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The HELP-LDL-apheresis multicentre study, an angiographically assessed trial on the role of LDL-apheresis in the secondary prevention of coronary heart disease. I. Evaluation of safety and cholesterol-lowering effects during the first 12 months

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Abstract. Fifty-one patients with coronary heart disease (CHD) and LDL-cholesterol levels $\geqslant 200$ mg dl^{-1} despite diet and drug therapy have been recruited into an angiographically controlled, multicentre, two-year study to evaluate HELP-LDL-apheresis in the secondary prevention of CHD. There were five drop-outs in the first year and 46 patients completed one year of therapy. An average of 2·79 l of plasma was treated per patient every 7·7 days. Treatment was well tolerated and the incidence of side effects was small (2·9% of treatments). Mean pre-/post-apheresis LDL-cholesterol levels decreased from 283/120 mg dl^{-1} at baseline to 207/78 mg dl^{-1} and 203/76 mg dl^{-1} after 6 and 12 months, respectively. Mean pre-/post-apheresis HDL-cholesterol levels rose significantly over the course of therapy from 40·5/36·6 mg dl^{-1} to 44·8/39·7 mg dl^{-1} and 48·2/41·3 mg dl^{-1} after 0, 6 and 12 months, respectively. No major derangement of pre-apheresis haemostasis nor of haematological or clinical chemical parameters had occurred after 12 months of treatment. The data from this study support the feasibility of HELP-LDL-apheresis as an adjunctive therapy for lowering cholesterol levels in CHD patients refractory to cholesterol-lowering drugs while substantially improving the HDL/LDL ratio.

Keywords. Cholesterol, coronary heart disease, fibrinogen, HDL-cholesterol, LDL-apheresis, LDL-cholesterol.

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Introduction

A causal link between plasma cholesterol and coronary heart disease (CHD) is now well established and conclusive evidence has been presented demonstrating that a cholesterol-lowering therapy can reduce the incidence of CHD and its associated effects. The results of the LRCC primary prevention trial [1], a randomized placebo-controlled double blind study with cholestyramine, revealed that for every 1% reduction in cholesterol there is a 2% reduction in the CHD incidence. The Helsinki Heart Study [2] further strengthened the view that lipid-lowering is effective in the primary prevention of CHD. While these two trails addressed the question of a lipid lowering therapy in the primary prevention of CHD, the CLAS study [3] has produced evidence confirming the benefit of such therapy in the secondary prevention of coronary disease. This randomized control study used a combination of diet, colestipol and nicotinic acid to achieve an average 43% reduction in the LDL-cholesterol levels of treated subjects. Angiographic evaluation after two years of treatment showed a highly significant improvement in the status of coronary lesions in the drug-treated group as compared to the placebo group.

Cholesterol lowering strategies to reduce the risk of CHD in the general population have now been proposed [4,5]. The cornerstone of such strategies is undoubtedly diet and where necessary drug therapy. With the advent of the HMG-CoA-reductase inhibitors [6–8], a new class of powerful lipid lowering drugs has been introduced with great potential for the treatment of hypercholesterolaemia. The use of these drugs is currently being evaluated whereby the long-

Table 1. Initial clinical and laboratory data of the patients recruited to the study and those completing 12 months of regular treatment

								Mean (SD)	
	n	M/F	MI	ACVB	Smoker*	Hypertension	Age (yr)	Total chol. [mg dl^{-1}]	Total triglyc. [mg dl^{-1}]
All patients recruited	51	34/17	28	22	36	17	44·4 (9·2)	386 (93)	161 (89)
Patients completing 12 months treatment	46	33/13	24	20	33	16	44·6 (8·0)	379 (92)	178 (106)

* Prior to diagnosis of CHD.

term safety will be of profound importance for their general application in the treatment of a chronic disease that only becomes clinically manifest after many years.

More radical measures for the treatment of hypercholesterolaemia such as partial ileal bypass [9,10], portocaval shunt [11], liver transplantation [12], plasma exchange [13–15] and LDL-apheresis [16–21] have been reported. Plasma exchange has proven to be particularly successful in the management of the severe hypercholesterolaemia of homozygous FH [22]. Several LDL-apheresis procedures with varying degrees of selectivity have subsequently been developed [16–21] and are at present being evaluated in clinical trials. While the use of such intensive therapies for the primary prevention of CHD will be largely restricted to the most severe forms of hypercholesterolaemia, their application as an adjunctive LDL-cholesterol lowering therapy in the secondary prevention of coronary heart disease remains an attractive therapeutic possibility. The combination of LDL-apheresis, together with diet and drugs, should allow a maximal lowering of LDL-cholesterol [23,24]. Besides LDL, the various apheresis procedures may also eliminate other potentially atherogenic factors, such as Lp(a) [25,26] and acutely improve the haemorheological status of the patient [27,28].

