Improved Cardiovascular Risk Profile and Renal Function in Renal Transplant Patients after Randomized Conversion from Cyclosporine to Tacrolimus

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Abstract. Cyclosporine is considered to contribute to the high cardiovascular morbidity and mortality in patients after renal transplantation. Tacrolimus may be more favorable in this respect, but controlled data are scarce. In this prospective randomized study in 124 stable renal transplant patients, the effects of conversion from cyclosporine to tacrolimus on cardiovascular risk factors and renal function were investigated. Follow-up was 6 mo. Statistical analysis was performed by ANOVA for repeated measurements. The serum creatinine level decreased from 137 ± 30 μmol/L to 131 ± 29 μmol/L ($P < 0.01$). Three months after conversion from cyclosporine to tacrolimus, mean BP significantly decreased from 104 ± 13 to 99 ± 12 mmHg ($P < 0.001$). Serum LDL cholesterol decreased from 3.48 ± 0.80 to 3.11 ± 0.74 mmol/L ($P < 0.001$,) and serum apolipoprotein B decreased from 1018 ± 189 to 935 ± 174 mg/L ($P < 0.001$). Serum triglycerides decreased from 2.11 ± 1.12 to 1.72 ± 0.94 mmol/L ($P < 0.001$). In addition, both rate and extent of LDL oxidation were reduced. The fibrinogen level decreased from 3638 ± 857 to 3417 ± 751 mg/L ($P < 0.05$). Plasma homocysteine concentration did not change. Three months after conversion, plasma fasting glucose level temporarily increased from 5.4 ± 1.3 mmol/L to 5.8 ± 1.9 mmol/L ($P < 0.05$). Conversion to tacrolimus resulted in a significant reduction of the Framingham risk score. In conclusion, conversion from cyclosporine to tacrolimus in stable renal transplant patients has a beneficial effect on renal function, BP, serum concentration and atherogenic properties of serum lipids, and fibrinogen.

During the past two decades, cyclosporine has proved to be a valuable immunosuppressive drug that has contributed to a significant reduction in the incidence of acute rejection after renal transplantation. However, cyclosporine also increases cardiovascular risk profiles (1). Ultimately, up to 63% of renal transplant patients die of cardiovascular disease (2). The increased cardiovascular risk profile as a result of cyclosporine is ascribed to both a quantitative increase in LDL particles and an increased oxidizability of the LDL particles (3–5). Use of cyclosporine is also associated with increased plasma lipoprotein(a) (Lp[a]) (6) and homocysteine levels (7), but these effects are not unequivocal (8). In addition, unfavorable effects on the fibrinolytic system by cyclosporine have been described (9). Apart from these disadvantageous effects of cyclosporine on several metabolic cardiovascular risk factors, cyclosporine leads to an elevation of BP (4). These side effects not only contribute to the high cardiovascular morbidity in renal transplant patients but also may lead to an accelerated loss of graft function (10–12).

Tacrolimus is like cyclosporine, a calcineurin inhibitor, with even more potent immunosuppressive properties. The use of tacrolimus after renal transplantation is associated with a less unfavorable effect on BP and serum lipid levels, but evidence from controlled studies is scarce (4,13). Little is known about the differential effects of cyclosporine and tacrolimus on established cardiovascular risk factors such as homocysteine, oxidizability of LDL particles, and fibrinogen levels. In the present study, we investigated the effect of conversion from cyclosporine to tacrolimus in renal transplant patients on the cardiovascular risk profile and on graft function in a multicenter, prospective, randomized design.

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Materials and Methods

Patients

To be eligible for the study, the patients had to be at least 1 yr posttransplantation with a stable endogenous creatinine clearance exceeding 20 ml/min. Immunosuppressive treatment at enrollment had to include cyclosporine with trough levels ranging from 50 to 200 ng/ml and prednisone, with or without azathioprine or mycophenolate mofetil.

