Kidney International, Vol. 51 (1997), pp. 1608–1612

Conversion from cyclosporine A to azathioprine treatment improves LDL oxidation in kidney transplant recipients

MARINUS A. VAN DEN DORPEL, HOSAM GHANEM, JACQUELINE RISCHEN-VOS, ARIE J. MAN IN'T VELD, HANS JANSEN, and WILLEM WEIMAR

Departments of Internal Medicine I and III, University Hospital Rotterdam and Department of Biochemistry, Erasmus University, Rotterdam, The Netherlands

Conversion from cyclosporine A to azathioprine treatment improves LDL oxidation in kidney transplant recipients. The use of the immunosuppressant cyclosporine A (CsA) after transplantation has been associated with less favorable plasma lipid profiles, which may contribute to the high incidence of cardiovascular morbidity and mortality in transplant recipients. Recent studies have suggested that oxidative modification of LDL plays an important role in the initiation and progression of atherosclerosis. It has also been demonstrated that CsA may facilitate lipid peroxidation *in vitro* and *in vivo*. Therefore, we determined several parameters of LDL oxidizability in renal transplant recipients who were switched from CsA to azathioprine (AZA)-based immunosuppressive treatment. The susceptibility of LDL to *in vitro* oxidation, LDL particle size, plasma titers of IgG and IgM antibodies against oxidized LDL and plasma LDL subclass patterns in 19 renal transplant recipients were determined during CsA treatment and 16 weeks after these patients were converted to AZA treatment. In addition, mean arterial pressure was recorded, and glomerular filtration rate and renal blood flow were estimated from the clearance of radiolabeled thalamate and hippurate. After conversion, the plasma concentrations of total cholesterol, LDL cholesterol and triglyceride decreased, while plasma HDL cholesterol did not change. During CsA therapy plasma LDL was significantly more susceptible to *in vitro* oxidation than during AZA, as reflected by a longer lag phase during *in vitro* oxidation (98.9 ± 24.3 vs. 114.7 ± 17.3 min, $P = 0.031$). In addition, the LDL size increased (236.5 ± 7.3 vs. 240.7 ± 6.8 nm, $P = 0.00001$), and the titers of IgM- and IgG-autoantibodies against oxidized LDL decreased significantly after patients were converted from CsA to AZA. The more atherogenic LDL subclass pattern B was present in 13 out of 19 patients during CsA. In five patients, pattern B changed into pattern A after conversion. The subclass B pattern was maintained in eight patients and subclass A pattern in six patients. In all patients the lag time of *in vitro* LDL oxidation increased, although the biggest changes were found in those patients in whom the LDL subclass changed from pattern B to pattern A. Mean arterial pressure decreased and renal function improved significantly after conversion. No correlation between parameters of lipid peroxidation and changes in blood pressure or renal function upon conversion, underlying renal disease, time since transplantation, or antihypertensive treatment was found. Our study demonstrates that treatment with CsA increases the susceptibility of LDL to *in vitro* oxidation, and also enhances the oxidation of LDL *in vivo*. In addition, conversion to AZA results in a more favorable lipid profile, which in combination with a lower arterial pressure and better renal function may decrease the risk for atherosclerosis. These factors may account for the cardiovascular complications during CsA treatment after organ transplantation, and also when CsA is used for other diseases.

Received for publication December 20, 1995
and in revised form November 27, 1996
Accepted for publication November 27, 1996

The use of cyclosporine A (CsA) has significantly improved graft survival after organ transplantation [1]. Unfortunately, patients treated with CsA often develop hypertension and hyperlipidemia [2–6]. These factors may contribute to the development of cardiovascular disease, which is the most important cause of death after renal transplantation [7]. It has been found that the use of CsA is associated with a more atherogenic plasma lipid profile [4, 8, 9]. CsA raises plasma cholesterol concentration, mainly by increasing the plasma low-density lipoprotein (LDL) cholesterol fraction, without affecting plasma high-density lipoprotein (HDL) cholesterol [3, 8]. CsA also influences lipoprotein properties and appears to increase lipid peroxidation [10]. The susceptibility of low density lipoproteins to oxidation is also increased by CsA, the lag phase of *in vitro* LDL oxidation being inversely correlated with the amount of CsA in LDL [11]. Moreover, in kidney transplant recipients on CsA the prevalence of small-dense LDL (corresponding with LDL subclass pattern B), which is more susceptible for oxidation than large LDL (corresponding with LDL subclass pattern A), is increased [12]. Since Taylor and coworkers demonstrated that overall lipid peroxidation is increased in kidney transplant recipients on CsA [13], these factors together may promote LDL oxidation *in vivo*. This is also suggested by the presence of increased autoantibodies against malondialdehyde LDL in kidney transplant recipients on CsA [12].