In order to assess the role of LDL-apheresis in the secondary prevention of CHD long-term multicentre trials are required. We therefore initiated a prospective, open multicentre study aimed at treating individuals with hypercholesterolaemia and CHD with an LDL-apheresis procedure based on the heparin-induced precipitation of LDL at acid pH, the so-called HELP system [20]. The aims of this study were threefold:

1 Evaluation of the long-term effects of regular HELP-LDL-apheresis on plasma lipoproteins and proteins.
2 Assessment of the efficacy, safety and practicability of long-term HELP-LDL-apheresis.
3 Evaluation of the coronary status of patients after two years of regular HELP-LDL-apheresis through coronary angiography.

Table 2. Classes of lipid-lowering drugs administered during the first 12 months of the HELP multicentre study

Drug type	No. of patients*
resins	35
fibrates	29
nicotinic acid	15
sitosterol	5

* Several patients were on multi-drug treatment.

This report describes the study design and the results of the first 12 months of regular HELP-LDL-apheresis.

Materials and methods

Study design

Ten centres participated in this study and 51 patients aged between 28 and 65 years were recruited (Table 1). To be eligible for treatment patients had to have clinically manifest coronary heart disease (CHD) with angiographically documented changes in several segments of the coronary arterial tree. A further criterion for selection was hypercholesterolaemia with LDL-cholesterol levels $\geqslant 200$ mg dl^{-1} despite diet and drug therapy. At the outset of this study, HMG-CoA-reductase inhibitors were still at the clinical trial stage in the FRG and so were excluded from our study design. Each centre was responsible for screening and selecting their own patients according to the study protocol and for maintaining the diet and drug therapy with which the patient had been treated prior to beginning LDL-apheresis. A list of the classes of lipid-lowering drugs that were administered to the patients during the first 12 months of the study is presented in Table 2.

The study protocol was approved by the ethics committee of the Faculty of Medicine, University of Göttingen. Written consent was obtained from all patients at the start of the study. Reasons for exclusion from the study included: haemorrhagic diathesis,

Table 3. Clinical and laboratory data on the five patients who were lost to follow-up in the study

Pat. no.	M/F	Age (yr)	LDL-C* initial	(mg dl^{-1}) final	Previous MI	AVCB no. of apheresis	Reasons for termination
6	F	18	540	230	−	−26	returned to native country
13	F	65	252	224	+	+25	>6 week break in treatment
30	F	47	337	245	+	−7	problems of vascular access
33	F	32	318	250	+	+14	fatal MI
42	M	49	230	232	+	−49	sudden cardiac death

* Pre-apheresis values

coagulation disorders, neoplasm, liver disease, severe cardiac insufficiency, cardiac valvular disease, apoplexy, dementia, non-compliance with dietary and drug therapy.

Coronary angiograms were planned for each patient prior to beginning HELP therapy and again after two years of regular treatment. Detailed guidelines for standardisation of the coronary angiography were included in the study protocol. Before and after each apheresis the following parameters were determined: total cholesterol, total triglycerides, LDL-cholesterol, HDL-cholesterol, prothrombin time, thrombin time, fibrinogen and plasminogen.

HELP-LDL-apheresis

The details of this procedure have been published elsewhere [20]. Briefly, plasma and blood cells are separated by a 0·55 μm plasma filter and the plasma is then continuously mixed with a sodium acetate buffer (pH 4·85) containing 100 U ml^{-1} heparin. The precipitate formed at this low pH is then removed by filtration through a 0·45-μm polycarbonate filter and the excess heparin in the filtrate is adsorbed to a DEAE-cellulose filter. Finally, physiologic pH is restored and excess fluid removed by bicarbonate dialysis/ultrafiltration before the treated plasma is mixed with the blood cells from the plasma filter and returned to the patient. Each centre was advised to treat between 2·5 and 3 litres of plasma at each visit and to document the exact volume treated as well as the treatment time. All centres were equipped with a Plasmat Secura (B. Braun Melsungen) and were regularly supplied with the necessary sterile disposable filters and tubing systems required. Centres were required to treat patients every seven days with the exception of holidays and illness. Patients who for any reason had to interrupt treatment for an interval of six weeks or more were excluded from the evaluation.

Laboratory parameters

Total cholesterol, HDL-cholesterol and triglycerides were determined with commercially available test kits (Boehringer, Mannheim). LDL-cholesterol was measured using the Quantolip LDL test kit (Immuno, Heidelberg). The haemostasiological and clinical chemical parameters were measured in the respective laboratories of each centre.

Statistics

The results were evaluated using exploratory data analysis. A global trend over the period of 0, 6 and 12 months was tested using the Friedman Rank Test at a significance level of 5%. Significant results were further subjected to Wilcoxon sign-rank tests to investigate differences between two time points (e.g. 0 and 12 months). The significance levels were adjusted by the Holm procedure [29].

