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Acute and Long-Term Effects of Low-Density Lipoprotein Apheresis on the Serum Concentrations of Vitamins E and A*

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Summary. Serum α-tocopherol and retinol concentrations were followed in four heterozygous adults and one homozygous child with familial hypercholesterolemia being treated by regular low-density lipoprotein (LDL) apheresis. Approximately 50% of plasma α-tocopherol was eliminated during a single apheresis procedure in the heterozygous adults, while a complete elimination of this vitamin along with LDLs was observed in the homozygous child. Absolute losses of α-tocopherol amounted to 13.4–22.5 mg/apheresis and are equivalent to the recommended dietary intake for 1.5 to 2 days. Despite these losses, no changes were observed either in serum α-tocopherol levels or in the ratio of α-tocopherol/total serum lipids after 12 months regular apheresis treatment. Serum retinol concentrations only showed a small decrease on apheresis, there being apparently no specific elimination of this vitamin. The absolute losses ranged from 42–422 μg/apheresis and were, therefore, much lower than the recommended dietary intake of the equivalent of 1500 μg retinol/day. It is concluded that no extra supplementation of these vitamins is required during LDL-apheresis therapy, although it may be advisable to monitor vitamin E status in patients on long-term, intensive therapy.

Key words: LDL apheresis – Vitamin E – Vitamin A – α-Tocopherol – Retinol

Repetitive intermittent plasmapheresis is an effective means for lowering low-density lipoprotein (LDL) cholesterol levels in the treatment of both the heterozygous and homozygous forms of familial hypercholesterolemia [12, 22–24, 28]. More recently, selective methods have been developed to continuously remove LDLs while allowing the return of the patient's own plasma. These specific procedures include binding to immobilized antibodies [8, 20], binding to immobilized dextran sulfate [9, 27], cascade filtration [9, 25], and precipitation with heparin at acidic pH [2, 7].

Although a large volume of data has accumulated on the use of these procedures to lower LDL levels, no attention has been paid to the effect of these treatment modalities on the fat-soluble vitamins and in particular vitamin E (α-tocopherol). This vitamin is transported by the plasma lipoproteins and although no specific lipoprotein functions as the sole carrier, a large proportion is to be found within the LDL fraction [6, 13]. Vitamin E is a strong antioxidant and one of its physiological functions is probably to protect biological systems and membranes against the deleterious effects of free radicals and peroxides.

Dietary vitamin A (retinyl ester) is transported by the chylomicrons from the intestine to the liver for storage. Most of the retinyl ester remains associated with the chylomicrons in plasma and there is only a 5%–10% transfer to other plasma lipoproteins [3, 26]. As needed, retinyl ester is hydrolysed in the liver to retinol which is then transported to the tissues bound to retinol-binding protein. Only a small amount of plasma retinol is associated with lipoproteins, notably the triglyceride-rich lipoproteins [3].

It might therefore be anticipated that LDL apheresis will effectively eliminate that fraction of plasma α-tocopherol bound to LDLs, but will not greatly influence plasma retinol concentrations. The present study was designed to investigate both the acute changes in plasma α-tocopherol and retinol concentrations after LDL apheresis using the

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Table 1. Clinical and laboratory data of the five subjects being treated by HELP-LDL apheresis

Patient	Sex	Age (years)	HELP-LDL apheresis		Mean LDL cholesterol levels (mg/dl)		
			Frequency per month	Months duration	Before therapy	During therapy	
						SA	EA
ES	F	44	3.9	20.5	323	210	62
HE	M	31	3.3	19	365	255	97
RK	M	46	2.9	16	252	156	76
JC	F	7	3.9	12.5	796	416	169
CG	F	28	4.3	12	240	157	39

SA, mean serum concentration at the start of apheresis; EA, mean serum concentration at the end of apheresis

HELP procedure [2, 7], as well as the long-term effects in patients who have been undergoing regular HELP-LDL apheresis for 12 months or more.

Materials and Methods

Patients

Five patients with severe type II hypercholesterolemia were included in this study (Table 1). The clinical details of three of these patients (ES, HE, and JK) have already been described [7].

