Safety and effectiveness of long-term LDL-apheresis in patients at high risk

[Therapy And Clinical Trials]

Thiery, Joachim; Seidel, Dietrich

Institute of Clinical Chemistry, University Hospital Großhadern, Munich, Germany
Correspondence to Dietrich Seidel, Institute of Clinical Chemistry, University Hospital Großhadern, Marchioninistrasse 15, 81377 Munich, Germany

Since its introduction more than 10 years agod, LDL-apheresis has gained much interest and has proven its clinical utility in patients who cannot be adequately treated by diet and drug therapy alone. A number of clinical studies have clearly demonstrated that regular LDL-apheresis not only favourably influences the progression of coronary artery disease, but also decreases the incidence of cardiovascular events and prolongs survival time of coronary patients at high risk. Both hypercholesterolemia and hyperfibrinogenemia show a high prevalence in heart transplant recipients and seem to cause direct effects on survival time. Heparin-mediated extracorporeal LDL-precipitation-LDL-apheresis has proven to be very successful in this group of patients, which may be caused by the simultaneous removal of LDL, lipoprotein (a) and fibrinogen, and also because LDL-apheresis decreases the susceptibility of LDL to oxidation. In addition, there is clear clinical and experimental evidence that LDL-apheresis rapidly improves the endothelial-mediated vasomotion. Not unexpectedly, there are differences in specificity and side-effects between the systems used and these deserve more attention for future routine clinical use. Curr Opin Lipidol 9:521–526.

Introduction

There is clear clinical evidence that a drastic lowering of plasma LDL-cholesterol concentrations reduces significantly the rate of total and coronary mortality as well as the incidence of cardiovascular events in high risk hypercholesterolemic patients [1•]. In most patients, standard diet and treatment with 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase-inhibitors are adequate to reach the recommended plasma LDL-cholesterol levels of 100 mg/dl and below. However, in addition to patients with the rare form of homozygous hypercholesterolemia, a small group of high risk patients remains with elevated LDL-cholesterol levels despite maximal cholesterol lowering drug therapy. This group also

comprises patients who do not tolerate therapy with statins. These patients can be treated with high effectiveness by LDL-apheresis, an extracorporeal procedure for selective removal of LDL from the circulation [2].

Clinical utility of long-term LDL-apheresis

Lupien *et al.* [3] were the first to attempt the selective removal of LDL because plasma exchange requires substitution of plasma fractions with its inherent danger of infections and allergic reactions. Subsequently, several LDL-apheresis procedures with varying degrees of selectivity and effectiveness have been developed, some of which have gained successful clinical application. The procedures most commonly available today are the LDL-immunoadsorption, using immobilized sheep antibodies to apoprotein B_{100} [4], LDL adsorption to dextran sulfate attached to cellulose (DSCAL) [5] and the heparin-mediated extracorporeal LDL-precipitation (HELP) [6]. In addition to the drastic reduction of plasma LDL and lipoprotein (a) by the apheresis systems only the HELP procedure results in significant changes of hemorheological factors because of its simultaneous removal of plasma fibrinogen.

The apheresis systems were introduced in the early 1980s: immunoadsorption in 1981, dextran sulfate adsorption in 1985 and the heparin-mediated extracorporeal LDL-precipitation in 1986. Biocompatibility and effectiveness of the three apheresis procedures were recently compared in a small prospective cross-over [7]. All three LDL-apheresis methods effectively decreased total and LDL-cholesterol between 60% and 75%. High density lipoproteins and albumin were significantly reduced by the immunoadsorption procedure (-27%), but not by the DSCAL-and HELP-apheresis. HELP reduced plasma fibrinogen significantly (-58%), LDL-immunoadsorption showed no effect. As an unexpected result, a significant reduction of fibrinogen by the DSCAL procedure (-40%) was observed. However, this observation could not be confirmed in a recent large long-term study using the same apheresis system [8••].