Results

A total of 51 patients with severe CHD and type IIa hypercholesterolaemia were recruited into the study (Table 1). Twenty-eight patients had suffered a previous myocardial infarction (MI) and 22 patients had received an aorta-coronary venous bypass (ACVB). Over two-thirds of the patients were smokers prior to the diagnosis of CHD. Forty-six patients completed 12 months of regular treatment while 5 patients were lost to the study during the first year (Table 3). Two suffered cardiac deaths two days or more after the previous HELP-LDL-apheresis procedure. Patient 33 suffered a fatal MI after anticoagulation therapy had been discontinued on account of an imminent dental appointment. Autopsy revealed a thrombotic stenosis of the venous bypass of the right coronary artery. Patient 42 died from sudden heart failure after strenuous exercise. His relatives refused an autopsy. Because of poor venous access patient 30 required a Cimino Bresica fistula for treatment. However, repeated occlusion of the shunt resulted in this patient having to be withdrawn from the study. Patient 13 had to interrupt apheresis treatment for a period of more than six weeks due to an operation and did not fulfil the study guidelines. Finally, patient 6 was lost to follow up because she returned to her native country before completing the study.

Technical details

During the first 12 months the average treatment frequency interval was 7·7 days (SD = 4·2), each patient being treated on average 47 times during the year. Actual treatment time not including initial preparation lasted 118 min (SD = 24) and on average 2·79 l (SD = 0·37) of plasma were treated at each visit. Table 4 summarizes in more detail the volume of plasma treated in relation to the total number of

Table 4. Number and frequency of aphereses in which a particular volume of plasma could be treated

Plasma volume treated	No. of aphereses	%
< 1·5	29	1·36
1·5 < 2·0	40	1·88
2·0 < 2·5	123	5·77
2·5 < 3·0	1939	90·99
Total	2131	100

Table 5. Clinical side-effects reported during HELP-LDL-apheresis

Side-effect	Frequency	No. of patients affected
Angina pectoris	22	9
Vagal reaction (Hypotension, bradycardia < 60/min, nausea)	12	11
Haematoma	6	3
Shunt occlusion†	5	4
Arrhythmia	4	4
Eye burning	4	4
Shivering	3	3
Collapse	3	3
Dyspnoe	2	2
Vertigo	1	1
Total	62	29*

* Several patients reported more than one side effect.
† Does not include the data of the patient who was lost to the study on account of repeated shunt occlusion.

aphereses. More than 2·5 l of plasma could be treated in over 90% of aphereses and more than 2 l in over 96% of aphereses.

Side effects

From a total of 2131 individual aphereses on the 46 patients completing 12 months of treatment, 62 cases of undesirable side effects were reported in 29 patients. These are listed in Table 5. Adverse clinical reactions were reported in only 2·9% of treatments and the reactions were generally of a minor nature and not uncommon to other extracorporeal treatment procedures [30–32]. No major life-threatening complication occurred during treatment. The two sudden cardiac deaths described above occurred during the treatment-free period.

During the course of therapy there was, however, a general improvement in the angina symptoms of our patients. At the outset of treatment 38 suffered from angina while only eight reported no symptoms. After six months of treatment 20 patients no longer experienced angina (Table 6).

Table 6. Number of patients reporting angina pectoris symptoms during the course of 12 months regular HELP-apheresis

Angina pectoris symptoms	Months of treatment 0	6	12
None	8	20	19
Present	38	26	24
No details	0	0	3

Lipids and lipoproteins

The levels of various lipid and lipoprotein parameters measured directly before an apheresis are compared after 6 and 12 months of regular therapy to the levels before the start of apheresis therapy in Table 7. Pre-apheresis total cholesterol values could be maintained at levels about 23% lower than those before therapy. This reduction was primarily due to a mean drop in LDL-cholesterol of 28%. The patients with the highest baseline LDL-cholesterol concentration before starting HELP treatment displayed the greatest percent reduction ($r = 0{\cdot}71$). The median LDL-cholesterol levels decreased progressively during the first 3–4 treatments but remained relatively stable thereafter at around 200–210 mg dl^{-1} (Fig. 1). The post-apheresis levels stabilized at values of around 80 mg dl^{-1}, representing a decrease of about 60% at each treatment. In contrast to LDL, mean pre-apheresis HDL-cholesterol levels rose by about 19% during the course of 12 months therapy. Interestingly, mean pre-apheresis triglyceride levels dropped by 19–20%.

Haemostasiological findings

Fibrinogen and plasminogen are two components of the coagulation/fibrinolytic system that are simultaneously precipitated with LDL during the HELP procedure. The mean fibrinogen level at the start of treatment was 308 mg dl^{-1} (Table 8). Similar to LDL-cholesterol, the pre-apheresis fibrinogen levels fell in the first 3–4 treatments and remained relatively constant over the course of therapy. The mean pre-apheresis levels at 6 and 12 months of 250 and 235 mg dl^{-1}, respectively, represent a reduction of around 19–24%. In contrast to LDL and fibrinogen, pre-apheresis plasminogen concentrations were unchanged after 6 and 12 months. This is presumably due to the more rapid turnover of plasminogen, concentrations generally returning to pre-apheresis levels within 2–3 days of the apheresis [33]. No derangement of pre-apheresis haemostasis was observed (Table 8) after 6 and 12 months of regular therapy. The average prothrombin and activated partial thromboplastin times remained unchanged. The values observed on completion of an apheresis procedure were typical for

