Acute and chronic effects on cholesterol biosynthesis of LDL-apheresis with or without concomitant HMG-CoA reductase inhibitor therapy

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Abstract To determine the acute and long-term effects of low density lipoprotein (LDL) reduction on cholesterol biosynthesis, we studied changes in the cholesterol precursors mevalonic acid (MVA) and lathosterol in patients with heterozygous familial hypercholesterolemia undergoing LDL-apheresis. Long-term LDL-apheresis in eight patients resulted in slight but insignificant increases in plasma MVA levels and lathosterol/cholesterol (L/C) ratios over 18 months. In short-term studies, six patients not on drugs and six patients treated with lovastatin or pravastatin had blood taken immediately before and after LDL-apheresis, and afterwards on days 1, 2, 3, 5, and 7. Plasma L/C ratios and MVA concentration did not change significantly on the first day after LDL-apheresis in those not on statin therapy (1.11 ± 0.08 vs. 1.40 ± 0.18, and 9.2 ± 1.3 vs. 9.1 ± 0.6 ng/ml, respectively) but increased in the statin-treated group (from 0.78 ± 0.09 to 1.55 ± 0.21, $P = 0.003$ and from 5.0 ± 0.7 to 11.0 ± 1.6 ng/ml, $P = 0.008$, respectively). There was no clear correlation between the changes in either of these precursors and the extent of reduction of total cholesterol by LDL-apheresis, but there was a strong inverse correlation with the post-apheresis LDL-cholesterol level ($r = -0.77$, $P = 0.002$ for L/C ratio; $r = -0.75$, $P = 0.003$ for MVA). Post-apheresis changes in L/C ratio and MVA were mutually correlated ($r = 0.68$. $P = 0.01$). We conclude that LDL-apheresis stimulates cholesterol biosynthesis transiently despite concomitant therapy with an HMG-CoA reductase inhibitor, the degree of stimulation being inversely related to the level to which the LDL-cholesterol was reduced.—**Pfohl, M., R. P. Naoumova, C. Klass, W. Knisel, B. Jakober, T. Risler, and G. R. Thompson.** Acute and chronic effects on cholesterol biosynthesis of LDL-apheresis with or without concomitant HMG-CoA reductase inhibitor therapy. *J. Lipid Res.* 1994. **35:** 1946–1955.

Supplementary key words familial hypercholesterolemia • lathosterol • mevalonic acid

Extracorporeal elimination of LDL-cholesterol by LDL-apheresis is a widely accepted therapeutic measure for patients with severe familial hypercholesterolemia (FH) (1). There is general but not universal agreement that cholesterol biosynthesis is increased after this procedure. Early data suggested an increased rate of cholesterol biosynthesis (2), without any change in apolipoprotein B synthesis (3) in FH patients undergoing plasmapheresis. Cholesterol balance studies in a homozygous FH child showed increased cholesterol synthesis during a period of biweekly plasmapheresis (4), and more recent observations showed a similar increase after LDL-apheresis (5, 6). In contrast, Gylling et al. (7) have reported recently that there were no significant changes in cholesterol synthesis during treatment with LDL-apheresis.

The quantification of cholesterol precursors in plasma or serum is an accepted method for assessing cholesterol biosynthesis in vivo. One of these precursors, 7-lathosterol, is well established as an index of whole-body cholesterol synthesis in humans (8–11). Its serum concentration is directly associated with hepatic HMG-CoA reductase activity (12). Serum lathosterol concentration appears to be elevated in FH patients, but ratios relative to serum cholesterol are similar (8, 13) or lower than normolipidemic subjects (14). The serum lathosterol concentration and lathosterol/cholesterol (L/C) ratio are independent of the fatty acid composition of the diet (9), but both are increased during treatment with drugs such as cholestyramine or colestipol (13, 15, 16), which stimulate cholesterol biosynthesis in the liver. In contrast, decreased serum lathosterol levels and L/C ratios have been observed during therapy with HMG-CoA reductase inhibitors in normolipidemic (17) and hyperlipidemic subjects (9, 13).

Abbreviations: VLDL, very low density lipoprotein; HDL, high density lipoprotein; LDL, low density lipoprotein; FH, familial hypercholesterolemia; L/C, lathosterol/cholesterol ratio; HMG, 3-hydroxy-3-methylglutaryl; MVA, mevalonic acid; MVL, mevalonolactone; GC-MS, gas chromatography-mass spectrometry; AUC, area under the curve.

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Measurement of plasma mevalonic acid (MVA) provides another useful index of cholesterol biosynthesis. The conversion of hydroxy-methyl-glutaryl-CoA (HMG-CoA) to MVA determines, under most circumstances, the rate of cholesterol biosynthesis (18). A diurnal rhythm of plasma MVA has been described in human subjects and a close correlation has been demonstrated between fasting MVA levels and whole body cholesterol synthesis, as measured by the sterol balance method (19, 20). Plasma MVA concentration, like lathosterol, is correlated with hepatic HMG-CoA reductase activity (21), and is decreased by HMG-CoA reductase inhibitors (18, 20). There is also a good correlation between plasma MVA and the rate of incorporation of deuterium into plasma free cholesterol (22).

To determine the effects of extracorporeal elimination of LDL-cholesterol on cholesterol biosynthesis, we have measured serum lathosterol and plasma MVA in patients with heterozygous FH undergoing LDL apheresis.

