

Renoprotective effects of atorvastatin compared with pravastatin on progression of early diabetic nephropathy

著者	Takazakura Akiko, Sakurai Masaru, Bando Yukihiro, Misu Hirofumi, Takeshita Yumie, Kita Yuki, Shimizu Akiko, Hayakawa Tetsuo, Kato Ken-ichiro, Kaneko Shuichi, Takamura Toshinari
journal or publication title	Journal of Diabetes Investigation
volume	6
number	3
page range	346-353
year	2015-05-01
URL	http://hdl.handle.net/2297/45713

doi: 10.1111/jdi.12296

Renoprotective effects of atorvastatin compared with pravastatin on progression of early diabetic nephropathy

Akiko Takazakura^{1†}, Masaru Sakurai^{3†}, Yukihiro Bando^{4†}, Hirofumi Misu^{1,2}, Yumie Takeshita¹, Yuki Kita¹, Akiko Shimizu⁵, Tetsuo Hayakawa⁵, Ken-ichiro Kato¹, Shuichi Kaneko¹, Toshinari Takamura^{1,2*}

Departments of ¹Disease Control and Homeostasis, and ²Comprehensive Metabolism, Kanazawa University Graduate School of Medical Sciences, Kanazawa, ³Department of Epidemiology and Public Health, Kanazawa Medical University, Uchinada, Ishikawa, ⁴Department of Internal Medicine, Fukui-ken Saiseikai Hospital, Fukui, Fukui, and ⁵Department of Internal Medicine, Toyama City Hospital, Toyama, Toyama, Japan

Keywords

Early diabetic nephropathy, Renoprotective effects, Statins

*Correspondence

Toshinari Takamura
 Tel.: +81-76-265-2233
 Fax: +81-76-234-4250
 E-mail address: ttakamura@m-kanazawa.jp

J Diabetes Invest 2015; 6: 346–353

doi: 10.1111/jdi.12296

ABSTRACT

Introduction: Several studies have shown that statins suppress the progression of diabetic nephropathy. However, few reports have directly compared the renoprotective effects between potent and conventional statins.

Materials and Methods: Patients with diabetic nephropathy, selected as those with a serum creatinine level of 0.9–1.5 mg/dL and simultaneously having either microalbuminuria or positive proteinuria, were randomly assigned to one of three groups: a conventional diet therapy group, a group given 10 mg of pravastatin and a group given 10 mg of atorvastatin. Renal function was evaluated before and after a 12-month period of therapy.

Results: The atorvastatin group had a significant decrease in low-density lipoprotein cholesterol at 3 months and thereafter compared with the other groups. The urinary albumin-to-creatinine ratio significantly decreased in the atorvastatin group; the degree of this decrease was significantly greater than that in the diet therapy group. The kidney function estimated with cystatin C (CysC) and the estimated glomerular filtration rate calculated from CysC were significantly preserved in the atorvastatin group compared with the pravastatin group. In a multivariate regression analysis, the use of atorvastatin was the only explanatory variable for the changes in CysC; this was independent of changes in low-density lipoprotein cholesterol.

Conclusions: Atorvastatin is more effective than pravastatin for the prevention of increase in CysC, and this renoprotective effect was considered to a result of the pleiotropic effect of atorvastatin independent of its lipid-lowering effect. This study was registered with UMIN (no. UMIN 000001774).

INTRODUCTION

Diabetes mellitus is a major cause of chronic kidney disease (CKD). Evidence has been growing that intensive glycemic control or antihypertensive therapy with antagonists of the renin–angiotensin system suppresses the progression of CKD in patients with diabetes^{1,2}. However, optimal management strategies for diabetic nephropathy have not been established³.

We previously reported that administration of a statin decreases albuminuria in a rat model of experimental diabetic nephropathy through pleiotropic effects⁴. To date, many lines of clinical evidence have reported that dyslipidemia is connected with the progression of kidney diseases^{5–7}, probably as a result of lipotoxicity⁸. Large-scale meta-analyses have shown that statin therapy has a protective effect against deterioration of renal function in CKD patients with baseline albuminuria above 30 mg/day^{9,10}.

Lipid-lowering therapy by statins might also be useful in reducing albuminuria in type 2 diabetic patients with an early stage of diabetic nephropathy¹¹. Furthermore, several lines of

†These authors contributed equally to this work.
 Received 12 February 2014; revised 19 September 2014; accepted 30 September 2014

evidence show that potent statins, such as atorvastatin and rosuvastatin, prevent deterioration of kidney function in diabetic patients with CKD^{12,13}. However, it has not been fully determined whether the renoprotective effect of statins is independent of their lipid-lowering action. In addition, no reports are available that directly compare conventional statins and potent statins in terms of their renoprotective effects against CKD in patients with diabetes. In the current study, we evaluated the effects of pravastatin and atorvastatin on renal outcomes in diabetic patients with early diabetic nephropathy.