Table 7. Serum lipid and lipoprotein concentrations after 0, 6 and 12 months regular HELP-LDL-apheresis

	Months		
	0	6	12
Total chol. ($mgdl^{-1}$)		***	
S mean (SD)	358 (89)	279 (41)	277 (41)
median	337	278	270
		***	
E mean (SD)	180 (57)	137 (23)	137 (26)
median	179·5	139	129
Triglyc. ($mgdl^{-1}$)		*	
S mean (SD)	184 (130)	149 (72)	148 (102)
median	150·5	128	122·5
		*	
E mean (SD)	113 (104)	94 (62)	93 (70)
median	78·5	76	69
LDL-chol. ($mgdl^{-1}$)		***	
S mean (SD)	283 (86)	207 (40)	203 (41)
median	255·5	204	197
		***	
E mean (SD)	120 (53)	78 (21)	76 (24)
E median	115·5	76	72
HDL-chol. ($mgdl^{-1}$)		***	
S mean (SD)	40·5 (11)	44·8 (9)	48·2 (11)
S median	41·0	43·8	48·5
		** *	
E mean (SD)	36·6 (14)	39·7 (11)	41·3 12·0
median	31·0	37·2	40·0

S = pre-apheresis concentrations.
E = post-apheresis concentrations.
* $p < 0{\cdot}05$; ** $p < 0{\cdot}01$; *** $p < 0{\cdot}001$; significances were only tested for the pre-apheresis concentrations.

anticoagulation therapy during extracorporeal treatment.

Clinical chemical findings

Electrolyte concentrations remained stable during the course of HELP therapy (Table 9). A small reduction in pre-apheresis total protein concentrations presumably reflects a drop in specific heparin-binding proteins such as fibrinogen and apo B. Two further heparin-binding proteins the complement factors C3 and C4 were also reduced by regular HELP treatment but concentrations remained within the normal limits. Pre-apheresis levels of plasma albumin were unaffected by regular treatment. Haematological parameters were also largely unaffected. The small drop in haemoglobin and erythrocyte concentrations may be due to small blood losses during the extracorporeal procedure and due to the regular blood sampling for control of laboratory parameters.

Discussion

Since the first report of De Gennes *et al.* [13] there have been numerous accounts on the use of plasmapheresis and plasma exchange for the treatment of hypercholesterolaemia. Thompson *et al.* in particular [14,15] have pioneered the long-term use of this therapy for the treatment of individuals with homozygous familial hypercholesterolaemia. Regular exchange on a weekly or twice monthly basis increased the life expectancy of treated homozygous siblings, improved their well-being, induced regression of xanthoma and arrested or slowed the progression of atherosclerosis [22]. Although plasma exchange in general is apparently well tolerated and free of serious side effects, fatalities have been described which were primarily due to the use of fresh frozen plasma [34]. Fewer allergic side effects were experienced when a plasma protein fraction was employed but regular use may still induce deficiency of essential plasma proteins not present in this fraction. The incidence of medically relevant undesirable side effects associated with plasma exchange has been set as 12% [32]. To obviate the need of an exogenous protein source several selective procedures have been developed for on-line removal of LDL from plasma. The most specific procedure, immunadsorption, utilizes immobilized antibodies against apo B to selectively remove all apo B containing lipoproteins while allowing the remaining plasma proteins to be returned to the patients [17]. This was one of the first LDL-apheresis systems to be described and has now been in clinical application for several years [35]. Using this procedure Hombach *et al.* [35] reported regression of stenosis demonstrated by standardized coronary angiography in a small number of patients treated for periods ranging from 5 to 54 months.

Cascade or double filtration plasmapheresis allows a separation of large molecular weight plasma components such as LDL from the majority of the other plasma proteins [18,21] through use of membrane filters with appropriate sieving coefficients. This procedure, however, results in the loss of other large molecular weight species such as HDL and IgM. Furthermore, the selectivity of the membrane decreases with time due to formation of a secondary membrane through deposition of proteins on the inner membrane surface. This can then lead to a substantial loss of albumin unless membrane fouling is restricted by dilution of the plasma and repeated back-flushing of the membrane.

The affinity of LDL for polysulphated polysaccharides provides a basis for two further techniques to remove LDL from plasma. These procedures appear to be intermediate beween immunoadsorption and

