Plasma lipid and apoprotein levels following plasmapheresis in a subject homozygous for familial hypercholesterolemia

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Summary. Plasma apoprotein levels return to levels near the baseline more rapidly than plasma cholesterol levels following plasmapheresis.

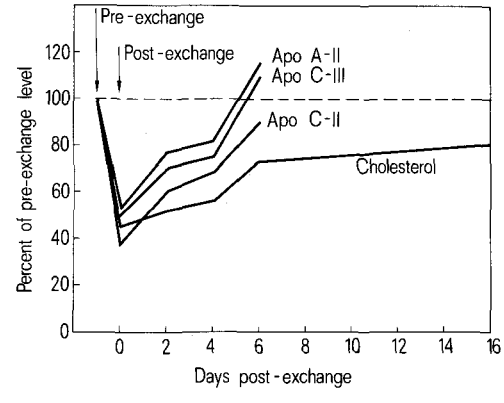
Familial hypercholesterolemia is an autosomal, dominant, inherited disorder of lipid metabolism, which is associated with a lack of high-affinity low density lipoprotein (LDL) receptors in fibroblasts, lymphocytes, and smooth muscle cells[2]. In this disorder, various studies have shown both a decrease in the fractional catabolic rate of LDL and a 2-3 fold overproduction of this lipoprotein[3-6]. In the homozygous form, the disease is characterized by extremely high levels of plasma cholesterol, tendonous xanthomas, corneal arcus and premature atherosclerosis. This condition does not respond successfully to conventional dietary or drug treatment with the result that novel methods of treatment have been explored. One such method is plasmapheresis, a process in which blood is removed continuously from the patient and separated into cellular and plasma components. The plasma is removed while the red cells and other components are returned to the patient. Since the plasma fraction contains the lipoproteins, this procedure results in a lowering of the plasma cholesterol level. Although the rate of increase of cholesterol and LDL following plasmapheresis has been investigated[4,7,8], the rate of increase of the plasma apoproteins has not been reported. This information could be of importance because the apoproteins are involved with the regulation of lipid metabolism, and it would seem desirable to know the effect of plasmapheresis on these apoproteins. In our laboratory, we have developed radioimmunoassays for apoA-II, apoC-II, and apoC-III, and are now reporting the plasma levels of these apoproteins in a homozygous patient following plasmapheresis.

Materials and methods. The patient, a 24-year-old white male, was diagnosed as homozygous for familial hypercholesterolemia at the age of 1½. He underwent total plasma exchange, during which 3.4 l of plasma were exchanged. Blood was removed from an antecubital vein, anticoagulated with citrate, and pumped through a NCI-IBM cell separator, model 2990. The patient's plasma was removed and an equal volume of normal serum albumin was returned to the patient along with the red blood cells, by means of the other antecubital vein[9]. Blood samples were obtained prior to and immediately following plasmapheresis and then at 2, 3, 6, and 16 days post-exchange. Plasma samples were analyzed for cholesterol using an enzymic method[10], and for apoA-II, apoC-II, and apoC-III by routine double-antibody radioimmunoassays[11-13]. All radioimmunoassays were performed in triplicate.

Results and discussion. The absolute levels of cholesterol and the apoproteins pre- and post-plasmapheresis are presented in the table along with the total plasma content of these factors in the patient, and the relative rates of increase are illustrated in figure 1. It is evident that the apoprotein levels returned to levels near the baseline by 6 days post-exchange, whereas cholesterol was still below the baseline level at 16 days post-exchange. This rate of return of cholesterol is somewhat slower than the 10-13 days found by Apstein et al.[7], whose homozygous patient underwent 5-9 day courses of plasmapheresis with the removal of 250-500 ml of plasma per day.

Based on circulating plasma levels and total plasma volume estimated on the basis of body weight[14], the amount of cholesterol removed from this patient during plasmapheresis was 15.24 g, leaving 12.88 g of cholesterol in the plasma compartment. By 6 days post-exchange, this compartment contained 20.52 g, an increase of 7.64 g. It is impossible to determine the origin of this cholesterol but it is likely that at least 3 sources were included: absorption from the diet, endogenous synthesis, and the re-equilibration of cholesterol from the tissues to the plasma. The latter process is important, for it is hoped that repeated plasmapheresis procedures would promote this re-equilibration, resulting in the clearing of cholesterol from peripheral tissues and a decrease in the total body cholesterol content.

