QUANTITATION OF THE TWO MAJOR APOPROTEINS OF HUMAN HIGH DENSITY LIPOPROTEINS BY SOLID PHASE RADIOIMMUNOASSAY

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Received 19 April 1978

1. Introduction

Serum high density lipoprotein (HDL) plays a major role in the transport and metabolism of cholesterol which is mediated through its interaction with the enzyme lecithin: cholesterol acyltransferase (LCAT) [1]. Evidence has also been presented that HDL facilitates the uptake of cholesterol from peripheral tissues and the clearance of cholesterol from arterial wall [2]. Furthermore, decreased HDL and HDL cholesterol levels were shown to be correlated to the incidence of coronary heart disease [3,4]. HDL contains two major apoprotein components which have been designated apoprotein A-I (apo A-I) and apoprotein A-II (apo A-II) [5–7]. These two protein moieties account for 90% of the total protein content of HDL [8], and are believed to be of central importance not only for the structural but also for the functional integrity of HDL. Apo A-I has been shown to activate LCAT in vitro [9] and to remove cholesterol from the aortic smooth muscle cells [10].

Rapid and precise measurement of apo A-I and apo A-II would be valuable in elucidating their structural and physiological functions. To this end we have raised antisera against human apo A-I and apo A-II and developed a specific solid phase radioimmunoassay for their quantitation.

2. Materials and methods

2.1. *Sera*

Sera were taken from normolipidemic males and females after a 12 h fast. The sera were delipidated by ether–ethanol [11] or by 1,1,3,3,-tetramethyl urea (TMU) [12].

2.2. *Preparation of lipoproteins and apoproteins*

HDL was isolated from individual donors by sequential ultracentrifugation [13] and repeatedly delipidated with 4 vol. acetone (twice), heptane (twice), ether–ethanol (1:1) (twice) and ether (once). The residue was finally dried under N_2. The delipidated HDL was chromatographed on Sephadex G-150 in 6 M urea, 0.03 M Tris–HCl, pH 8.2 [14], and the fractions containing both apo A-I and apo A-II, respectively, were rechromatographed on DEAE cellulose in 6 M urea [7,15,16]. Purity of isolated

apo A-I and apo A-II was assessed by polyacrylamide gel electrophoresis in urea.

2.3. *Preparation of antisera against apo A-I and apo A-II*

Adult sheep were immunized with 0.5 mg apo A-I and apo A-II, respectively, in complete Freund's adjuvant. They were boosted with intervals of 2 weeks with the same antigen dose. Bleedings were taken after 6 weeks with intervals of 2 weeks. Anti-apo A-I antibodies were isolated with an immunoadsorbent consisting of apo A-I which was coupled to cyanogen bromide-activated Sepharose 4B using the procedure in [17]. Antisera against apo A-II were passed over the apo A-I Sepharose column in order to adsorb anti-apo A-I antibodies. Both antibody preparations were monospecific as tested by immunoelectrophoresis and by double diffusion in agar.

2.4. *Radioimmunoassay (RIA)*

For coupling to bromoacetylcellulose (BAC) the γ-globulin fraction of the anti-apo A-II antiserum was precipitated by ammoniumsulphate at 35% saturation. Isolated anti-apo A-I antibodies or the γ-globulin fraction of the anti-apo A-II antiserum were coupled to BAC as described in [18]. Apo A-I and apo A-II were trace-labeled with ^{125}I by the chloramine-T method [19].

The binding inhibition assay was set up as follows: To 140 μl RIA buffer (375 ml 0.2 M phosphate, pH 7.5, 100 ml EDTA 0.1 M, 10 ml NaN_3 2%, 10 ml BSA 22%, 500 ml NaCl 0.9%, 5 ml Tween 20) 50 μl BAC-conjugate, 10 μl dilutions of unlabeled antigens and 50 μl ^{125}I-labeled antigen was added. The mixture was incubated overnight at 4°C. After 3 washes with RIA buffer the test tubes containing the sedimented BAC conjugate were counted in a gamma counter (Nuclear, Chicago). All tests were done in duplicate.

2.5. *Other procedures*

Protein concentrations were determined by the method in [20].

