候群(成人例)の診療指針．日腎会誌 2002；44：751-761.
8. Sugiyama H, Yokoyama H, Hiroshi Sato H, et al. Japan Renal Biopsy Registry：The first nationwide, web-based, and prospective registry system of renal biopsies in Japan. Clin Exp Nephrol(in press)
9. Nationwide and long-term survey of primary glomerulonephritis in Japan as observed in 1,850 biopsied cases. Research Group on Progressive Chronic Renal Disease. Nephron 1999；82：205-213.
10. Li LS, Liu ZH. Epidemiologic data of renal diseases from a single unit in China：analysis based on 13,519 renal biopsies. Kidney Int 2004；66：920-923.
11. Gesualdo L, Di Palma AM, Morrone LF, Strippoli G, Schena FP. The Italian experience of the national registry of renal biopsies. Kidney Int 2004；66：890-894.
12. Rychlik I, Jancova E, Tesar V, et al. The Czech registry of renal biopsies. Occurrence of renal diseases in the years 1994-2000. Nephrol Dial Transplant 2004；19：3040-3049.
13. Covic A, Schiller A, Volovat C, et al. Epidemiology of renal disease in Romania：a 10 year review of two regional renal biopsy databases. Nephrol Dial Transplant 2006；21：419-424.
14. Malafronte P, Mastroianni-Kirsztajn G, Betonico GN, et al. Paulista registry of glomerulonephritis：5-year data report. Nephrol Dial Transplant 2006；21：3098-3105.
15. Polito MG, Moura LA, et al. An overview on frequency of renal biopsy diagnosis in Brazil：clinical and pathological patterns based on 9,617 native kidney biopsies. Nephrol Dial Transplant 2010；25：490-496.
16. Chang JH, Kim DK, Kim HW, et al. Changing prevalence of glomerular diseases in Korean adults：a review of 20 years of experience. Nephrol Dial Transplant 2009；24：2406-2410.
17. Korbet SM, Genchi RM, Borok RZ, Schwartz MM. The racial prevalence of glomerular lesions in nephritic adults. Am J Kidney Dis 1996；27：647-651.

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ARTICLE

Long-term effect of modification of dietary protein intake on the progression of diabetic nephropathy: a randomised controlled trial

D. Koya · M. Haneda · S. Inomata · Y. Suzuki · D. Suzuki · H. Makino · K. Shikata · Y. Murakami · Y. Tomino · K. Yamada · S. I. Araki · A. Kashiwagi · R. Kikkawa · on behalf of the Low-Protein Diet Study Group

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Abstract
Aims/hypothesis There is currently insufficient evidence to recommend a low-protein diet for type 2 diabetic patients with diabetic nephropathy. We assessed whether a low-protein diet could prevent the progression of diabetic nephropathy.
Methods This was a multi-site parallel randomised controlled trial for prevention of diabetic nephropathy progression among 112 Japanese type 2 diabetic patients with overt nephropathy. It was conducted in Japan from 1 December 1997 to 30 April 2006. The participants were randomly assigned using a central computer-generated schedule to either low-protein diet (0.8 g $kg^{-1} day^{-1}$) and normal-protein diet (1.2 g $kg^{-1} day^{-1}$), and were followed for 5 years. The participants and investigators were not blinded to the assignment. The primary outcomes were the annual change in estimated GFR and creatinine clearance, the incidence of doubling of serum creatinine and the time to doubling of baseline serum creatinine.

Other members of the Low-Protein Diet Study Group are listed in Electronic supplementary material

Electronic supplementary material The online version of this article (doi:10.1007/s00125-009-1467-8) contains supplementary material, which is available to authorised users.

D. Koya (✉)
Division of Endocrinology and Metabolism,
Kanazawa Medical University,
Uchinadacho, Kahokugun,
Ishikawa 920-0293, Japan
e-mail: koya0516@kanazawa-med.ac.jp

M. Haneda
Department of Medicine, Asahikawa Medical College,
Asahikawa, Japan

S. Inomata
Akita Medical Center,
Akita, Japan

Y. Suzuki
School of Health Sciences, Factory of Medicine,
Niigata University,
Niigata, Japan

D. Suzuki
Department of Medicine, Tokai University,
Isehara, Japan

H. Makino · K. Shikata
Department of Medicine and Clinical Science,
Okayama University Graduate School of Medicine,
Okayama, Japan

Y. Murakami · S. I. Araki · A. Kashiwagi · R. Kikkawa
Shiga University of Medical Science,
Otsu, Japan

Y. Tomino
Division of Nephrology, Juntendo University,
Tokyo, Japan

K. Yamada
Metabolic Disease Clinic,
Chiba, Japan

Results The study was completed by 47 (84%) of 56 participants in the low-protein diet group and 41 (73%) of 56 participants in the normal-diet group. During the study period, the difference in mean annual change in estimated GFR between the low-protein diet and the normal-protein diet groups was −0.3 ml min^{-1} 1.73 m^{-2} (95% CI −3.9, 4.4; p=0.93). The difference in mean annual change in creatinine clearance between the low-protein diet and the normal-protein diet groups was −0.006 ml s^{-1} 1.73 m^{-2} (95% CI −0.089, 0.112; p=0.80). A doubling of serum creatinine was reached in 16 patients of the low-protein group (34.0%), compared with 15 in the normal-protein group (36.6%), the difference between groups being −2.6% (95% CI −22.6, 17.5; p=0.80). The time to doubling of serum creatinine was similar in both groups (p=0.66).
Conclusions/interpretation It is extremely difficult to get patients to follow a long-term low-protein diet. Although in the low-protein group overall protein intake was slightly (but not significantly) lower, it did not confer renoprotection.