In this study Gordon *et al.* described the long-term effects of LDL-apheresis in a trial with a follow-up of 5 years in 49 hypercholesterolemic patients (10 homozygous familial hypercholesterolemia, 39 heterozygous familial hypercholesterolemia [8••]). The study consisted of a 22-weeks controlled treatment period with an optional follow-up phase. Lipid lowering drug therapy was continued. A total of 3902 LDL-apheresis treatments were performed with a frequency of one procedure every 11±5 days for homozygotes and 14±6 days for heterozygotes. Twenty of the 49 patients discontinued the LDL-apheresis during the study period for reasons which were usually not attributed to complications of the therapy or to worsening of cardiovascular disease. The most common causes for discontinuation were inconvenience of the treatment and financial

reasons. Adverse clinical events occurred in 142 of the 3902 LDL-apheresis treatments performed (4%). The most common event was hypotension (0.9%).

It was reported previously [9] that the negative charges of dextran sulfate used for LDL-apheresis initiate the intrinsic coagulation pathway producing large amounts of bradykinin. Recent findings indicate that bradykinin generated during DSCAL-apheresis has pathophysiological effects via significant activation of the prostaglandin system (e.g. prostaglandin E_2) [10•]. This is of clinical relevance in some patients treated with the DSCAL procedure, since concomitant treatment with angiotensin-converting enzyme-inhibitors can lead to severe anaphylactoid reactions during DSCAL treatment by an excessive increase in bradykinin levels [11]. Such reactions were not observed during long-term HELP-apheresis or LDL-immunoadsorption.

The acute reductions of LDL-cholesterol (76% per DSCAL procedure) in both heterozygotes and homozygotes were maintained throughout the long-term follow-up of Gordon *et al.* [8••]. There were only minimal changes from baseline in pretreatment LDL- and HDL-cholesterol levels at years 2 and 4 in the patients with heterozygous familial hypercholesterolemia. However, there was a reduction in pretreatment LDL-cholesterol from a baseline level of 480 mg/dl to 410 mg/dl and 352 mg/dl at years 2 and 4, respectively, in patients with homozygous familial hypercholesterolemia.

A decrease of pretreatment LDL-cholesterol levels during long-term LDL-apheresis of a homozygous patient was also reported in a previous study using the HELP procedure [12]. It was also demonstrated that HMG-CoA reductase inhibitors are an effective adjunctive therapy in homozygous patients, once LDL cholesterol levels have already been lowered by regular LDL-apheresis [12,13•].

In the long-term study using the DSCAL system, 24 cardiovascular events in 49 patients occurred during the 5 years before initiation of LDL-apheresis (6.3 events/1000 patient-months of follow-up), whereas only seven events occurred during the 5 years of apheresis (3.5 events/1000 patient-months of follow-up). Although this remarkable 44% reduction in the rate of cardiovascular events was not statistically significant, the study was not designed and powered to measure clinical events and end points [8••]. We found a comparable clinical outcome in a 5 years follow-up observation trial using the HELP procedure. In 186 high risk patients with pre-existing coronary heart disease and hypercholesterolemia 55 myocardial infarctions occurred during 10 years before undergoing HELP-LDL-apheresis; 26 myocardial infarctions occurred 5 years before start of apheresis, whereas only four infarctions occurred during the 5 years follow-up of HELP-LDL-apheresis. This remarkable reduction of cardiovascular events in patients with pre-existing coronary heart disease is giving proof of the excellent clinical effectiveness of LDL-apheresis. Its

clinical utility and benefit to the patient is further substantiated by the low number of deaths as a result of heart disease in a large group of patients treated with the HELP procedure from July 1995 to August 1998. Four hundred and two hypercholesterolemic patients with coronary heart disease were treated with more than 27 000 apheresis procedures for 678±315 days and a treatment frequency of 10.7±3.7 days. Only six out of 402 patients at high coronary risk died under the HELP-apheresis treatment in this time period, five patients from coronary heart disease and one patient for unknown reasons. These results clearly demonstrate that regular LDL-apheresis not only favourably influences the progression of coronary artery disease [14,15,16•], but also decreases the incidence of cardiovascular events and prolongs the survival time of patients with coronary heart disease.

Recently, a new LDL adsorber-system with polyacrylate coated polyacrylamide gel (DALI) was described, which can eliminate LDL from whole blood without prior plasma separation [17•,18•]. In two small pilot studies biocompatibility and efficacy of the system were tested. According to the recommendations of the supplier, the DALI procedure should not be performed in patients with concomitant angiotensin-converting enzyme-inhibitor therapy because of the risk of severe hypotensive reactions. LDL-cholesterol was reduced by 45% and lipoprotein (a) by 43%. HDL-cholesterol, fibrinogen levels and blood cell counts were not significantly changed. However, the results of controlled clinical trials in larger patient populations have to be expected before routine clinical application of this interesting new technique can be recommended.