MATERIALS AND METHODS

The present research project was a prospective study spanning 12 months, and was a randomized, open-label, clinical study. Patients were recruited from 2008 through 2010 from Kanazawa University Hospital, Toyama City Hospital and Fukui-ken Saiseikai Hospital. The enrolment criteria were as follows: the participants had to (i) be diabetic patients currently being seen on an outpatient basis, who had not been treated with statin therapy within 3 months of the start of the study; (ii) had a low-density lipoprotein cholesterol (LDL-C) of 120–150 mg/dL two consecutive times; and (iii) had a blood serum creatinine (Cr) level of 0.9–1.5 mg/dL simultaneously with a test result of either microalbuminuria (≥ 30 mg/gCr) or positive proteinuria (qualitative) more than twice. Microalbuminuria and proteinuria were derived from proper laboratory tests. Participants could have either type 1 or type 2 diabetes, could be any age between 18 and 80 years, and could be either sex. Exclusion criteria were as follows: (i) poor blood pressure (BP) control (160/100 mmHg or higher); (ii) glycemic control that was either poor or unstable (the most recent glycated hemoglobin [HbA1c; National Glycohemoglobin Standardization Program (NGSP) value] was 10% or higher, or change in HbA1c over the most recent 3 months was 2% or higher); (iii) patients with hematuria; (iv) patients with a urinary tract infection; and (v) patients with a past history of atherosclerosis, such as coronary heart disease, stroke and peripheral artery disease. Informed consent to the study was obtained in writing from all study participants. The research protocol was approved by the ethics committee of each facility involved, and the study was carried out in compliance with the Declaration of Helsinki.

Figure S1 summarizes the participants' progress through the trial from the perspective of the Consolidated Standards of Reporting Trials criteria for randomized trials. After a prior assessment, 120 patients were randomly assigned to one of three groups: a group that would be treated with diet therapy, a group that would be given a statin (pravastatin) and a group that would be treated with a potent statin (atorvastatin). The subjects' identification number on their medical chart determined their group. Over the 12 months of the study, 43 of the patients would be provided with dietary guidance (the diet therapy group). The dietary guidance was provided by a national registered dietitian at the hospitals at least once every 3 months. The dietitian checked the meals and gave the

participants some advice about the diet for diabetic nephropathy, such as restriction of protein (~0.8 g/kg) and salt intake (<6 g/day). The other 28 patients would be given 10 mg/day of pravastatin in addition to being provided with dietary guidance (the pravastatin group), and 35 patients would be given 10 mg/day of atorvastatin in addition to dietary guidance (the atorvastatin group).

Blood and urine samples were collected at minimum intervals of 3 months. If a patient began dialysis during the 12 months of the monitoring period, a final evaluation of that patient was carried out at that point. It was decided that, as a rule, the medications listed here would not be added to a patient's regimen during the monitoring period: therapeutic agents for the treatment of hyperlipidemia other than statins (fibrates, ezetimibe, nicotinic acid, probucol, resins, ethyl icosapentate formulations and others) and medications having an effect of reducing proteinuria, such as dipyridamole. Administration of the following would be carried out only at the minimum necessary level during the monitoring period: non-steroidal anti-inflammatory drugs and antibiotics that affect renal function. It was decided that standard therapies consisting primarily of angiotensin-converting enzyme (ACE) inhibitors or angiotensin II receptor blockers (ARBs) would be given with the aim of keeping BP under 130/80 mmHg (or under 125/75 mmHg if proteinuria 1 g/day or higher was observed). Adjustments for glycemic control would be made as necessary with oral medications or insulin dosages, in addition to diet and exercise programs, to keep HbA1c under 6.9% (NGSP) according to the guidelines of the Japan Diabetes Society¹⁴.

Measurements

Height, bodyweight, BP, fasting blood values, and urine values were measured at baseline and at every consultation for 3 months. Body mass index (BMI) was calculated as weight/height² (kg/m²). Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and triglycerides (TGs) were measured using commercially available kits. LDL-C was calculated by the Friedewald method. HbA1c was measured using the Tosoh G8 analyzer (Tosoh, Tokyo, Japan) at Kanazawa University Hospital, the Tosoh G7 analyzer (Tosoh, Tokyo, Japan) at Toyama City Hospital and the ADAMS A1c HA-8180 analyzer (ARK-RAY, Kyoto, Japan) at Fukui-ken Saiseikai Hospital. Quality control of HbA1c measurements was carried out using the standard certified by the Japanese Diabetes Society (JDS). HbA1c values were converted to NGSP values using the formula provided by the JDS: HbA1c (NGSP) = 1.02 × HbA1c (JDS) + 0.25¹⁵.