Oxidative modification of LDL is thought to play a crucial role in the onset and progression of atherosclerotic disease [14]. Oxidized LDL contains novel epitopes, which act as chemoattractants for monocyte and macrophage migration, thus stimulating the formation of foam cells [15]. Oxidized LDL appears to be toxic for endothelial cells. Galle and coworkers found that after acute dosing of CsA to rats, the development of CsA-mediated renal dysfunction was associated with lipid peroxidation [16]. Furthermore, oxidized low density lipoproteins inhibit the activity of inducible NO-synthase inactivated macrophages *in vitro* [17, 18]. This reduction in NO production may influence cell-to-cell interactions and vasomotor tone. In previous studies, conversion from CsA to azathioprine (AZA)-based immunosuppression led to the reduction of plasma triglyceride and cholesterol levels [19]. As plasma triglycerides are strongly related to LDL size and LDL size is related to the susceptibility of LDL to oxidation, LDL oxidation may also be influenced during conversion from CsA to AZA.

To investigate the effects of conversion of CsA to AZA on lipid

peroxidation, we determined plasma lipid profiles and parameters of *in vitro* and *in vivo* LDL oxidation in renal transplant recipients before and 16 weeks after discontinuation of CsA and replacement by AZA. In addition, renal hemodynamic parameters and arterial pressure were measured on both occasions.

Methods

Patients

Patients were recruited among renal transplant recipients who were enrolled in a prospective randomized clinical trial, which was designated to evaluate the effects of two different immunosuppressive regimens, that is, prednisone combined with CsA or with AZA, on long-term graft function and incidence of rejection episodes. For this trial all CsA-treated renal transplant recipients between 18 and 65 years of age, who were six months or longer after transplantation, were randomly allocated to either continuation of CsA treatment, or conversion from CsA- to AZA-based immunosuppression. Patients with diabetes mellitus, proteinuria over 3 grams per day, previous acute graft rejection, or histological evidence of chronic graft rejection were excluded. The first 19 consecutive patients fulfilling these criteria and willing to give written informed consent were studied.

Study design

The first study was performed while patients were on CsA and the second study 16 weeks later, when patients were on AZA therapy. During both studies the patients used the same dose of prednisone. All antihypertensive medication (beta blockers in 13 patients and calcium channel blockers in 8 patients) was discontinued three days prior to both study sessions. After the first study AZA was started at a dose of 2 mg/kg daily. Two weeks later the CsA dose was reduced to 50%, and the prednisone dose was increased to 30 mg daily. After another two weeks CsA was withdrawn, and the prednisone was tapered to the baseline dose within three weeks. If necessary, AZA dose adjustments were made according to the white blood cell count and hematocrit.

On both study days patients arrived in the cardiovascular research laboratory after an overnight fast. At arrival they were weighed and subsequently a small catheter (Venflon; Viggo Spectramed, Helsingborg, Sweden) was inserted in a forearm vein of each arm. One catheter was used for the infusion of radiolabeled thalamate and hippuran for renal function studies, and the other catheter was used for blood sampling. The study protocol was approved by the local Medical Ethics Committee.

Measurements

Renal hemodynamics. Effective renal plasma flow (ERPF) and glomerular filtration rate (GFR) were estimated from the clearance of ^{131}I-hippuran and ^{125}I-thalamate as desribed earlier [20]. During the renal function studies arterial pressure was measured by an automated oscillometric device (AccuTorr2; Datascope Corp., Montvale, NJ, USA). Renal blood flow (RBF) was calculated by dividing effective renal plasma flow by (1 − hematocrit). Before the start of the renal function studies a blood sample was taken for the determination of CsA 12-hour trough blood levels (CycloTrac SP; IncStar Corp., Stillwater, MN, USA).