SUBJECTS AND METHODS

Subjects

We studied 12 patients in Germany with heterozygous FH and coronary heart disease who had failed to respond sufficiently well to dietary treatment and combined drug therapy (total cholesterol > 7.5 mmol/l). Basal data of the patients are presented in **Table 1.** All had one or more clinical manifestations and/or complications of FH, i.e., tendon xanthoma, angina pectoris, and previous myocardial infarction. Seven patients had undergone coronary by-pass surgery, one of them (patient no. 2) twice. Patients 1, 2, and 5–12 were treated with cardioselective beta-blocking agents; all but one (no. 4) also received acetylsalicylic acid, 100–250 mg per day. The study was approved by the Ethical Committee of the University of Tübingen according to the declaration of Helsinki. Informed consent was obtained from each patient.

Study design

The study was divided into three main parts. In the first part we studied long-term changes in serum lipids, lipoproteins, lathosterol, and plasma MVA in eight patients (nos. 1–8) treated with LDL-apheresis at weekly intervals over a period of 18 months without concomitant lipid-lowering medication; this was designated the "long-term study." In the second part of the study we investigated the acute effects of LDL-apheresis on the above mentioned parameters in six patients without HMG-CoA reductase inhibitor (statin) therapy ("short-term off statin"), and in the third part the acute effects of LDL-apheresis in another six patients who received concomitant statin therapy ("short-term on statin").

Long-term study. All eight patients were treated with LDL-apheresis once a week over a period of at least 18 months. Five were treated by immunoadsorptive apheresis, and three by dextran sulfate apheresis. All lipid-lowering drugs were discontinued at least 4 weeks before starting LDL-apheresis. Patients were advised to maintain a lipid-lowering diet (< 200 mg cholesterol/d, 30% of daily energy intake as fat, with equal contributions from saturated, monounsaturated, and polyunsaturated fatty acids). During the study period patients did not take any drugs influencing lipid metabolism other than statins, as indicated. The body weight of the patients remained stable throughout the study period.

Blood samples for the determination of serum total cholesterol, VLDL-, LDL-, and HDL-cholesterol, triglyceride, and lathosterol, and for plasma MVA were

TABLE 1. Patients' characteristics

Patient no.	Age	BMI	Sex	Serum Cholesterol		Clinical Characteristics[a]
				Untreated	On Diet	
	yr	*kg/m²*		*mmol/l*	*mmol/l*	
1	45	30.8	f	16.6	12.0	TX, MI, CABG
2	54	25.6	m	13.8	9.0	TX, MI, CABG
3	61	20.7	f	10.9	9.3	MI, CABG
4	31	28.9	m	20.6	9.3	TX, AP, MI
5[b]	27	24.3	m	14.1	12.6	AP
6	36	23.6	m	12.4	10.0	TX, MI, CABG
7	58	25.9	m	12.7	9.0	MI, CABG
8[b]	58	24.9	m	13.5	8.6	AP
9[b]	43	25.8	m	15.2	10.7	TX, MI, CABG
10[b]	39	27.3	m	15.9	11.3	TX, AP
11[b]	38	24.6	m	13.9	10.0	TX
12[b]	44	25.7	m	13.3	10.3	TX, MI, CABG

[a] TX, tendon xanthoma; AP, angina pectoris; MI, myocardial infarction; CABG, coronary artery bypass grafting.
[b] On lovastatin or pravastatin during short-term study.

taken before and at 6, 12, and 18 months after starting LDL-apheresis.

Short-term studies. After at least 12 months of LDL-apheresis, blood samples were taken from twelve patients during a random week immediately before and after LDL-apheresis, and additionally on 1, 2, 3, 5, and 7 days after LDL-apheresis. Six patients were not on any lipid-lowering drug therapy, the other six were treated with either lovastatin or pravastatin in a dose of 20 mg twice daily. The morning medication was given after the blood samples had been taken. Statin therapy was initiated at least 6 months before the short-term study was performed.

LDL-apheresis

Immunoadsorptive apheresis was carried out according to Stoffel, Borberg, and Greve (23), using a continuous flow blood cell separator IBM Cobe 2997 linked with an automatic adsorption-desorption device ADA TM Medicap (Ulrichstein, Germany) equipped with adsorption columns containing polyclonal anti-apoB-antibodies from sheep coupled to Sepharose C1-4B gel (LDL-Therasorb TM, Baxter, Munich, Germany). Dextran sulfate apheresis was performed as described by Yokoyama et al. (24), using an MA-01 module (Kanegafuchi, Osaka, Japan) with a membrane filter plasma separator (Sulflux TM) and two adsorption columns (Liposorber LA 15 TM) containing cellulose beads covalently linked with dextran sulfate. Anticoagulation was achieved with heparin and citrate during immunoadsorption and by heparin alone during dextran sulfate apheresis.

Biochemical analysis

All samples, except those taken immediately after LDL-apheresis, were obtained between 8:00 and 9:00 AM after an overnight fast. The samples immediately after LDL-apheresis ("post-apheresis") were taken between 1:00 and 2:00 PM. After clotting, the tubes were centrifuged at 3000 rpm for 10 min and the serum was aspirated and stored at 4°C for lipid determination within the next 3 days. Serum for the determination of 7-lathosterol was immediately stored at −70°C. Plasma for the determination of MVA was taken from EDTA-blood and immediately frozen at −70°C.