Serum cystatin C (CysC) was measured using nephelometry at Kanazawa University Hospital, the gold colloid aggregation method at Toyama City Hospital and the latex agglutination turbidimetric immunoassay at Fukui-ken Saiseikai Hospital; that is, in different ways at the different facilities. However, according to in-house test reports, there is an extremely strong correlation between nephelometry and the gold colloid aggregation

method ($r = 0.996$), and between the gold colloid aggregation method and the latex agglutination turbidimetric immunoassay ($r = 0.999$). The urinary albumin-to-Cr ratio (U-Alb/Cr) was measured using the first morning urine. Albumin was measured using immunonephelometry. The estimated glomerular filtration rate (eGFR) was calculated using the following formula recommended for Japanese patients¹⁶: $eGFR \text{ (mL/min/1.73 m}^2\text{)} = 194 \times Cr^{-1.094} \times age^{-0.287} \times 0.739$ (if female). The eGFR using serum CysC (eGFR_{cys}) was calculated using the following formula recommended for Japanese patients¹⁷: $eGFR\text{-cys (mL/min/1.73 m}^2\text{)} = (104 \times Scys^{-1.019} \times 0.996^{Age} \times 0.929$ [if female]) - 8.

Statistical Analyses

Characteristics of the baseline profiles were compared among the three groups using the χ^2 -test for categorical variables, and one-way analysis of variance (ANOVA) and the *post-hoc* test (Bonferroni method) for continuous variables. In this intervention study, we used the changes in renal function (eGFR, U-Alb/Cr, CysC) over the 12-month period for primary outcome. Analysis of covariance (ANCOVA) with repeated measurements and the *post-hoc* test (Bonferroni method) were used to evaluate the differences in changes of LDL-C and renal function among the three groups. Multiple linear regression analyses were used to evaluate the factors that affected the 12-month changes in CysC. Age, sex, baseline bodyweight, baseline systolic BP, use of ACE inhibitors or ARBs during follow up, use of pravastatin and use of atorvastatin, and 12-months' changes in LDL-C, HbA1c, systolic BP and bodyweight were used as explanatory variables. Statistical analyses were carried out using the Japanese version of the Statistical Package for the Social Sciences (SPSS version 11.0; SPSS Japan Inc., Tokyo, Japan). A *P*-value of <0.05 was taken as statistically significant.

RESULTS

Baseline Clinical Characteristics

Table 1 shows the baseline clinical characteristics according to the different lipid-lowering therapies. There were no statistical differences in sex, diabetes type, age, BMI, duration of diabetes, systolic BP, use of ACE inhibitors or ARBs, HbA1c, TGs, HDL-C, or diabetes treatment method among the three groups. However, TC and LDL-C were higher in the atorvastatin group than in the diet therapy group.

Changes in Lipids and Other Metabolic Variables

Table 1 shows the differences in the clinical characteristics between the measurements at baseline and at 12 months. In the diet therapy group, no significant differences were observed in lipids, systolic BP and HbA1c at 12 months. In the pravastatin group and the atorvastatin group, significant decreases were observed in TC ($P = 0.024$ for the pravastatin group and $P < 0.001$ for the atorvastatin group) and in LDL-C ($P = 0.002$ for the pravastatin group and $P < 0.001$ for the atorvastatin group). In contrast, no significant differences were observed in

HDL-C, TGs, systolic BP and HbA1c during the follow-up period.

The pravastatin and atorvastatin groups showed significant reductions in LDL-C at 12 months compared with the baseline. In the atorvastatin group, LDL-C was significantly lower at 3 months and thereafter compared with the other two groups.

Renal Outcomes

The means of eGFR (mL/min/1.73 m²) were 63.6 ± 16.8 at baseline and 61.3 ± 16.3 at 12 months. There was no patient who required dialysis treatment during the follow-up period.

Figure 1a shows the percent changes in $\log(U\text{-Alb/Cr})$ among the three groups. A significant decrease in $\log(U\text{-Alb/Cr})$ was seen at 12 months in the atorvastatin group, and the values were significantly lower compared with the diet therapy group.

Figure 1b shows the changes in eGFR according to the three categories of lipid-lowering therapy. Compared with the diet and pravastatin groups, the atorvastatin group showed significant improvement in eGFR at 3 months; however, no differences were observed at 6 months and after among the three groups. As shown in Figure 1c, CysC was significantly lower in the atorvastatin group at 6 months and at 12 months compared with the pravastatin group. As shown in Figure 1d, eGFR_{cys} (eGFR calculated from CysC) was significantly improved in the atorvastatin group at 12 months compared with the pravastatin group.

Cystatin C was significantly lower in the atorvastatin group at 6 months and at 12 months compared with the pravastatin group. These results suggest that atorvastatin had a beneficial effect on the change in CysC.

We used multivariate regression analysis to review possible connections with other factors that might affect the change in CysC. In multivariate regression analysis, the use of atorvastatin was the only explanatory variable for the changes in CysC; this was independent of changes in LDL-C, as well as of sex, age, bodyweight, systolic BP, use of ACE inhibitors or ARBs and changes in HbA1c (Table S1). The results were similar when we evaluated the associations in the 102 patients after excluding four patients (one in the diet therapy group, one in the pravastatin group and two in the atorvastatin group). We also used multivariate regression analysis to review possible connections with other factors that might affect the change in eGFR_{cys} among the three groups (Table S1). 'The use of atorvastatin' and 'percent change in LDL-C at 12 months' were the explanatory variable for the changes in eGFR_{cys}.