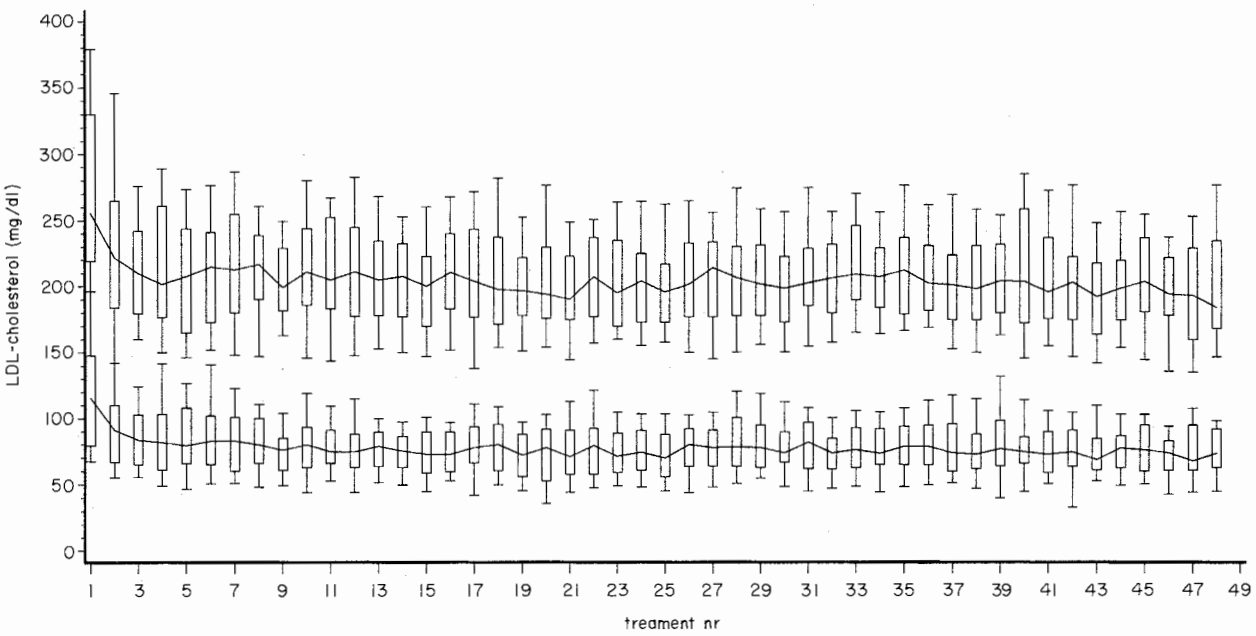

Figure 1. Pre- and post-apheresis concentrations during the course of 12 months regular HELP-LDL-apheresis in 46 patients. Data are presented as box plots for consecutive treatments; the limits defined by the box plots are the 10, 25, 75 and 90 percentiles. The median (50 percentile) values are linked by solid lines.

cascade filtration in their selectivity. Lupien *et al.* were the first to describe a selective LDL-apheresis procedure in which blood was mixed with heparin agarose beads to adsorb LDL [16]. After filtration the blood was then reinfused to the patient. More recently a disposable affinity column of dextran sulphate immobilised to cellulose has been introduced to allow on-line removal of LDL from plasma.

A novel approach has exploited the fact that LDL can be precipitated with heparin in the absence of cations at low pH. This procedure has been termed the Heparin Extracorporeal LDL-Precipitation (HELP) System. After precipitation of LDL the precipitate is removed by filtration and the pH of the plasma is restored by bicarbonate dialysis. In addition to LDL a limited number of other heparin-binding plasma proteins and lipoproteins are eliminated by this procedure. The lipoprotein Lp(a) is precipitated almost as efficiently as LDL itself [26]. This is, therefore, at present one of the most potent systems for reducing levels of this lipoprotein. Fibrinogen is also coprecipitated along with LDL and Lp(a) [20]. The reduction in the levels of this protein and also in LDL induces acute and chronic improvements in the haemorheological parameters of patients being treated by the HELP procedure [27]. Plasma exchange in which plasma protein fraction lacking LDL and fibrinogen was substituted has also been shown to provide beneficial rheological effects [36,37].

The present multicentre study was initiated to test the safety and efficacy of long-term regular HELP-LDL-apheresis in the treatment of refractory hypercholesterolaemia of patients with clinically documented coronary heart disease. A major goal is assessment of the coronary status of our patients by means of standardized coronary angiography after two years of regular intensive treatment. This report describes the results after one year of treatment. During the first 12 months 5 of 51 (9·8%) patients were lost to follow-up. Two patients suffered fatal cardiac deaths. These deaths did not occur during or on the day of treatment. In one case, a female patient who was on anticoagulant therapy required dental treatment. She suffered a fatal MI due to stenosis of the venous bypass after having been taken off this therapy prior to the dental appointment. The other patient died suddenly from heart failure after strenuous exercise. Since an autopsy was refused by the relatives, the exact cause of death could not be ascertained. Although these deaths cannot necessarily be linked to apheresis treatment, nor can we also exclude an association. However, it should be noted that the study collective represents a group of patients at extremely high risk on account of their existing coronary heart disease.