Cholesterol and triglyceride levels were determined with standard enzymatic techniques (Boehringer Mannheim) using autoanalyzers. HDL-cholesterol was measured after precipitation of VLDL and LDL using manganous chloride and phosphotungstate (25). LDL-cholesterol was calculated by the formula of Friedewald, Levy, and Fredrickson (26). Apolipoprotein B was determined nephelometrically (27).

Serum concentrations of 7-lathosterol were determined essentially by the method described by Wolthers et al. (28), using a 3700 Varian gas chromatograph (Varian Instruments Group, Palo Alto, CA) equipped with an OV 1 fused silica capillary column (Macherey-Nagel, Düren, Germany). N_2 was used as carrier gas. Lathosterol is reported in μmol/l and also as μmol/mmol cholesterol to correct for the effects of apheresis in removing the lipoprotein particles that transport both cholesterol and lathosterol in plasma (9).

Plasma MVA was analyzed using a modified version of the method described by Scoppola et al. (20). MVA was extracted from 1 ml of plasma after addition of 5 ng of [$^2H_3$]mevalonolactone (MVL) to each sample as internal standard. The sample was diluted 1:1.5 with water and incubated with Dowex 50 (H^+) for 1 h after which it was rinsed with water to increase recovery. Nonpolar lipids were removed by washing in 1 ml of hexane. MVL was extracted over ice from the aqueous solution into 20 ml of dichloromethane–propan-2-ol 9:1 (v/v). After purification on an Lc-Si silica cartridge, MVL was reconverted to free MVA by incubation with 100 μl of di-isopropylethylamine–water 1:2 (v/v) for 1 h at room temperature. MVA was converted to its 3,5-bis(trifluoromethyl)benzyl ester by addition of 30 μl of acetonitrile, 10 μl of di-isopropylethylamine, and 20 μl of 3,5-bis(trifluoromethyl)benzylbromide 10%. After 30 min at room temperature the reagents were removed under a stream of nitrogen. The trimethylsilyl ester derivative was then prepared by incubation with 30 μl of 50% bis(trimethylsilyl)-trifluoroacetamide overnight at room temperature. Excess reagent was removed under nitrogen, and the samples were reconstituted in 20 μl of decane for GC–MS. Derivatives were chromatographed on a DB 5 capillary GC column (15 m, Jones Chromatography) using helium as a carrier gas. The column was held at 150°C for 1 min, followed by a linear temperature gradient of 20°C per min to 300°C. A Grob injector was used in the splitless mode at 250°C; the mass spectrometry was performed as described by Scoppola et al. (20). The normal range, obtained from 21 healthy persons between 8 and 9 AM, was 6.1 ± 2.6 ng/ml (mean ± SD), with a between assay C of V of 6.7%.

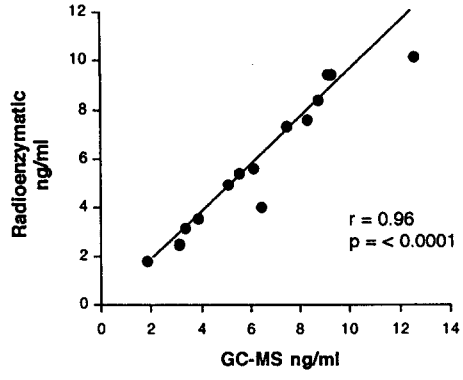

Fig. 1. Split sample comparison of 15 samples of plasma assayed by radioenzymatic assay (15) and by gas chromatography-electron capture mass spectrometry. The radioenzymatic assays were kindly performed by Drs. A. Pappu and R. Illingworth, Oregon Health Sciences University, to whom samples were shipped frozen on dry ice.

TABLE 2. Changes in serum lipids and lipoproteins during long-term LDL-apheresis

	Baseline	On LDL Apheresis For 6 Months	On LDL Apheresis For 12 Months	On LDL Apheresis For 18 Months	P^a
	mmol/l		*mmol/l*		
Total cholesterol	10.5 ± 0.7	7.8 ± 0.3	7.4 ± 0.3	7.3 ± 0.1	<0.0001
LDL-cholesterol	8.8 ± 0.7	6.2 ± 0.2	5.5 ± 0.3	5.6 ± 0.2	<0.0001
HDL-cholesterol	1.1 ± 0.1	1.0 ± 0.1	1.2 ± 0.2	1.1 ± 0.1	n.s.
VLDL-cholesterol	0.7 ± 0.1	0.6 ± 0.2	0.8 ± 0.2	0.7 ± 0.2	n.s.
Triglycerides	2.7 ± 0.5	3.1 ± 0.6	2.4 ± 0.6	2.4 ± 0.4	n.s.

All values are pre-apheretic values and are given as means ± SEM; n = 8.
[a]ANOVA baseline vs. 18 months; n.s., not significant.

As shown in **Fig. 1** a split sample comparison of this method showed excellent agreement with the radioenzymatic assay used by Pappu and Illingworth (15).