JC is a 7-year-old girl with homozygous familial hypercholesterolemia (FH) documented by tissue culture to be of the receptor-defective type. CG is a young woman aged 28 years with the heterozygous form of FH. At the age of 27 she suffered two myocardial infarctions. She has angiographically documented severe, diffuse, three-vessel coronary artery disease.

Help-LDL Apheresis

The HELP procedure has been described in detail elsewhere [7]. In the case of the adults, 3000 ml plasma is regularly cleared of LDLs, while 1000 ml plasma is treated from the homozygous child at each session.

Analytical Methods

Blood samples were collected at the beginning (SA) and end (EA) of each apheresis procedure for laboratory analysis.

Serum retinol and α-tocopherol concentrations were determined simultaneously after extraction into an organic matrix using an isocratic high-performance liquid chromatography (HPLC) procedure according to Nierenberg [16] with slight modifications (D. Niedman, unpublished results). Retinyl acetate and α-tocopherol acetate were used as internal standards. The within-run coefficients of variation were 2.3% and 3.3% and the between-run coefficients were 6.1% and 3.3% for retinol and α-tocopherol, respectively.

Serum total cholesterol, total triglycerides, and total phospholipids were measured using enzymatic test kits (Wako, Neuss). LDL cholesterol was determined using a precipitation procedure (Quantolip-LDL, Immuno, Heidelberg) based on dextran sulfate [1]. HDL cholesterol was also measured by means of a precipitation method (Boehringer, Mannheim)

Results

Clinical and laboratory data of the five subjects studied are presented in Table 1. Duration on HELP therapy ranges from 12 to 20.5 months with a frequency of around three to four aphereses per month. Mean LDL-cholesterol concentrations are shown for each patient both prior to and during therapy with HELP-LDL apheresis. While on apheresis therapy, the LDL-cholesterol levels fluctuate between a maximum observed immediately before (SA) and a minimum measured immediately after (EA) apheresis. Between each apheresis LDL levels increase exponentially towards the pretreatment values [21]. Regular apheresis in our patients has led to a 30%–48% reduction in the pretreatment levels (SA), compared with the values observed before starting HELP therapy.

Acute Effect of HELP-LDL Apheresis on α-Tocopherol and Retinol Concentrations

To determine the effect of a single LDL apheresis on α-tocopherol and retinol, their serum concentrations were determined both before and after a treatment procedure. The mean values obtained from 10 aphereses in each patient are shown in Table 2, along with the corresponding LDL- and

Table 2. Acute effect of LDL apheresis on both retinol and α-tocopherol concentrations as well as LDL and HDL cholesterol concentrations. The mean serum concentrations at the start (SA) and end (EA) of an apheresis ($n=10$) are presented; 3000 ml plasma was treated at each apheresis in the four adults and 1000 ml in the case of the homozygous child (JC)

Patient	P_V[a] ml	α-Tocopherol (mg/l)		Retinol (μg/l)		LDL cholesterol (mg/dl)		HDL cholesterol (mg/dl)	
		SA	EA	SA	EA	SA	EA	SA	EA
ES	2117	16.2±1.6	7.3±1.5	339±75	246±40	197±22	49±13	52±5	46±6
HE	3078	17.8±2.5	10.5±1.9	616±97	479±82	253±39	86±14	69±5	62±5
RK	3206	14.4±4.1	9.3±1.5	651±80	568±40	141±18	55±5	52±5	47±8
JC	1190	25.7±5.4	11.0±4.3	206±26	171±23	418±57	170±39	22±5	14±3
CG	2026	12.1±1.1	5.5±0.9	386±36	279±26	150±5	35±5	47±5	39±4

[a] P_V = plasma volume of the patient

HDL-cholesterol concentrations. We were then able to calculate the efficiency of the elimination of these vitamins and lipoproteins using the following exponential equation [7, 21]:

$$\frac{C_E}{C_0} = e^{-\frac{kP_E}{P_V}} \quad (1)$$

where C_E is the plasma concentration at the end of apheresis, C_0 is the plasma concentration at the start of apheresis, P_E is the volume of plasma treated, P_V is the plasma volume of the patient, and k is a factor equal to or smaller than 1, depending upon the efficiency with which the vitamin or lipoprotein is removed from the plasma in the extracorporal system. The plasma volumes of our patients were calculated from regression equations involving body weight and height [14, 17]. After substituting C_E, C_0, P_E, and P_V in Eq. 1, the k values shown in Table 3 were derived for α-tocopherol, retinol, and LDL and HDL cholesterol.