3. Results

Figure 1 represents the standard curve for the displacement of ^{125}I-labeled apo A-I from BAC–anti-

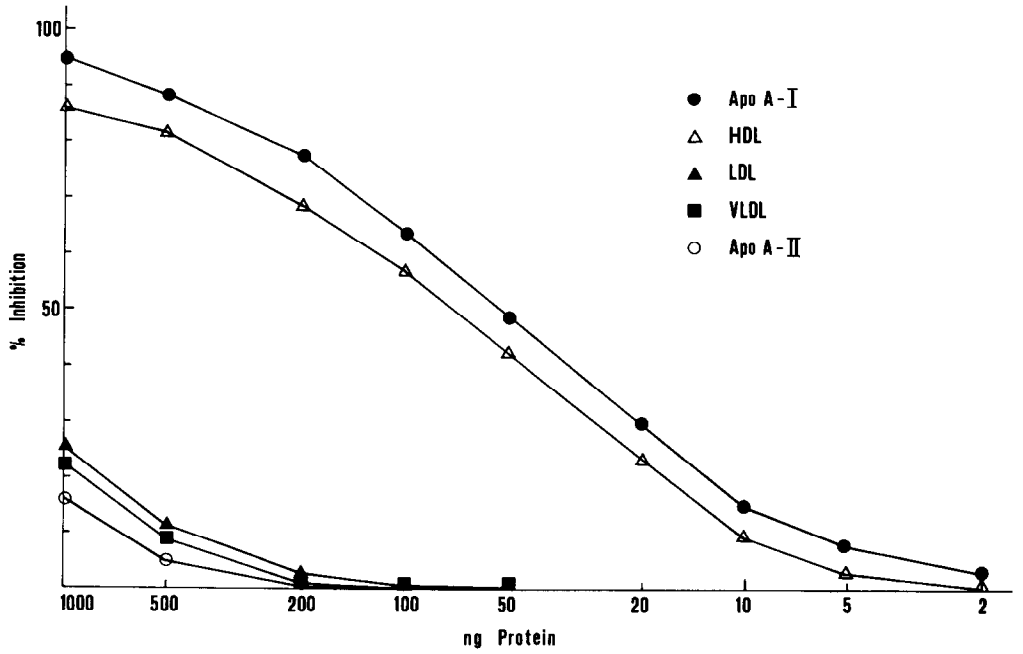

Fig.1. Displacement of ^{125}I apo A-I from BAC coupled anti-apo A-I by apo A-I (●), HDL (×), LDL (▲), VLDL (■) and apo A-II (○).

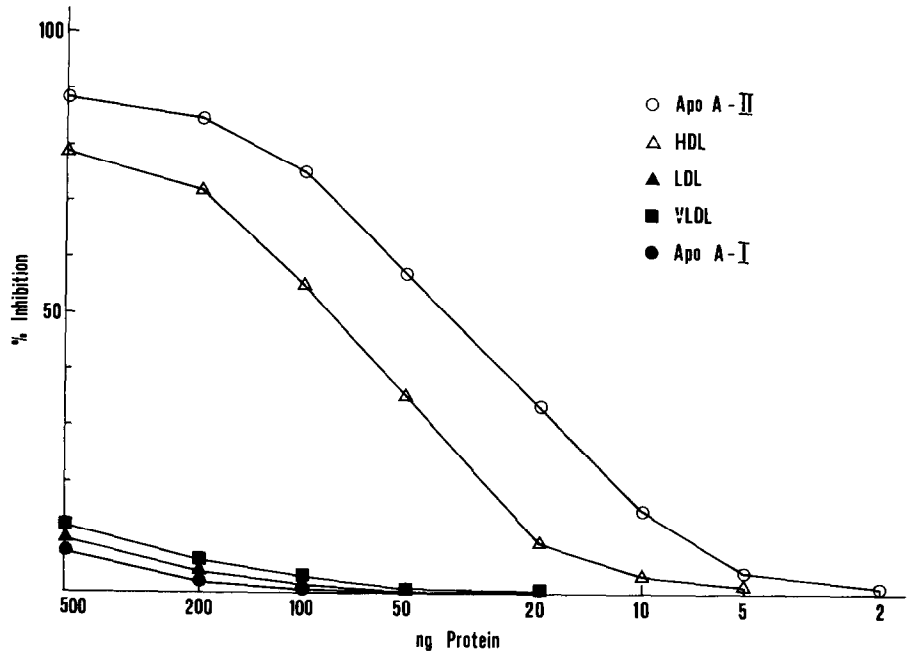

Fig.2. Displacement of ^{125}I apo A-II from BAC coupled anti-apo A-II by apo A-II (○), HDL (×), LDL (▲), VLDL (■) and apo A-I (●).

apo A-I by unlabeled apo A-I and the displacement of ^{125}I-labeled apo A-I by various unlabeled inhibitors such as very low density lipoprotein (VLDL), low density lipoprotein (LDL), delipidated and native HDL, and apo A-II. Only apo A-I and HDL were effective inhibitors indicating that the assay was specific for apo A-I. The assay was linear from 10–100 ng apo A-I.