Study Protocol

This prospective, randomized, open-label study was performed in four renal transplant centers. After stratification for center, patients were randomized in a 1:1 manner to either continuation of cyclosporine or conversion to tacrolimus. Total follow-up was 6 mo. Evaluation took place at baseline and after 3 and 6 mo. Target drug trough levels were 50 to 200 ng/ml for cyclosporine and 5 to 8 ng/ml for tacrolimus. Lipid-lowering drug treatment and antihypertensive treatment preferably remained unchanged. Adjustment of lipid-lowering medication was allowed when the serum total cholesterol level was below 4.0 mmol/L or exceeded 6.5 mmol/L or when the serum triglyceride level exceeded 4.0 mmol/L. Changes in antihypertensive medication were allowed when the systolic BP was >160 mmHg or <120 mmHg or when the diastolic BP was >90 mmHg or <70 mmHg. BP was measured in supine position manually after 5 min of rest in all participating patients. In patients from three of four participating centers, BP was also measured using an automated device (Dinamap, Critikon, Tampa, FL) during 30 min. The diagnosis of diabetes was based on the fasting glucose levels, with a cutoff value of 7.0 mmol/L, measured on two separate occasions, in accordance with the American Diabetes Association criteria (14). Framingham risk scores were calculated using age, smoking behavior, the presence of diabetes, fasting LDL and HDL cholesterol levels, and systolic and diastolic BP (15). The study was approved by the medical ethics committee of each participating center. All patients gave written informed consent.

Laboratory Procedures

All blood was drawn after an overnight fast, during morning hours in sitting position. The blood sample for determination of the parameters of fibrinolysis was drawn after releasing the tourniquet to avoid hemolysis.

Serum lipids and lipoproteins (by a combined ultracentrifugation-precipitation method), Lp(a) (by a commercially available RIA), and total apolipoprotein B (by immunonephelometry) were determined as described previously (16). The oxidizability of LDL was determined at baseline and after 3 mo of follow-up by registration of the 234-nm absorbance of dienes produced by copper added to isolated LDL (17). Both EDTA-plasma samples of each person were stored at −80°C (with 10 μl of 600 g/L saccharose solution per milliliter of plasma as cryoprotectant) and analyzed in the same assay. The sample for determination of plasma homocysteine was placed on ice until centrifugation and frozen storage. The plasma homocysteine concentration was measured using an HPLC procedure, with reverse-phase separation and fluorescence detection (18). Antigen levels of the various components of the plasminogen activator system were determined by ELISA (19). The cyclosporine level was determined in whole blood with a fluorescence-polarization immunoassay (TDx/TDx FLx cyclosporine monoclonal whole-blood assay; Abbott Laboratories, Chicago, IL), and the tacrolimus level was determined using a microparticle enzyme immunoassay (IMx Tacrolimus II Assay; Abbott Laboratories).

Statistical Analyses

Statistical analysis was performed using the SPSS package, version 9.0. Values are given as mean ± SD in case of a normal distribution or otherwise as median and interquartile range. Statistical analysis was performed using one-way ANOVA for repeated measurements. When the ANOVA revealed a statistically significant difference, post hoc comparisons were performed using Bonferroni correction for multiple comparisons.

For parameters that were determined at only two time points, *t* test was used in case of normal distribution; otherwise, Wilcoxon signed ranks test was used. For correlations, Pearson product moment correlation coefficient was used, and for analysis of proportions, Fisher exact test was applied. Results are analyzed on an intention-to-treat basis for renal function and BP, whereas the metabolic parameters are analyzed in the patients who remained on study medication during follow-up. $P < 0.05$ was considered statistically significant.

Results

Between February 1999 and September 2000, 124 patients were included in the study. Baseline characteristics are shown in Table 1. Sixty-four patients were converted to tacrolimus (conversion group), and 60 patients remained on cyclosporine (control group). There were no baseline differences between both groups in serum creatinine level, BP, or any metabolic parameter. Mean baseline cyclosporine trough level was 129 ± 42 ng/ml in the control group *versus* 130 ± 42 ng/ml in the conversion group (NS). In the control group, the cyclosporine trough level remained unchanged during follow-up. In the conversion group, the mean tacrolimus trough level was 7.4 ± 1.8 ng/ml after 3 mo and 7.4 ± 1.9 ng/ml after 6 mo of follow-up. Baseline prednisone dose was 0.10 ± 0.04 mg/kg in the conversion group and 0.10 ± 0.04 mg/kg in the control group; baseline azathioprine dose was 0.88 ± 0.40 mg/kg (n =

Table 1. Baseline patient demographics

	Cyclosporine (n = 60)	Tacrolimus (n = 64)
Time after RTX (yr)	6.6 ± 3.7	5.7 ± 2.7
Gender (m/f)	34/26	41/23
Age (yr)	50 ± 13	50 ± 14
Race		
white	57 (95%)	60 (94%)
black	0	0
other	3 (5%)	4 (6%)
Original renal disease		
chronic glomerulonephritis	17 (28%)	20 (31%)
chronic pyelonephritis	5 (8%)	11 (17%)
polycystic kidney disease	13 (22%)	13 (20%)
diabetic nephropathy	3 (5%)	1 (2%)
otherwise	19 (32%)	17 (27%)
unknown	3 (5%)	2 (3%)
Diabetes		
type 1	5 (8%)	2 (3%)
type 2	3 (5%)	6 (9%)

Mean ± SD; no significant differences. RTX, renal transplantation.