DISCUSSION

The present study compared the renoprotective effects in diabetic patients with mild CKD during 1 year between three lipid-lowering therapies: diet therapy, pravastatin and atorvastatin. Atorvastatin, but not pravastatin, significantly decreased the U-Alb/Cr from the baseline, and the degree of this decrease

Table 1 | Baseline and follow-up clinical features of laboratory markers by treatment for hyperlipidemia

	Diet therapy group			Pravastatin group			Atorvastatin group		
	0 M	12 M	<i>P</i>	0 M	12 M	<i>P</i>	0 M	12 M	<i>P</i>
No. patients	43			28			35		
Men: Women	38:5			25:3			29:6		
Type 1: type 2 diabetes	1:42			1:27			2:33		
Age (years)	63 ± 11			63 ± 11			60 ± 11		
Diabetes duration (years)	8.0 ± 8.4			8.4 ± 6.1			6.4 ± 3.5		
ACEI or ARBS (+: -)	20:23	20:23	1.000	14:14	14:14	1.000	17:18	18:17	0.085
Diabetes treatment (Diet: OHA: insulin)	5:30:8	5:30:8	1.000	3:14:11	3:14:11	1.000	7:21:7	7:21:7	1.000
BMI (kg/m ²)	24.3 ± 3.2	24.7 ± 3.3	0.200	23.4 ± 3.0	23.9 ± 3.2	0.154	23.8 ± 3.3	23.7 ± 2.9	1.000
Systolic blood pressure (mmHg)	128 ± 12	129 ± 12	1.000	129 ± 13	125 ± 16	0.385	129 ± 14	126 ± 13	0.557
HbA1c (NGSP) (%)	7.3 ± 0.8	7.4 ± 1.0	1.000	7.4 ± 1.1	7.4 ± 1.1	1.000	7.7 ± 1.5	7.7 ± 1.5	1.000
TC (mg/dL)	186 ± 27	193 ± 34	0.508	193 ± 32	175 ± 27	0.024	205 ± 35†	153 ± 24	<0.001
LDL-C (mg/dL)	109 ± 25	109 ± 25	1.000	118 ± 28	98 ± 21	0.002	127 ± 25†	74 ± 16	<0.001
HDL-C (mg/dL)	50 ± 14	52 ± 19	0.127	49 ± 10	48 ± 11	1.000	49 ± 13	51 ± 15	0.333
TG (mg/dL)	134 ± 62	154 ± 136	0.404	146 ± 87	154 ± 87	1.000	153 ± 79	137 ± 92	0.812
eGFR (mL/min/1.73 m ²)	61.4 ± 16.0	58.3 ± 13.0	0.077	66.3 ± 17.0	63.5 ± 17.0	0.313	64.0 ± 18.0	63.2 ± 18.0	1.000
eGFR _{cys} (mL/min/1.73 m ²)	90.1 ± 26.9	88.3 ± 27.0	0.706	83.1 ± 20.1	79.1 ± 21.9	0.091	92.5 ± 27.9	94.9 ± 29.5	0.471
Cystatin C (mg/L)	0.89 ± 0.30	0.90 ± 0.28	1.000	0.92 ± 0.19	0.99 ± 0.28	0.053	0.87 ± 0.23	0.85 ± 0.21	1.000
Log(U-Alb/Cr)	1.4 ± 0.8	1.5 ± 0.8	0.625	1.6 ± 1.1	1.5 ± 1.2	1.000	1.3 ± 0.6	1.1 ± 0.7	0.041

Data values are mean ± standard deviation. The χ^2 -test for categorical variables and one-way analysis of variance for continuous variables were used for the analyses. Repeated measures analysis of covariance was used to compare the details between 0 month and 12 months. ACEI, angiotensin-converting enzyme inhibitors; ARBS, angiotensin II receptor blockers; BMI, body mass index; Diet, diet therapy; eGFR, estimated glomerular filtration rate; eGFR_{cys}, estimated glomerular filtration rate using serum cystatin C; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; insulin, insulin therapy; LDL-C, low-density lipoprotein cholesterol; Log(U-Alb/Cr), log-transformed urinary albumin/creatinine; M, months; OHA, oral hypoglycemic agent; TC, total cholesterol; TG, triglyceride. †*P* < 0.05 vs diet therapy group by *post-hoc* test (Bonferroni).

was significantly greater compared with the diet therapy. Although there were no significant differences in the changes of eGFR among the three groups, atorvastatin significantly preserved CysC levels and eGFR_{cys} compared with pravastatin. These findings showed that the renoprotective effects are different between atorvastatin and pravastatin, and that atorvastatin is more useful than pravastatin for its renoprotective effect in diabetic patients with early diabetic nephropathy.