Statistical methods

Data are presented as means ± standard error of the mean (SEM). Statistical significance of differences during treatment was evaluated by multivariate analysis of variance for repeated measures (ANOVA). The relationship between the changes in the L/C ratio and MVA and the reduction of total cholesterol, the post-apheresis cholesterol, and the post-apheresis LDL-cholesterol were explored by univariate regression analysis. The area under the curve (AUC) for L/C ratio and MVA was determined using the trapezoid rule, using the preapheretic value as an extrapolated baseline. The relationships between AUC and the reduction in total cholesterol, and the postapheretic LDL-cholesterol were evaluated by univariate regression analysis. $P \leq 0.05$ was considered to be statistically significant.

RESULTS

Long-term study

Mean pre-apheresis serum total and LDL-cholesterol levels were significantly reduced by 26% and 30% respectively after 6 months, by 30% and 38% after 12 months, and by 31% and 36% after 18 months of LDL apheresis (**Table 2**). There were no significant changes in HDL-cholesterol, VLDL-cholesterol, and triglycerides. After a slight increase at 6 months, serum lathosterol concentration was significantly decreased after 12 and 18 months (**Table 3**). L/C ratios and plasma MVA were within the normal range at baseline but showed a tendency to increase over time; however, this trend was not statistically significant.

Short-term studies

Acute treatment with LDL-apheresis resulted in a marked decrease of total and LDL-cholesterol, which was succeeded by a characteristic rebound during the following week (**Table 4, Fig. 2A**). Immediately after LDL-apheresis, levels of HDL-cholesterol, VLDL-cholesterol, and triglycerides were all slightly reduced, but had returned to baseline by the next day. During the remainder of the week the levels of all these variables were similar to pre-apheresis values (Table 4).

Concomitant statin treatment resulted in significantly lower levels of total cholesterol, LDL-cholesterol, and VLDL-cholesterol throughout the study period. The post-apheresis rebound of LDL-cholesterol was suppressed by statin treatment; during the first 2 days after the procedure, LDL-cholesterol rose by 1.6 ± 0.2 mmol/l in the statin-treated group compared with 2.4 ± 0.5

TABLE 3. Changes in serum lathosterol, lathosterol/cholesterol ratio, and plasma mevalonic acid during long-term LDL-apheresis

	Baseline	On LDL Apheresis For 6 Months	On LDL Apheresis For 12 Months	On LDL Apheresis For 18 Months	P^a
Lathosterol (μmol/l)	10.6 ± 1.3	11.8 ± 1.3	7.4 ± 0.8	8.3 ± 1.8	0.0115
L/C ratio[b]	1.01 ± 0.07	1.51 ± 0.17	1.04 ± 0.10	1.14 ± 0.24	n.s.
Mevalonic acid (ng/ml)	4.8 ± 0.7	6.0 ± 0.9	6.5 ± 1.2	7.1 ± 1.0	n.s.

All values are pre-apheretic values and are given as means ± SEM; n = 8.
[a]ANOVA baseline vs. 18 months; n.s., not significant.
[b]Ratio calculated as lathosterol (μmol/l)/cholesterol (mmol/l).

TABLE 4. Short-term changes in serum lipids and lipoproteins after LDL-apheresis without or with concomitant stain therapy

	LDL Apheresis							Effects of	
	Pre	Post	Day 1	Day 2	Day 3	Day 5	Day 7	Apheresis[a]	Statin[a]
				mmol/l				*P*	
Total cholesterol									
Off statin	9.0 ± 0.5	3.4 ± 0.1	5.4 ± 0.2	6.4 ± 0.5	6.9 ± 0.5	7.9 ± 0.5	8.5 ± 0.3		
On statin	8.0 ± 0.5	2.9 ± 0.2	3.8 ± 0.3	4.6 ± 0.3	5.5 ± 0.5	6.5 ± 0.6	7.3 ± 0.3	<0.0001	<0.0001
HDL-cholesterol									
Off statin	1.2 ± 0.1	1.0 ± 0.1	1.1 ± 0.2	1.0 ± 0.2	1.1 ± 0.2	1.0 ± 0.2	1.1 ± 0.2		
On statin	1.0 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.2 ± 0.2	n.s.	n.s.
VLDL-cholesterol									
Off statin	1.2 ± 0.3	0.8 ± 0.2	1.2 ± 0.2	1.3 ± 0.2	1.2 ± 0.2	1.5 ± 0.4	1.2 ± 0.2		
On statin	1.1 ± 0.3	0.8 ± 0.2	0.8 ± 0.2	0.9 ± 0.2	0.8 ± 0.2	0.9 ± 0.2	0.7 ± 0.1	n.s.	0.0035
Triglycerides									
Off statin	2.7 ± 0.5	2.0 ± 0.3	2.5 ± 0.5	2.5 ± 0.5	2.6 ± 0.5	3.2 ± 0.9	2.7 ± 0.6		
On statin	2.6 ± 0.5	2.0 ± 0.5	2.6 ± 0.4	2.5 ± 0.4	2.4 ± 0.4	2.8 ± 0.5	3.3 ± 0.6	n.s.	n.s.

All values are expressed as means ± SEM. There were six subjects off statin and another six on statin. Samples were taken immediately pre LDL-apheresis (pre), post apheresis (post), and on days 1, 2, 3, 5, and 7 after LDL-apheresis.
[a]ANOVA pre-apheresis vs. day 7; n.s., not significant.

mmol/l in the group without statin ($P = 0.031$). HDL-cholesterol and serum triglycerides were unaffected by statin treatment but VLDL-cholesterol was reduced significantly (Table 4).

