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Low Density Lipoprotein Apheresis Improves Regional Myocardial Perfusion in Patients With Hypercholesterolemia and Extensive Coronary Artery Disease

The LDL-Apheresis Atherosclerosis Regression Study (LAARS)

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Objectives. **In a randomized study we evaluated the effect of biweekly low density lipoprotein (LDL) apheresis plus simvastatin versus medication alone on regional myocardial perfusion.**

Background. **In patients with severe hypercholesterolemia, diet and lipid-lowering drugs are often insufficient to achieve optimal LDL cholesterol values. Low density lipoprotein apheresis is a very effective lipid-lowering therapy. Assessment of regional myocardial perfusion enables evaluation of the functional state of the coronary circulation.**

Methods. **We studied 42 patients with severe hypercholesterolemia and extensive coronary artery disease who were randomized to diet and simvastatin with or without biweekly LDL apheresis. Regional myocardial perfusion was assessed by digital subtraction angiography with videodensitometric calculation of hyperemic mean transit time (HMTT) of contrast medium at baseline and after 2 years of therapy.**

Results. **Low density lipoprotein cholesterol decreased by 63% (to 3.0 mmol/liter) in the LDL apheresis group and by 47% (to 4.1 mmol/liter) in the medication group. Paired HMTT measurements were assessed in 43 regions in the LDL apheresis group and 35 regions in the medication group. In the LDL apheresis group, regional HMTT decreased over 2 years from 3.35 ± 1.18 (mean ± SD) to 2.87 ± 0.82 s (−14%, p = 0.001), whereas no change in the medication group was observed: 2.95 ± 1.06 to 2.96 ± 0.90 s (p = NS). In the patient-based comparison, the mean change in HMTT was −0.45 s (−14%, p = 0.01) in the LDL apheresis group and −0.05 s (−2%, p = NS) in the medication group, respectively. Only exercise-induced ischemia improved in the LDL apheresis group.**

Conclusions. **Biweekly LDL apheresis plus simvastatin decreased time-averaged LDL cholesterol levels by an additional 31% (1.1 mmol/liter) compared with medication alone. After 2 years of therapy, regional myocardial perfusion improved in the LDL apheresis group and remained unchanged in the medication group. Thus, aggressive reduction of LDL cholesterol has a favorable effect on regional myocardial perfusion and alleviates ischemia.**

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Low density lipoprotein (LDL) cholesterol levels often remain elevated in patients with familial hypercholesterolemia (FH) despite diet and cholesterol-lowering drugs. High LDL cholesterol levels are strongly associated with premature development of coronary artery disease, especially in men with an inherent risk of coronary death (1). Low density lipoprotein apheresis is considered a valuable therapeutic option for patients with homozygous FH and for patients with coronary artery disease and hypercholesterolemia refractory to diet and drugs (2–4). It has been shown in a number of nonrandomized studies (2,5–8) that plasma exchange or LDL apheresis led to regression or slowing of progression of coronary atherosclerotic lesions and resolution of xanthomas. The use of plasma exchange was also associated with increased survival among patients with homozygous FH compared with their untreated homozygous siblings (9). In one recently published randomized trial in patients with FH (10), biweekly LDL apheresis versus drug therapy for 2 years showed no difference in coronary anatomy as measured by quantitative coronary angiography (QCA) (10). In the LDL-Apheresis Atherosclerosis Regression Study (LAARS) (11), there was also no difference in quantitatively assessed coronary anatomy, but patients undergoing LDL apheresis had a significant improvement in exercise variables versus patients on drug therapy alone. Questions

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Abbreviations and Acronyms

DSA = digital subtraction angiography
FH = familial hypercholesterolemia
HMTT = hyperemic mean transit time (of contrast medium)
LAARS = LDL-Apheresis Atherosclerosis Regression Study
LAD = left anterior descending coronary artery
LCx = left circumflex coronary artery
LDL = low density lipoprotein
QCA = quantitative coronary angiography
RCA = right coronary artery
ROI = region of interest