In agreement with our earlier findings [7], the mean value of the factor k for LDLs was approximately 1 in each of the patients studied, indicating complete removal of this lipoproteins from the patient's plasma in the extracorporal system. In the homozygous child, α-tocopherol was eliminated with the same efficiency as LDLs, reflecting the fact that LDLs represent the vast proportion of the plasma lipoproteins in this patient. For the heterozygous patients, k values for α-tocopherol were relatively constant, ranging from 0.47 to 0.56. Thus, approximately 50% of the serum α-tocopherol is apparently eliminated with the LDLs in these patients. Retinol on the other hand showed much lower k values of between 0.15 and 0.26, indicating only a low clearance by the HELP procedure.

From the mean pre- and posttreatment serum concentrations of the two vitamins and knowing the plasma volumes of the patients, it was also possible to quantitate the average losses due to a single apheresis. The amounts are shown in Table 4. Losses of α-tocopherol were between 13.4 and 22.5 mg at each session, corresponding to 1.5–2 days of the recommended dietary intake [5]. There was a large variation in the losses of retinol, ranging from 42 to 422 μg, the absolute amount depending upon the plasma concentration.

Table 3. Efficiency of the elimination of α-tocopherol, retinol, LDL, and HDL through HELP-LDL apheresis. The factor k was derived by substituting the results from Table 2 into Eq. 1

Patient	k			
	α-Tocopherol	Retinol	LDL cholesterol	HDL cholesterol
ES	0.56	0.23	0.98	0.09
HE	0.54	0.26	1.11	0.11
RK	0.47	0.15	1.00	0.11
JC	1.01	0.22	1.07	0.54
CG	0.53	0.22	0.98	0.13

Table 4. Mean total losses of retinol and α-tocopherol due to a single apheresis treatment of 3000 ml plasma in four adults or 1000 ml plasma in a homozygous child

Patient	α-Tocopherol (mg)	Retinol (μg)
ES	18.8	197
HE	22.5	422
RK	16.4	266
JC	17.5	42
CG	13.4	217

Long-Term Changes in α-Tocopherol and Retinol during HELP-LDL Apheresis Therapy

α-Tocopherol and retinol concentrations were monitored every 6 months during the course of

Table 5. Long-term effect of weekly LDL apheresis therapy with the HELP system on serum α-tocopherol and retinol concentrations as well as on the ratio of α-tocopherol to total serum lipids

Patient	α-Tocopherol (mg/l)				Ratio α-tocopherol/ total serum lipids (mg/g)				Retinol (μg/l)			
	0	6	12	18	0	6	12	18	0	6	12	18 (months)
ES	18.0	14.8	16.0	17.8	2.11	2.14	2.28	2.65	228	279	282	404
HE	25.4	17.9	18.3	19.1	2.12	2.01	2.66	2.24	648	637	425	662
RK	11.2	10.3	18.9		1.29	1.24	3.18		791	521	743	
JC	16.5	19.6	25.7		1.08	3.12	2.93		143	214	248	
CG	16.0	11.9	13.8		2.53	2.32	2.61		445	350	436	
Mean	17.4	14.9	18.5		1.83	2.17	2.73		451	400	427	
±SD	5.1	3.9	4.5		0.61	0.67	0.34		273	175	196	