10) in the conversion group and 0.95 ± 0.47 mg/kg ($n = 15$) in the control group. In the control group, two patients used mycophenolate mofetil 2000 mg/d. The dosages of prednisone, azathioprine, and mycophenolate mofetil remained unchanged throughout the study period.

In the control group, two patients died, one of a cardiac event and one of sepsis. In this group, an additional three patients were withdrawn from the study for the following reasons: patient's wish to switch to tacrolimus, patient's wish to stop prednisone, and one patient lost to follow-up. In the conversion group, one patient died of a cardiac event. In this group, six patients were withdrawn from the study as a result of worsening of preexistent diabetes ($n = 1$), new-onset diabetes ($n = 1$), headache ($n = 2$), allergic skin reaction to tacrolimus ($n = 1$), and progression of preexistent proteinuria ($n = 1$). A renal graft biopsy in the last patient, taken 4 mo after conversion to tacrolimus, showed signs of chronic allograft nephropathy.

Renal Function and BP

After conversion from cyclosporine to tacrolimus, no acute rejections occurred. After 6 mo of follow-up, conversion from cyclosporine to tacrolimus resulted in a significant decrease in the serum creatinine level from 137 ± 30 to 131 ± 29 μmol/L (Table 2). Urinary protein excretion showed a small but significant increment in the cyclosporine group, whereas it remained unchanged in the tacrolimus group.

At 3 mo of follow-up, the manually measured systolic and diastolic BP were significantly decreased in the tacrolimus group from 144 ± 21 mmHg to 138 ± 18 mmHg and 84 ± 12 mmHg to 80 ± 11 mmHg, respectively (Table 2). These changes were no longer significant after 6 mo of follow-up. These results did not change when only patients who remained on study medication during follow-up were analyzed.

Lipid Profile

After conversion from cyclosporine to tacrolimus, serum total and LDL cholesterol, triglycerides, and apolipoprotein B levels decreased, whereas HDL cholesterol and Lp(a) level did not change (Table 3). Entering the use of a statin as a covariate revealed a significant influence of statin usage on the change in LDL cholesterol level after conversion. In the tacrolimus group, patients who were taking statins had a lower baseline LDL cholesterol level compared with patients who were not using statins (3.20 ± 0.60 mmol/L *versus* 3.86 ± 0.90 mmol/L; $P < 0.01$), and there was no significant reduction in LDL cholesterol level 6 mo after conversion to tacrolimus in patients who were using statins (0.22 ± 0.66 mmol/L; $P = 0.31$ for comparison with baseline), compared with a significant reduction in patients who were not using statins (0.65 ± 0.64 mmol/L; $P < 0.001$ for comparison with baseline).

At 3 mo of follow-up, tacrolimus-treated patients showed a significant increase in the resistance to *in vitro* oxidation of the LDL particles, as shown by an increment in the lag time for oxidation and decrements in the appearance rate of dienes and the total amount of diene formation. The reduction in oxidizability of the LDL particle was not dependent on the concomitant use of statins.

In two cyclosporine-treated patients, statins were started and in one patient the dosage was increased. In the tacrolimus group, the statin was stopped in one patient and the dosage was reduced in another patient.

Glucose Regulation

In the tacrolimus group, the plasma fasting glucose level showed an increase after 3 mo of follow-up. After 6 mo of follow-up, the glucose level was not different from the baseline value (Table 4). When diabetic and nondiabetic patients were analyzed separately, there were no significant changes in glu-