remain about 1) the cause of this functional improvement, 2) the methods for evaluating the potential benefits in aggressive lipid-lowering therapy, and 3) the impact on patient care. Traditionally, clinical end points, such as improved survival and reduction of major cardiac events, are the best proof of effect in lipid-lowering trials but require numerous patients over a long period of time (12,13). Quantitative coronary angiography is the present reference standard for evaluating lipid-lowering therapy in coronary artery disease (14–16). However, application of QCA has several limitations, such as the diffuse character of coronary artery disease, complexity of stenosis geometry, foreshortening in nonorthogonal views, insufficient contrast staining and limited resolution of the radiographic chain (16–18). Moreover, there seems to be a dissociation between the results of angiographic studies and clinical outcome. Lipid-lowering therapy reduces the incidence of cardiovascular events to a greater extent than the proportionate changes in plaque dimensions suggest (15,19). Reduction of hypercholesterolemia retards the progression of coronary atherosclerosis by depleting the lipid core of the plaque and deminishing the inflammatory process; not the change in angiographic lumen size but the process of plaque stabilization appears to decrease the risk of disruption with fewer clinical events (20). The endothelium plays a major role in regulating the vascular smooth muscle tone. Endothelial dysfunction can result in an impaired blood flow of the large and small coronary arteries (21–25). Dysfunction of endothelium-mediated vasomotion is associated with atherosclerosis, hypercholesterolemia and smoking in adults and is already present in the systemic arteries of children with heterozygous FH (21–26). Lipid-lowering therapy improves the endothelium-mediated vasomotion after relatively short periods of time (27–29). The functional improvement of the coronary circulation precedes structural improvement (30). The cumulative effects of all anatomic and functional abnormalities of the epicardial coronary arteries in combination with possible dysfunction of conduit and resistance vessels are responsible for the ultimate effect of atherosclerosis on myocardial perfusion. In this setting, it should be emphasized that minor changes in lumen diameter of the coronary arteries have a major effect on vascular resistance, according to Poisseuille's law.

To evaluate the functional state of the coronary circulation after aggressive lipid-lowering therapy with LDL apheresis, we assessed regional myocardial perfusion by means of digital subtraction angiography (DSA) followed by videodensitometric calculation of the hyperemic mean transit time (HMTT) of contrast medium in the LAARS trial.

Methods

The purpose of the study was to compare the effects of aggressive lipid-lowering treatment by LDL apheresis versus conventional treatment on the anatomy and functional state of the coronary tree. The trial design, patient characteristics, methodology of lipid measurements, QCA and exercise testing have been described previously (11). Briefly, consenting patients with extensive coronary artery disease and hypercholesterolemia were randomized to diet and 40 mg of simvastatin once daily with or without biweekly LDL apheresis. The inclusion criteria were 1) male patients between 18 and 70 years old; 2) extensive coronary atherosclerosis demonstrated by cardiac catheterization; 3) total serum cholesterol ≥8.0 mmol/liter or LDL cholesterol ≥5.8 mmol/liter and serum triglycerides ≤5.0 mmol/liter during a standard lipid-lowering diet. The ethics committee of the University Hospital of Nijmegen approved the study. The study cohort included 42 male patients with a mean (±SD) age of 52.0 ± 9.2 years (range 29 to 69). The elevated lipid levels were due to heterozygous FH in 32 patients (76%), 16 in each group. In the remaining 10 patients, the diagnosis was primary hypercholesterolemia in 8 (19%) and familial combined hypercholesterolemia in 2 (5%). In the LDL apheresis group, 17 patients had three-vessel disease, and 4 had two-vessel disease, whereas in the medication group 19 patients had three-vessel disease, and 2 had two-vessel disease (a vessel was considered *diseased* when there was ≥50% diameter stenosis in a major branch). Before entry into the study, all patients had a 2-month run-in period. Antianginal medication was continued, but lipid-lowering medication was stopped. If total cholesterol remained elevated above the lower limit for inclusion into the study, cardiac catheterization and exercise testing were carried out. After cardiac catheterization, patients were randomized in a stratified way, which took into account the total cholesterol and lipoprotein(a) levels, age and history of coronary bypass surgery. Antianginal medication was continued at the same doses during the trial. If adaptation in medication was necessary during the study, the original prescription, if possible, was restored before the follow-up cardiac catheterization. The follow-up cardiac catheterization was performed 1 month after the last LDL apheresis. Low density lipoprotein apheresis was performed with an automated system MA-01 (Kanegafuchi Chemical Industry Co. Ltd, Osaka, Japan) with two small-sized dextran sulfate cellulose columns that were used and regenerated alternately in one procedure, permitting continuous apheresis (11). Before the start of the LDL apheresis, all patients received 10,000 U of heparin. A volume of 5,000 ml (~1.5 plasma volume) was treated per session.