Blood sampling. Blood samples were drawn into EDTA-containing polypropylene tubes (final concentration EDTA, 1 mg/ml) on ice. Plasma was obtained by low speed centrifugation at 4°C. To the plasma, sucrose (10 μl sucrose solution per ml plasma) was added to a final concentration of 0.6% to prevent LDL aggregation [21]. Plasma was stored at −80°C until use.

Separation of plasma lipoproteins

Low density lipoproteins were isolated by density gradient ultracentrifugation as described by Redgrave, Roberts and West [22]. To prevent oxidation of the lipoproteins during ultracentrifugation, 0.1 mM EDTA and 0.005% thiomersal were added to the gradient solutions. Before use, the solutions were gassed by nitrogen to remove the oxygen. All runs were for 24 hours at 15°C and 40,000 rpm in an SW 41 TI Beckman rotor using polyallomere tubes. HDL cholesterol was determined after precipitation of apoB containing lipoproteins with heparin/$MnCl_2$ [23]. The plasma LDL cholesterol concentration was calculated using the Friedewald et al formula [24].

Low density lipoprotein oxidation

LDL oxidation experiments were carried out using the procedure of Esterbauer [25] monitoring the generation of lipid peroxidation products in the presence of 1.66 mM $CuCl_2$ at 254 nm, as described before [12]. The lag time was defined as the interval between initiation of the reaction and the intercept of the tangent of the slope of the absorbance curve with the time scale axis expressed in minutes.

LDL size and subclass determination

The LDL subclass patterns were identified by electrophoresis on 2 to 16% PAGE gels, as described by Austin et al [26]. The gels were prepared with an LKB 11300 Ultrograd gradient mixer [27]. In each gel, reference sera with known a subclass were applied to lane one and six of a total 12 lanes. The gels were stained with Oil Red O for lipid and the subclass pattern determination. For the determination of the LDL size, a set of standard proteins with known hydrated diameters was run on the same gel as the samples. The standard proteins (HMW electrophoresis calibration kit; Pharmacia, Piscataway, NJ, USA) were thyroglobulin (170 Å), ferritin (122 Å) and catalase (104 Å). The gels were stained with Coomassie Brilliant Blue R 250. The center of the most prominent LDL band was marked on the gel. The migration distance of the bands from the top of the gel was measured. The average LDL particle diameter was estimated from a quadratic extrapolation of a plot of the logarithm of the diameter of the standards versus the migration distance of the standards [28]. The subclass pattern A was assigned to LDL particles with a diameter $>$ 238 nm and the subclass pattern B to LDL particles with a diameter $<$ 238 nm. The cutoff level was set at 238 nm because this produced the best separation of the two subclasses on the gels after electrophoresis [29].

Autoantibodies against malondialdehyde-modified LDL

Plasma titers of IgG and IgM autoantibodies against MDA-modified LDL were determined by ELISA as described elsewhere [29]. The antibody titer was defined as the absorbance of the wells coated with MDA-LDL divided by the absorbance of the wells coated with native LDL for each plasma sample [30].

Other analytical methods

Plasma cholesterol and triglycerides (Boehringer Mannheim, Mannheim, Germany) and creatinine (Sigma Diagnostics, St.

Table 1. Clinical characteristics of 19 renal transplant recipients before and after conversion from CsA to AZA

	CsA	AZA	*P*
Male/female	13/6		
MAP[a] *mm Hg*	116 ± 14	109 ± 12	0.0003
Post-Tx time *months*	24 ± 5	27 ± 5	
Body weight *kg*	75.7 ± 15.4	76.9 ± 15.2	0.757
Body mass index kg/m^2	24.9 ± 3.5	25.1 ± 3.3	0.696
Prednisone dose *mg/day*	9.6 ± 2.1	9.6 ± 2.1	0.967
CsA dose *mg/kg/day*	5.5 ± 1.7	—	
CsA 12-hr trough level *ng/ml*	250 ± 66	—	
AZA dose *mg/kg/day*	—	1.8 ± 0.4	
GFR[b] *ml/min*	50 ± 14	57 ± 19	0.047
RBF[c] *ml/min*	377 ± 97	431 ± 150	0.042
RVR[d] *mm Hg/ml/min*	0.346 ± 0.108	0.283 ± 0.116	0.016
Antihypertensives per patient	1.3 ± 0.7	0.3 ± 0.6	0.0001