HELP therapy was well-tolerated by the patients and adverse side effects were registered in only a small number of cases. The most frequent side effect reported during a HELP procedure, angina pectoris, was obviously related to the underlying heart disease. The second most frequent side effect, vagal reaction, is also associated with other extracorporeal procedures. The total incidence of side effects in all treatments (2·9%) compares favourably with the figure of 12% reported for plasma exchange in general [32]. Of the 38 patients who suffered from angina at the start of the HELP program, 12 reported no symptoms after six months of treatment. This improvement may well be linked with the acute and long-term rheological

Table 8. Haemostesiological parameters after 0, 6 and 12 months regular HELP-LDL-apheresis

	Months		
	0	6	2
Fibrinogen ($mgdl^{-1}$)		***	
S mean (SD)	308 (98)	250 (70)	235 (78)
median	280	249	235
		***	
E mean (SD)	147 (62)	137 (39)	96 (42)
median	130	98	79
Plasminogen ($mgdl^{-1}$)			
S mean (SD)	10·0 (1·9)	10·6 (1·5)	10·7 (1·7)
median	10·1	10·9	10·4
E mean (SD)	5·3 (1·6)	5·8 (3·4)	5·0 (1·1)
median	5·0	4·9	4·9
Prothrombin time (%)			
S mean (SD)	86·0 (22·3)	86·8 (24·0)	86·4 (22·2)
median	93·5	97·5	95·4
E mean (SD)	46·1 (15·8)	44·4 (14·7)	41·6 (12·6)
median	47·0	46·3	43·7
Thrombin time (sec)			
S mean (SD)	15·8 (2·6)	14·7 (3·7)	14·9 (3·6)
median	16·2	15·5	15·8
E mean (SD)	20·4 (9·4)	20·1 (7·4)	21·7 (7·9)
median	18·0	19·0	21·0
PTT (sec)			
S mean (SD)	31·9 (6·3)	29·4 (5·0)	29·9 (4·7)
median	29·0	28·5	30·3
E mean (SD)	65·0 (33·6)	64·5 (26·8)	55·5 (15·5)
median	54·0	53·0	54·7

S = pre-apheresis concentrations.
E = post-apheresis concentrations.
* $p < 0{\cdot}05$; ** $p < 0{\cdot}01$; *** $p < 0{\cdot}001$; significances were only tested for the pre-apheresis concentrations.

changes that accompany the reduction in both LDL and fibrinogen levels [27].

Although several coagulation factors were also precipitated, repeated treatment led to no derangement of pre-apheresis haemostasiological parameters. Post-apheresis values were typical for the heparin-anticoagulation necessary for such extracorporeal procedures. The absorption of the excess heparin from the precipitation reaction by the heparin adsorber has proven to be completely adequate. Although fibrinogen is effectively eliminated post-apheresis values remained above the value of 60 mg dl^{-1} that guarantees effective coagulation. Other coagulation factors appear to rapidly recover their pre-apheresis values within one to three days of apheresis [33]. Analysis of the lipid data revealed that HELP-LDL-apheresis induced improvements in the lipids of our patients. Mean total cholesterol and LDL-cholesterol levels were effectively maintained at reduced levels compared to the pre-therapeutic values. Due to the nature of apheresis these levels fluctuate between post-apheresis 'lows' and pre-apheresis 'highs'. For this particular patient collective the regular treatment of 2·79 l of plasma every 7·7 days led to a mean 28% reduction in pre-apheresis LDL-cholesterol levels as compared to the basal values at the outset of HELP therapy. There was a strong negative correlation between the extent of this reduction and the initial pre-apheresis concentration. At each apheresis there was an average 60% reduction in LDL levels. The pre- and post-apheresis levels remained relatively constant after an initial reduction during the first three to four procedures. This is in agreement with the hypothesis that an acute reduction in LDL levels does not alter the synthetic or catabolic rates of this lipoprotein [38]. HDL-cholesterol levels on the other hand showed a definite increase during the course of HELP therapy. Such changes have been observed after regular immunosorbant LDL-apheresis [39] and therefore do not appear to be specific for any particular apheresis procedure.

Table 9. Clinical chemical and haematological parameters after 0, 6 and 12 months regular HELP-LDL-apheresis

	Months		
	0	6	12
Sodium (mmol/l)	140·5 (2·6)	139·9 (2·7)	141·6 (2·6)
Potassium (mmol/l)	4·18 (0·40)	4·19 (0·59)	4·11 (0·38)
Calcium (mmol/l)	2·37 (0·13)	2·31 (0·13)	2·33 (0·15)
Total protein (g/l)	73·1 (7·0)	68·2 (5·5)	67·8 (4·3)
Albumin (g/l)	47·7 (8·1)	46·0 (9·3)	46·6 (9·3)
C3-compl. (%)	95 (25)	83 (26)	83 (26)
C4-compl. (%)	33 (14)	21 (7)	21 (8)
Hemoglobin (g/dl)	14·1 (1·8)	31·2 (1·9)	13·0 (1·9)
Hematocrit (%)	42·1 (5·5)	39·7 (5·7)	39·2 (5·7)
Erythrocytes (T/l)	4·63 (0·61)	4·46 (0·60)	4·47 (0·56)
Leucocytes (G/l)	6·5 (1·71)	6·61 (1·75)	6·54 (2·00)
Thrombocyte (G/l)	241 (80)	264 (69)	261 (74)

Although our patient had normal triglyceride levels, triglyceride concentrations also fell on regular treatment and could be maintained at values 19% lower than those at the outset of the therapy.