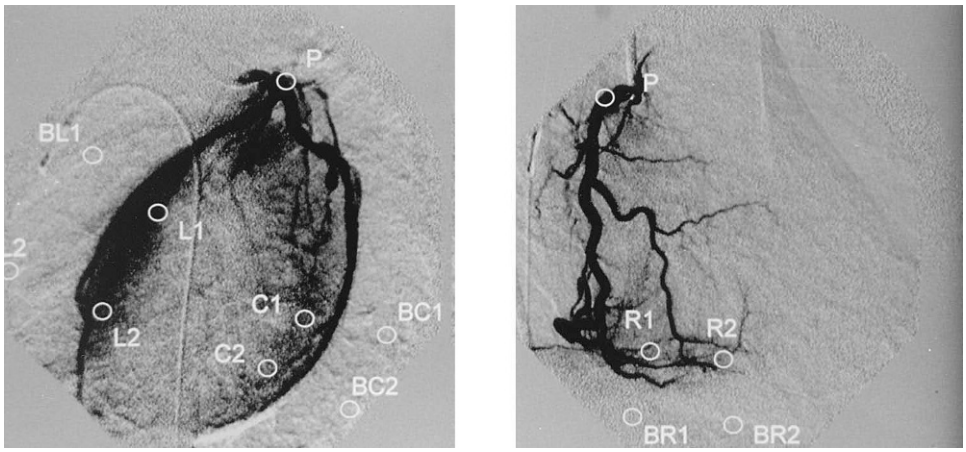


Figure 1. Example of the positioning of the ROIs in one of the subtracted images of the coronary arteries early after contrast injection. **Left panel**, LAD in the 60° left anterior oblique projection. **Right panel**, RCA in the 30° right anterior oblique projection. B = background ROI; C1,2 = ROIs 1 and 2 in perfusion area of LCx; L1,2 = ROIs 1 and 2 in perfusion area of LAD; P = ROI at catheter tip; R1,2 = ROIs 1 and 2 in perfusion area of RCA.

Assessment of regional myocardial perfusion. We used the method of DSA, followed by videodensitometry and calculation of the HMTT of contrast to compare the maximal regional myocardial perfusion in the areas of the left anterior descending coronary artery (LAD), left circumflex coronary artery (LCx) and right coronary artery (RCA) at baseline and at follow-up. This method has been developed in our laboratory and has been validated in model studies, animal experiments and in humans (31–34). Before cardiac catheterization, patients were trained to hold their breath with use of a nose clamp at maximal inspiration for 15 to 20 s. After completion of angiography of the LAD, the left Judkins catheter with a 7F tip (Cordis) was left in place for the DSA protocol, in which a 60° left anterior oblique projection was used. After angiography of the RCA, the protocol was repeated in the 30° right anterior oblique projection. Meticulous care was taken to repeat the follow-up DSA under exactly the same circumstances, with the same medication.

At the start of the procedure, a 5F stimulation catheter was positioned in the right atrium. During DSA, the heart was triggered in synchrony with the radiographic pulses, slightly above its inherent rate, to provide a strictly regular heart rhythm. Iohexol-350 (Nycomed AS, Oslo, Norway), a nonionic low osmolar contrast agent was used. For all studies, 6 ml of contrast medium was injected, using a power injector at a speed of 4 ml/s. Twenty-five seconds after administration of 8 to 12 mg of papaverine (depending on the size of the vascular bed), the patient was instructed to hold his breath, and image acquisition was started 5 s later followed by contrast injection. One image/heart cycle was obtained just before the onset of the QRS complex. Image acquisition was performed on a Bicor radiograph system connected to a Digitron-3 computer for digital subtraction angiography (Siemens AG, Erlangen, Germany). Images were generated with the automatic brightness control switched off after the fourth image in every study to enable density calculation. Subsequently, the images were digitized in a 512 × 512 matrix with 1,024 density levels. The image quality was checked immediately after image acquisition, and if necessary the imaging protocol was repeated a maximum of three times.

Image and data processing were performed off-line using a Sun SPARC station 10 (Sun Microsystems, Inc.) with programmed Khoros software. Circular regions of interest (ROIs) of 200 to 600 pixels were constructed over the tip of the coronary catheter to record the contrast injection and over the myocardium supplied by the respective vessels (Fig. 1). The ROIs were chosen at the myocardial level on predefined localizations, carefully avoiding overlap with major side branches and veins. Close to the myocardial ROI, a background ROI was chosen to analyze changes in background density (Fig. 1). Time–density curves were generated by sampling the averaged pixel density within a ROI in consecutive images and, subsequently, these curves were fitted with a gamma-variate function. In case of deviating background density, the fitted curves of the myocardial ROIs were corrected with the corresponding background ROI. The HMTT is the difference in the mean transit time of the contrast medium at the myocardial ROI and at the injection site ROI (ROI at catheter tip). Because of the pressure dependency of flow during maximal hyperemia, HMTTs were corrected for a normalized mean aortic blood pressure of 100 mm Hg for comparison between the two studies. This correction was performed by multiplying the mean transit time in seconds by the ratio $\bar{Pa}/100$, where $\bar{Pa}$ is the actual mean blood pressure during image acquisition. If a pressure curve showed a pressure drop or wedging during hyperemia, indicating that the catheter influenced maximal flow, the study was excluded from further analysis. Myocardial areas that were subjected to a Q wave myocardial infarction or occlusion of the proximal vessel were excluded from analysis.