[a] Mean arterial pressure
[b] Glomerular filtration rate/m^2 body surface area
[c] Renal blood flow
[d] Renal, vascular resistance

Table 2. Plasma lipid concentrations before and after conversion from CsA to AZA

	CsA	AZA	*P*
Total cholesterol *mmol/liter*	5.9 ± 0.9	5.0 ± 0.7	0.002
LDL cholesterol *mmol/liter*	3.7 ± 0.7	3.0 ± 0.6	0.001
HDL cholesterol *mmol/liter*	1.1 ± 0.4	1.2 ± 0.4	0.340
Total triglyceride *mmol/liter*	2.4 ± 1.0	1.7 ± 0.6	0.001

Table 3. Parameters of *in vitro* and *in vivo* lipid peroxidation before and after conversion from CsA to AZA

	CsA	AZA	*P*
LDL particle size *nm*	236.5 ± 7.3	240.7 ± 6.8	0.00001
LDL subclass pattern A/B	6/13	11/8	0.191
Lag time *min*	98.9 ± 24.3	114.7 ± 17.3	0.031
IgG anti-MDA-LDL titer	1.24 ± 0.15	1.19 ± 0.14	0.028
IgM anti-MDA-LDL titer	2.02 ± 0.29	1.78 ± 0.19	0.0001

Louis, MO, USA) were determined using commercially available test kits.

Statistical analysis. All data are presented as means ± SD. Data before and after conversion were compared by a paired Student's *t*-test. Correlations between variables were calculated using the Pearson correlation test. The level of significance was set at $P < 0.05$.

Results

Patient characteristics

The clinical characteristics of the patients during both treatment periods are shown in Table 1.

Effect of conversion on plasma lipids and lipoproteins

After conversion total cholesterol, LDL cholesterol and plasma triglycerides decreased significantly. Plasma HDL cholesterol concentration was stable (Table 2).

Table 4. Relationship between the plasma concentration of total cholesterol, LDL and HDL cholesterol and triglycerides, and the change in LDL subclass pattern following conversion from CsA to AZA

	LDL subclass change upon conversion		
	A to A	B to A	B to B
Number of patients	6	5	8
Triglyceride *mM*			
CsA	1.96 ± 0.44	1.94 ± 0.55	2.96 ± 1.22
AZA	1.74 ± 0.52	1.07 ± 0.13	2.17 ± 0.59
P value	0.140	0.020	0.049
Total cholesterol *mM*			
CsA	5.72 ± 1.02	6.28 ± 0.69	6.00 ± 0.94
AZA	4.93 ± 0.89	4.44 ± 0.67	5.14 ± 0.61
P value	0.212	0.003	0.092
LDL cholesterol *mM*			
CsA	3.60 ± 0.70	4.23 ± 0.51	3.50 ± 0.69
AZA	3.15 ± 0.62	2.66 ± 0.64	3.08 ± 0.56
P value	0.274	0.003	0.204
HDL cholesterol *mM*			
CsA	1.23 ± 0.33	1.16 ± 0.51	0.98 ± 0.38
AZA	1.38 ± 0.51	1.30 ± 0.53	1.08 ± 0.37
P value	0.529	0.663	0.582

Table 5. Relationship between the lag time of copper chloride-induced *in vitro* LDL oxidation and the change in LDL subclass pattern following conversion from CsA to AZA

LDL subclass change	Number of patients	Lag time CsA	Lag time AZA	*P* value
A to A	6	122 ± 30	131 ± 19	0.508
B to A	5	84 ± 18	115 ± 10	0.014
B to B	8	90 ± 11	102 ± 10	0.044

Effect of conversion on LDL-particle size and subclass distribution

The mean diameter of LDL particles increased significantly (236.5 ± 7.3 nm vs. 240.7 ± 6.8 nm, $P = 0.00001$; Table 3). The number of patients which had LDL subclass pattern A, representing the less oxidizable large-size LDL, increased from 6 to 11 out of 19 patients. None of the patients changed from subclass A to subclass B, or had a smaller LDL size after conversion. The changes of plasma lipid concentrations were closely related to the LDL subclass pattern before conversion. In patients whose subclass pattern changed from B to A, the LDL cholesterol, total cholesterol and triglycerides fell much more than in patients who had pattern A or B both before and after conversion (Table 4).