The initial results from this multicentre trial attest that HELP-LDL-apheresis is a safe and efficient procedure for effectively lowering cholesterol and in particular LDL-cholesterol levels when applied on a regular basis. Compliance has been good in this group of high risk patients and complications have in general been minor and uncommon. Our data support the feasibility of such apheresis procedures as an adjunctive therapy for obtaining an aggressive lowering of cholesterol levels in high risk patients while substantially improving the HDL/LDL ratio. It remains to be established to what extent such aggressive therapy can improve the coronary status of our patients. The results of the angiographic evaluation will be reported in due course after all patients have completed 24 months of regular HELP therapy.

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References

1 Lipid Research Clinics Program. The lipid research coronary primary prevention trial results II. The relationship of reduction in incidence of coronary heart disease to cholesterol lowering. J Amer med Ass 1984;251:365–74.
2 Frick MH, Elo O, Haapa K, Heinonen OP, Heinsalmi P, Helo P, Huttunen JK, Kaitaniemi P, Koskinen P, Mannienen V, Maenpaa H, Malkonen M, Mantaari M, Narola S, Pasternack A, Pikkarainen J, Romo M, Sjoblom T, Nikkila EA. Helsinki Heart Study: Primary prevention trial with gemfibrozil in middle-aged men with dyslipidemia. N Engl J Med 1987;317:1237–45.
3 Blankenhorn DH, Nessim SA, Johnson RL, Sanmarco ME, Azen SP, Cashin-Hemphill L. Beneficial effects of combined Colestipol-Niacin therapy on coronary atherosclerosis and coronary venous bypass grafts. J Amer med Ass 1987;257:3233–40.
4 Study Group, European Atherosclerosis Society. Strategies for the prevention of coronary heart disease: a policy statement of the European Atherosclerosis Society. Eur Heart J 1987;8:77–88.
5 The Expert Panel. Report of the National Cholesterol Education Program Expert Panel on detection, evaluation and treatment of high blood cholesterol in adults. Arch Intern Med 1988;148:36–39.
6 Endo A, Kuroda M, Tanzawa K. Competitive inhibition of 3-hydroxy-methyl-glutaryl coenzyme A reductase by ML-236A and ML-236B fungal metabolites, having hypercholesterolemic activity. FEBS Lett, 1976;72:323–6.
7 Alberts AW. HMG-CoA-reductase inhibitors – the development. Atherosclerosis Rev 1988;18:123–31.
8 Havel RJ, Hunninghake DB, Illingworth DR, *et al.* Lovastatin (Mevinolin) in the treatment of heterozygous familial hypercholesterolemia: a multicentre study. Ann Intern Med 1987;107:609–15.
9 Buchwald H. Lowering of cholesterol adsorption and blood levels by ileal exclusion. Circulation 1964;29:713–20.
10 Koivisto P, Miettinen TA. Long-term effects of ileal bypass on lipoproteins in patients with familial hypercholesterolaemia. Circulation 1984;70:290–6.
11 Starzl TE, Chase HP, Ahrens EH, McNamara DJ, Bilheimer DW, Schaefer EF, Rey J, Porter KA, Stein E, Francavilia A, Benson LN. Portocaval shunt in patients with familial hypercholesterolemia. Ann Surg 1983;198:273–83.
12 Starzl LE, Bilheimer DW, Bahnson HT, Shaw BW, Hardesty RL, Griffith BP, Iwatsuki S, Zitelli BJ, Gartner JC, Malatack JJ, Urbach AH. Heart-liver transplantation in a patient with familial hypercholesterolemia. Lancet 1984;1:1382–3.
13 De Gennes J, Touraine R, Maunard B *et al.* Formes homozygotes cutaneotendineuses de xanthomatose hypercholesterolemique dans une observation familiale exemplaire. Essai de plasmapherese à titre de traitement heroique. Bull Mem Soc Hop Paris 1976;118:1377–1402.
14 Thompson GR, Lowenthal R, Myant NB. Plasma exchange in the management of homozygous familial hypercholesterolemia. Lancet 1975;1:1208–11.
15 Thompson GR, Barbir M, Okabayashi K, Trayner I, Larkin S. Plasmapheresis in familial hypercholesterolemia. Arteriosclerosis 1989; (suppl. 1):1152–7.
16 Lupien PJ, Moojani S, Award J. A new approach to the management of familial hypercholesterolemia: removal of plasma cholesterol based on the principle of affinity chromatography. Lancet 1976;1:1261–5.
17 Stoffel W, Borberg H, Greve V. Application of specific extracorporeal removal of low density lipoprotein in familial hypercholesterolemia. Lancet 1981;II:1005–7.
18 von Bayer H, Schwerdtfeger R, Schwartzkopf W, Schrig R, Kochinke F, Marx M, Schutten D. Selective removal of low density lipoproteins (LDL) by plasmapheresis combined with selective plasma-protein ultracentrifugation (SPU). Plasma Ther Transfus Technol 1983;4:447–58.
19 Yokoyama S, Hayashi R, Satatni M, Yamamoto A. Selective removal of low density lipoprotein by plasmapheresis in familial hypercholesterolemia. Arteriosclerosis 1985;5:613–22.