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Keywords Albuminuria · Diabetic nephropathy · eGFR · Low-protein diet · Proteinuria

Abbreviations

ACE-I	ACE inhibitors
ARBs	Angiotensin II receptor blockers
eGFR	Estimated GFR
ESRD	End-stage renal disease
MDRD	Modification of Diet in Renal Disease study

Introduction

Diabetic nephropathy develops in 40% of patients with diabetes and, in spite of progress in new treatment for diabetes and anti-hypertensive drugs, is the leading cause of end-stage renal disease (ESRD) worldwide [1–3]. Diabetic nephropathy is also closely associated with higher cardiovascular mortality rates [4]. Therefore, additional efforts are needed to arrest the progression of diabetic nephropathy.

A low-protein diet slows the progression of renal disease and improves survival in patients with various glomerulopathies, including diabetic kidney disease [5]. Clinically, a meta-analysis suggested that low-protein diet lowers the incidence of ESRD or death in patients with non-diabetic nephropathies [6]. Another meta-analysis of 108 patients with type 1 diabetes in five studies (mean follow-up 4.5–35 months) showed the benefit of low-protein diet in slowing the progression of diabetic nephropathy [7]. Indeed, a low-protein diet is recommended as nutritional management of diabetic nephropathy [8], although there is insufficient evidence to suggest that such a diet improves renal dysfunction [9, 10]. The landmark study of non-diabetic kidney disease, the Modification of diet in renal disease study (MDRD), also failed to reach a conclusion regarding the benefits of a low-protein diet in reducing risk of ESRD or death [11, 12]. Furthermore, extended follow-up after the MDRD trial also failed to show a significant benefit of low-protein diet in slowing the development of ESRD and all-cause mortality [13].

To explore the uncertainties on effectiveness of low-protein diet, we conducted a randomised controlled trial to determine the effect of low-protein diet on the progression of renal dysfunction and albuminuria in type 2 diabetic patients with overt nephropathy.

Methods

Study design This was a multi-site randomised controlled trial for prevention of diabetic nephropathy progression among 112 type 2 diabetic patients, who were aged 30 to 70 years and had overt nephropathy. The trial was conducted from 1 December 1997 to 30 April 2006. After a baseline run-in period (3 months), the patients were monitored for 5 years. The protocol was approved by the institutional review boards of each centre. All participating patients provided written, informed consent. Before the present study, 41 diabetic patients with overt nephropathy had been randomly assigned to normal protein intake (n=21) and low protein intake (n=20) groups. This 1 year feasibility trial was completed by 34 patients. Daily protein intake in the feasibility study was 1.22±0.25 g $kg^{-1}day^{-1}$ (normal) and 0.92±0.43 g $kg^{-1}day^{-1}$ (low protein) and the difference was statistically significant (p<0.05). Based on these data, sample size for the present study was calculated. To achieve 90% power with a 5% significance level, we found that least 31 participants per group would be necessary. To account for drop-out due to trial duration, a 100 participants (50 per group) were planned for analysis.

Participants The participants were Japanese men and women, aged 30 to 70 years. All had type 2 diabetes (defined according to World Health Organization criteria) of at least 5 years duration and were being treated by diet or by diet plus oral hypoglycaemic agents or insulin injection. Other inclusion criteria were: (1) urinary protein excretion more than 1 g/day but less than 10 g/day; (2) urinary albumin excretion rate of more than 200 μg/min at least twice in a 1 year period; (3) serum creatinine below

176 μmol/l; (4) at least simple diabetic retinopathy; and (5) on normal-protein diet (1.2 g kg^{-1} day^{-1}). Potential participants were excluded if they had: type 1 diabetes; other renal diseases; body weight less than 80% of ideal body weight; clinically significant illness such as congestive heart failure, hepatic disease, recent myocardial infarction and stroke, and urinary tract infection; or if they were being treated with a low-protein diet (0.8 g $kg^{-1}day^{-1}$) and/or ACE inhibitors (ACE-I) or angiotensin II receptor blockers (ARBs). Hypertension was defined as blood pressure ≥140/90 mmHg or use of anti-hypertensive drugs.

Randomisation and intervention During the 3 month screening period, the participants continued to take a normal-protein diet (1.2 g $kg^{-1}day^{-1}$) and their usual medications. They were then randomly assigned at a central location to follow either a low-protein diet (0.8 g $kg^{-1}day^{-1}$) or a normal-protein diet (1.2 g $kg^{-1}day^{-1}$) with the appropriate energy intake for each participant without masking.

The methods of minimisation for allocation were applied according to age, sex, serum creatinine, estimated GFR (eGFR), and urinary albumin and protein levels during the screening period. Both groups were instructed to meet the registered dietitian for 30 min every 3 months to assess and counsel dietary issues. After randomisation we followed the participants for approximately 3.5 years (1–5 years). Every 3 months, all participants completed a 3 day food record to assess daily protein, energy and sodium intake. For this purpose, we used the fourth revised and enlarged edition of *Standard tables of food composition in Japan* [14]. The dietary protein intake was also assessed by urinary urea nitrogen excretion during 24 h urine collection every 3 months, using the formula of Maroni et al. [15]. To achieve dietary protein goals, dietary regimens were modified every 3 months or more as needed. The estimated protein intake during the study represents the mean of all measurements after randomisation.