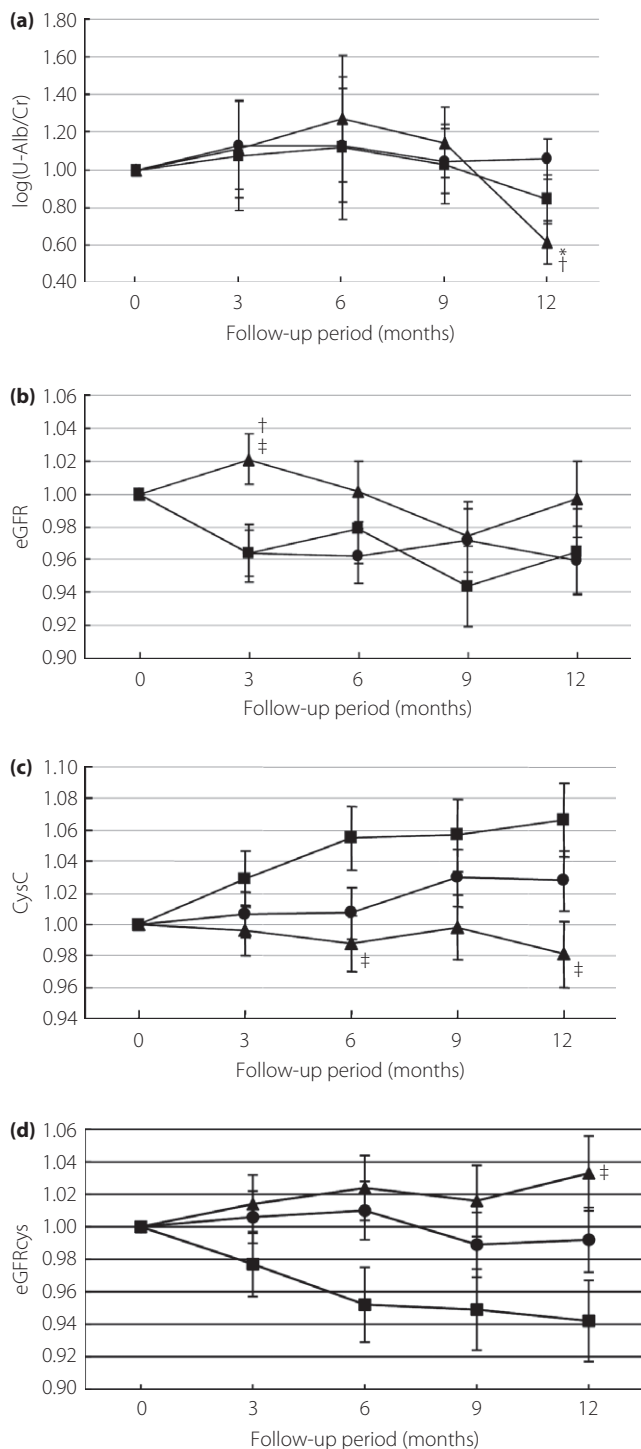
Some studies have reported that atorvastatin has a protective effect on renal function^{9,10,12,18–21}. In the Greek Atorvastatin and Coronary-heart-disease Evaluation (GREACE) study, aggressive administration of atorvastatin induced an increase in Cr clearance (CrCl) after 6 weeks, and the effect was continued for up to 48 months¹⁸. However, in the present study, eGFR did not change during the 12 months in the atorvastatin group, as well as in the diet group and pravastatin group. Our study findings are in concert with a previous single arm study²², that in patients with CKD, administration of rosuvastatin, one of the other strong statins, at a dose of 2.5 mg for 24 weeks, significantly decreased U-Alb/Cr and CysC, but did not change eGFR.

These differences in the results might due to the low dose of statins used in the present study. In our study, we used 10 mg/day of atorvastatin, whereas, in the GREACE study, atorvastatin

was started at 10 mg/day, and the dose was titrated up to a maximum of 80 mg/day (mean dose 24 mg/day)¹⁸. The Treating to New Targets study showed that atorvastatin administration increased eGFR in a dose-dependent manner, and the increase was significantly greater in patients who received a high dose (80 mg/day) compared with those who received a low dose (10 mg/day)¹⁹.

Differences in the renal function of the study participants might also affect the results. In the GREACE study, renoprotective effects have been particularly prominent with respect to early-stage renal disease. The Collaborative Atorvastatin Diabetes Study also showed that atorvastatin improves eGFR in patients with type 2 diabetes with albuminuria, but not in those with normo-albuminuria¹². Similar to the atorvastatin, pravastatin also prevented the decrease in eGFR in patients with moderate CKD (eGFR <40 mL/min/1.73 m²), but not in those with mild CKD (eGFR >60 mL/min/1.73 m²)^{23, 24}. In our study patients, mean eGFR (61.4–66.3 mL/min/1.73 m²) was relatively preserved even though they had albuminuria/proteinuria. This might relate to the result that eGFR did not change in our study participants.

Furthermore, the short follow-up period may also affect the results. The Protection Against Nephropathy in Diabetes with Atorvastatin trial reported that in patients with type 2 diabetes



with early renal disease, no statistical difference was observed in renal function between those taking high- (80 mg/day) or low-dose (10 mg/day) atorvastatin over 2.1 years²⁵. However, the study periods of this trial and our present study are shorter than those of the GREACE study and the Treating to New Targets study. Therefore, it is difficult to compare these results directly.