Normal ranges ($n = 70$): α-tocopherol, 8.7–22.2 mg/ml (median, 11.4 mg/ml); retinol, 326–883 mg/ml (median, 563 mg/ml)

regular HELP therapy. No major changes were observed (Table 5) in the levels of these two vitamins over 12 months in our five subjects. Plasma tocopherol concentrations were generally above the 50th percentile of a normal collective, in keeping with the hyperlipidemia of these patients. Even after 18 months treatment in two subjects, serum tocopherol concentrations were relatively unchanged compared to pretherapeutic values. The ratio of vitamin E to total plasma lipids has been suggested as a more suitable indicator of nutritional adequacy for this vitamin [11]. This is due to the fact that vitamin E occurs almost exclusively in the plasma lipoproteins and as serum lipid concentrations increase, vitamin E appears to partition out of the cellular membrane compartment and into the circulating fraction [4]. Thus, in vitamin E-deficient states [18, 19], the vitamin E to total plasma lipids ratio is lower than in normal healthy controls. No decreases were observed in the α-tocopherol/total serum lipids ratio of our five patients during HELP therapy. The values remained relatively constant in three of the patients, even after 18 months treatment in two of them (ES and HE). In the remaining two patients (RK and JC), low α-tocopherol/total serum lipids ratios were present before starting HELP-LDL apheresis therapy. These ratios, however, were still greater than the value of 0.8 mg/g which is considered to be the lower limit indicating an adequate nutritional status of vitamin E [11]. Rather than a further deterioration occurring during long-term LDL apheresis, the values in fact increased without the need for additional dietary supplementation.

Retinol concentrations were also unaffected after 12 and 18 months of regular apheresis treatment. Even though low serum concentrations were seen in two of our patients (ES and JC) before starting regular apheresis, the concentrations did not decrease further, but rather increased during therapy.

Discussion

Plasma exchange and selective LDL apheresis are being increasingly used for the treatment of severe hypercholesterolemia. As yet, however, no information has been published on the effect of such procedures on the fat-soluble vitamins, and in particular on vitamin E which is transported to a large extent in plasma by LDLs [6, 13]. The function of this vitamin appears to be that of an antioxidant [5], probably preventing the oxidation of polyunsaturated fatty acids and other important compounds such as vitamins A and C, as well as inhibiting the formation of peroxides and free radicals. As such it could play a protective role in the atherosclerotic process. We, therefore, considered it of importance to determine whether or not regular LDL elimination would influence the serum levels of this vitamin.

In the heterozygous FH patients on HELP-LDL apheresis, the elimination of α-tocopherol was about one-half that of LDLs, presumably reflecting the fact that approximately 50% of the serum α-tocopherol is transported in the LDLs. This proportion is somewhat lower than the value of 63% reported for persons with type IIa hypercholesterolemia [6]. In the case of the homozygous child, however, α-tocopherol was eliminated as efficiently as LDLs from plasma on HELP treatment. Since LDL levels are massively elevated in this child and HDL levels are extremely low, the former will make up the bulk of the plasma lipo-

proteins and will therefore be the predominant carriers of vitamin E in this child.

These results demonstrate that significant quantities of vitamin E can be lost on LDL apheresis. In terms of absolute amounts, the losses in our patients ranged from 13.4 to 22.5 mg α-tocopherol at each apheresis. This is equivalent to the recommended dietary intake [5] for 1.5 to 2 days (8 mg/day for women, 10 mg/day for men). Although our patients are being treated by HELP-LDL apheresis every 7 days, with the exception of holidays and illness, there was no evidence of the development of a vitamin E deficiency after 12 or 18 months' regular treatment. Not only did the serum α-tocopherol levels remain fairly constant during treatment, but more importantly the ratio of serum α-tocopherol to total serum lipids [11], a more suitable indicator of the vitamin E status, did not deteriorate. It would therefore appear that the vitamin E losses due to LDL apheresis are adequately compensated by the nutritional intake of our patients, none of whom were on additional vitamin supplementation. Nevertheless, it should be noted that there are usually substantial reserves of vitamin E in the tissues and it may take several years for a vitamin E deficiency to become manifest. Furthermore, if the patient is receiving a diet rich in polyunsaturated fat, then there will be an increased need for vitamin E [10]. Therefore, depending upon the individual case, and the frequency of treatment, it may be relevant to periodically control the vitamin E status of those patients who are on regular LDL apheresis.

In contrast to vitamin E, LDLs do not contribute significantly to the transport of vitamin A either in the form of retinyl ester or retinol. Consequently, retinol was not eliminated by HELP-LDL apheresis to any great extent and the total losses on a single apheresis of between 42–442 μg are much lower than the recommended daily dietary intake of the equivalent of 1500 μg retinol [15].

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