Institute, National Institutes of Health Grant No. HL 17269, and a grant by the National Institutes of Health 1RO1 HL-24759-01. During the conduct of this work J.P.M. was on leave of absence from the Department of Medicine, University Hospital of South Manchester, Manchester, U.K. and he is grateful to the Medical Research Council of England for a Travelling Fellowship.

Manuscript received 27 August 1979 and in revised form 15 April 1980.

REFERENCES

1. Miller, G. J., and N. E. Miller. 1975. Plasma-high-density-lipoprotein concentration and development of ischaemic heart disease. *Lancet* **1:** 16–19.
2. Gordon, T., W. P. Castelli, M. C. Hjortland, W. B. Kannel, and T. R. Dawber. 1977. High density lipoprotein as a protective factor against coronary heart disease. The Framingham study. *Am. J. Med.* **62:** 707–714.
3. Karlin, J. B., and A. H. Rubenstein. 1979. Serum lipoprotein quantification by immunochemical methods. *In* The Biochemistry of Atherosclerosis. A. M. Scanu, R. W. Wissler, and G. S. Getz, editors. Dekker, New York. 189–227.
4. Schonfeld, G., and B. Pfleger. 1974. The structure of human high density lipoprotein and the levels of apolipoprotein A-I in plasma as determined by radioimmunoassay. *J. Clin. Invest.* **54:** 236–246.
5. Karlin, J. B., D. J. Juhn, J. I. Starr, A. M. Scanu, and A. H. Rubenstein. 1976. Measurement of human high density lipoprotein apolipoprotein A-I in serum by radioimmunoassay. *J. Lipid Res.* **17:** 30–37.
6. Fainaru, M., R. J. Havel, and T. E. Felker. 1976. Radioimmunoassay of apolipoprotein A-I of rat serum. *Biochim. Biophys. Acta.* **446:** 56–68.
7. Schonfeld, G., B. Pfleger, and R. Roy. 1975. Structure of human high density lipoprotein reassembled in vitro. *J. Biol. Chem.* **250:** 7943–7950.
8. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
9. Manual of Laboratory Operations. Lipid Research Clinics Program. Vol. 1. Lipid and lipoprotein analysis. 1974. DHEW Publication (NIH) 75–628.

10. Havel, R. J., H. A Eder, and J. H. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34:** 1345–1353.
11. Weber, K., and M. Osborn. 1969. The reliability of molecular weight determination by dodecyl sulfate-polyacrylamide gel electrophoresis. *J. Biol. Chem.* **244:** 4406–4412.
12. Laurell, C-B. 1972. Electroimmunoassay. *Scand. J. Clin. Lab. Invest.* **29:** Suppl. 124, 21–37.
13. Kane, J. P., T. Sata, R. L. Hamilton, and R. J. Havel. 1975. Apoprotein composition of very low density lipoproteins of human serum. *J. Clin. Invest.* **56:** 1622–1634.
14. Forte, T. M., R. W. Nordhausen, A. V. Nichols, G. Endemann, P. Miljanich, and J. J. Bell-Quint. 1979. Dissociation of apolipoprotein A-I from porcine and bovine high density lipoproteins by guanidine hydrochloride. *Biochim. Biophys. Acta.* **573:** 451–463.
15. Albers, J. J., P. W. Wahl, V. G. Cabana, W. R. Hazzard, and J. J. Hoover. 1976. Quantitation of apolipoprotein A-I of human plasma high density lipoprotein. *Metabolism.* **25:** 633–644.
16. Avogaro, P., G. Cazzolato, G. B. Bon, G. B. Quinci, and M. Chinello. 1978. HDL-cholesterol, apolipoproteins A_1 and B. Age and index body weight. *Atherosclerosis,* **31:** 85–91.
17. Mao, S. J. T., A. M. Gotto, Jr., and R. L. Jackson. 1975. Immunochemistry of human plasma high density lipoproteins. Radioimmunoassay of apolipoprotein A-II. *Biochemistry.* **14:** 4127–4131.
18. Schonfeld, G., R. A. Bradshaw, and J-S. Chen. 1976. Structure of high density lipoprotein. The immunologic reactivities of the COOH- and NH_2-terminal regions of apolipoprotein A-I. *J. Biol. Chem.* **251:** 3921–3926.
19. Schonfeld, G., J-S. Chen, and R. G. Roy. 1977. Use of antibody specificity to study the surface disposition of apoprotein A-I on human high density lipoproteins. *J. Biol. Chem.* **252:** 6655–6659.
20. Fainaru, M., M. C. Glangeaud, and S. Eisenberg. 1975. Radioimmunoassay of human high density lipoprotein A-I. *Biochim. Biophys. Acta.* **386:** 432–443.
21. Shepherd, J., C. J. Packard, J. R. Patsch, A. M. Gotto, Jr., and O. D. Taunton. 1978. Metabolism of apolipoproteins A-I and A-II and its influence on the high density lipoprotein subfraction distribution in males and females. *Eur. J. Clin. Invest.* **8:** 115–120.
22. Caslake, M. J., E. Farish, and J. Shepherd. 1978. Metabolism of apolipoprotein A-I in healthy young adults. *Metabolism.* **27:** 437–447.
23. Glickman, R. M., and P. H. R. Green. 1977. The intestine as a source of apolipoprotein A_1. *Proc. Nat. Acad. Sci. USA.* **74:** 2569–2573.
24. Curry, M. D., P. Alaupovic, and C. Alan Seunram. 1976. Determination of apolipoprotein A and its constitutive A-I and A-II polypeptides by separate electroimmunoassays. *Clin. Chem.* **22:** 315–322.
25. Reman, F. C., and A. Vermond. 1978. The quantitative determination of apolipoprotein A-I (apo-lp-Gln I) in human serum by radial immunodiffusion assay (RID). *Clin. Chim. Acta.* **87:** 387–394.
26. Mao, S. J. T., J. P. Miller, A. M. Gotto, and J. T. Sparrow. 1980. The antigenic structure of apolipoprotein A-I in human high-density lipoproteins: radioimmunoassay using surface-specific antibodies. *J. Biol. Chem.* **255:** 3448–3453.