Although no conclusions regarding synthetic rates of the apoproteins can be made without data on catabolic rates,



Plasma cholesterol and apoprotein levels before and after plasmapheresis

	Cholesterol		Apo A-II		Apo C-II		Apo C-III	
	mg/dl	Total plasma* (g)	mg/dl	Total plasma* (mg)	mg/dl	Total plasma* (mg)	mg/dl	Total plasma* (mg)
Pre-exchange	740	28.12	21.8	828.4	1.62	61.56	4.99	189.6
Post-exchange	339	12.88	11.5	437.0	0.80	30.40	1.85	70.3
2 days post-exchange	378	14.36	16.9	642.2	0.98	37.24	3.58	136.0
3 days post-exchange	426	16.19	17.9	680.2	1.11	42.18	3.76	142.9
6 days post-exchange	540	20.52	25.4	965.2	1.44	54.72	5.52	209.8
16 days post-exchange	675	25.65	ND		ND		ND	

* Based on total plasma volume estimated as 4.4% of b.wt; ND, not determined.

the average amount of apoprotein synthesized in excess of the amount catabolized over the 6-day period can be calculated from the plasma levels, assuming a plasma volume of 3.8 l[14]. It thus appears that there was a total body increment of 88 mg/day of apoA-II, 4 mg/day of apoC-II, and 23 mg/day of apoC-III as calculated from the table.

The finding that apoprotein levels return to levels near the baseline before the plasma cholesterol achieves its pre-exchange level is significant, as it suggests that plasmapheresis can be repeated at intervals that would permit successive decrements in the plasma cholesterol and a decrease in total body cholesterol while permitting recovery of plasma apoA-II, apoC-II, and apoC-III levels. This observation requires further documentation both in additional patients and also for the other apoproteins. We are currently engaged in work that would permit this to be done.

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Human plasma dopamine-β-hydroxylase: oxygen and thermal stability[1]

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Summary. There are wide individual variations in the thermal stability of human plasma dopamine-β-hydroxylase (DBH). Thermal stability variations have proven of value in biochemical genetic studies of many enzymes. The development of DBH thermolability depends on the exposure of plasma to oxygen. This observation may help to elucidate the biochemical basis of the genetic regulation of DBH.

Dopamine-β-hydroxylase (E.C. 1.14.17.1, DBH) catalyzes the conversion of dopamine to the neurotransmitter norepinephrine, is localized to catecholamine containing vesicles in sympathetic nerves and the adrenal medulla, and is found circulating in blood[2]. Plasma DBH has often been measured because of the possibility that it might reflect the status or function of the sympathetic nervous system[2]. However, it is now known that most of the variance in basal human plasma DBH enzymatic activity and immunoreactive protein is due to the effects of a single genetic locus, *DBH*[3-9]. Recent studies have shown that, in addition to variation in basal enzyme activity, there are wide individual variations in the thermal stability of human plasma DBH[7,10]. Variations in thermal stability reflect variations in protein structure and have been helpful in the study of genetic polymorphisms of a variety of enzymes[6,11-13].

Approximately 10% of a randomly selected population has thermolabile plasma DBH. The trait of thermolability is a characteristic of the DBH molecule itself, and has a significant familial aggregation[10]. Family studies indicate that although this characteristic is probably inherited, it does not segregate with the alleles at the locus *DBH* that control basal enzyme activity[10,13,14].

Thermolability of DBH cannot be demonstrated with freshly obtained plasma, but rather it is 'developed' after plasma is incubated at 37 °C for 18-24 h in vitro. This step must then be followed by thermal inactivation at 55 °C for 20 min, a procedure required for the 'expression' of thermolability[10].

Elucidation of the biochemical mechanism responsible for the development of the characteristic of thermolability would contribute significantly to our understanding of individual biochemical differences in human DBH and might prove useful in studies of the structural gene of this important catecholamine biosynthetic enzyme. We have studied the possibility that exposure to oxygen during the development of thermolability might be the critical element in uncovering individual variations in the thermal stability of human plasma DBH.

Heparinized blood samples were obtained from selected laboratory personnel known to have either thermolabile or thermostable DBH and from 99 consecutive, randomly selected blood donors at the Mayo Clinic Blood Bank. Preincubation for the development of the characteristic of thermolability was performed by incubating 200 µl of each sample at 37 °C for 18 h in stoppered 2 ml vacutainer tubes. The gas in the vacutainer was changed by evacuating the tube for 20 sec under a vacuum of 20 inches of mercury, followed by introducing nitrogen, oxygen or air into the tube through a 25 gauge needle for 20 sec with 10 psi of pressure. The procedure for changing the gas in the tube was then repeated a 2nd time. After the preincubation step, plasma was diluted with water 1:50 (vol:vol) and 200 µl aliquots were placed in reaction tubes. The thermal inactivation step necessary to bring about the expression of thermolability was performed by incubation of the diluted plasma at 55 °C for 20 min. Control samples were kept on ice at 4 °C during the thermal inactivation step. DBH