Figure 1 | Time-course of changes in urinary albumin excretion (U-Alb/Cr), estimated glomerular filtration rate (eGFR), serum cystatin C (CysC) and estimated glomerular filtration rate using serum cystatin C (eGFRcys) from baseline across the three groups of cholesterol treatment. Data values are mean \pm standard error. Black circles, diet therapy group; black squares, pravastatin group; black triangles, atorvastatin group. Repeated measures analysis of covariance and the *post-hoc* test (Bonferroni) was used for the analysis. * $P < 0.05$ vs baseline values. † $P < 0.05$ vs the diet therapy group. ‡ $P < 0.05$ vs the pravastatin group. Log(U-Alb/Cr), log-transformed urinary albumin/creatinine.

In the present study, U-Alb/Cr was significantly improved in the atorvastatin group, but not in the pravastatin group, at 12 months compared with the baseline. More strikingly, the change in CysC was significantly smaller, and eGFRcys was significantly higher in the atorvastatin group compared with the pravastatin group. To date, there are no reports that have compared the preventive effect on renal dysfunction progression between atorvastatin and other statins using CysC and eGFRcys as indices.

To date, eGFR and U-Alb/Cr calculated from serum creatinine (sCr) is commonly used for the diagnosis of CKD. However, because sCr does not change in a mild to moderate decrease in creatinine clearance (CrCl), eGFR and U-Alb/Cr calculated by sCr might not be effective to evaluate the renal function in a relatively early stage of CKD. In recent years, CysC has been tested as a better marker that detects an early decrease of CrCl in mild CKD. CysC is a low molecular mass serum protein that is freely filtered by the glomerulus and reabsorbed by the proximal kidney tubule. Serum Cr increases when CrCl decreases to 31–50 mL/min, whereas CysC starts to increase from a milder CrCl decrease of 51–70 mL/min²⁶. Because eGFR is calculated based on the serum Cr, it does not reflect a mild decrease in CrCl in patients with relatively high CrCl. Thus, using the CysC and eGFRcys as indices for renal function could detect slight changes in CrCl, especially in patients with an early stage of CKD. Therefore, in the present study, we compared the differences of these markers for renal function. Together with the discussion here, longer study is required to conclude the effect of atorvastatin on eGFR calculated from sCr.

We postulate the reasons for the discrepancy between a significant, but subtle, effect on U-Alb/Cr and the obvious effect throughout the study period on CysC of atorvastatin beyond pravastatin. First, the Cr serum level is affected by diet, inflammation, age, sex, muscle mass, exercise and other parameters, whereas the CysC serum level is not affected by these factors²⁷. Indeed, the standard deviation was much larger in U-Alb/Cr than CysC in the present study. Second, the discrepancy might suggest the specific mechanism underlying the protective effect of atorvastatin on renal function; statin might more preferentially improve GFR rather than hyperfiltration or size/charge barriers of the glomerular basement membrane. It is postulated

that statins gradually increase GFR as a result of their pleiotropic actions, such as endothelial vasodilation (and the increase in circulating plasma volume accompanying it), regression of atheromatous renovascular stenosis, regression of intimal hyperplasia in arcuate arteries or arteriosclerosis progression delay in afferent arterioles¹⁸. Future study evaluating the effect of atorvastatin stratified by impaired GFR-dominant and albuminuria/proteinuria-dominant types of diabetic nephropathy might test this hypothesis. Interestingly, a similar result was reported as a protective effect of an Nrf2 activator bardoxolone methyl on kidney function in CKD with type 2 diabetes²⁸. Patients receiving bardoxolone methyl had a significant increase in eGFR and had a slight, but significant, increase in the U-Alb/Cr as compared with placebo at 24 and 52 weeks. Therefore, the effect of statins on the Keap1/Nrf2 system in the kidney might be a novel issue worth investigating. Indeed, several statins were reported to activate Nrf2 signaling in the liver²⁹, embryonic fibroblasts³⁰, vascular smooth muscle cells³¹ and brain³².

The present results from multiple regression analyses showed that the use of atorvastatin affected CysC independently of its cholesterol-lowering effect. The previous prospective studies in diabetic patients with progressive renal disease compared the renoprotective effect of atorvastatin and rosuvastatin for CKD patients²⁰, and showed that the renoprotective effect was stronger in atorvastatin (80 mg/day) than high-dose rosuvastatin (40 mg/day), whereas rosuvastatin had stronger cholesterol-lowering effects. These results suggest that the renoprotective effect of atorvastatin would be due to pleiotropic effects beyond its lipid-lowering effect.

The strength of the present study is that it is a direct comparison between atorvastatin and pravastatin with respect to their effect on renal function. There has been no previous report that directly compared the effect of potent statins and conventional statins on the progression of CKD in patients with diabetes. Furthermore, there have been no reports that compared the preventive effect on renal dysfunction progression between atorvastatin and other statins using CysC as an index. Previous studies^{23,24} suggest that the renoprotective effect of pravastatin is limited to moderate to severe kidney disease, and that atorvastatin has a superior effect on early diabetic nephropathy through its pleiotropic effect. Such properties of the different classes of statins might underlie the advantage of atorvastatin over pravastatin in the renoprotective effect, because the subjects enrolled in the present study had relatively mild CKD.