Laboratory tests Blood and urine samples were brought to the central laboratory (SRL, Tokyo, Japan) and each clinical parameter was measured using the Hitachi 7170 analyzer (Hitachi High-Technologies, Tokyo, Japan) unless otherwise specified. GFR was estimated using the following modified MDRD formula for Japanese participants [16]: eGFR (ml min^{-1} 1.73 m^{-2})=175×[serum creatinine (μmol/l)/88.4]$^{-1.154}$×[age (years)]$^{-0.203}$×0.741×(0.742 if female), where serum creatinine estimated by an enzymatic method was calibrated. Creatinine clearance from a 24 h timed urine collection was calculated and corrected to a body surface area of 1.73 m^2. Urinary excretion of protein and albumin was measured every 3 months in 24 h timed urine samples using an immunoturbidity assay and a pyrogallol red–molybdate complex (LX60000; Eiken Chemical Co., Tokyo, Japan), respectively. Urinary nitrogen was measured by an enzymatic ultraviolet method every 3 months. Blood samples were obtained every 3 months to measure: renal function (blood urea nitrogen, creatinine, Na, K, Cl, uric acid) by an autoanalyser; lipids (total cholesterol, triacylglycerol, HDL-cholesterol) by an enzymatic colorimetric method and a direct inhibition method, respectively; transferrin by an immunoturbidity assay (BN-II; Dade Boehring, Marbug, Germany); serum glucose by a glucose oxidase method; and HbA_{1c} by ion exchange HPLC (ADAMS A1c HA-8160; Aarkray, Kyoto, Japan).

Outcomes The primary outcomes were: (1) the annual change in eGFR and creatinine clearance; (2) the incidence of doubling of serum creatinine; and (3) the time to doubling of baseline serum creatinine. The secondary outcomes included the proportion of patients with ESRD requiring haemodialysis and the annual changes in urinary protein and albumin excretion. Quality of life was assessed annually using the SF-36 [17].

The secondary analysis, which was not based on a direct comparison of randomised groups, was performed to assess the biological dose–response relationship between actual protein intake and progression of type 2 diabetic nephropathy.

Statistical analysis An independent data and safety monitoring board monitored the study. The Lan–DeMets alpha spending-function method was used to adjust for interim analyses once a year. Four formal interim analyses were performed during the study period. The p value for one interim analyses was set at $p=0.01$. Data handling and trial management were coordinated centrally by EPS (Tokyo, Japan).

The mean dietary protein intake between the low- and normal-protein diet groups was analysed using Wilcoxon's rank sum test. Dietary protein intake in the low- and normal-protein diet groups during the study was analysed by repeated measures ANOVA.

Analyses of the primary and secondary outcomes were performed according to the intention-to-treat principle; we included data from all randomised patients with the exception of the 24 participants lost or excluded between randomisation and study termination (Fig. 1). For continuous variables, the mean and standard deviation were calculated. Because of the skewed deviation, values for albuminuria and proteinuria are given as medians and interquartile ranges. In calculating the slopes of the rates of change of eGFR and creatinine clearance, linear regression analysis was used and included the data of patients who reached an endpoint. A minimum of 1 year follow-up with at least two measurements of serum and urinary creatinine

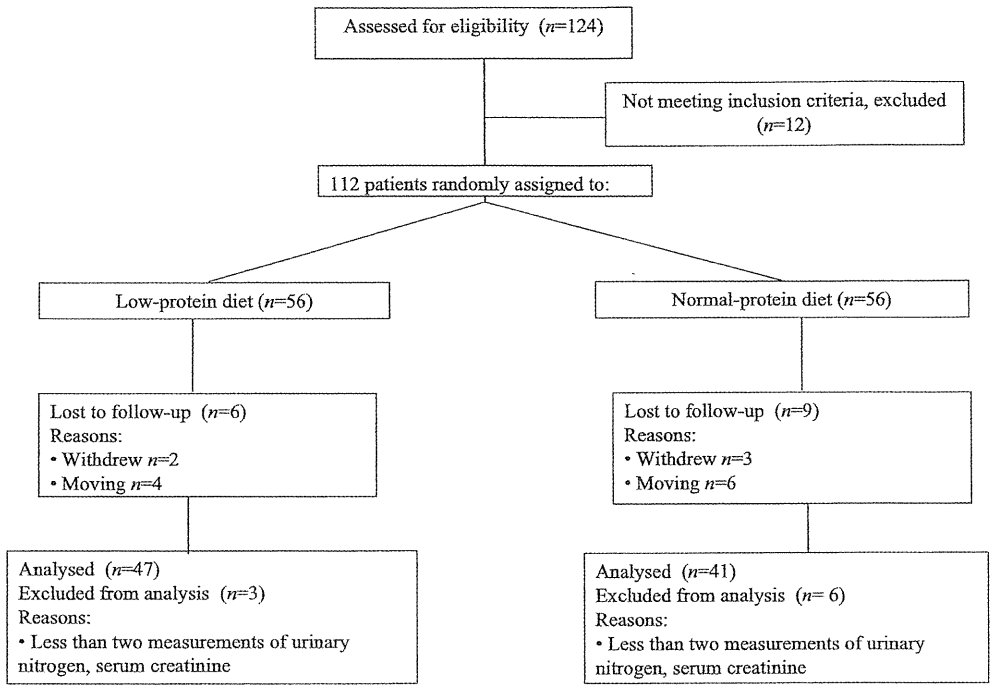


Fig. 1 Design of the trial. Fifteen patients were lost during follow-up because they moved away or withdrew informed consent within 1 year of follow-up. Nine patients were excluded from analysis because they had less than two measurements of urinary nitrogen excretion and serum creatinine