For final analysis, paired time–density curves of sufficient quality without artifacts were selected by a cardiologist (W.R.M.A.) who had no knowledge of treatment allocation. Figure 2 shows an example of paired time–density curves of an ROI. The final data base was composed, and subsequent HMTTs were calculated and corrected for blood pressure, as stated before. Regional myocardial perfusion was assessed by averaging the comparative HMTTs (one to two ROIs) in the area supplied by the LAD, LCx and RCA to one value/perfusion area per session. Perfusion areas supplied by bypass

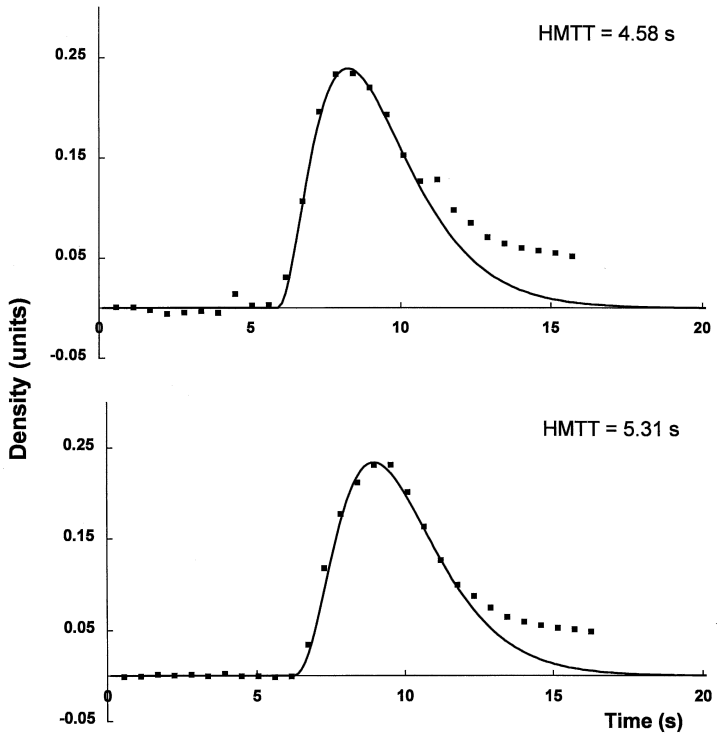


Figure 2. Example of paired time–density curves of ROI at baseline (**upper panel**) and at follow-up (**lower panel**). The HMTT values are calculated from these curves, where the onset of time is derived from the time–density curve of the respective ROI at the injection site (not shown).

grafts were also imaged by the DSA protocol, carefully avoiding evaluation of perfusion areas with competitive flow through native coronary artery and bypass graft. The ROIs of perfusion areas supplied by bypass grafts were classified as part of the original coronary artery region. Analysis of the effect of both lipid-lowering treatments on myocardial perfusion was calculated from HMTT values derived from individual ROIs from a regional- and patient-based assessment of HMTT. The patient-based evaluation was performed by averaging the regional HMTT values to one value per patient as a global estimate of myocardial perfusion. Analysis of the patient-based HMTT values was performed both with and without correction for change in hematocrit. For this correction the HMTT values at follow-up were subtracted by the percent change in hematocrit, assuming a linear relation between hematocrit and blood viscosity in this narrow range of actual hematocrit values (35).

Statistical analysis. Comparisons of prespecified continuous variables within groups were made by two-sided paired *t* tests and between-group analysis by unpaired *t* tests for normally distributed data. For categoric comparison, a chi-square test was performed when appropriate. Correlations were tested with the Pearson correlation coefficient. For all hypothesis tests, a two-sided p value <0.05 was considered significant. Results are expressed as mean value ± SD, unless otherwise indicated.

Results

Forty-two male patients were randomized into two groups of 21 patients in our center. The results in both groups for lipid metabolism, QCA, exercise testing and clinical events have been described elsewhere (11). In short, LDL cholesterol decreased in the LDL apheresis group from 7.78 ± 1.86 mmol/liter to a time-averaged value of 2.95 ± 1.13 mmol/liter and from 7.85 ± 2.34 to 4.13 ± 1.58 mmol/liter in the medication group. Quantitative coronary angiography showed no changes in mean segment diameter and minimal obstruction diameter within and between the two groups. At exercise tests, in the LDL apheresis group versus the medication group, time to onset of 1-mm (0.1 mV) ST segment depression was prolonged by 39%, and maximal ST segment depression was halved. Clinical characteristics and patient-based study data are shown in Table 1.