Serum apolipoprotein B was reduced by LDL-apheresis from 202 ± 23 mg/dl to 62 ± 5 mg/dl without statin and from 192 ± 12 mg/dl to 54 ± 9 mg/dl with statin ($P < 0.0001$), followed by the typical rebound during the subsequent days. Again, the statin-treated group showed significantly lower levels throughout the observation period and the post-apheretic rebound was significantly suppressed ($P < 0.01$). The ratio of LDL-cholesterol to apoB (calculated as LDL-cholesterol [mg/dl]/apoB [mg/dl]) was acutely reduced by LDL apheresis (from 1.28 ± 0.07 to 1.02 ± 0.08 without statin and from 1.18 ± 0.08 to 0.76 ± 0.10 with statin), but returned to pre-apheretic values within 7 days. During that period, the ratio was significantly lower in the statin-treated group compared to the group without statin ($P < 0.0001$).

Serum lathosterol was reduced acutely by LDL-apheresis (Fig. 2B), but showed a quicker rebound than LDL-cholesterol. Based on the calculated plasma volumes, the mass of lathosterol removed by LDL-apheresis was 19.3 ± 1.9 μmol in the patients without additional statin treatment, and 10.2 ± 1.4 μmol in the patients with additional statin treatment ($P < 0.01$). The rate of reappearance of lathosterol during the rebound period was 2.5 ± 0.3 μmol per day without statin and 2.0 ± 0.4 μmol per day with statin therapy (not significant). The L/C ratio increased on the first day after LDL-apheresis; this increase was significantly more marked in the statin-treated group (Fig. 2C). The increase in the L/C ratio on day 1 reflected both an increase in lathosterol, as evidenced by a significant rise in the lathosterol:apoB ratio ($P < 0.0001$), as well as a decrease in cholesterol. However, LDL-cholesterol:apoB ratios on day 1 were only 10% (off statin) and 15% (on statin) lower than pre-apheresis values. Thus, assuming that most of the lathosterol in plasma is transported by LDL (16), changes in LDL composition may have exaggerated, but were not solely responsible for, the rise in the L/C ratio after LDL apheresis.

Mean plasma MVA levels were almost twice as high in the group off statin than in those on statin before LDL apheresis (9.2 ± 1.3 v 5.0 ± 0.7 ng/ml; $P < 0.001$). MVA remained stable in the non-statin-treated group, but rose significantly on the first day after LDL-apheresis in the statin-treated group (Fig. 2D). Changes in the L/C ratio and plasma MVA were not significantly correlated with the reduction in serum total cholesterol (**Fig. 3A/B**), which reflects the amount of cholesterol removed by LDL-apheresis, but correlated inversely with the post-apheresis level of LDL-cholesterol (Fig. 3C/D). As **Fig. 4** shows, the changes in L/C ratio and plasma MVA were also significantly correlated with each other.

There was no significant correlation between the AUC of the L/C ratio or plasma MVA between day 1 and day 7 and the reduction in total cholesterol ($r = -0.29$, $P = 0.17$ for L/C ratio, $r = -0.01$, $P = 0.50$ for plasma MVA), but there was a clear inverse correlation between the AUC of the L/C ratio and the postapheretic LDL-cholesterol ($r = -0.61$, $P = 0.01$). However, the AUC of plasma MVA and the postapheretic LDL-cholesterol were not significantly correlated ($r = -0.26$, $P = 0.20$). The reason for this discrepancy is unclear.