Table 2. Renal function and BP

	Cyclosporine ($n = 60$)			Tacrolimus ($n = 64$)		
	Baseline	3 Mo	6 Mo	Baseline	3 Mo	6 Mo
Serum creatinine (μmol/l)	143 ± 48	147 ± 48	146 ± 53	137 ± 30	136 ± 32	131 ± 29†
Proteinuria (g/24 hr)	0.20 (0.10–0.52)		0.22† (0.11–0.70)	0.20 (0.08–0.50)		0.14 (0.08–0.44)
Body weight (kg)	75.0 ± 14.0		74.8 ± 14.3	77.8 ± 15.8		76.5 ± 15.1‡
BP manually						
mean (mmHg)	102 ± 10	104 ± 11	104 ± 11	104 ± 13	99 ± 12‡	101 ± 13
systolic (mmHg)	140 ± 15	144 ± 18	142 ± 18	144 ± 21	138 ± 18†	140 ± 21
diastolic (mmHg)	83 ± 9	84 ± 10	85 ± 10	84 ± 12	80 ± 11‡	82 ± 10
BP Dynamap						
mean (mmHg)	105 ± 13	105 ± 11	106 ± 10	106 ± 14	101 ± 9	102 ± 11
systolic (mmHg)	141 ± 17	142 ± 14	144 ± 14	141 ± 19	135 ± 15	137 ± 18
diastolic (mmHg)	83 ± 9	82 ± 9	83 ± 9	82 ± 10	79 ± 8	79 ± 9
No. of antihypertensive drugs	1.9 ± 1.0	1.9 ± 1.0	2.0 ± 1.0	2.1 ± 1.1	2.0 ± 1.1	2.2 ± 1.1

Mean ± SD or median and interquartile range.
* $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$ for comparison with baseline. Dynamap BP was determined in 84 patients from three participating centers.

Table 3. Parameters of lipid metabolism

	Cyclosporine ($n = 60$)			Tacrolimus ($n = 64$)		
	Baseline	3 Mo	6 Mo	Baseline	3 Mo	6 Mo
Total cholesterol (mmol/L)	5.86 ± 0.83	5.79 ± 0.70	5.80 ± 0.88	5.79 ± 0.85	5.26 ± 1.07‡	5.31 ± 0.89‡
LDL cholesterol (mmol/L)	3.63 ± 0.74	3.49 ± 0.68	3.54 ± 0.81	3.48 ± 0.80	3.09 ± 0.75‡	3.11 ± 0.74‡
HDL cholesterol (mmol/L)	1.43 ± 0.45	1.42 ± 0.50	1.40 ± 0.52	1.42 ± 0.43	1.41 ± 0.40	1.42 ± 0.41
Triglycerides (mmol/L)	1.88 ± 0.91	2.04 ± 1.12	2.01 ± 0.94	2.11 ± 1.12	1.77 ± 1.10†	1.72 ± 0.94‡
Apolipoprotein B (mg/L)	1068 ± 199	1056 ± 190	1069 ± 223	1018 ± 189	938 ± 160‡	935 ± 174‡
Lp(a) (U/L§	144.5 (51.3–478.3)	187.5 (65.5–521.3)	193.0 (77.5–537.5)	216.5 (94.3–564.0)	223.5 (99.8–511.3)	258.0 (120.0–627.0)
No. of patients on lipid-lowering drugs	22 (37%)	24 (40%)	24 (40%)	35 (55%)	34 (53%)	34 (53%)
In vitro oxidizability of LDL¶						
Lag time (min)	80.2 ± 10.8	80.2 ± 9.2		81.0 ± 10.6	83.2 ± 10.6*	
Diene appearance rate (nmol/mg LDL protein per min)	13.9 ± 2.2	14.0 ± 2.5		13.8 ± 1.9	13.3 ± 1.9†	
Diene production (nmol/mg LDL protein)	616.8 ± 60.4	614.5 ± 73.8		606.1 ± 59.2	587.6 ± 60.2†	

Mean ± SD or median and interquartile range.
* $P < 0.05$; † $P < 0.01$; ‡ $P < 0.001$ for comparison with baseline.
§ Conversion-factor: 1 U/L is approximately 0.7 mg/l (phenotype dependent).
¶ Determined in two participating centers (41 cyclosporine patients and 42 tacrolimus patients).