Hyperemic mean transit time. The DSA protocol for calculation of HMTT was performed in all 42 patients. In the LDL apheresis group, 19 patients completed the 2 years of LDL apheresis; 2 stopped because of progression of angina pectoris after 3 and 10 months, respectively. Both patients underwent coronary artery bypass graft surgery. In the medication group, one patient underwent bypass surgery after 22 months of treatment. The HMTT studies of these patients at follow-up were considered as end of study measurements. One patient in the LDL apheresis group who was admitted to the hospital for unstable angina pectoris 12 months after the start of the LDL apheresis underwent percutaneous transluminal coronary angioplasty of the LAD and the RCA. After coronary angioplasty, LDL apheresis was continued, but the two perfusion areas were excluded from assessment of regional myocardial perfusion. In 35 patients (83% [18 in the LDL apheresis group, 17 in the medication group]), comparative data from

Table 1. Clinical Characteristics and Patient-Based Study Data

Pt No./Age (yr)	Group	Prestudy Event	LDL Chol (mmol/liter)		ΔMSD (mm)	ΔMOD (mm)	ΔST Time (s)	ΔHMTT (s)
			Base	During				
1/29	L	/	12.09	5.67	0.39	0.20	−420	0.17
2/59	M	I/C	7.01	3.74	0.14	0.15		−0.09
3/67	M	A/C	6.62	3.90	0.11	0.05	0	0.97
4/59	M	I/	7.05	4.26	0.06	0.04		1.46
5/52	M	I/	5.94	3.58	−0.14	−0.12		−1.58
6/43	L	NQ/	5.45	2.65	−0.30	−0.24	−30	−0.19
7/69	M	I/CP	6.28	3.06	0.11	−0.09		
8/67	M	I/P	6.51	2.69	−0.42	−0.25		2.57
9/46	L	/	6.63	3.00	−0.12	−0.09	−360	−0.62
10/65	L	/	6.97	3.76	0.05	0.07		−0.14
11/46	L	I/C	7.17	2.76	0.23	0.19		
12/46	M	A/	6.11	3.57	−0.08	−0.08	60	−0.75
13/66	L	I/	6.97	2.14	−0.08	−0.16	−299	
14/58	M	/C	6.43	3.47	0.07	0.12		
15/53	L	I/C	6.80	1.98	−0.09	−0.14	−360	−0.38
16/40	M	/	9.90	5.32	0.26	0.20	120	0.00
17/58	L	I/C	6.71	2.03	0.00	−0.13	−60	−0.51
18/52	L	A/C	11.36	5.22	−0.33	−0.06	23	−0.06
19/47	M	I/C	12.86	5.62	−0.29	−0.09		−0.41
20/30	L	A/C	10.75	3.69	−0.03	−0.06	−330	0.41
21/50	L	A/C	7.60	3.12	0.04	0.20		
22/45	M	NQ/	9.20	4.95	0.07	0.10	0	−0.21
23/56	M	A/C	11.65	4.93	0.17	0.07		−0.62
24/44	M	I/	6.59	3.43	0.18	0.06	360	−0.14
25/57	L	I/P	7.06	2.09	−0.02	−0.06	−165	−1.09
26/56	M	I/C	5.92	2.76	0.08	0.05	−120	−1.58
27/50	L	I/	10.84	3.36	−0.06	−0.04		−0.30
28/42	L	A/	7.60	2.51	0.20	0.17		0.45
29/63*	L	IA/C	6.57	2.09				−1.27
30/49	M	/CP	8.30	4.29	0.00	0.03		−1.50
31/52	M	I/	7.60	4.14	−0.01	0.02	60	0.22
32/54	L	I/C	6.28	2.10	0.10	0.09	−240	−1.51
33/42	L	/	7.03	2.00	−0.14	−0.11		−0.50
34/56	M	A,A/C	6.68	3.26	−0.06	−0.08	0	0.59
35/56	L	A/C	6.66	2.03	−0.11	−0.13	−55	−1.34
36/46	L	/	8.84	4.02	0.00	0.03	−67	−0.36
37/49	M	NQ/	7.31	4.48	0.07	−0.01		
38/50	M	A/CP	5.83	2.79				0.24
39/62	L	I/PC	6.36	2.59	−0.03	0.03	−60	−1.37
40/54	L	I/	6.38	2.27	0.03	−0.01	−88	0.57
41/66	M	A/	7.16	3.16	−0.02	−0.07	−60	
42/42	M	I/P	13.90	9.29	0.20	0.15	5	−0.03

*Patient underwent repeat coronary artery bypass graft surgery (C) 12 weeks after start of low density lipoprotein (LDL) apheresis and died postoperatively due to low cardiac output. A = anterior myocardial infarction; Base = baseline; During = mean LDL cholesterol level during trial (for LDL apheresis group [L], data are time-averaged values [for conversion to mg/dl, multiply by 38.7]); I = inferior myocardial infarction; M = medication; NQ = non-Q wave myocardial infarction; P = percutaneous transluminal coronary angioplasty; Pt = patient; ΔHMMT = change in hyperemic mean transit time; ΔMOD = change in minimal obstruction diameter (baseline vs. follow-up); ΔMSD = change in mean segment diameter from quantitative coronary angioplasty measurements (baseline vs. follow-up); ΔST Time = change in time to 1-mm (0.1 mV) ST segment depression.

the first study and subsequent follow-up were available. Paired data were missing in seven patients because of 1) insufficient image quality from one session in two patients and from both sessions in a third patient with chronic airway obstruction; 2) loss of stored digital images from one session in two patients; and 3) no comparable DSA in two patients.

