PLASMA APOLIPOPROTEIN A-I LEVELS AFTER THORACIC DUCT DRAINAGE IN THE RAT

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1. Introduction

The major apolipoprotein of high density lipoproteins (HDL), apolipoprotein A-I (apo A-I) is also a dominant apoprotein in rat chylomicrons [1–7], and the polar surface structures of the chylomicrons contribute a significant amount of material to circulating HDL [8,9]. Immunofluorescent studies on intestinal tissue [10,11], and data on lypmh lipoprotein labelling after administration of a radioactive amino acid pulse [12–15] indicate that the mucosa actively synthesizes apo A-I, apo A-IV and apo B, but only small amounts of C-peptides and apo E. The presence of apo A-I in human intestinal mucosa [16] has been correlated to an active synthesis of apo A-I in tissue culture of human small intestinal biopsies [17].

In contrast apo E is the major apoprotein of nascent lipoproteins secreted by the liver, which also contain significant amounts of newly synthesized apo B and apo C, and much smaller proportions of apo A-I and A-IV than the intestinal lipoproteins [18–20].

In the present study we examined the effects of thoracic duct drainage on the plasma lipid- and apo A-I levels. The purpose was to see if the drainage creates an apo A-I-deficient state, which could be a useful experimental model, or whether regulatory processes that compensate for the significant apo A-I loss could be revealed.

2. Materials and methods

Thoracic duct cannulations were performed on male white Sprague-Dawley rats weighing 250–300 g and the animals were treated after operation as described earlier [21]. The animals had a gastric fistula through which they were fed 0.9–1.2 ml of corn oil in divided doses on the day after operation. On the third day they were not given any fat. All animals had a continuous infusion of 2.5 ml h^{-1} of 0.5% saline/2.5% glucose/0.05% potassium chloride, but did not receive any protein or amino acids during the experimental period.

Apo A-I was isolated from the HDL fraction obtained by zonal ultracentrifugation of rat plasma [22]. Apo A-I was separated from other apolipoproteins (A-IV, E and C) by gel filtration on screened Sephadex G-200, superfine [23] in 6 M guanidine hydrochloride (column size: 1.6 × 95 cm). The gel filtration was repeated twice in order to remove traces of apo E.

Antiserum against apo A-I was raised in rabbits by multisite intracutaneous injections of 200 μg antigen in complete Freund's adjuvant. The animals were boosted twice with 3 weeks' interval. The antiserum was absorbed with rat LDL and albumin before use.

The content of apo A-I in serum and lymph was determined by single radial immunodiffusion after previous delipidation of the samples with tetramethyl urea as described by Albers et al. [24]. Rat serum frozen at −70°C was used as standard. All apo A-I levels are given as per cent of the standard serum level.

Cholesterol and triacylglycerols were determined by the Boehringer-Mannheim enzymatic kit methods, and phospholipids according to Bartlett [25]. Protein was determined by the method of Lowry et al. [26].

3. Results

The apo A-I concentrations in serum were not lowered after thoracic duct drainage but were on average 28% higher than the concentrations in the control sera (table 1), obtained from animals which

Table 1
Plasma lipid- and apolipoprotein A-I levels in normal and thoracic duct cannulated rats

	Normals	Thoracic drained rats
Plasma apo-A-I concentration (%)	97.7 ± 4.4 (n = 7)	125.9 ± 5.5[a] (n = 11)
Serum protein concentration (mg ml^{-1})	70.8 ± 1.8	53.3 ± 3.3[a]
Apo-A-I concentration in lymph (%)		29.9 ± 1.9
Serum cholesterol (mg 100 ml^{-1})	58.7 ± 4.4	66.5 ± 6.9[n.s.]
Serum lipidsoluble P (μg ml^{-1})	84.3 ± 4.7	55.9 ± 3.9[a]
Serum triacylglycerol S (mg 100 ml^{-1})	31.4 ± 2.6	29.7 ± 2.3[n.s.]

[a] Difference statistically significant ($P < 0.01$) as estimated by the Student's t-test N.S. = not significant

Measurements on the thoracic duct cannulated animals were performed 72 h after operation. Values for apolipoprotein A-I concentrations are expressed as % of the concentration in control serum

had been fasted over night. Since the apo A-I concentrations in lymph was 20–30% of that in the control sera, and the lymph flow during the experimental period (72 h) was 53–130 ml, the total apo A-I drainage during the experimental period amounted to 2–4 plasma volumes of apo A-I. Also the plasma cholesterol level was somewhat increased in the cannulated rats (table 1), whereas the triacylglycerol levels were about the same as in the fasted controls, and the plasma phospholipid levels were somewhat lowered.

Although the animals were undoubtedly in a protein catabolic state during the experiment, since they did not receive protein or amino acids and lost 15–20 g weight, the lymphatic apo A-I output did not decrease with time (fig.1). The protein catabolic state, and the significant loss of protein by the lymph caused, however, a decrease in the levels of total plasma protein (fig.2.)