during the study period were aggregated in the slope analysis. Primary outcome values between groups were assessed by an analysis of covariance model, with low-protein diet as a factor and baseline urinary protein, serum creatinine, HbA_{1c}, systolic blood pressure and daily protein intake, in addition to age and sex, as covariates. The incidence of doubling of serum creatinine was compared with the χ^2 test. The times to doubling of baseline serum creatinine and its components were compared by Kaplan–Meier survival curves and the log-rank test. Baseline serum creatinine was adjusted using Cox proportional hazards models with terms for the diet assignment. Secondary outcomes were compared with the χ^2 test (for non-parametric data) or repeated measures ANOVA (for continuous data).

In secondary analysis, the differences between achieved protein intake and renal functions were determined using Pearson's correlation coefficient and Spearman's rank/correlation coefficient. To identify the factors associated with the doubling of serum creatinine, the potential risk factors such as systolic blood pressure, protein intake, sodium intake, HbA_{1c} and total cholesterol were included in the Cox proportional hazards model, adjusting for sex, age, urinary albumin excretion and serum creatinine.

All statistical tests were two-sided. For the final analysis of the primary endpoints and all other endpoints, a p value of 0.05 or less was considered to indicate significance. Data were analysed using SAS 8.2 (Statistical Analysis System, Cary, NC, USA).

Results

Participants The baseline characteristics of the 112 type 2 diabetic participants with nephropathy who underwent randomisation were similar between low-protein diet and normal-protein diet groups (Table 1). The study was completed by 47 of the 56 (84%) participants in the low-protein diet group and by 41 of the 56 (73%) participants in the normal-protein diet group (Fig. 1). In both groups, the reasons for dropping out were: loss of follow-up due to moving (ten participants); withdrawal of informed consent (five participants); and less than two measurements of dietary protein intake and of serum and urinary creatinine during the study period (nine participants).

Dietary assessment At randomisation, there was no difference in mean dietary protein intake between the two diet groups as assessed by a 3 day food record and a dietitian (low-protein 1.0±0.3 vs normal-protein 1.1±0.2 g $kg^{-1}day^{-1}$) and by estimates using 24 h urinary nitrogen excretion (1.0±0.2 vs 1.0±0.2 g $kg^{-1}day^{-1}$, respectively). During the study, the mean protein intake from the food record was significantly different between low- and normal-protein intake group (0.9±0.2 vs 1.1±0.2 g $kg^{-1}day^{-1}$, respectively, $p<0.0001$), while the protein intake derived from 24 h urinary nitrogen excretion was similar between the two group (1.0±0.2 vs 1.0±0.2 g $kg^{-1}day^{-1}$, respectively, $p=0.16$). The mean protein intake estimated by urinary nitrogen excretion in the low-protein diet group was lower than that in the normal-protein group during the study period, but the difference was

Table 1 Baseline characteristics of the participants

Variable	Low-protein diet (n=56)	Normal-protein diet (n=56)
Age (years)	57.5±7.8	56.3±8.7
Male sex, n (%)	33 (58.9)	33 (57.1)
Height (cm)	160.4±8.5	160.7±7.8
Weight (kg)	63.8±10.7	62.9±10.5
Systolic blood pressure (mmHg)	138±21	137±16
Diastolic blood pressure (mmHg)	77±11	77±12
Serum creatinine (μmol/l)	91.9±50.4	98.1±45.1
eGFR (ml min^{-1} 1.73 m^{-2})	63.5±26.9	61.1±23.7
Urinary albumin (μg/min)	488 (214–1,359)	527 (325–1,364)
Urinary protein (g/day)	1.1 (0.4–3.2)	1.2 (0.5–2.9)
HbA_{1c} (%)	7.8±1.5	7.5±1.7
Total cholesterol (mmol/l)	5.7±1.1	5.8±1.3
Triacylglycerol (mmol/l)	1.8±0.9	1.8±0.9
With hypertension (%)	63.0	68.6

Unless otherwise stated, values are mean±SD or medians (interquartile range)