Low density lipoprotein oxidation

The mean lag time to copper-induced oxidation increased from 99 ± 24 minutes during CsA, to 115 ± 17 minutes during AZA, indicating that during AZA, LDL was significantly less susceptible to *in vitro* oxidation. The largest increase in lag time upon conversion occurred in patients whose subclass pattern changed from B to A. When the LDL subclass pattern did not change, the lag time remained stable (pattern A to A), or increased slightly (pattern B to B; Table 5).

Autoantibodies against MDA-modified LDL

The concentration and titer of IgM autoantibodies against MDA-LDL fell significantly after conversion (−27% and −16%, respectively; Table 3). Both before and after conversion patients

with subclass A had a lower IgM antibody concentration than patients with subclass B. The largest decrease in antibody concentration occurred in patients in which the LDL subclass pattern changed from B to A (54.9 ± 18.5 vs. 32.1 ± 8.4 μg/ml, $P = 0.0365$). The antibody concentration dropped also in the patients in which the LDL subclass pattern A did not change (34.7 ± 8.5 vs. 23.4 ± 4.5 μg/ml, $P = 0.0170$). In patients with a subclass B pattern before and after conversion, IgM antibody concentration was elevated and remained unchanged (48.7 ± 9.1 vs. 41.8 ± 11.6 μg/ml, $P = 0.2075$).

Discussion

Cardiovascular disease is the most important cause of death in renal transplant recipients [7]. This may be related to pretransplant renal replacement therapy, the underlying renal disease that may be accompanied by elevated blood pressure, or to other factors. Elevated serum cholesterol and hypertension are well-known side-effects of CsA, which occur in a significant proportion of patients. Therefore, the use of CsA may worsen the cardiovascular risk profile of transplant patients.

Recently, it has become clear that the atherogenicity of LDL is, apart from their plasma concentration, dependent on chemical modifications such as oxidation [14, 15]. Oxidized LDL particles are avidly taken up by scavenger receptors that are present on endothelial cells and macrophages and may induce foam cell formation *in vivo* [32]. Oxidized LDL may trigger immune reactions in the vessel wall, promote gene expression of cell adhesion molecules in arterial cells and impair endothelial vasodilator function [32–34].

Our study demonstrates that conversion from CsA to AZA leads to a longer lag time of *in vitro* LDL oxidation, indicating that LDL particles are less susceptible to oxidative modification during AZA. *In vitro* studies have shown that CsA induces lipid peroxidation both in liver and renal microsomes [10]. Recently it has been demonstrated that the oxidizability of LDL is strongly correlated to CsA blood concentrations, suggesting that CsA may facilitate LDL oxidation [11]. Increased lipid peroxidation was also found in CsA-treated heart transplant recipients [37]. In our study there was no significant correlation between CsA 12-hour trough blood concentrations and any of the lipid peroxidation parameters. The observed effects on LDL oxidation may, apart from a direct influence of CsA, be caused by the increase in LDL size following conversion. It has been shown that small LDL particles, corresponding with pattern B, are more susceptible to oxidation than large LDL particles, which correspond with pattern A [36]. A number of our patients shifted from pattern B to A, while none of them shifted from pattern A to B. Therefore, besides the loss of the pro-oxidant effect of CsA, the significant increase in LDL size may have contributed to the decrease in LDL oxidizability *in vitro*.

It is well known that patients with an elevated plasma triglyceride are much more likely to express LDL subclass pattern B, and have small-dense LDL particles [36, 39]. We also found that patients with a subclass B pattern had a higher plasma triglyceride level, and had the largest decrease after conversion. The increased plasma triglyceride concentrations associated with the use of CsA may therefore be an alternative explanation for the changes in lipid peroxidation we have found.