Effects of LDL-apheresis on LDL-oxidation

Oxidative modification of low density lipoproteins induces endothelial dysfunction such as induction of cell adhesion molecules, decrease of vasomotion and increased procoagulant activity [19•]. Modified and oxidized LDL are taken up by macrophages in the vessel wall and can lead to cholesterol storage with the formation of foam cells. Recently, several studies have demonstrated that LDL-apheresis alters the susceptibility of LDL to oxidation. LDL-apheresis rapidly improves the resistance of LDL to oxidation by a significant prolongation of the lag-time of copper-induced LDL-oxidation as estimated by the conjugated-diene absorbance at 234 nm [20,21,22•]. The precise mechanism of this effect is not clear. One explanation could be a possible change in the composition of LDL particle size distribution as a result of the removal of small dense particles, which are more susceptible to oxidation [23]. Another cause contributing to the enhanced resistance of LDL particles after apheresis may be a rise of antioxidative agents in the LDL-particles.

We have recently demonstrated [20] that plasmalogen phospholipids, which have been previously shown to enhance the oxidative resistance of LDL [24], significantly increased after HELP-apheresis. In contrast, the

vitamin E content of LDL was not altered by LDL-apheresis, whereas the total plasma vitamin E concentrations were significantly decreased by the concomitant removal of vitamin E with the LDL-particles from the circulation [20]. In a recent study including 26 patients undergoing HELP-apheresis, the resistance of LDL to oxidation (lag-time) after HELP-apheresis was closely related to a significant increase of plasmalogen phospholipids in the LDL-particles, whereas the association between the LDL-[alpha]-tocopherol content and the lag-time of LDL-oxidation was only weak [25•]. It can be suggested that the enrichment of LDL with plasmalogen phospholipids after apheresis prevents the formation of LDL-lyso-phosphatidylcholine, which has been shown to mediate many effects of oxidized LDL [19•]. The most pronounced plasmalogen effects were observed immediately after LDL-apheresis, and declined throughout the next 48 h.

Fatty acid composition of plasma lipoproteins can be altered by LDL-apheresis. An increase of oleic acid and a remarkable decrease in arachidonic acid after apheresis were reported [26], which may contribute to the decreased susceptibility of the post-apheresis-LDL particles to oxidation. Decreased LDL lipid peroxidation was also demonstrated in the long-term follow-up during repeated LDL-apheresis (DSCAL) in patients with homozygous familial hypercholesterolemia [22•].

These data provide substantial experimental evidence that LDL-apheresis not only decreases the plasma pool of LDL, but also alters the susceptibility of LDL to oxidation by compositional changes of the lipoprotein particles.

Effects of LDL-apheresis on vasomotion

There is new experimental and clinical evidence that hypercholesterolemia and elevated lipoprotein (a) levels can lead to an impairment of endothelium-dependent vasomotion [27,28•]. It has been suggested that oxidized LDL can impair the signal transduction between endothelial cell surface receptors and nitric oxide production leading to an inadequate production and availability of nitric oxide, which is followed by reduced vasodilatation, and that these effects can be ameliorated by lipid-lowering therapy [29]. Recently, it was demonstrated that a single LDL-apheresis shows a beneficial effect of the acetylcholine-induced endothelial-dependent vasomotion in peripheral arteries [30•,31••].

In patients with hypercholesterolemia LDL-apheresis significantly improved forearm blood flow in response to acetylcholine without changes in the endothelium independent vasodilatation response to sodium nitroprusside. The production of nitric oxide was significantly potentiated. Furthermore, plasma levels of oxidized LDL decreased significantly after apheresis and showed an inverse correlation to the degree of acetylcholine-induced vasodilatation and nitric oxide production. Thus, it

can be assumed that the fast and drastic removal of LDL from the circulation, as well as the enhanced resistance of LDL to oxidation by apheresis, may potentiate the production of endothelium-derived relaxing factors. Interestingly, the plasma endothelin levels increased after the apheresis despite the amelioration of endothelial function [31••]. Recently, a case of a patient was reported [32•] who showed a remarkable improvement of myocardial perfusion after 6 months of HELP-apheresis as assessed by exercise thallium-201 single-photon emission computed tomography, accompanied by an improvement in radionuclide ventriculography parameters. This patient, a 69-year-old woman with familial hypercholesterolemia, had suffered from myocardial infarction and underwent coronary bypass surgery. She was treated first for 20 months with immunoadsorption LDL-apheresis. Thereafter, the treatment was changed to the HELP procedure. The marked improvement in myocardial perfusion after 6 months of HELP-apheresis indicates that the additional reduction of plasma fibrinogen may contribute to the better perfusion of the myocardium by favourably influencing blood rheology.