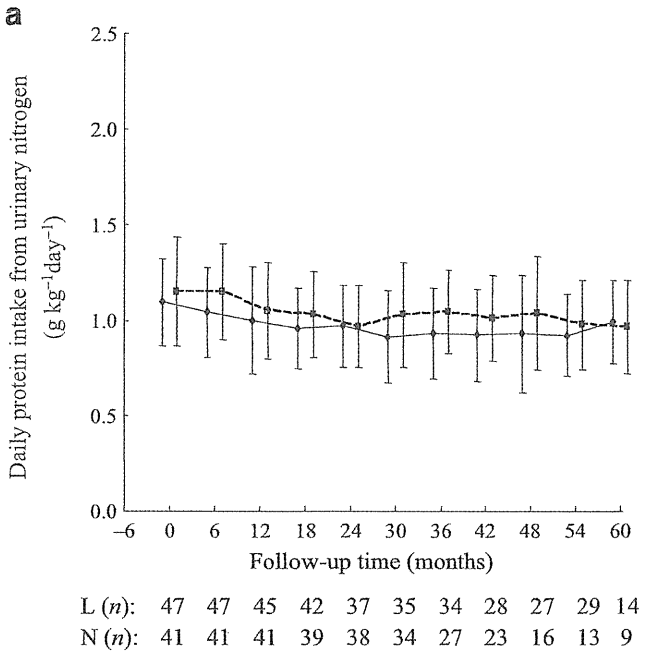
not significant (p=0.14) (Fig. 2a). This was in contrast to the significant difference between the two groups based on food record (p<0.0001) (Fig. 2b).

Primary outcomes The mean annual change in eGFR was −6.1±6.5 ml min^{-1} 1.73 m^{-2} for the low-protein diet group, compared with −5.8±5.7 ml min^{-1} 1.73 m^{-2} for the normal-protein diet group; the difference between the two groups was −0.3 ml min^{-1} 1.73 m^{-2} and not significant (95% CI −3.9, 4.4; p=0.93). The mean annual change in creatinine clearance was −0.163±0.159 ml s^{-1} 1.73 m^{-2} for the low-protein diet group, compared with −0.157± 0.125 ml s^{-1} 1.73 m^{-2} for the normal-protein diet group; the difference between the two groups was −0.006 ml s^{-1} 1.73 m^{-2} and also not significant (95% CI −0.089, 0.112; p=0.80). A doubling of serum creatinine was reached in 16 patients of the low-protein diet group (34.0%), as compared with 15 in the normal-protein diet group (36.6%), with a difference between the two groups of −2.6% (95% CI −22.6, 17.5; p=0.80). The time to doubling of serum creatinine was similar in both groups (p=0.66) (Fig. 3). The hazard ratio for the doubling of serum creatinine by Cox regression was 0.42 (95% CI 0.042, 4.22) for the low-protein diet group.

Secondary outcomes The proportion of patients with ESRD was 6.4% in the low-protein diet group, compared with 7.3% in the normal-protein diet group, with a difference between the two groups of −0.9% (95% CI −0.11, 0.10; p= 0.86). During the study period, the level of albuminuria in the low-protein diet group was not different from that in the normal-protein diet group (Fig. 4a). The level of proteinuria was also similar (Fig. 4b).

Associations of achieved protein intake with eGFR and creatinine clearance The secondary analysis, which was not based on a direct comparison of randomised groups, was performed to assess the biological dose–response relationship between actual protein intake and the progression of diabetic nephropathy in type 2 diabetes, without adjustment for other covariates. The lower protein intake, which was calculated by urinary nitrogen excretion (Fig. 5a) and the 3 day food record (Fig. 5b), was not associated with a slower deterioration of GFR. The correlational analysis using the annual change in creatinine clearance was also not conclusive with regard to the efficiency of low-protein diet, as measured by urea nitrogen excretion (p=0.22) (Fig. 5c) and dietary record (p=0.71) (Fig. 5d). In the multivariate model, adjusted for systolic blood pressure, protein and sodium intake, HbA_{1c} and serum total cholesterol during the study, systolic blood pressure was independently associated with the doubling of serum creatinine (Table 2).

Adverse events and quality of life During the study, one participant of the low-protein diet group died due to tuberculosis-linked sepsis and one participant of the normal-protein diet group died due to acute myocardial infarction. The difference in body weight between baseline and end of follow-up was 0.9 kg in the low-protein diet group and 0.2 kg in the normal-diet group, which was not significantly different between the two groups. During the study period, there was also no significant difference between the two groups in total energy (108.8±18.4 vs 113.8±15.9 kJ $kg^{-1}day^{-1}$) and sodium intake (7.7±2.1 vs 7.9±2.0 g/day) as determined from the 3 day food record. Furthermore, the level of transferrin was not significantly



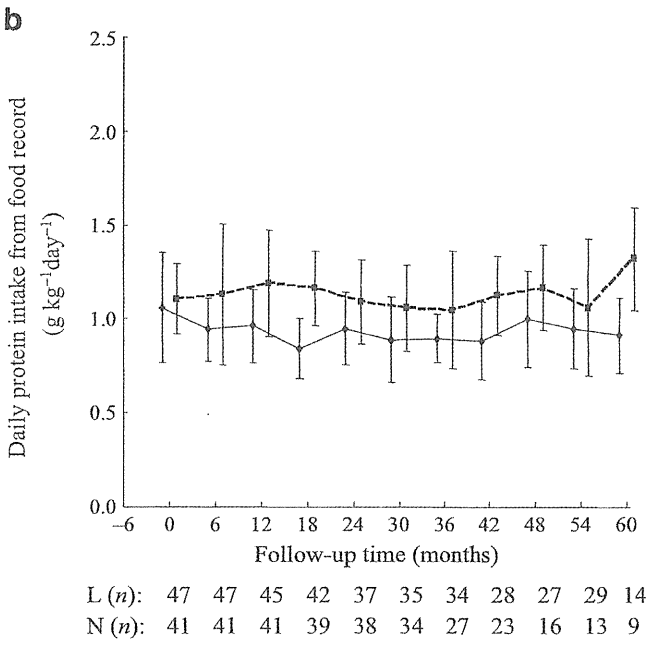


Fig. 2 Dietary protein intake in the low-protein diet (continuous lines) and normal-protein diet (dashed lines) groups estimated (**a**) from urinary nitrogen excretion and (**b**) from 3 day food record during the study. L (*n*), low-protein diet group (*n* participants); N (*n*), normal-protein diet group (*n* participants). Data are mean±SD