20 Eisenhauer T, Armstrong VW, Wieland H, Fuchs C, Scheler F, Seidel D. Selective removal of low density lipoproteins (LDL) by precipitation at low pH: first clinical application of the HELP system. Klin Wochenschr 1987;65:161–8.
21 Homma Y, Mikami Y, Tamachi H, Nakaya N, Nakamura H, Goto Y: Comparison of selectivity of LDL removal by double filtration and dexran-sulfate cellulose column plasmapheresis, and changes of subfractionated plasma lipoproteins after plasmapheresis in heterozygous familial hypercholesterolemia. Metabolism 1987;36:419–23.
22 Thompson GR, Miller JP, Breslow JL. Improved survival of patients with homozygous familial hypercholesterolemia treated with plasma exchange. Br Med J 1985;291:1671–3.
23 Thompson GR, Ford J, Jenkinson M, Trayner I. Efficacy of mevinolin as adjuvant therapy for refractory familial hypercholesterolemia. Q J Med 1986;60:803–11.
24 Thiery J, Armstrong VW, Eisenhauer T, Adam R, Janning G, Creutzfeldt W, Kreuzer H, Seidel D. Combination of Simvastatin and heparin-induced extracorporeal LDL/fibrinogen precipitation (HELP) in the treatment of hypercholesterolemia in CAD patients. In: Atherosclerosis VIII, Crepaldi G, Gotto AM, Manzato E, Baggio G eds. Amsterdam: Excerpta Medica, 1989: 831–35.
25 Schenck I, Keller Ch, Hailer S, Wolfram G, Zöllner N. Reduction of Lp(a) by different methods of plasma exchange. Klin Wochenschr 1988;66:1197–1201.
26 Armstrong VW, Schleef J, Thiery J, Muche R, Schuff-Werner P, Eisenhauer T, Seidel D. Effect of HELP-LDL-apheresis on serum concentrations of human lipoprotein(a): kinetic analysis of the post-treatment return to baseline levels. Eur J Clin Invest 1989;19:235–40.
27 Schuff-Werner P, Schütz E, Seyde WC, Eisenhauer T, Janning G, Armstrong VW, Seidel D. Improved haemorheology associated with a reduction in plasma fibrinogen and LDL in patients being treated by heparin-induced extracorporeal LDL precipitation (HELP). Eur J Clin Invest 1989;19:30–7.
28 Kleophas W, Leschke H, Tschöpe D, Martin J, Schauseil J, Schottenfeld J, Strauer BE, Gries FA. Akute Wirkungen der extrakorporalen LDL-Cholesterin und Fibrinogen-Elimination auf Blutrheologie und Mikrozirkulation. Dtsch Med Wschr 1990;115:3–7.
29 Holm S. A simple sequentially rejective multiple test procedure. Scand J Statist 1979;6:65–70.
30 Blagg CR. Acute complications associated with hemodialysis. In: Drukker W, Parsons FM, Mahler JF eds. Replacement of renal function by dialysis, 2nd ed., Martinus Nijhoff Medical Division, Boston, The Hague, Dordrecht, Lancaster, 1983; 611–29.
31 Council on Scientific Affairs. Current status of therapeutic plasmapheresis and related techniques. Report of the AMA Panel on therapeutic plasmapheresis. J Amer med Ass 1985;253:819–25.
32 Sutton DM, Nair RC, Roch G, and the Canadian Apheresis Study Group. Complications of plasma exchange. Transfusion 1989;29:124–7.
33 Schuff-Werner P, Schütz E, Reitemeyer F, Oppermann M, Eisenhauer T, Armstrong VW, Köstering H, Götze O, Seidel D. Heparin extracorporeal LDL precipitation (HELP): Rheological, hemostasiological and immunological effects. In: Gotto AM, Mancini M, Richter WO, Schwant P eds. Treatment of severe hypercholesterolemia in the prevention of coronary heart disease. Basel: Karger Verlag, 1990; 196–204.
34 Huestis DW. Mortality in therapeutic haemapheresis. Lancet 1983;1:1043.
35 Hombach V, Borberg H, Gadzkowski A, Oette K, Stoffe W. Regression der Koronarsklerose bei familiärer Hypercholesterinämie IIa durch spezifische LDL-Apherese. Dtsch med Wschr 1986;111:1709–15.
36 Kilpatrick D, Fleming J, Clyne C, Thompson GR. Reduction of blood viscosity following plasma exchange. Atherosclerosis 1979;32:301–6.
37 Rubba P, Postiglione A, Scarpato N, Iannuzzi A, Mancini M. Improved reactive hyperemia test after plasma exchange in familial hypercholesterolemia. Atherosclerosis 1985;56:237–42.
38 Soutar AK, Myant NB, Thompson GR. Metabolism of apolipoprotein B containing lipoproteins in familial hypercholesterolemia. Effects of plasma exchange. Atherosclerosis 1979;32:315–27.
39 Parker TS, Gordon BR, Saal SD, Rubin AL, Ahrens EH. Plasma high density lipoprotein is increased in man when low density lipoprotein (LDL) is lowered by LDL-apheresis. PNAS 1986;83:777–81.