Similar findings were reported in the LDL-Apheresis Atherosclerosis Regression Study (LAARS) comparing the effects of biweekly LDL-apheresis (DSCAL) plus simvastatin versus simvastatin alone on regional myocardial perfusion assessed by digital subtraction angiography (hyperemic transit time of contrast medium) [33]. After 2 years of treatment, regional myocardial perfusion improved only in the LDL-apheresis group and remained unchanged in the medication group.

Very recently, a significant 30% improvement in coronary vasodilatation capacity was reported within 24 h after a single HELP-apheresis treatment as assessed by positron emission tomography (PET) [34••]. In nine patients with coronary heart disease and hypercholesterolemia, PET was carried out immediately before and 18–20 h after HELP-apheresis. LDL-cholesterol levels before the first and second PET examinations were 194±38 mg/dl and 81±20 mg/dl, respectively, plasma fibrinogen levels 258±42 mg/dl and 144±39 mg/dl, respectively. Myocardial blood flow following dipyridamole administration increased after LDL-apheresis from 173–226 ml/min per 100 g ($P<0.01$). Calculated coronary flow reserve was improved from 1.91–2.48 ($P<0.02$). In addition, the minimum coronary resistance fell from 0.61–0.43 mmHg/min per 100 g per ml ($P<0.01$) [34••].

This interesting finding gives additional clinical evidence that short-term LDL-apheresis using the HELP procedure enhances the myocardial perfusion by an immediate improvement of impaired endothelial-dependent vasodilatation and additive hemorheologic effects. Therefore, the short- and long-term application of LDL-apheresis opens new therapeutic perspectives, particularly for patients with end-stage coronary artery disease, unstable angina and otherwise untreatable coronary heart disease. However, differences in the systems must be considered.

Clinical utility of long-term LDL-apheresis in heart transplant recipients

The major cause of morbidity and mortality in heart transplant recipients (HTX) in long-term follow-up is the development of accelerated graft vessel disease with an incidence of 5% to 10% per year [35]. The precise pathogenesis of graft vessel disease is still uncertain. However, at least three main factors appear to be involved: (1) multiple rejection episodes with T-lymphocyte infiltration into the coronary arteries; (2) elevated plasma fibrinogen levels leading to impaired microcirculation and inadequate oxygen supply in the graft tissue; and (3) elevated plasma LDL-cholesterol levels leading to the development of atherosclerotic lesions with accumulation of cholesterol-loaded foam cells. Both hypercholesterolemia and hyperfibrinogenemia show a high prevalence in HTX-recipients [36,37]. Recently, two randomized clinical trials demonstrated an impressive survival benefit in HTX-recipients by lowering LDL-cholesterol plasma levels by statins [38,39•]. However, because of possible adverse interactions of statins in conjunction with the regular immunosuppressive therapy, the application of adequate doses of statins in transplant recipients with severe hypercholesterolemia is limited. However, there are no efficient drugs which normalize elevated plasma fibrinogen levels to strongly enhance the risk of graft vessel disease in these patients. In order to reduce the plasma fibrinogen and LDL-cholesterol concentrations and to minimize the development of graft vessel disease we initiated a long-term 'intention to treat' study using the HELP procedure [40]. To date, more than 1000 HELP treatments in 21 HTX-patients were performed and well-tolerated. Compared to baseline values, the mean interval LDL-cholesterol levels were reduced by 50%, plasma fibrinogen by 38% and lipoprotein (a) by 32%. Blood cyclosporine A levels were not significantly affected by the HELP procedure, although a small amount of cyclosporine A, which binds to LDL (less than 5% of the plasma level) is eliminated together with the LDL particles by apheresis. However, this did not affect the therapeutic blood levels. In a recent pilot study consisting of 10 heart transplant recipients with elevated plasma fibrinogen levels, patients were treated with simvastatin and in addition with regular HELP-apheresis for 3 years. Only one patient developed significant graft vessel disease as assessed by annual coronary angiography. In comparison, seven out of 10 HTX-patients in a matched control group without apheresis treatment showed signs of transplant vasculopathy [41•].