different between the two groups during the study period (p=0.83). There were no significant differences in health-related quality of life between the two groups during the study period, as measured by several SF-36 subscales (physical function, social function, physical role, emotional role, mental health, energy, pain and general health perceptions; p>0.1).

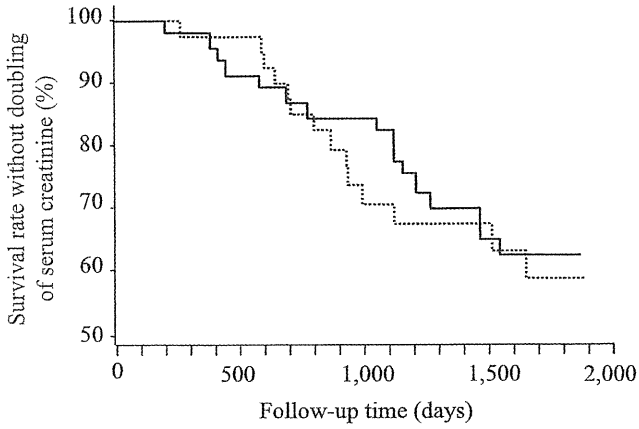


Fig. 3 Kaplan–Meier estimates of the primary endpoints from the study entry to time of doubling of baseline serum creatinine. The time to doubling was similar in both low-protein diet (continuous line) and normal-protein diet (dashed line). p=0.66 by logrank test

Discussion

We found that the low-protein diet was not associated with a better renal outcome than a normal-protein diet in patients with type 2 diabetes. Low-protein diet did not slow the rate of progression of nephropathy as estimated not only by the incidence of doubling of serum creatinine, but also by the time to doubling of serum creatinine concentration, compared with the normal-protein diet group. The mean annual change in eGFR and creatinine clearance was also similar between the two groups. The secondary analysis, which assessed the association between the rate of progression of diabetic nephropathy and the achieved protein intake, also failed to find a beneficial effect. Based on the time-dependent Cox proportional hazards model, no renal benefit of low-protein diet was observed, although systolic blood pressure significantly influenced the progression of diabetic nephropathy. We thus interpret these results to indicate that a low-protein diet is probably not renoprotective in patients with type 2 diabetic nephropathy.

In a long-term study similar to ours, Pijls et al. reported that protein restriction is neither feasible nor efficacious [18], although they had recruited type 2 diabetic patients with microalbuminuria (30–300 mg/day) and relatively high albuminuria within the normo-albuminuric range (albuminuria >20 mg/day or detectable urinary albumin, i.e. albumin concentration >6.5 mg/l). In contrast, Hansen et al. performed a 4 year prospective, controlled trial with concealed randomisation to compare the decline in GFR and development of ESRD or death in type 1 diabetes patients with advanced diabetic nephropathy comparable to our participants [19]. Their usual-protein diet group consumed 1.02 g kg^{-1}day^{-1} as compared with 0.89 (range