Table 4. Glucose, parameters of fibrinolysis, and plasma homocysteine

	Cyclosporine ($n = 60$)			Tacrolimus ($n = 64$)		
	Baseline	3 Mo	6 Mo	Baseline	3 Mo	6 Mo
Glucose (mmol/L)	5.5 ± 1.5	5.3 ± 1.2	5.2 ± 1.1	5.4 ± 1.3	5.8 ± 1.9*	5.6 ± 1.8
nondiabetic	5.1 ± 0.6	5.0 ± 0.8	5.1 ± 0.7	5.1 ± 0.9	5.4 ± 0.8	5.3 ± 1.0
diabetic	8.6 ± 2.9	7.4 ± 2.1	6.4 ± 2.5	8.0 ± 1.9	9.2 ± 4.4	8.9 ± 3.4
HbA1c (%)	5.6 ± 0.5	5.6 ± 0.5	5.6 ± 0.4	5.8 ± 0.9	6.1 ± 1.7	6.1 ± 1.4
nondiabetic#	5.5 ± 0.4	5.6 ± 0.4	5.5 ± 0.3	5.6 ± 0.4	5.5 ± 0.5	5.6 ± 0.5
diabetic	7.9 ± 0.6	7.6 ± 0.6	7.6 ± 0.7	8.8 ± 1.7	9.2 ± 1.2	8.5 ± 1.0
Fibrinogen (mg/L)	3543 ± 679	3569 ± 681	3637 ± 708	3638 ± 857	3367 ± 763‡	3417 ± 751*
tPA (ng/mL)	3.80 ± 15.68	3.29 ± 1.42	3.22 ± 1.37	4.07 ± 2.71	3.65 ± 1.78	3.68 ± 2.04
PAI-I (ng/ml)	26.82 ± 15.68	33.23 ± 20.78	34.95 ± 19.13	30.04 ± 20.57	30.38 ± 17.68	31.65 ± 18.29
tPA-PAI complex (ng/ml)	8.38 ± 4.77	8.20 ± 4.03	7.77 ± 4.56	10.19 ± 7.42	9.53 ± 6.37	9.79 ± 6.57
uPA (ng/ml)	1.22 ± 0.37	1.27 ± 0.41	1.23 ± 0.40	1.26 ± 0.35	1.17 ± 0.37*	1.16 ± 0.33*
Homocysteine (μmol/l)	18.6 (16.3–22.1)	19.0 (14.6–21.6)	18.1 (14.5–21.2)	18.4 (14.7–23.7)	18.0 (13.9–24.7)	16.6 (14.6–21.1)

Mean ± SD or median and interquartile range.
* $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$ for comparison with baseline.
# Determined in all patients from one center (17 cyclosporine patients and 18 tacrolimus patients). tPA, tissue type plasminogen; PAI-I, plasminogen activator inhibitor type I.

cose level in either group during follow-up. HbA1c levels were determined in one center ($n = 35$), and there were no significant changes after conversion to tacrolimus. When diabetic and nondiabetic patients were analyzed separately, again there were no significant changes in HbA1c level in either group after conversion to tacrolimus.

Six months after conversion from cyclosporine to tacrolimus, four patients (6%) had developed new-onset diabetes, *versus* two patients in the control group (3%; NS). In the conversion group, the new-onset diabetes could be treated with oral antidiabetic drugs in two cases, and in the other two patients, only dietary measures were necessary, resulting in plasma fasting glucose levels persistently below 6 mmol/L and an HbA1c below 6.1%. Both patients who were put on antidiabetic medication wanted to be taken off the tacrolimus, and they were converted from tacrolimus to azathioprine. No acute rejections occurred. In one patient, the oral antidiabetic medication could be stopped 4 mo after withdrawal of tacrolimus; in the second patient, the oral antidiabetic medication was continued in a low dosage. In three of six tacrolimus-treated

patients with preexistent type 2 diabetes, the dose of the oral antidiabetic medication had to be increased, *versus* zero of three patients in the control group. HbA1c level did not change in either group during follow-up.

Other Metabolic Cardiovascular Risk Factors

Baseline homocysteine level correlated significantly with baseline serum creatinine value ($r = 0.43$, $P < 0.001$). During follow-up, there were changes neither in plasma homocysteine levels (Table 4) nor in serum folic acid concentration (data not shown).

After conversion from cyclosporine to tacrolimus, the plasma fibrinogen level and the plasma urokinase type plasminogen activator (uPA) level decreased. There were no changes in the plasma levels of plasminogen activator inhibitor type I, tissue type plasminogen, and tissue type plasminogen–plasminogen activator inhibitor type I complex (Table 4).