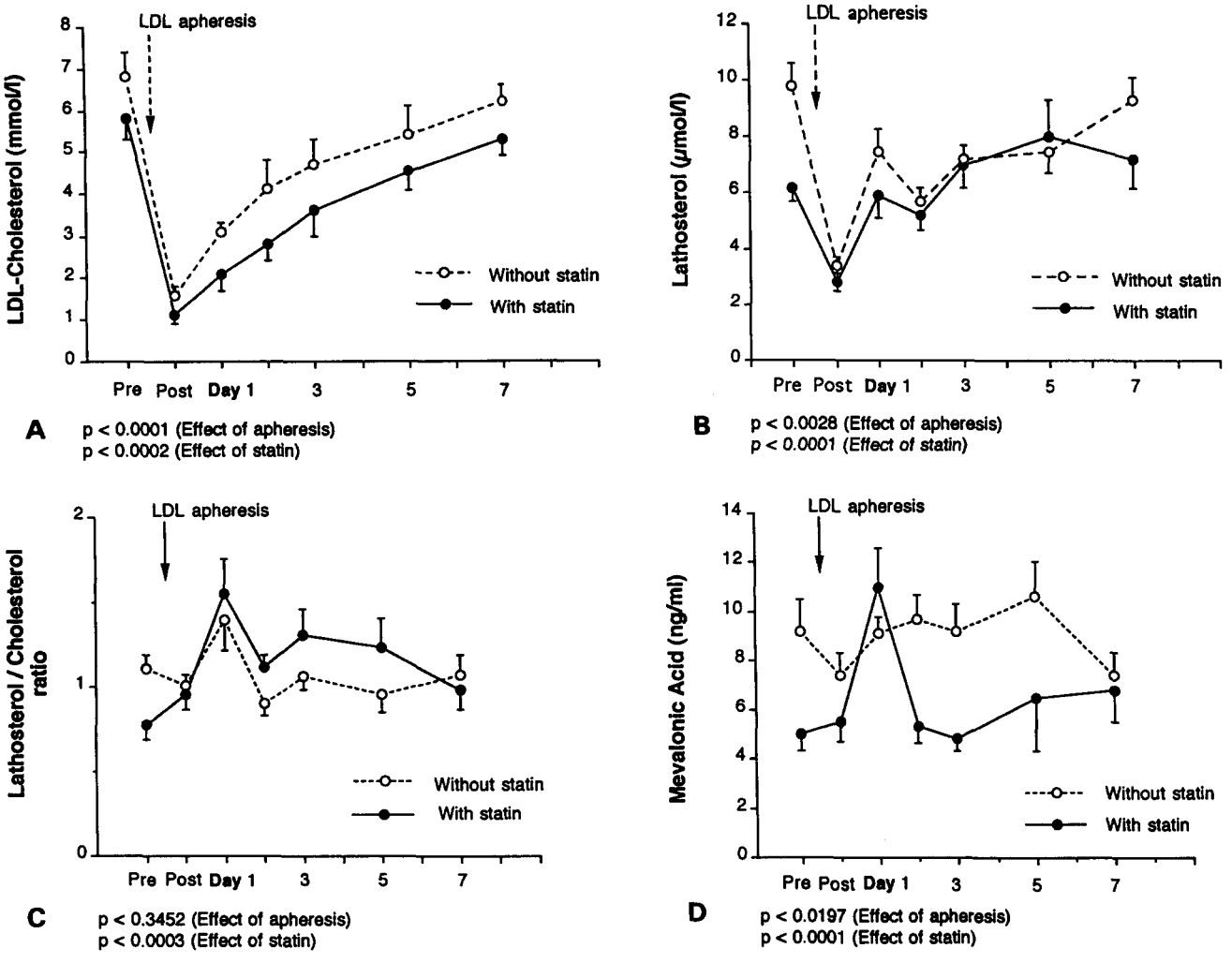

Fig. 2. Short-term changes after LDL apheresis in (A) serum LDL-cholesterol, (B) lathosterol, (C) lathosterol/cholesterol (L/C) ratio, and (D) plasma MVA. Samples were taken immediately before (pre), and after (post) LDL-apheresis and on days, 1, 2, 3, 5, and 7. L/C ratio was calculated as lathosterol μmol per l/cholesterol mmol per l.

DISCUSSION

The aim of this study was to examine the acute and chronic effects of LDL-apheresis on cholesterol biosynthesis in FH. We measured serum lathosterol as a marker of whole-body cholesterol biosynthesis, but because lathosterol is transported by lipoproteins and is thus partly removed from plasma along with cholesterol by the LDL-apheresis procedure itself, we expressed our results as the ratio of lathosterol:cholesterol. We used plasma MVA as a second parameter of cholesterol biosynthesis as it is free from this potential source of bias. In addition, although both MVA and lathosterol are well-established indices of the rate of cholesterol synthesis (8–22), they differ in being the respective products of early and late steps on the cholesterol biosynthetic pathway. Despite the partial removal of VLDL- and LDL-associated lathosterol by the procedure, changes in the L/C ratio and plasma MVA after LDL-apheresis were in general well correlated (Fig. 4). However, although both these variables are convenient to use under non-steady-state conditions they are at best only semi-quantitative indices of cholesterol synthesis.

LDL-apheresis removes LDL-cholesterol and, less efficiently, VLDL-cholesterol from plasma. In our patients long-term treatment resulted on average in a reduction of pre-apheresis levels of LDL-cholesterol by 30% to 38%. Pre-apheresis values underestimate the efficacy of LDL-apheresis, as they represent the maximum level of LDL-cholesterol between consecutive procedures. A better index is the integrated mean under the rebound curves which, in the short-term studies, revealed overall reductions in LDL-cholesterol of 56% below baseline levels with LDL-apheresis alone and of 65% with concomitant