There were several limiting factors in the present study. First, CysC was measured in different ways at the different facilities. However, according to in-house test reports, there is an extremely strong correlation between nephelometry and the gold colloid aggregation method, and between the gold colloid aggregation method and the latex agglutination turbidimetric immunoassay. As a result, we postulate that as long as CysC is measured using the same method, there is a strong possibility

that similar results can be obtained. Second, the number of patients happened to be different in the three groups because we used the participants' identification number on their medical chart to determine their group. However, baseline characteristics, such as sex, age and blood pressure, were not different among the three groups, and the results observed in the present study are independent of these confounding factors, which affect the changes in renal function. Finally, kidney biopsy was not carried out in all cases. In particular, three patients out of 14 patients with macroalbuminuria were not complicated with diabetic retinopathy. We ruled out malignancy-associated membranous nephropathy and other nephritis syndrome clinically in these patients.

In conclusion, the present study showed that atorvastatin inhibits the elevation of CysC in diabetic patients to a greater extent than pravastatin. In addition, this renoprotective effect was considered to be the result of the pleiotropic effect of atorvastatin independent of its lipid-lowering effect. For lipid management in diabetic patients, atorvastatin is more effective than pravastatin in terms of preventing increase in CysC.

ACKNOWLEDGMENTS

We thank all of the medical and nursing staff at the Kanazawa University Hospital, Toyama City Hospital, and Fukui-ken Saiseikai Hospital for their dedication and commitment to collecting and examining the patients' blood and urine samples. We thank the registered dietitian for developing the diet therapy, based on guidelines. TT is the guarantor of this work and, as such, had full access to all of the data in the study, and takes responsibility for the integrity of the data and the accuracy of the data analysis. SK has received research grants from Daiichi-Sankyo, Pfizer and Astellas. No other potential conflicts of interest relevant to this article are reported.

REFERENCES

1. Patel A, MacMahon S, Chalmers J, *et al.* Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. *N Engl J Med* 2008; 358: 2560–2572.
2. Mann JF, Schmieder RE, McQueen M, *et al.* Renal outcomes with telmisartan, ramipril, or both, in people at high vascular risk (the ONTARGET study): a multicentre, randomised, double-blind, controlled trial. *Lancet* 2008; 372: 547–553.
3. Fioretto P, Dodson PM, Ziegler D, *et al.* Residual microvascular risk in diabetes: unmet needs and future directions. *Nat Rev Endocrinol* 2010; 6: 19–25.
4. Ota T, Takamura T, Ando H, *et al.* Preventive effect of cerivastatin on diabetic nephropathy through suppression of glomerular macrophage recruitment in a rat model. *Diabetologia* 2003; 46: 843–851.
5. Adinortey MB, Gyan BE, Adjimani J, *et al.* Dyslipidaemia Associated with Type 2 Diabetics with Micro and Macrovascular Complications among Ghanaians. *Indian J Clin Biochem* 2011; 26: 261–268.