Framingham Risk Score

After conversion to tacrolimus, a significant reduction of the Framingham risk score was observed at 3 mo of follow-up but no longer at 6 mo after conversion ($P = 0.13$). Entering the use of a statin as covariate revealed a significant influence of statin usage on the change in the Framingham risk score after conversion. In the patients who were not using a statin, the Framingham risk score significantly decreased from 4.0 ± 6.1 to 2.6 ± 6.3 at 6 mo ($P < 0.001$), whereas in patients who were using a statin, the Framingham risk score did not change (6.0 ± 4.1 at baseline and 6.1 ± 4.6 at 6 mo; Figure 1).

Discussion

The grossly enlarged cardiovascular risk in renal transplant patients is related to a combination of partly related risk factors, such as hypertension, hyperlipidemia, hereditary risk, diabetes, physical inactivity, obesity, hyperuricemia, hyperhomocysteinemia, and hyperparathyroidism. For addressing the pressing challenge in renal transplantation of improving patient survival and long-term graft function, this broad spectrum of risk factors has to be optimized, without putting the graft function at risk. In this prospective, randomized, controlled trial, conversion from cyclosporine to tacrolimus in stable renal transplant patients resulted in an improvement in a number of cardiovascular risk factors, together with an improvement in renal function without risking acute rejection.

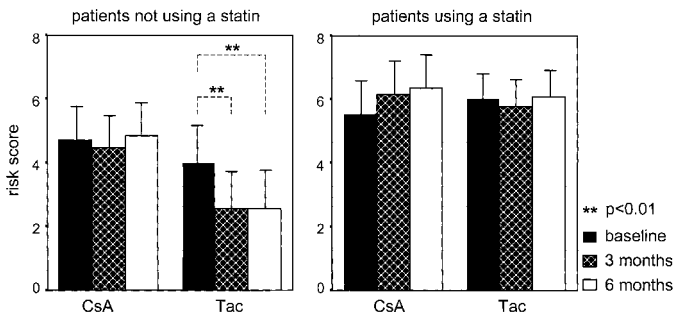

Figure 1. Framingham risk score (mean and SEM) on cyclosporine (CsA) *versus* tacrolimus (Tac).

After conversion to tacrolimus, the serum LDL cholesterol level is reduced and the LDL particles are less susceptible to oxidation. Cyclosporine increases serum LDL cholesterol level by inhibiting the synthesis of LDL receptors in the liver, thereby interfering with the LDL receptor–mediated catabolism in the liver (20). The improvement in serum LDL cholesterol level after conversion to tacrolimus might be due to the withdrawal of this inhibition of the LDL receptor production. However, this quantitative improvement of LDL cholesterol is achieved only in patients who are not treated with statins. In patients who use statins, baseline LDL cholesterol already is reduced and no further reduction is observed after conversion from cyclosporine to tacrolimus, at least not in this subgroup with a limited number of patients. The atherogenicity of LDL cholesterol depends not only on its serum concentration but also on the oxidizability of the particles (21). Therefore, it is important that conversion from cyclosporine to tacrolimus reduced not only the serum concentration of LDL cholesterol but also the oxidizability of the LDL particle. Both the increment in the lag time before initiation of oxidation of LDL and the lower rate of oxidation reflect increased resistance of LDL against oxidation. This reduction in oxidizability occurred in patients both with and without the use of statins, and, therefore, conversion to tacrolimus was beneficial for both patient groups. The reduced oxidizability of the LDL particles is likely to be associated with the concurrent decrease in serum triglycerides after conversion to tacrolimus. Reduced levels of serum triglycerides and apolipoprotein B are associated with an altered, less dense composition of the LDL particles (22). These lighter LDL particles contain more lipids compared with the protein component, resulting in conformational changes with a diminished access for free radicals and pro-oxidants such as copper to cause oxidation of the fatty acids (23). Indeed, the lipid-enriched lower-density LDL subfractions have been shown to be more resistant against oxidation (24). Furthermore, lighter LDL particles are more easily cleared from the circulation by the high-affinity LDL receptor, leading to a shorter plasma residence time, during which the particle is susceptible to *in vivo* oxidation (25). In line with our findings, an increased lag time for oxidation in patients on tacrolimus compared with patients on cyclosporine was recently reported (26). In contrast, Varghese *et al.* (27) reported that LDL from tacrolimus-treated patients was more susceptible to oxidation compared with cyclosporine-treated patients. However, in that cross-sectional study, the blood level of cyclosporine was very low, averaging 87 ± 35 ng/ml, which is below the limit of 120 ng/ml above which accelerated oxidation of isolated LDL was found (5).