The oxidation of LDL *in vivo* is reflected by the presence of autoantibodies against oxidized LDL [38]. We found a significant decrease in plasma concentration of IgM antibodies against oxidatively modified LDL following conversion, especially in patients who had an increase of the lag time to *in vitro* LDL oxidation. This suggests that during CsA LDL is more susceptible to oxidation, which may lead to increased LDL oxidation *in vivo*. One may argue that AZA may directly impair antibody production. However, in a previous study we have found that during CsA the production of antibodies against influenza vaccination was inhibited, rather than during AZA [40].

The increased susceptibility to oxidation during CsA was accompanied by a reduction of renal blood flow and glomerular filtration rate. Recently it has been reported that in uninephrectomized rats treated with different doses of CsA, lipid peroxidation products in renal tissue homogenates and renal vascular resistance were dose-dependently increased. Both could be prevented by the coadministration of the antioxidant vitamin E, suggesting that oxidative processes play a causative role in the pathogenesis of CsA-induced renal injury [41]. However, other investigators were unable to confirm these findings in humans [42]. In a cross sectional study, Maggi et al showed that in hemodialysis patients the titer of autoantibodies directed against oxidatively-modified LDL is increased when compared to healthy controls, suggesting that enhanced LDL oxidation in present *in vivo* [43]. In our study no relationship between absolute values or changes in renal function, in blood pressure or duration of pretransplant renal replacement therapy and any of the lipid peroxidation parameters was present.

Only a few of our study patients developed symptomatic cardiovascular disease during the post-transplant period. Obviously, our study population was too small to allow definitive conclusions about the association between the prevalence of cardiovascular events and lipid peroxidation *in vivo*.

In conclusion, this study shows that the susceptibility of LDL to *in vivo* and *in vitro* oxidation appears to be increased during CsA administration when compared to AZA. In view of recent reports of the importance of oxidatively modified LDL in the pathogenesis of atherosclerosis, our findings offer an additional explanation for the high cardiovascular morbidity and mortality after renal transplantation. The molecular mechanism of our observations is still unresolved. Further studies of the effects of CsA on lipid peroxidation and its implications for atherogenesis are needed.

Reprint requests to M.A. van den Dorpel, M.D., Department of Internal Medicine I, University Hospital Rotterdam, Dr. Molewaterplein 40, 3015 GD Rotterdam, The Netherlands.

References

1. European Multicentre Trial Group: Cyclosporin in cadaveric renal transplantation: 5-year follow-up of a multicentre trial. *Lancet* ii:506–509, 1987
2. Kasiske BL, Tortorice KL, Heim-Duthoy KL, Awni WM, Rao KV: The adverse impact of cyclosporin on serum lipids in renal transplant patients. *Am J Kidney Dis* 17:700–707, 1991
3. Versluis DJ, Wenting GJ, Weimar W: Alterations in lipid and carbohydrate metabolism attributable to cyclosporine A in renal transplant recipients. (letter) *Br Med J* 292:272, 1986
4. Raine AEG, Carter R, Mann JI, Morris PJ: Adverse effect of cyclosporin on plasma cholesterol in renal transplant recipients. *Nephrol Dial Transplant* 3:458–463, 1988
5. Curtis JJ: Hypertension and kidney transplantation. *Am J Kidney Dis* 7:181–196, 1986
6. Chapman JR, Marcen R, Ariaz M, Raine AEG, Dunnill MS, Morris PJ: Hypertension after renal transplantation: A comparison