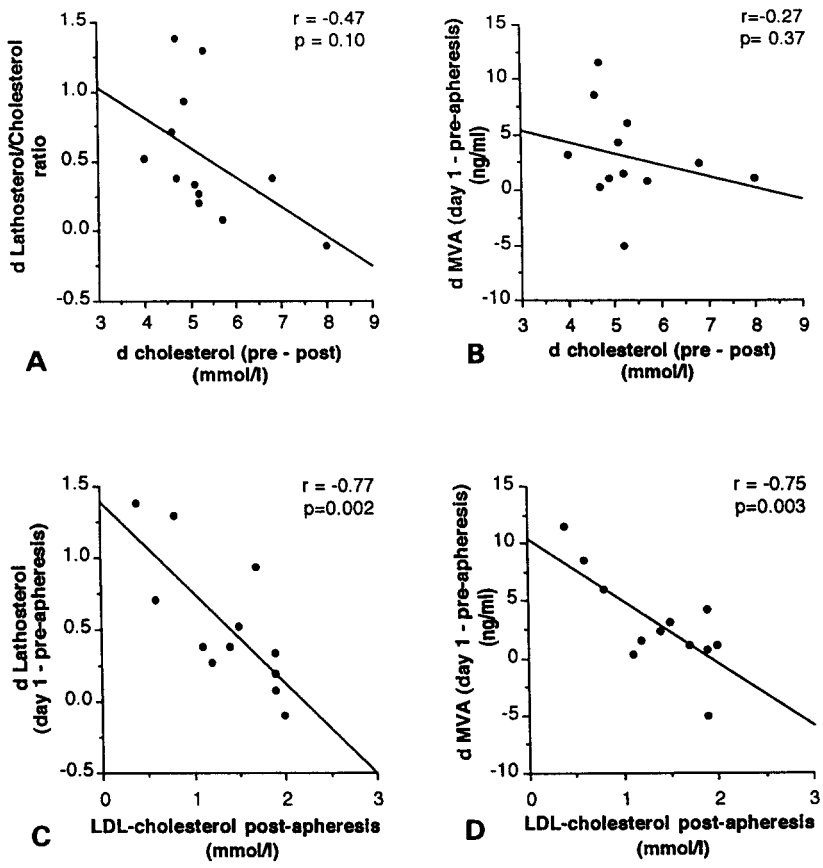

Fig. 3. Correlations between post-apheresis changes in lathosterol/cholesterol (L/C) ratio and plasma MVA and changes in serum total and LDL-cholesterol after LDL-apheresis. There was no significant correlation between the increase in L/C ratio (A) and plasma MVA (B) on the first day after LDL-apheresis as compared with the pre-apheresis values and the reduction of total serum cholesterol. However, changes in L/C ratio (C) and plasma MVA (D) did correlate inversely with the post-apheresis serum LDL-cholesterol.

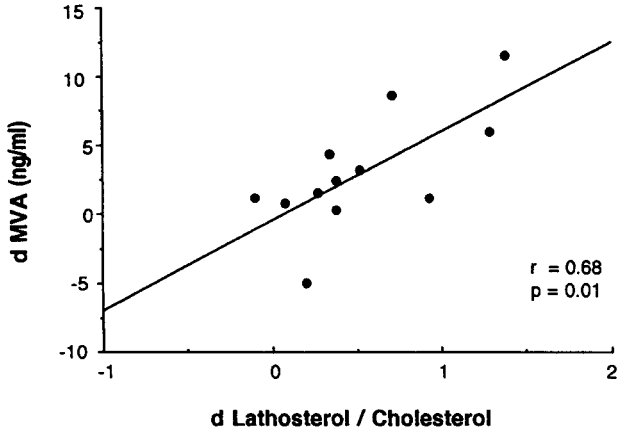

Fig. 4. Correlation of changes in lathosterol/cholesterol (L/C) ratio and plasma MVA after LDL apheresis. Both parameters are calculated as value on day 1 after LDL-apheresis minus pre-apheresis value; d latho/chol = difference in L/C ratio; d MVA = difference in plasma MVA.

statin treatment. Although the additional effect of statins on LDL seems relatively slight, it should be remembered that our patients were selected for LDL-apheresis therapy because of their inadequate response to drug therapy.

Long-term repetitive LDL-apheresis resulted in a slight but insignificant increase in cholesterol biosynthesis, based on changes in the L/C ratio and plasma MVA. This lack of any obvious increase in cholesterol biosynthesis may be due to several factors. First, the measurements of L/C ratio and plasma MVA were performed in plasma samples taken immediately before the LDL-apheresis procedure, thus reflecting the cholesterol biosynthesis rate at an interval of 1 week after the last procedure. Second, although plasma MVA increased by 48% over the period of 18 months, due to inter-individual variations this increase failed to reach significance. However, our long-term results are in accordance with those of Gylling et al. (7). Their study has been published so far only as an abstract but did not show any long-term changes in

cholesterol biosynthesis during biweekly treatment with LDL-apheresis plus lovastatin and a resin, as judged by the ratio of cholesterol precursors to serum total cholesterol. However, these authors do not appear to have studied the short-term effects of LDL-apheresis, and therefore may have overlooked the transient increase in cholesterol biosynthesis that we observed.

Each LDL-apheresis procedure removes a substantial amount of cholesterol from plasma. Lowering the serum cholesterol by 5.2 mmol/l removes approximately 6 g of cholesterol from a patient with a plasma volume of 3 l (6). Without replacement of the latter via mobilization of tissue cholesterol or de novo synthesis, the body cholesterol stores would be quickly depleted. That a reduction in body cholesterol stores occurs during long-term treatment with LDL-apheresis is well exemplified by the observed regression of tendon xanthoma (29). Analogous changes in the composition of atherosclerotic lesions are suggested by anecdotal observations in a hypercholesterolemic patient who died after more than 6 years of extracorporeal LDL-elimination (30).

The lack of change in L/C ratio and plasma MVA during long-term LDL-apheresis does not exclude the possibility that LDL-apheresis has acute effects on cholesterol biosynthesis. In contrast to dietary and drug treatment, LDL-apheresis is an intermittent procedure, reducing LDL-cholesterol concentration to very low levels immediately after each procedure. There is also a reduction in the concentration of VLDL particles, which is partly due to the activation of lipoprotein lipase caused by heparinization of the patients (31). Plasma lathosterol levels were reduced by 61% immediately after LDL apheresis but the L/C ratio and plasma MVA were unchanged. However, both of the latter subsequently failed to show the expected decrease in accordance with their known diurnal rhythm (11, 19, 20); on the contrary there was a tendency for an increase in both variables on the day after apheresis, which was significant in the statin-treated group ($P < 0.001$). Although there was a strong inverse correlation between the post-apheresis LDL-cholesterol level and the increase in L/C ratio or MVA in plasma on the day after LDL-apheresis there was no obvious correlation with the decrement in serum total cholesterol, which reflects the amount of cholesterol removed (Fig. 3).