We previously showed that tacrolimus trough level reduction from 9.5 to 6.4 ng/ml did not result in a reduction of total and LDL cholesterol and triglycerides (28), which makes it unlikely that the improvement in the serum lipid profile in our conversion group is related to a relatively low level of tacrolimus in our patients. Also, it is unlikely that the improvement in renal function in the conversion group can account for the

improvement in the lipid profile, because the lipid profile was already improved at 3 mo, whereas renal function was improved only after 6 mo. Also, there was no correlation between the reduction in LDL cholesterol or in triglyceride levels and the serum creatinine level after conversion to tacrolimus (data not shown).

Diabetes is a major cardiovascular risk factor and is also associated with an increased risk of developing graft failure (29). Although tacrolimus has been reported to be diabetogenic (30,31), this risk is predominantly present in the initial period after transplantation (32). After lowering the dose of tacrolimus and withdrawal of steroids, most patients can be weaned off the diabetic medication (33). In this study, performed in a population that predominantly consisted of white patients, only a small and temporary increase in fasting glucose level was present at 3 mo after conversion. When diabetic and nondiabetic patients were analyzed separately, there was no significant change in glucose level in either group during follow-up. This, however, could be related to reduced power to detect a difference as a result of a smaller population. We know from previous studies that tacrolimus influences glucose metabolism by reducing pancreatic insulin secretion in a dose-dependent manner (28). Initially, also an increase in insulin resistance has been reported (32), but this is the result of the coadministration of steroids (28). At standard maintenance trough levels, no difference between cyclosporine and tacrolimus could be determined with regard to their diabetogenic properties (34). Therefore, in the majority of patients, conversion from cyclosporine to tacrolimus is safe with regard to the development of diabetes. The risk of developing diabetes after conversion to tacrolimus is restricted to patients who already experience a disturbed glucose tolerance before therapy and might be increased in black and Hispanic patients, who have a greater predisposition to develop posttransplant diabetes (30,32,35).

In 77% of our patients, the fasting homocysteine level exceeded the advised upper level of 15 μmol/L. An impaired renal function is supposed to be an important factor contributing to hyperhomocysteinemia after renal transplantation (7), and there are conflicting data on the role of cyclosporine (7,8). Conversion from cyclosporine to tacrolimus did not result in a significant reduction of the fasting homocysteine level.

The observed reduction in fibrinogen levels after conversion to tacrolimus might also be important in reducing cardiovascular disease, because a high fibrinogen level is an important and independent cardiovascular risk factor (36). Also, in a cross-sectional study, high fibrinogen levels have been associated with chronic renal allograft dysfunction (37). With regard to determinants of the fibrinolytic system, a reduction in the level of the plasma uPA level was observed in the tacrolimus group. The plasma levels of the other determinants of the fibrinolytic system remained unchanged after conversion. uPA is involved not only in fibrinolysis but also in tissue remodeling during wound healing. High levels of uPA are associated with a higher degree of restenosis after coronary artery angioplasty (38). However, there are no data indicating that reduction of the uPA level has a role in the primary prevention of cardiovascular disease.

The manually measured systolic and diastolic BP were decreased at 3 mo after conversion from cyclosporine to tacrolimus by 7 $\pm$ 18 mmHg and 5 $\pm$ 10 mmHg, respectively. In agreement with these findings, a recent study in healthy volunteers showed that in contrast to cyclosporine, tacrolimus did not increase BP (39). However, in the current study, the decline in BP tended to wane with time and was no longer significant at 6 mo of follow-up. Measurement of BP with an automated device showed a similar trend, although the differences did not reach statistical significance, probably because of the smaller number of patients in whom the BP was determined automatically. No change in antihypertensive medication or in tacrolimus trough level that could account for the diminished effect of conversion to tacrolimus on BP after 6 mo occurred.

The Framingham risk score was used to integrate the differential effects of cyclosporine and tacrolimus on several important components of the cardiovascular risk profile, namely serum lipids, BP, and diabetes. Conversion to tacrolimus resulted in a significant reduction of the Framingham risk score. This reduction, however, was apparent only in patients who were not using statins, indicating that reduction of the LDL cholesterol level is the main factor influencing the risk score after conversion to tacrolimus. The Framingham risk score was developed in an American population that did not have specific underlying diseases and has been validated in several other geographic and ethnic populations (40). Probably, renal transplant patients will benefit from a reduction of the Framingham risk score. However, the risk score has never been validated in patients who have renal disease, and because renal patients experience other contributing pathogenic mechanisms, (*e.g.*, hyperparathyroidism, secondary hyperlipidemia, characterized by hypertriglyceridemia and small, dense LDL particles), it is unlikely that the prediction of the absolute risk of coronary heart disease from the Framingham risk score can be applied directly to these patients. Another drawback of this risk score is that it does not take into account several more recently recognized cardiovascular risk factors, such as the oxidizability of the LDL particles and the fibrinogen level.