The respective data for apo A-II are displayed in fig.2. The monospecificity of the assay for apo A-II is indicated by the fact that only apo A-II and HDL could effectively displace ^{125}I-labeled apo A-II from BAC–anti-apo A-II. This assay was linear from 10–100 ng for apo A-II.

Denaturation of HDL with TMU did not significantly affect its inhibitory capacity in both assays, whereas delipidation with ether–ethanol gave values which were about 15% lower than values obtained with native HDL or with native sera.

The data obtained for a collective of 20 normal males and 20 normal females from 20–60 years old is given in table 1. From day to day analysis (n = 10) gave variation coefficients of 7% for the assay of apo A-I and 9% for apo A-II.

Table 1
Concentration of apo A-I and apo A-II in normal sera

	Apo A-I		Apo A-II	
	Mean ± SD (mg/dl)	Range (mg/dl)	Mean ± SD (mg/dl)	Range (mg/dl)
Males (n = 20)	109 ± 13	92 – 129	32 ± 4	25 – 44
Females (n = 20)	124 ± 24	98 – 155	37 ± 7	25 – 51

4. Discussion

We describe here two sensitive and rapid solid phase radioimmunoassays for the two major apoproteins of HDL apo A-I and apo A-II. The assays are sensitive in the range 10–100 ng and they are specific for apo A-I and apo A-II, respectively. From day to day analysis gave variation coefficients of 7% for apo A-I and 9% for apo A-II indicating appropriate precision for both assays. The analysis time was in the order of 15 h which is considerably faster than previously reported techniques for the measurement of apoproteins of HDL.

Quantitation of apo A-I using a double antibody radioimmunoassay [21–24] or the immunodiffusion technique [25] has already been described. These authors determined comparable values for apo A-I but noted different behaviour upon delipidation. While delipidation was not necessary to measure total apo A-I in plasma [24], only 5% of the radiolabeled apo A-I displaced by delipidated plasma was displaced when delipidation with organic solvents was omitted [21]. Without denaturation only 60–80% assayable apo A-I was measured [22]. Heating to 52°C was used for denaturation [22]. Only about 30% apo A-I was assayable in native sera as compared to sera delipidated with an ether–ethanol mixture [23]. In our assay for apo A-I denaturation with TMU did not significantly alter the values and seems to be unnecessary. Delipidation of sera or HDL with an ether–ethanol mixture, however, decreased the inhibitory capacity to about 85% values obtained with native sera or HDL. The decrease in inhibitory capacity is probably due to irreversible denaturation of the protein with delipidation by organic solvents. The different data observed by the various groups of investigators might be due to differences in antigenic specificity and affinity of the antibodies used. Our antisera were raised in sheep instead of rabbits used by the other authors. They were obtained after repeated biweekly boosting the animals over a period of several months. They probably represent a high affinity population of antibodies.

Quantitation of apo A-II so far has only been performed in [23]. A radioimmunoassay for apo A-II to study antigenic reactive regions of apo A-II has been developed [26]. However, serum apo A-II levels were determined [26]. The apo A-II concentration in native HDL was 1.4-times greater than that of apo HDL [26]. Similar values of apo A-II in native and in sera delipidated with ether–ethanol were reported [23], which is in marked contrast to the findings with apo A-I [23]. In our assay delipidation with organic solvents decreased the inhibitory capacity of sera and HDL in the assay for apo A-II to about the same extent as in the assay for apo A-I. Apart from the differences noted with delipidation our values for apo A-I and apo A-II are in reasonable agreement with the other data known so far.

It now seems possible to quantitate both major apoproteins of HDL in a rapid and easy way and to assess their physiological role. Preliminary results from this laboratory indicate that patients with coronary heart disease have decreased levels of apo A-I while the apo A-II levels appear to be slightly increased. These data strengthen the physiological importance of the two major apoproteins of HDL.

Acknowledgements

The authors wish to thank Miss E. Ischi and Miss I. Hanicka for excellent technical assistance. This work was supported by the Swiss National Science Foundation and the Deutsche Forschungsgemeinschaft SFB 90.

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