The reduction in serum total cholesterol did not correlate with the calculated AUC of the L/C ratio and of plasma MVA during the week after LDL-apheresis whereas the postapheretic LDL-cholesterol was inversely correlated with the AUC of the L/C ratio but not with the AUC of plasma MVA. The results suggest that the acute reduction in LDL-cholesterol concentration caused by this procedure results in a transient up-regulation of cholesterol biosynthesis within 20 h, which was most marked when LDL-cholesterol was reduced below 1 mmol/l. It is known that the activity of the key enzyme of cholesterol biosynthesis, HMG-CoA reductase, can be altered rapidly by regulation at the transcriptional, translational, as well as at the protein mass level (32). These regulatory steps are mainly sterol-dependent (32), and our findings suggest a strong inverse correlation between the plasma LDL-cholesterol concentration and cholesterol biosynthesis in vivo.

Because the most pronounced increases in cholesterol precursors occurred after LDL apheresis in those patients who were also treated with a statin, it might be argued that statins caused a paradoxical up-regulation of cholesterol synthesis. Chronic statin treatment is known to increase the amount of HMG-CoA reductase in liver cells (17), and de-inhibition of the enzyme by removal of active metabolites of lovastatin or pravastatin from plasma during LDL-apheresis, although unlikely, has not been excluded. Alternatively, it may be that the increased mass of HMG-CoA reductase during statin therapy is more easily up-regulated when LDL-cholesterol is acutely lowered. Additionally, post-apheretic LDL-cholesterol levels were appreciably lower in the patients on statin treatment (1.1 $\pm$ 0.2 mmol/l) than in those without statins (1.6 $\pm$ 0.2 mmol/l), which would further stimulate the process.

Transient up-regulation of cholesterol biosynthesis after LDL-apheresis was not reflected in the rate of rebound of VLDL- and LDL-cholesterol. There was no significant correlation between the increase of plasma MVA on day 1 after LDL-apheresis and increases in VLDL- and LDL-cholesterol on the first and second days ($r = 0.359$, $P = 0.874$). However, the rates of rebound of both VLDL-cholesterol (Table 4) and LDL-cholesterol (Fig. 2A) were significantly reduced in the statin-treated group. Presumably this reflects an increase in the fractional catabolic rate of LDL and VLDL remnants and/or decreased secretion of apoB-containing lipoproteins by statin therapy, secondary to inhibition of cholesterol synthesis (33). In the latter context it is noteworthy that the overall effect of statin in decreasing MVA was highly significant whereas that of LDL apheresis in increasing MVA was less marked (Fig. 2D).

In vitro measurements of hepatic cholesterol biosynthesis and hepatic uptake of LDL in vivo have been shown to change in parallel (34), and it has recently been documented that hepatic mRNA levels for the LDL receptor and HMG-CoA reductase show coordinate regulation in mice (35). Up-regulation of LDL receptor activity is one of the main effects of treatment with HMG-CoA reductase inhibitors and bile acid sequestrants (17, 36, 37); these drugs play an important role enhancing the effectiveness of LDL-apheresis. Another factor influencing the rate of rebound of cholesterol during the first 2 days after LDL-apheresis is the influx of extravascular cholesterol into plasma (38).

Compared with the marked increase in cholesterol

precursor sterols (13) and urinary MVA (15) observed during treatment with resins, extracorporeal removal of LDL-cholesterol stimulates cholesterol biosynthesis to a lesser extent. Presumably this reflects the intermittent character of LDL-apheresis therapy, which causes acute but transient decreases in LDL- and VLDL-cholesterol. The subsequent increase in cholesterol biosynthesis does not appear to be associated with any increase in apolipoprotein B production, judging from the results of studies in FH patients and normal subjects following plasma exchange (3) and HELP-apheresis (39).

In conclusion, our study suggests that the acute reduction of LDL-cholesterol induced by LDL-apheresis transiently stimulates cholesterol biosynthesis in patients with FH. The extent of stimulation is dependent on the depth of the post-apheresis level of LDL-cholesterol reached in plasma and is not suppressed by concomitant statin treatment. The transient increase in cholesterol synthesis did not influence the rate of rebound of VLDL and LDL-cholesterol after LDL apheresis. In contrast, apart from a brief escape on day 1, statin therapy markedly decreased cholesterol synthesis throughout the week after LDL apheresis and reduced the rate of rebound of both VLDL and LDL-cholesterol; this suggests an inhibitory effect on secretion of apoB-containing lipoproteins. This conclusion was also reached by Koizumi et al. (33) who showed that the kinetic model of Apstein et al. (40), which assumes that LDL synthesis remains constant, could not explain the effect of pravastatin on LDL rebound solely in terms of an increase in the fractional catabolic rate of LDL. The relevance of these findings to the regulation of cholesterol and lipoprotein synthesis in normal subjects remains to be shown.

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