Evaluation of myocardial perfusion based on individual *ROIs.* In total, 121 comparative ROIs were available, 68 in the LDL apheresis group and 53 in the medication group (mean 3.5 ± 1.7/patient). One hundred three ROIs were generated in regions supplied by native coronary arteries and 18 supplied by bypass grafts. On the basis of the ROIs, the average HMTT

Table 2. Regional Hyperemic Mean Transit Time Values in the Three Myocardial Perfusion Areas

	LAD Region	LCx Region	RCA Region	All Regions
LDL apheresis group	n = 17	n = 18	n = 7	n = 42
Baseline HMTT (s)	3.28 (0.97)	2.93 (0.66)	4.61 (1.85)	3.35 (1.18)
Follow-up HMTT (s)	2.81 (0.86)	2.66 (0.67)	3.57 (0.79)	2.87 (0.82)
p value	0.02	0.08	0.11	0.001
Medication group	n = 15	n = 13	n = 7	n = 35
Baseline HMTT (s)	2.78 (0.78)	3.15 (1.22)	2.95 (1.35)	2.95 (1.06)
Follow-up HMTT (s)	2.75 (0.81)	3.11 (1.07)	3.14 (0.79)	2.96 (0.90)
p value	0.88	0.93	0.74	0.96
Difference (p value)*	0.17	0.51	0.14	0.04

*Difference in mean change between low density lipoprotein apheresis and medication group (*t* test). Data presented are mean value (SD). HMTT = hyperemic mean transit time; LAD = left anterior descending coronary artery; LCx = left circumflex coronary artery; RCA = right coronary artery.

decreased by 0.59 ± 1.01 s in the LDL apheresis group ($p < 0.0001$) and by 0.10 ± 1.16 s ($p = 0.52$) in the medication group (0.48-s between-group difference, $p = 0.01$).

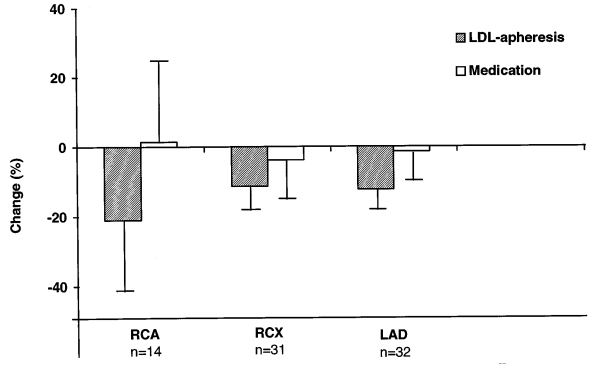
Evaluation of regional myocardial perfusion (Table 2). Baseline HMTT values for the LDL apheresis and medication groups were 3.35 ± 1.18 s (n = 42) and 2.95 ± 1.06 s (n = 35), respectively, and were statistically not different ($p = 0.52$). The relative changes in HMTT in the three perfusion areas of the LAD, LCx and RCA are shown in Figure 3. All three coronary perfusion areas in the LDL apheresis group contributed equally to the final result.

Patient-based analysis. In the patient-based analysis the average HMTT value in the LDL apheresis group (n = 18) was 3.19 ± 0.78 s at baseline and 2.74 ± 0.68 s at follow-up (mean difference −0.45 s [−14%], $p = 0.01$). In the medication group (n = 17), the average HMTT value was 2.99 ± 0.84 s at baseline and 2.94 ± 0.77 s at follow-up (mean difference −0.05 s [−2%], $p = 0.85$), for a mean difference in change between the two groups of 0.40 s ($p = 0.19$).

Hematocrit measured at cardiac catheterization in the LDL apheresis group was 0.44 ± 0.03 at baseline and 0.43 ± 0.04 liter/liter at follow-up ($p = 0.23$) and 0.45 ± 0.03 and 0.45 ± 0.03 liter/liter, respectively, in the medication group ($p = 0.44$). For correction of possible changes in blood viscosity due to changes in hematocrit, the mean patient-based HMTT at follow-up catheterization was corrected by the percent change in hematocrit from baseline to follow-up catheterization. In the LDL apheresis group (n = 18), the average HMTT corrected for hematocrit change decreased from 3.19 ± 0.78 s at baseline to 2.78 ± 0.69 s at follow-up ($p = 0.008$) and from 2.99 ± 0.84 s at baseline to 2.97 ± 0.84 s at follow-up in the medication group ($p = 0.94$). The mean difference in HMTT between the LDL apheresis group and the medication group after correction for hematocrit was 0.38 s ($p = 0.22$), rather than 0.40 s without correction for hematocrit changes.