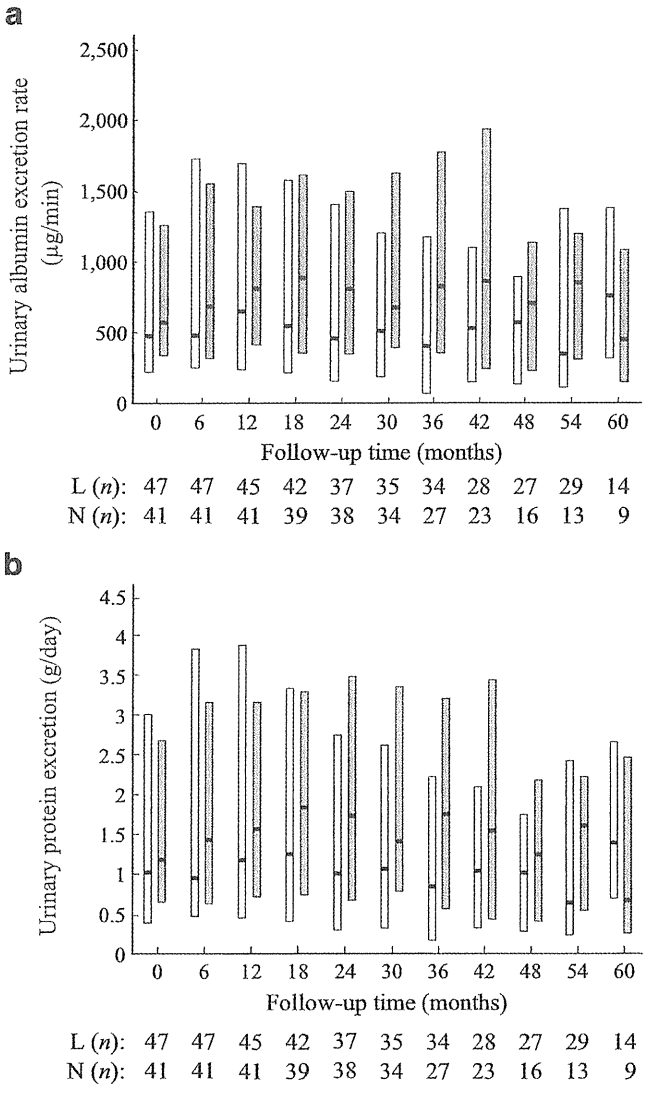


Fig. 4 The effect of low-protein diet (white columns) and normal protein intake (grey columns) on albuminuria (**a**) and proteinuria (**b**). Boxes indicate 25th and 75th percentiles of albuminuria or proteinuria. Horizontal lines indicate median. L (*n*), low-protein diet group (*n* participants); N (*n*), normal-protein diet group (*n* participants)

0.83–0.95) g $kg^{-1}day^{-1}$ in the low-protein diet group, a protein intake similar to our groups. However, in contrast to our findings, Hansen et al found that type 1 diabetic patients suffering from progressive diabetic nephropathy experienced a beneficial effect of moderately restricted dietary protein on the development of ESRD or mortality rates. The discrepancy might be due to the different types of diabetes and/or use of antihypertensive drugs, with almost 90% of patients in their study taking ACE-I. In our study, patients were instructed not to take ACE-I and/or ARBs, as these had not been approved for the treatment of diabetic nephropathy in Japan when this study was completed.

The prescribed protein intake in the low-protein group in our study (approximately 0.8 g $kg^{-1}day^{-1}$) resulted in a mean achieved protein intake of about 1.0 g $kg^{-1}day^{-1}$, as estimated by urinary nitrogen excretion, which was not statistically different from protein intake in the normal-protein diet. Since diabetic patients have to accept other restrictions to their diet regimen [4, 8, 20], compliance to an additional low-protein diet could be reduced. The achieved level of long-term dietary protein restriction may reflect everyday life in an outpatient clinic set-up. Therefore, we cannot directly address the issue of whether the effects of lower protein intake such as 0.8 g $kg^{-1}day^{-1}$, the amount recommended in a nutritional statement by the American Diabetes Association (2008) [8], would be beneficial for type 2 diabetic patients with nephropathy. Non-adherence to the prescribed low-protein diet would result in underestimation of the true beneficial effect of the low-protein diet in the present study. However, it is not reasonable to assume that a lower protein intake equal to or less than 0.8 g $kg^{-1}day^{-1}$ would reduce the risk of progression of diabetic nephropathy, because the relationship between achieved protein intake (0.55–1.6 g $kg^{-1}day^{-1}$) and annual rate of eGFR decline as well as creatinine clearance decline also failed to produce any benefits for low-protein diet in our study. The MDRD, moreover, also failed to reach a conclusion on this issue [11–13]. Indeed, the recent long-term follow-up of the MDRD provides evidence that even very low protein diet, supplemented with keto acids and amino acids, increased the risk of death without the benefit of delaying progression of kidney diseases [21].

In the present study, we found that systolic blood pressure, rather than other variables such as blood glucose control, daily protein intake and sodium intake, played a major role in accelerating the progression of diabetic nephropathy during the follow-up period. Our results suggest that blood pressure control results in inhibition of progression of diabetic nephropathy [4, 22]. Furthermore, coexisistence of hypertension and type 2 diabetes is well known to accelerate the risk not only of development and progression of diabetic nephropathy, but also of cardiovascular disease outcome [22–25], meaning that control of high blood pressure is a major protective strategy against renal and cardiovascular outcomes in patients with diabetic nephropathy. Indeed, recent guidelines recommend treating type 2 diabetic patients with antihypertensive drugs, if their blood pressure is in the high-normal (previously normal) range (130–139/85–90 mmHg), and sometimes even if blood pressure is in the normal and/or low prehypertensive range (120–129/80–85 mmHg) [26, 27].

Although previous experimental data suggested that the effects of low-protein diet, similar to treatment with an ACE-I or ARBs, are mediated through blockade of the renal renin–angiotensin system [28, 29], dietary protein