4. Discussion

The main conclusion of the present study is, that a complete drainage of thoracic duct lymph does not lead to lowered plasma-apo A-I concentrations but

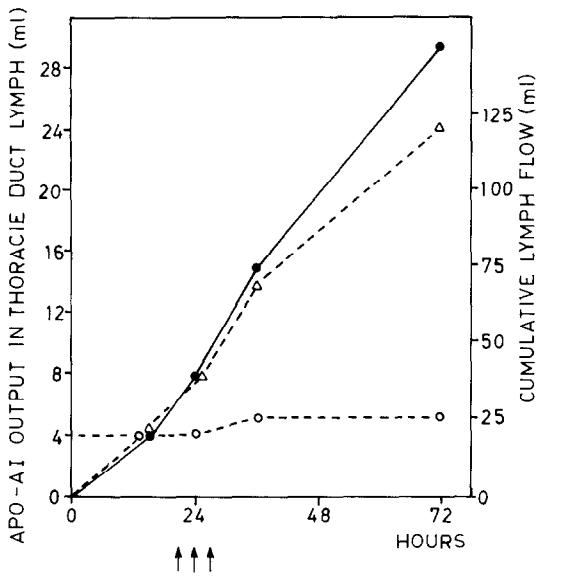

Fig.1. Time course for apolipoprotein A-I output in thoracic duct lymph. The apolipoprotein A-I output is expressed as multiples of the apo-A-I content in 1 ml control serum. Apo-A-I concentration is expressed as % of that in control serum. At each arrow 300 μl corn oil was fed through the stomach fistula. Values are from one representative animal. ○——○, Apolipoprotein-A-I concentration; △——△, cumulative lymph flow; ●——●, cumulative apolipoprotein-A-I output.

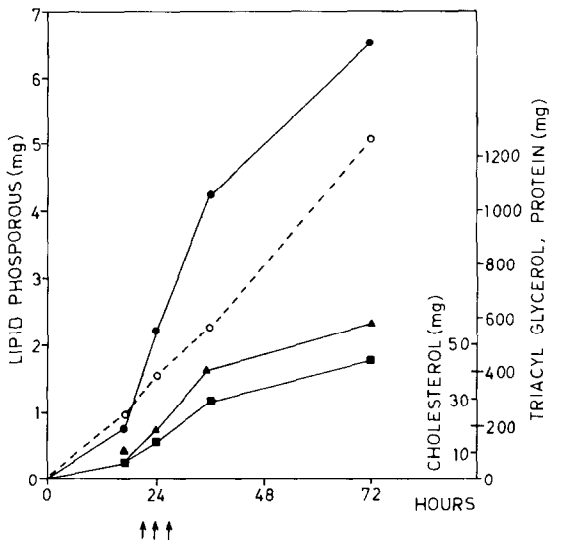

Fig.2. Time course for the output of lipids and total protein in thoracic duct lymph. Values are from the same animal as in fig.1. At each arrow 300 μl corn oil was fed through the gastric fistula. ■——■, Chloresterol; ▲——▲, triacylglycerol; ○——○, protein; ●——●, lipid phosphorus.

Table 2
Total losses of protein and lipids in the lymph

	Drainage in 72 h thoracic duct cannulated rats
Total lymph flow (ml)	98.0 ± 10.5
Protein (mg)	1294 ± 167
Phospholipid (μg P)	6212 ± 857
Cholesterol (mg)	32.9 ± 3.9
Triacylglycerol (mg)	971 ± 201
Apo-A-I (ml serum)	29.1 ± 3.5

The losses of protein, lipids and apolipoprotein A-I in the lymph drained for 72 h after the thoracic duct cannulation were measured. The drainage of apolipoprotein A-I was calculated as 'ml of control serum cleared from apolipoprotein A-I' as follows: Total lymph flow in 72 h (ml) × lymph apolipoprotein A-I concentration as % of that in control serum × 1/100

rather to an increase. The average amount of apo A-I drained in the lymph per 24 h corresponded to that present in 10 ml plasma, i.e. approximately one plasma volume (table 2). In normal rats the apo A-I turnover occurs with a $t_{1/2}$ of about 10 h [27–29]. Our values for the loss of apo A-I in the thoracic duct lymph were thus of the order 80–85% of those reported for the whole normal plasma turnover. The loss of other proteins by the lymph was significant, but was less than half of that of apo A-I when expressed as plasma volumes. Yet the total plasma protein concentration was decreased by about 30%. The increased apo A-I concentration could thus not be explained by hemoconcentration due to dehydration of the animals. Instead the increased plasma-apo A-I levels indicate the presence of a mechanism compensating for the significant loss by the lymph. The most likely hypothesis is that an increased synthesis occurs in the drained animals. Such an increase might be due to the release of a feedback control of hepatic synthesis linked to the catabolism of HDL or of apo A-I in the liver. There is, however, evidence that the intestine may secrete apo A-I not only into lymph but also directly into blood [15]. It has thus not been ruled out that thoracic duct drainage may lead to an increased intestival synthesis, and secretion of apo-lipoprotein A-I into both blood and lymph. In addition there might be a decreased catabolism of plasma HDL-apolipoprotein in thoracic duct cannulated rats. The experimental testing of these different possible explanations might provide significant information about the mechanisms by which the plasma apo A-I and the HDL levels are regulated.

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