6. Keane WF, Tomassini JE, Neff DR. Lipid abnormalities in patients with chronic kidney disease: implications for the pathophysiology of atherosclerosis. *J Atheroscler Thromb* 2013; 20: 123–133.
7. Afzali B, Haydar AA, Vinen K, *et al.* Beneficial effect of statins on the kidney: the evidence moves from mouse to man. *Nephrol Dial Transplant* 2004; 19: 1032–1036.
8. Vaziri ND. Lipotoxicity and impaired high density lipoprotein-mediated reverse cholesterol transport in chronic kidney disease. *J Ren Nutr* 2010; 20(5 Suppl): S35–S43.
9. Sandhu S, Wiebe N, Fried LF, *et al.* Statins for improving renal outcomes: a meta-analysis. *J Am Soc Nephrol* 2006; 17: 2006–2016.
10. Douglas K, O'Malley PG, Jackson JL. Meta-analysis: the effect of statins on albuminuria. *Ann Intern Med* 2006; 145: 117–124.
11. Kimura S, Inoguchi T, Yokomizo H, *et al.* Randomized comparison of pitavastatin and pravastatin treatment on the reduction of urinary albumin in patients with type 2 diabetic nephropathy. *Diabetes Obes Metab* 2012; 14: 666–669.
12. Colhoun HM, Betteridge DJ, Durrington PN, *et al.* Effects of atorvastatin on kidney outcomes and cardiovascular disease in patients with diabetes: an analysis from the Collaborative Atorvastatin Diabetes Study (CARDS). *Am J Kidney Dis* 2009; 54: 810–819.
13. Abe M, Maruyama N, Okada K, *et al.* Effects of lipid-lowering therapy with rosuvastatin on kidney function and oxidative stress in patients with diabetic nephropathy. *J Atheroscler Thromb* 2011; 18: 1018–1028.
14. Japan Diabetes Society. Treatment Guide for Diabetes. Tokyo, Japan, Bunkodo, 2007.
15. Kashiwagi A, Kasuga M, Araki E, *et al.* International clinical harmonization of glycated hemoglobin in Japan: From Japan Diabetes Society to National Glycohemoglobin Standardization Program values. *J Diabetes Invest* 2012; 3: 39–40.
16. Matsuo S, Imai E, Horio M, *et al.* Revised equations for estimated GFR from serum creatinine in Japan. *Am J Kidney Dis* 2009; 53: 982–992.
17. Horio M, Imai E, Yasuda Y, *et al.* GFR estimation using standardized serum cystatin C in Japan. *Am J Kidney Dis* 2013; 61: 197–203.
18. Athyros VG, Mikhailidis DP, Papageorgiou AA, *et al.* The effect of statins versus untreated dyslipidaemia on renal function in patients with coronary heart disease. A subgroup analysis of the Greek atorvastatin and coronary heart disease evaluation (GREACE) study. *J Clin Pathol* 2004; 57: 728–734.
19. Shepherd J, Kastelein JJ, Bittner V, *et al.* Intensive lipid lowering with atorvastatin in patients with coronary heart disease and chronic kidney disease: The TNT (Treating to New Targets) Study. *J Am Coll Cardiol* 2008; 51: 1448–1454.
20. de Zeeuw D. Different renal protective effect of atorvastatin and rosuvastatin diabetic and non-diabetic renal patients with proteinuria. Results of the PLANET trials 2010. *European Renal Association-European Dialysis and Transplant Association Congress, Munich, 2010.*
21. Bianchi S, Bigazzi R, Caiazza A, *et al.* A controlled, prospective study of the effects of atorvastatin on proteinuria and progression of kidney disease. *Am J Kidney Dis* 2003; 41: 565–570.
22. Abe M, Maruyama N, Y Yoshida, *et al.* Efficacy analysis of the lipid-lowering and renoprotective effects of rosuvastatin in patients with chronic kidney disease. *Endocr J* 2011; 58: 663–674.
23. Tonelli M, Moyé L, Sacks FM, *et al.* Effect of pravastatin on loss of renal function in people with moderate chronic renal insufficiency and cardiovascular disease. *J Am Soc Nephrol* 2003; 14: 1605–1613.
24. Tonelli M, Isles C, Craven T, *et al.* Effect of pravastatin on rate of kidney function loss in people with or at risk for coronary disease. *Circulation* 2005; 112: 171–178.
25. Rutter MK, Prais HR, Charlton-Menys V, *et al.* Protection Against Nephropathy in Diabetes with Atorvastatin (PANDA): a randomized double-blind placebo-controlled trial of high- vs. low-dose atorvastatin(1). *Diabet Med* 2011; 28: 100–108.
26. Shimizu-Tokiwa A, Kobata M, Ito H, *et al.* Serum cystatin C is a more sensitive marker of glomerular function than serum creatinine. *Nephron* 2002; 92: 224–226.
27. Newman DJ. Cystatin C. *Ann Clin Biochem* 2002; 39: 89–104.
28. Pergola PE, Raskin P, Toto RD, *et al.* BEAM Study Investigators. Bardoxolone methyl and kidney function in CKD with type 2 diabetes. *N Engl J Med* 2011; 365: 327–336.
29. Habeos IG, Ziros PG, Chartoumpekis D, *et al.* Simvastatin activates Keap1/Nrf2 signaling in rat liver. *J Mol Med (Berl)* 2008; 86: 1279–1285.
30. Chartoumpekis D, Ziros PG, Psyrogiannis A, *et al.* Simvastatin lowers reactive oxygen species level by Nrf2 activation via PI3K/Akt pathway. *Biochem Biophys Res Commun* 2010; 396: 463–466.
31. Makabe S, Takahashi Y, Watanabe H, *et al.* Fluvastatin protects vascular smooth muscle cells against oxidative stress through the Nrf2-dependent antioxidant pathway. *Atherosclerosis* 2010; 213: 377–384.
32. Du Y, Zhang X, Ji H, *et al.* Probuco and atorvastatin in combination protect rat brains in MCAO model: upregulating Peroxiredoxin2, Foxo3a and Nrf2 expression. *Neurosci Lett* 2012; 509: 110–115.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1 | The variables associated with 12-month change in cystatin C and estimated glomerular filtration rate using serum cystatin C.

Figure S1 | Consolidated Standards of Reporting Trials flow diagram. Summary of recruitment, randomization and participants' progress through the trial. After a prior assessment, 120 patients were randomly assigned to one of three groups: a group that would be treated with diet therapy, a group that would be given a statin (pravastatin) and a group that would be treated with a potent statin (atorvastatin). The patients' identification number on their medical chart determined their group. Over the 12 months of the study, 43 of the patients would be provided with dietary guidance (the diet therapy group). The other 28 patients would be given 10 mg/day of pravastatin in addition to being provided with dietary guidance (the pravastatin group), and 35 patients would be given 10 mg/day of atorvastatin in addition to dietary guidance (the atorvastatin group).