of cyclosporin and conventional immunosuppression. *Transplantation* 43:860–864, 1987
7. BRAUN WE: Long-term complications of renal transplantation. *Kidney Int* 37:1363–1378, 1990
8. BALLANTYNE CM, PODET EJ, PATSCH WP, HARATI Y, APPEL V, GOTTO AM, YOUNG JB: Effects of cyclosporin therapy on plasma lipoprotein levels. *JAMA* 262:53–56, 1989
9. WEBB AT, REAVELEY DA, O'DONNELL M, O'CONNOR B, SEED A, BROWN EA: Does cyclosporin increase lipoprotein(a) concentrations in renal transplant patients? *Lancet* 341:268–70, 1993
10. INSELMANN G, BARTH A, ENGEMANN R, HEIDEMANN HT: Cyclosporine A-induced lipid peroxidation in human liver microsomes and its influence on cytochrome P-450. *Eur J Clin Invest* 21:461–465, 1991
11. APANAY DC, NEYLAN JF, RAGAB MS, SGOUTAS DS: Cyclosporine increases the oxidizability of low-density lipoproteins in renal transplant recipients. *Transplantation* 58:663–669, 1994
12. GHANEM H, VAN DEN DORPEL MA, WEIMAR W, MAN IN'T VELD AJ, EL-KANNISHY MH, JANSEN H: Increased low density lipoprotein oxidation in stable kidney transplant recipients. *Kidney Int* 49:488–493, 1996
13. TAYLOR JE, SCOTT N, HILL A, BRIDGES A, HENDERSON IS, STEWART WK, BELCH JJF: Oxygen free radicals and platelet and granulocyte aggregability in renal transplant patients. *Transplantation* 55:500–530, 1990
14. STEINBERG D, PARTHASARATHY S, CAREW TE, KHOO JC, WITZTUM JL: Beyond cholesterol: Modifications of LDL that increase its atherogenicity. *N Engl J Med* 320:916–924, 1989
15. PALINSKI W, YLÄ-HERTTUALA S, ROSENFELD ME, BUTLER SW, SOCHER SA, PARTHASARATHY S, CURTISS LK, WITZTUM JL: Antisera and monoclonal antibodies specific for epitopes generated during oxidative modification of low density lipoprotein. *Arteriosclerosis* 10:325–335, 1990
16. GALLE J, SCHENCK I, SCHOLLMEYER P, WANNER C: Cyclosporine and oxidized lipoproteins affect vascular reactivity: Influence of the endothelium. *Hypertension* 21:315–321, 1993
17. YANG X, CAI B, SCIACCA RR, CANNON PJ: Inhibition of inducible nitric oxide synthase in macrophges by oxidized low-density lipoproteins. *Circ Res* 74:318–328, 1994
18. TANNER FC, NOLL G, BOULANGER CM, LÜSCHER TF: Oxidized low density lipoproteins inhibit relaxations of porcine coronary arteries; role of scavenger receptor and endothelium-derived nitric oxide. *Circulation* 83:2012–2020, 1991
19. VERSLUIS DJ, WENTING GJ, DERKX FHM, SCHALEKAMP MADH, JEEKEL J, WEIMAR W: Who should be converted from cyclosporine to conventional immunosuppression in kidney transplantation and why? *Transplantation* 44:387–389, 1987
20. ZIETSE R, BLANKESTIJN PJ, POS B, BALK AHMM, DERKX FHM, WEIMAR W, SCHALEKAMP MADH: Optimising glomerular filtration rate and effective renal plasma flow measurements using a simple pharmacokinetic model. *Clin Nephrol* 43:26–34, 1995
21. KLEINVELD HA, HAK-LEMMERS HLM, STALENHOEF AFH, DEMACKER PNM: Improved measurement of low-density lipoprotein susceptibility to copper-induced oxidation: Application of a short procedure for isolating low-density lipoprotein. *Clin Chem* 38:2066–2072, 1992
22. REDGRAVE TG, ROBERTS DCK, WEST E: Separation of plasma lipoproteins by density gradient ultracentrifugation. *Anal Biochem* 65:42–49, 1975
23. BURSTEIN M, SCHOLNICK HR, MORFEN R: Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. *J Lipid Res* 11:583–595, 1970
24. FRIEDEWALD WT, LEV RI, FREDRICKSON DS: Estimation of low density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 18:499–502, 1972
25. ESTERBAUER H, STRIEGL G, PUHL H, ROTHENEDER M: Continuous monitoring of *in vitro* oxidation of human LDL. *Free Radic Res Commun* 6:67–75, 1989