In a recent secondary prevention trial Park *et al.* [42•] demonstrated that chronic LDL-apheresis with the HELP procedure can lead to regression of pre-existing graft vessel disease. Initially, all HTX-patients ($n=8$) had survived at least 2 years after transplantation, but had already developed graft vessel disease. Mean LDL-cholesterol levels were 210 mg/dl despite treatment with pravastatin. During a treatment consisting of 22 months of weekly HELP-apheresis, the mean plasma LDL-cholesterol, lipoprotein (a)

and fibrinogen levels fell from 210, 35 and 312 mg/dl to mean interval values between two apheresis treatments of 126, 25 and 260 mg/dl, respectively. The quantitative analysis of coronary angiograms performed 16 months before, at the time of initiation and 22 months after start of apheresis treatment showed a significant improvement of the luminal diameter of the stenosis.

The results of these pilot studies suggest that long-term LDL-apheresis with the HELP procedure is a safe and effective measure to prevent the development and to induce regression of graft vessel disease in heart transplant recipients at high risk.

New therapeutic applications of LDL-apheresis

The impressive and safe cholesterol lowering effects in coronary and in heart transplant patients, as well as the improvement of endothelial function, hemorheology and LDL-oxidation, open additional therapeutic applications in patients suffering from other ischemic manifestations: e.g. impaired cerebrovascular flow reserve, stroke, acute retinal ischemia, sudden loss of hearing, early occlusion of bypass grafts, acute pancreatitis and periphereal vascular disease. Aggressive cholesterol lowering with LDL-apheresis and statins can also reduce the extent of periphereal vascular disease in the carotids as well as in the arteries of the lower limbs [43,44,]. In addition, a recent pilot study with short-term LDL-apheresis using the HELP system in patients with sudden loss of hearing showed first promising results suggesting the clinical utility of LDL-apheresis in microvascular disorders [45•].

Conclusion

Several trials designed to assess the effect of long-term LDL-apheresis in combination with statins in the therapy of chronic coronary heart disease clearly demonstrated clinical benefits. More recent studies have also shown that graft vascular disease after heart transplantation can be prevented by chronic extracorporeal removal of LDL and fibrinogen from plasma. In addition, there is clear clinical and experimental evidence that LDL-apheresis rapidly improves endothelial-mediated vasomotion and decreases the susceptibility of LDL to oxidation. Because of differences in the technology of the various systems available, side-effects deserve future attention and more clinical experience may provide further insights into the acute effects of LDL-apheresis on both plaque stabilization and improvement of microcirculatory disorders.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest

•• of outstanding interest

1. •Braunwald E. Shattuck lecture–cardiovascular medicine at the turn of the millenium: triumphs, concerns, and opportunities. N Engl J Med 1997;337:1360–1369. This review provides an overview concerning the prevention of coronary artery disease and the decrease in coronary mortality by effective modification of risk factors, particularly by drastic reduction of plasma LDL cholesterol levels.

2.Gordon BR, Saal SD. Current status of low density lipoprotein-apheresis for the therapy of severe hyperlipidemia. Curr Opin Lipidol 1996;7:381–384.

3.Lupien PJ, Moorjani S, Awad J. A new approach to the management of familial hypercholesterolemia: removal of plasma-cholesterol based on the principle of affinity chromatography. Lancet 1976;1:1261–1265.

4.Stoffel W, Borberg H, Greve V. Application of specific extracorporeal removal of low density lipoproteins in familial hypercholesterolemia. Lancet 1981;2:1005–1007.

5.Yokoyama S, Hayashi R, Satani M, Yamamoto A. Selective removal of low density lipoprotein by plasmapheresis in familial hypercholesterolemia. Arteriosclerosis 1985;5:613–622.

6.Eisenhauer Th, Armstrong VW, Wieland H, Fuchs C, Nebendahl K, Scheler F, Seidel D. Selective continuous elimination of low density lipoproteins (LDL) by heparin precipitation: first clinical application. ASAIO-Trans 1986;32:104–107.