The improvements in the cardiovascular risk profile as described above not only are important for the cardiovascular morbidity and mortality but also are likely to be of benefit for long-term graft function. Chronic allograft nephropathy (CAN) is the main cause of late graft loss in renal transplantation. The pathogenesis of CAN is multifactorial. The initiating factors are probably mainly immunologic, whereas the perpetuating factors are considered to be largely nonimmunologic, including hyperlipidemia and hypertension (41). Accordingly, increased levels of LDL cholesterol and triglycerides are associated with the occurrence of CAN after renal transplantation (11,42). Oxidatively modified LDL cholesterol is a chemotactic factor for monocytes and macrophages, both in vascular endothelium and in renal glomeruli, and it may cause activation of endothelial cells, smooth muscle cells, mesangial cells, and macrophages (43–45). Bosmans *et al.* (10) showed deposition of LDL and oxidized LDL in a mesangiocapillary way in the glomeruli, in endothelial cells, and in the interstitial space. The amount of oxidized LDL immunostaining was related to the

increase in the density of macrophages in the tubulointerstitial compartment and to the extent of interstitial fibrosis (10). Therefore, the reduction of LDL cholesterol and the improvement of LDL oxidation by tacrolimus may be of benefit for long-term graft survival.

Some studies report that tacrolimus as part of the initial maintenance immunosuppressive therapy leads to a significantly improved long-term renal graft survival compared with cyclosporine, but this effect of tacrolimus is not unequivocal (31,46). In the analysis of Hariharan *et al.*, tacrolimus was not a significant factor contributing to the recently observed improvements in graft half-life (47). Possible mechanisms by which tacrolimus could induce an improvement in graft survival include stronger immunosuppression leading to a reduction in the incidence and severity of acute rejections (30,31), differential effects on renal hemodynamics leading to diminished renal vasoconstriction on tacrolimus (39), a reduction in BP and serum lipid levels (4), and a reduction in interstitial fibrosis inducing cytokines such as TGF-β (48). On the basis of this knowledge, it is of interest to see that conversion from cyclosporine to tacrolimus in a later stage after renal transplantation still can lead to an improvement in renal function. In view of the improvement in renal function, the question arises whether equipotent dosages of cyclosporine and tacrolimus have been used. In the participating centers, where mainly patients of white origin are treated, the standard target tacrolimus trough level after 1 yr posttransplantation ranges from 5 to 8 ng/ml (49). Our mean tacrolimus trough level was lower than the level that was achieved in several previously performed long-term studies, ranging from 8.5 ng/ml to approximately 12 ng/ml, but also our cyclosporine level was low compared with these studies, in which levels ranging from 174 ng/ml to approximately 180 ng/ml were achieved (30,50). Our tacrolimus and cyclosporine trough levels were comparable with the levels in the study of Mayer *et al.*, (31) who achieved mean trough levels of 7.3 ng/ml and 119 ng/ml for tacrolimus and cyclosporine, respectively. In our population, the maintenance tacrolimus dose was sufficient to prevent acute rejections in all patients. Longer follow-up of our patients will disclose whether the improvement in graft function is sustained.

In conclusion, this study demonstrates that conversion from cyclosporine to tacrolimus results in an improved cardiovascular risk profile, with regard to serum levels as well as atherogenic properties of the lipid profile, the serum fibrinogen level, and BP. Furthermore, renal function improves. These findings are likely to be of importance in reducing the incidence of cardiovascular disease and may benefit graft survival. The diabetogenic effect of tacrolimus was of clinical relevance in a minor subset of patients, particularly those who already had an impaired glucose tolerance before conversion to tacrolimus. Overall, when long-term treatment with calcineurin inhibitors is required, tacrolimus is preferable to cyclosporine for optimization of the cardiovascular risk profile and graft function, except perhaps in patients who are known to have an impaired glucose tolerance.

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