26. AUSTIN MA, BRESLOW JL, HENNEKENS CH, BURING JE, WILLETT WC, KRAUSS RM: Low density lipoprotein subclass patterns and the risk of myocardial infarction. *JAMA* 260:1917–1921, 1988
27. JANSEN H, HOP W, VAN TOL A, BRUSCHKE AVG, BIRKENHÄGER JC: Hepatic lipase and lipoprotein lipase are not major determinants of low density lipoprotein subclass pattern in human subjects with coronary heart disease. *Atherosclerosis* 107:45–54, 1994
28. CORESH J, KWITEROVICH PO, SMITH HH, BACHORIK PS: Association of plasma triglyceride concentration and LDL particle diameter, density, and chemical composition with premature coronary artery disease in men and women. *J Lipid Res* 34:1687–1697, 1993
29. JANSEN H, GHANEM H, KUYPERS HSAM, BIRKENHÄGER JC: Autoantibodies against malondialdehyde-modified LDL are elevated in subjects with an LDL subclass B pattern. *Atherosclerosis* 115:225–262, 1995
30. PALINSKI W, YLÄ-HERTTUALA S, ROSENFELD ME, BUTLER SW, SOCHER SA, PARTHASARATHY S, CURTISS LK, WITZTUM JL: Antisera and monoclonal antibodies specific for epitopes generated during oxidative modification of low density lipoprotein. *Arteriosclerosis* 10:325–335, 1990
31. MOORE R, THOMAS D, MORGAN E, WHEELER D, GRIFFIN P, SALAMAN J, REES A: Abnormal lipid and lipoprotein profiles following renal transplantation. *Transplant Proc* 25:1060–1061, 1993
32. FOGELMAN AM, SHECHTER I, SAEGER J, HOKOM M, CHILD JS, EDWARDS PA: Malondialdhyde alteration of low density lipoproteins leads to cholesteryl ester accumulation in human monocyte-macrophages. *Proc Natl Acad Sci USA* 77:2214–2218, 1980
33. BERLINER JA, TERRITO M, SEVANIAN A, RAMIN S, KIM JA, RANSHAD B, ESTERSON M, FOGELMAN AM: Minimally modified low density lipoprotein stimulates monocyte endothelial interactions. *J Clin Invest* 85:1260–1266, 1990
34. MOREL DW, HESSLER JR, CHISOLM GM: Low density lipoprotein cytotoxicity induced by free radical peroxidation of lipids. *J Lipid Res* 24:1070–1076, 1983
35. DE GRAAF J, HAK-LEMMERS HL, HECTORS MC, DEMACKER PN, HENDRIKS JC, STALENHOEF AFH: Enhanced susceptibility to *in vitro* oxidation of the dense low density lipoprotein subfraction in healthy subjects. *Arteriosclerosis Thromb* 11:298–306, 1991
36. CHAIT A, BRAZG RL, WASHINGTON S, TRIBBLE DL: Susceptibility of small, dense, low density lipoproteins to oxidative modification in subjects with the atherogenic lipoprotein phenotype pattern B. *Am J Med* 94:350–356, 1993
37. CHANCERELLE Y, DE LORGERIL M, VIRET R, CIRON B, DUREAU G, RENAUD S, KERGONOU J-F: Increased lipid peroxidation in cyclosporine-treated heart transplant recipients. *Am J Cardiol* 68:813–816, 1991
38. CHAIT A: Methods for assessing lipid and lipoprotein oxidation. *Curr Opin Lipid* 3:389–394, 1992
39. AUSTIN MA, BRESLOW JA, HENNEKENS CH, BURING JE, WILLETT WC, KRAUSS RM: Low density lipoprotein subclass patterns and the risk of myocardial infarction. *JAMA* 260:1917–1921, 1988
40. VERSLUIS DJ, BEYER WEP, MASUREL N, WENTING GJ, WEIMAR W: Impairment of the immune response to influenza vaccination in renal transplant recipients by cyclosporine but not azathioprine. *Transplantation* 42:376–379, 1986
41. WANG C, SALAHUDEEN AK: Lipid peroxidation accompanies cyclosporine nephrotoxicity: Effects of vitamin E. *Kidney Int* 47:927–934, 1995
42. STEIN CM, LONGMIRE AW, MINTON TA, ROBERTS LJ II, PINCUS T, MORROW JD: Cyclosporine-induced alterations in renal function are not associated with lipid peroxidation. *Transplantation* 58:386–388, 1994
43. MAGGI E, BELLAZZI R, GAZO A, SECCIA M, BELLAMO G: Autoantibodies against oxidatively-modified LDL in uremic patients undergoing dialysis. *Kidney Int* 46:869–876, 1994