7.Schaumann D, Welch-Wichary M, Voss A, Schmidt H, Olbricht CJ. Prospective cross-over comparisons of three low-density lipoprotein (LDL)-apheresis methods in patients with familial hypercholesterolemia. Eur J Clin Invest 1996;26:1033–1038.

8. ••Gordon BR, Kelsey SF, Dau PC, Gotto AM Jr, Graham K, Illingworth DR. Long-term effects of low-density lipoprotein apheresis using an automated dextran sulfate cellulose adsorption system. Liposorber Study Group. Am J Cardiol 1998;81:407–411. This study reports on the long-term safety, lipid lowering and clinical efficacy of LDL-apheresis using a dextran sulfate cellulose adsorption column technique for a 5-year period in patients with heterozygous and homozygous familial hypercholesterolemia who were inadequately controlled by drug therapy. The impressive decrease of cardiovascular events during the period of apheresis treatment compared with the high number of events during the 5 years before initiation of LDL-apheresis clearly demonstrates the clinical benefit of long-term apheresis in patients at high risk.

9.Kojima S, Harada-Shiba M, Nomura S, Kimura G, Tsushima M, Kuramochi M. Effect of nafamostat mesilate on bradykinin generation during low-density lipoprotein apheresis using a dextran sulfate cellulose column. ASAIO-Trans 1991;37:644–648.

10. •Kojima S, Ogi M, Yoshitomi Y, Kuramochi M, Ikeda J, Naganawa M, Hatakeyama H. Changes in bradykinin and prostaglandins plasma levels during dextran-sulfate low-density apheresis. Int J Artif Organs 1997;20:178–183. This study supports the view that in patients taking angiotensin-converting enzyme inhibitors, the anaphylactoid reaction occurring during apheresis using dextran sulfate cellulose adsorption column technique may be caused by an excessive

rise in bradykinin levels via activation of the prostaglandin system, particularly of prostaglandin E2.

11.Olbricht CJ, Schaumann D, Fischer D. Anaphylactoid reactions, LDL-apheresis with dextran sulphate, and ACE inhibitors. Lancet 1992;340:908–909.

12.Thiery J, Walli AK, Janning G, Seidel D. Low-density lipoprotein plasmapheresis with and without lovastatin in the treatment of the homozygous form of familial hypercholesterolemia. Eur J Pediatr 1990;149:716–721.

13. •Marais AD, Naumova RP, Firth JC, Penny C, Neuwirth CK, Thompson GR. Decreased production of low density lipoprotein by atorvastatin after apheresis in homozygous familial hypercholesterolemia. J Lipid Res 1997;38:2071–2078. A new study on the mechanism of action of atorvastatin in patients with homozygous familial hypercholesterolemia undergoing LDL-apheresis suggesting that atorvastatin decreases the rate of produciton of LDL.

14.Schuff-Werner P, Gohlke H, Bartmann U, Baggio G, Corti MC, Dinsenbacher A. The HELP-LDL-apheresis multicentre study, an angiographically assessed trial on the role of LDL-apheresis in the secondary prevention of coronary heart disease. II. Final evaluation of the effect of regular treatment on LDL-cholesterol plasma concentrations and the course of coronary heart disease. The HELP-Study Group. Eur J Clin Invest 1994;24:724–732.

15.Kroon AA, Aengevaeren WR, van der Werf T, Uijen GJ, Reiber JH, Bruschke AV, Stalenhoef AF. LDL-Apheresis Atherosclerosis Regression Study (LAARS). Effect of aggressive versus conventional lipid lowering treatment on coronary atherosclerosis. Circulation 1996;93:1826–1827.

16. •Donner MG, Richter WO, Schwandt P. Long term effect of LDL-apheresis on coronary heart disease. Eur J Med Res 1997;2:270–274. A study comparing the long-term safety and efficacy of three LDL-apheresis procedures.

17. •Bosch T, Schmidt B, Kleophas W, Gillen C, Otto V, Passlick-Deetjen J, Gurland HJ. LDL hemoperfusion – a new procedure for LDL apheresis: first clinical application of an LDL adsorber compatible with human whole blood. Artif Organs 1997;21:977–982. This is a pilot study demonstrating the clinical tolerance and biocompatibility of the DALI-apheresis system, which removes LDL from the whole blood without previous plasma separation.

18. •Stefanutti C, DiGiacomo S, Vivenzio A. Comparison of different LDL-apheresis techniques. Artif Organs 1998;21:66–71. Study of different LDL-apheresis procedures including the recent DALI system.