There were no significant correlations between the change in percent as well as absolute changes in HMTT with the mean lipid levels during the trial [total cholesterol, LDL cholesterol, high density lipoprotein cholesterol, ratio of LDL to high density lipoprotein cholesterol, lipoprotein(a)] or relative and absolute changes in lipid levels. No significant correlations were found between percent changes as well as absolute changes in HMTT and minimal obstruction diameter, mean segment diameter and time to 1-mm ST segment depression. Classification of the patients into two groups (improvement vs. no change in or impairment of time to 1-mm ST segment depression at exercise test) showed a decrease in HMTT of 16 ± 21% in the group with improvement versus an increase in HMTT of 4 ± 19% in the group with no change or impairment ($p = 0.04$). The change in hematocrit at cardiac catheterization was not related to the time to 1-mm ST segment depression or to the change in HMTT.

Figure 3. Bar graphs showing mean percent change in HMTT ± SEM in the regions of the LAD, LCx (ramus circumflexus [RCX]) and RCA, respectively, for the LDL apheresis group versus the medication group.



Discussion

Although the LAARS trial showed no significant change in quantitatively assessed coronary anatomy in both groups, the results of the HMTT measurements showed an increase in myocardial perfusion in the LDL apheresis group, whereas the medication-only group remained in stable condition. This improvement in myocardial perfusion in the LDL apheresis group is in agreement with the improved ischemic threshold that has been previously reported (11,36). These results show a discrepancy between anatomic and physiologic variables in the assessment of coronary atherosclerosis. The improved functional status in the LDL apheresis group was accompanied by a lower LDL cholesterol level (1.1 mmol/liter) than in the medication-only group. To explain the improvement in myocardial perfusion, we hypothesized that one or more of the following mechanisms may be operative:

1. *Recovery of endothelial nitric oxide production and release in the conduit vessels.* Recent studies in animals and humans (21–25,37) associate hypercholesterolemia and increased Lp(a) levels with impaired endothelium-dependent vasodilation. Lipid lowering during 6 months to 1 year may enhance endothelial function, as assessed with acetylcholine (27–29). Improvement in endothelial function may enhance exercise-

induced dilation of the large epicardial coronary arteries (21,38). Short-term cholesterol lowering with LDL apheresis directly improves endothelium-dependent relaxation of the epicardial coronary arteries, suggesting a direct effect of LDL cholesterol (39). The more pronounced lipid lowering in the LDL apheresis group in this study may result in better recovery of endothelial function and may thus improve the ischemic threshold at exercise testing. Assessment of HMTT reveals a time variable that is inversely related to maximal myocardial perfusion. Because of the method of assessment of HMTT (intracoronary papaverine and sublingual isosorbide dinitrate), it is unlikely that endothelium-dependent vasodilation is involved in the improved regional myocardial perfusion observed in this study.

2. *Remodeling of the conduit vessel wall.* Progression or regression of coronary artery stenosis may be associated with complex shape changes or remodeling (40), in which the integrated hemodynamic effects on the coronary circulation may differ from the changes in minimal obstruction diameter and mean segment diameter. According to Glagov et al. (41) and Stiel et al. (42), progression of coronary artery disease leads initially to compensatory dilation. In the setting of regression of atherosclerotic plaques, this process might be reversed, with remodeling of the vessel wall without a significant effect on lumen cross-sectional area of the epicardial coronary arteries. However, the dilation of the remodeled coronary arteries in response to papaverine may be enhanced, and maximal myocardial perfusion may be increased. This view is supported by a recent publication (43) in which the epicardial response to intracoronary nitroglycerin administration decreased significantly with increasing atherosclerotic wall thickening. Next to obstruction of the vascular lumen, atherosclerosis of the arterial wall also has an endothelium-independent effect on coronary vasomotion and flow. Decrease in atherosclerotic thickening of the arterial wall without a direct effect on the lumen size of the vessel may explain the observation that functional improvement precedes anatomic regression of atherosclerosis.

3. *Improved arteriolar vasodilation.* Resistance vessels are spared from the development of overt atherosclerosis. However, relaxation of these vessels may be reduced in patients with high LDL cholesterol levels due to alterations in endothelial cell function, as established in primates (23). Endothelium-dependent arteriolar relaxation was impaired, whereas endothelium-independent responses were nearly identical (23). In the human forearm model, the impairment of microvascular vasodilator function in patients with hypercholesterolemia is endothelium-dependent only (25); thus, improvement in endothelium-independent arteriolar vasodilation as a mechanism for our results is highly speculative. Another hypothesis, introduced by Gould et al. (44), is that the coronary flow response induced by direct effect of an arteriolar vasodilator may be augmented by further improvement in flow-mediated, endothelium-dependent epicardial coronary artery vasodilation. However, this effect was not likely to occur in this study, where patients received isosorbide dinitrate for epicardial coronary artery vasodilation before angiography. Maximal epicardial vasodilation after sublingual isosorbide dinitrate is known (45,46) to persist for 30 to 60 min, which was the time needed in this study to perform angiography and DSA.