19. •Berliner J, Leitinger N, Watson A, Huber J, Fogelman A, Navab M. Oxidized lipids in atherogenesis: formation, destruction and action. Thrombo Haemost 1997;78:195–199. An excellent review on the role of oxidatively modified lipoproteins in atherogenesis.

20.Bräutigam C, Engelmann B, Reiss, Reinhardt U, Thiery J, Richter WO, Brosche T. Plasmalogen phospholipids in plasma lipoproteins of normolipidemic donors

and patients with hypercholesterolemia treated by LDL apheresis. Atherosclerosis 1996;119:77–88.

21.Leitinger N, Pirisch C, Blazek I, Endler G, Sinzinger H. Decreased susceptibility of low-density lipoproteins to in-vitro oxidation after dextran-sulfate LDL-apheresis treatment. Atherosclerosis 1996;126:305–312.

22. •Napoli C, Ambrosio G, Scarpato N, Corso G, Palumbo G, D'Armiento FP. Decreased low-density lipoprotein oxidation after repeated selective apheresis in homozygous familial hypercholesterolemia. Am Heart J 1997;133:585–595. This study supports previous observations that LDL-apheresis reduces the susceptibility of LDL to oxidation.

23.de Graaf J, Hak-Lemmers HL, Hectors MP, Demnacker PN, Hendriks JC, Stalenhoef AF. Enhanced susceptibility to *in vitro* oxidation of the low density lipoprotein subfraction in healthy subjects. Arterioscler Thromb 1991;11:298–306.

24.Engelmann B, Bräutigam C, Thiery J. Plasmalogen phospholipids as potential protectors against lipid peroxidation of low density lipoproteins. Biochem Biophys Res Commun 1994;204:1235–1242.

25. •Hahnel D, Thiery J, Engelmann B. Plasmalogens contribute to the enhanced oxidation resistance of LDL after LDL-apheresis (HELP). European Atherosclerosis Society 70th EAS Congress, Geneva, Switzerland, 6–9 September [abstract]. This study demonstrates for the first time that the augmented resistance of LDL following LDL-apheresis is closely related to the increase in LDL plasmalogen contents.

26.Engelmann B, Bräutigam C, Kulschar R, Duhm J, Prenner E, Hermetter A. Reversible reduction of phospholipid bound arachidonic acid after low density lipoprotein apheresis. Evidence for rapid incorporation of plasmalogen phosphatidylethanolamine into the red blood cell membrane. Biochim Biophys Acta 1994;1196:154–164.

27.Zeiher AM, Drexler H, Saurbier B, Just H. Endothelium-mediated coronary blood flow modulation in humans. Effects of age, atherosclerosis, hypercholesterolemia, and hypertension. J Clin Invest 1993;92:652–662.

28. •Schachinger V, Halle M, Minners J, Berg A, Zeiher AM. Lipoprotein (a) selectively impairs receptor-mediated endothelial vasodilator function of the human coronary circulation. J Am Coll Cardiol 1997;30:927–934. This interesting study shows for the first time that elevated lipoprotein(a) levels are associated with an impaired vasodilator capacity of the coronary circulation.

29.Anderson TJ, Meredith IT, Yeung AC, Frei B, Selwyn AP, Ganz P. The effect of cholesterol-lowering and antioxidant therapy on endothelium-dependent coronary vasomotion. N Engl J Med 1995;332:488–493.

30. •Stadler RW, Ibrahim SF, Lees RS. Peripheral vasoactivity in familial hypercholesterolemic subjects treated with heparin-induced extracorporeal LDL-precipitation (HELP). Atherosclerosis 1997;128:241–249. This study indicated

that long-term LDL-apheresis improves brachial artery vasoactivity after a 5 min forearm occlusion.

31. ••Tamai O, Matsuoka H, Itabe H, Wada Y, Kohno K, Imaizumi T. Single LDL apheresis improves endothelium-dependent vasodilation in hypercholesterolemic humans. Circulation 1997;95:76–82. This interesting pilot study demonstrated an improvement in endothelium-dependent vasomotion in the human forearm vessel of patients with hypercholesterolemia by a single session of LDL-apheresis.