4. *Changes in blood rheology.* Improved myocardial perfusion might be due to changes in blood rheology as a result of repeated LDL apheresis (47,48). Because of this observation, assessment of HMTT in the present study was performed 1 month after final LDL apheresis to avoid a direct effect of LDL apheresis on viscosity. In LDL apheresis over dextran sulfate cellulose columns, plasma fibrinogen, a determinant of viscosity, is lowered acutely by 26% to 35%, returning to pretreatment levels between 2 and 7 days (11,49). Seven days after LDL apheresis plasma viscosity is unchanged, but blood viscosity is still reduced, independent of the change in hematocrit (48). Rigidity of red blood cells is increased in patients with high LDL cholesterol values, causing elevated blood viscosity. Low density lipoprotein apheresis improves the erythrocyte deformability, but how long this effect lasts after multiple procedures is unknown. In the study of Rubba et al. (48), 3 weeks after the last of six procedures for LDL apheresis, peak blood flow in the leg was significantly increased, whereas that in the forearm was unchanged. During our study, there was a small decrease in hematocrit in the LDL apheresis group, but at cardiac catheterization the hematocrit levels were not significantly different within and between both groups. The results of HMTT analysis after correction for change in hematocrit were virtually unchanged; we therefore decided to use the original data set for the entire analysis.

In our opinion, two of the four potential mechanisms are the most attractive explanations for the improved myocardial perfusion in the LDL apheresis group. 1) *The concept of the remodeling of the vessel wall.* It is conceivable that although lumen dimensions have not (yet) changed significantly, the total atherosclerotic burden of the vessel wall has decreased ("reversed Glagov sequence"), conditioning the vessel wall for an increased response to a muscle relaxant such as papaverine. In our study, lumen dimensions were assessed after administration of isosorbide dinitrate but without papaverine, whereas HMTT measurements were performed both after isosorbide dinitrate and papaverine. 2) *The improved rheologic condition caused by LDL apheresis.* The circumstantial evidence that viscosity remained unchanged between baseline and follow-up catheterization because of unchanged hematocrit and unchanged fibrinogen might be insufficient. Whole-blood viscosity is determined by many other factors, such as erythrocyte deformability and triglyceride and total cholesterol levels. Decreased whole-blood viscosity might have contributed to the improved maximal myocardial perfusion.

Further lipid-lowering studies should use intracoronary echocardiography to evaluate the total atherosclerotic burden and the existence of a "reversed Glagov sequence," combined with analysis of the vascular reaction to papaverine. Furthermore, analysis of whole-blood viscosity is needed to understand the basic interactions between lipid-lowering, viscosity,

endothelium-dependent and endothelium-independent vascular responses and myocardial perfusion.

Limitations of the study. The HMTT is inversely related to flow if the vascular volume remains constant: t = V/F, where t = mean transit time; F = flow; and V = vascular volume between the site of injection and point of measurement. Assessment of HMTT was based on the assumption that vascular volume after intracoronary papaverine administration was maximal and equal at baseline and follow-up cardiac catheterization. Theoretically, if vascular volume were smaller because of atherosclerosis and flow remained the same, a shorter mean transit time could also be found that erroneously could be interpreted as favorable. This type of error is very unlikely because vascular volume is mainly determined by arterioles and capillaries, vessels that are not involved in the atherosclerotic process. In addition, the unchanged mean segment diameter in this study supports the assumption that the vascular volume of the conduit vessels remained unchanged.

In this study, regional myocardial perfusion was assessed as the primary end point for flow evaluation. For patient-based HMTT values, regions from one patient were averaged to one HMTT value. However, in some patients only one region was available; in one patient even based on only one ROI. In the other patients, eight ROIs in three myocardial perfusion areas were available (including bypass evaluation). Therefore, patient-based HMTT values are not always representative of the entire myocardial perfusion in that patient.

Conclusions. Biweekly LDL apheresis plus simvastatin improved regional myocardial perfusion in patients with severe hypercholesterolemia and extensive coronary artery disease. In the control group, treated with lipid-lowering drugs only, regional myocardial perfusion remained stable. The improvement in myocardial perfusion is in agreement with that in ischemic threshold at exercise testing. The additional decrease in LDL cholesterol levels from LDL apheresis may be one explanation for the functional improvement in coronary circulation, although rheologic changes cannot be ruled out. Functional improvement may be due to endothelium-dependent or endothelium-independent changes, or both, in the coronary circulation. Further studies will be needed for elucidation of the exact mechanism. Calculation of the regional myocardial perfusion by DSA with videodensitometric calculation of the HMTT is feasible for evaluation of lipid-lowering trials. This method requires some minor modifications of the radiographic equipment and intensive instructions for the patient for breath holding. Assessment of regional myocardial perfusion in lipid-lowering studies has the benefit of combined evaluation of the epicardial coronary tree as well as the microcirculation.

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