32. •Jovin IS, Taborski U, Müller KD, Müller-Berghaus G. Improvement of myocardial perfusion after low-density lipoprotein apheresis treatment. Eur Heart J 1997;18:531–532. This case report describes an improvement in myocardial perfusion after drastic reduction of plasma LDL and fibrinogen using the HELP procedure.

33.Aengevaeren WR, Kroon AA, Stalenhoef AF, Uijen GJ, van der Werf T. Low density lipoprotein apheresis improves regional myocardial perfusion in patients with hypercholesterolemia and extensive coronary artery disease. LDL-Apheresis Atherosclerosis Regression Study (LAARS). J Am Coll Cardiol 1996;28:1696–1704.

34. ••Mellwig KP, Baller D, Gleichmann U, Moll D, Betker S, Weise R, Notohamiprodjo G. Improvement of coronary vasodilatation capacity through single LDL-apheresis. Atherosclerosis 1998;139:173–178. This elegant study demonstrated for the first time that the acute reduction of LDL, lipoprotein(a) and fibrinogen by a single HELP-apheresis procedure leads to an improvement in coronary vasodilatation assessed by PET recording in patients with severe hypercholesterolemia and coronary artery disease.

35.Gao SZ, Schroeder JS, Alderman EL, Hunt SA, Valantine HA, Wiederhold V, Stinson EB. Prevalence of accelerated coronary artery disease in heart transplant survivors. Comparison of cyclosporine and azathioprine regimens. Circulation 1989;80:100–105.

36.Ballantyne CM, Rodovancevic B, Farmer JA, Frazier OH, Chandler L, Payton-Ross C. Hyperlipidemia after heart transplantation: report of a 6-year experience, with treatment recommendations. J Am Coll Cardiol 1992;19:1315–1321.

37.Hunt BJ, Segal H, Yacoub M. Hemostatic changes in heart transplant recipients and their relationship to accelerated coronary sclerosis. Transplantation 1993;55:309–315.

38.Kobashigawa JA, Katznelson S, Laks H, Johnson JA, Yeatman L, Wang XM, Chia D. Effect of pravastatin on outcomes after cardiac transplantation. N Engl J Med 1995;333:621–627.

39. •Wenke K, Meiser B, Thiery J, Nagel D, von Scheidt W, Steinbeck G. Simvastatin reduces graft vessel disease and mortality after heart transplantation: a four year randomized trial. Circulation 1997;96:1398–1402. In a 4-year prospective randomized study with heart transplant recipients,

simvastatin treatment led to a significant survival benefit compared with dietary measures alone.

40.Wenke K, Thiery J, Arndtz N, Meiser BM, Seidel D, Reichart B. Treatment of hypercholesterolemia and prevention of coronary artery disease after heart transplantation by combination of low dose simvastatin and HELP-LDL-apheresis. Transplant Proc 1992;24:2674–2676.

41. •Jaeger BR, Meiser B, Nagel D, Überfuhr P, Thiery J, Brandl U. Aggressive lowering of fibrinogen and cholesterol in the prevention of graft vessel disease after heart transplantation. Circulation 1997;96:154–158. This pilot study indicated that LDL-apheresis using the HELP procedure can prevent the development of graft vessel disease in heart transplant patients at high risk.

42. •Park JW, Merz M, Braun P. Regression of transplant coronary artery disease during chronic low-density lipoprotein apheresis. J Heart Lung Transplant 1997;16:290–297. This study showed for the first time that long-term LDL-apheresis using the HELP procedure can lead to regression of transplant coronary artery disease in heart transplant recipients.

43.Kroon AA, van Asten WN. Effect of apheresis of low density lipoprotein on peripheral vascular disease in hypercholesterolemic patients with coronary artery disease. Ann Intern Med 1996;125:945–954.

44.Takagi M, Yamada T, Yamagucji H, Hashiyada H, Narimatsu M, Shibata R. The role of low-density lipoprotein apheresis as postoperative care of bypass grafting for chronic arterial occlusion. Cardiovasc Surg 1996;4:459–465.

45. •Suckfüll M, Thiery J, Wimmer C, Mees K, Schorn K. Hypercholesterolemia and hyperfibrinogenemia in sudden deafness. Laryngorhinootologie 1997;76:453–457. This study indicated that drastic lowering of plasma LDL-cholesterol and fibrinogen is an effective tool in the treatment of patients with sudden hearing loss and impaired microcirculation.