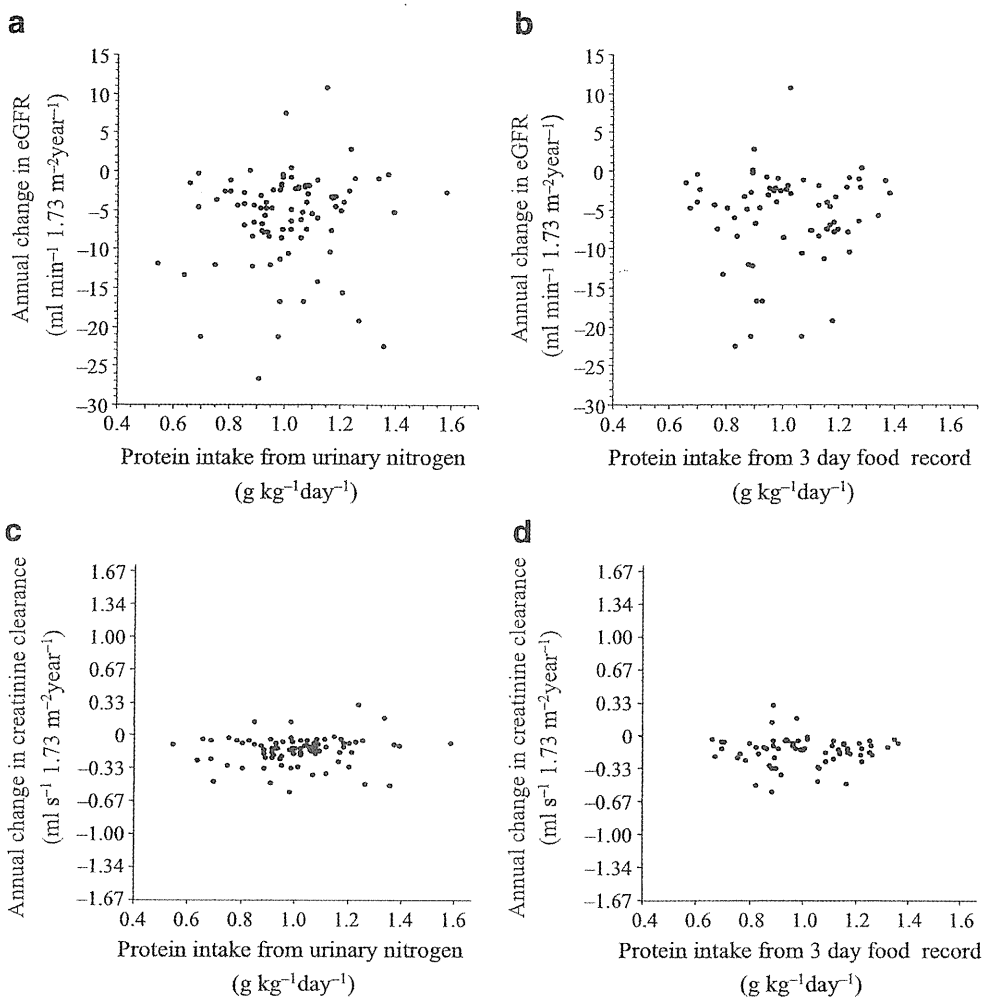


Fig. 5 Correlation between achieved protein intake estimated (**a**) from urinary nitrogen excretion and the annual change in eGFR, and (**b**) from 3 day food record and the annual change in eGFR. **c** Correlation between achieved protein intake, estimated from urinary nitrogen excretion and (**d**) from from 3 day food record, and the annual change in creatinine clearance. The p value was calculated using Spearman's rank correlation coefficient

restriction in the present study, where patients were not on ACE-I or ARBs, did not seem to act through the renin–angiotensin system. At present, adding ACE-I or ARB to multifactorial intervention could reduce the progression of diabetic nephropathy, as reported in several studies [30–35]. Interestingly, a recent report by Parving et al. showed that without restriction of dietary salt or protein, the use of the renin inhibitor, aliskiren, in combination with an ARB efficiently reduces urinary albuminuria in diabetic patients with overt proteinuria [36].

In summary, it is extremely difficult to get patients to follow a long-term low-protein diet, and although overall protein intake was slightly (but not significantly) lower, it did not confer renoprotection. Our data may shed the light on the dietary management of diabetic nephropathy. One possible result is that protein restriction may not remain a main nutritional recommendation in clinical practice, because we now have a most valuable therapeutic strategy for reducing progression of diabetic nephropathy as well as cardiovascular events and mortality rates by using intensive multifactorial interventions such as lifestyle management, ACE-I or ARBs, and lipid-lowering drugs, as reported in the Steno-2 study [32, 33]. Without additional data, we must continue to base decisions on the current balance of evidence for and against the efficacy and safety of dietary protein restriction.

Table 2 Hazard ratios of factors associated with the doubling of serum creatinine

Variable	Hazard ratio (95% CI)[a]	p value
Systolic blood pressure (mmHg)	1.1 (1.02–1.14)	0.012
Protein intake (g kg^{-1}day^{-1})	1.8 (0.07–44.64)	0.73
Sodium intake (g/day)	0.9 (0.72–1.14)	0.41
HbA_{1c} (%)	0.9 (0.59–1.23)	0.49
Total cholesterol (mmol/l)	1.0 (1.0–1.01)	0.49

[a] The multivariate model was adjusted for the following baseline variables: sex, age, urinary albumin excretion and serum creatinine

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

References

1. Ritz E, Orth SR (1999) Nephropathy in patients with type 2 diabetes mellitus. N Engl J Med 341:1127–1133
2. United Renal Data System 2006 (2006) Annual data report. Available from www.usrds.org/slides_2006.htm, accessed in June 2009
3. Nakai S, Masakane I, Akiba T et al (2008) An overview of dialysis treatment in Japan (as of Dec. 31, 2006). J Jpn Soc Dial Ther 41:1–28
4. Sasso FC, De Nicola L, Carbonara O et al (2006) Cardiovascular risk factors and disease management in type 2 diabetic patients with diabetic nephropathy. Diabetes Care 29:498–503
5. Mandayam S, Mitch WE (2006) Dietary protein restriction benefits patients with chronic kidney disease. Nephrology (Carlton) 11:53–57
6. Kasiske BL, Lakatua JD, Ma JZ, Louis TA (1998) A meta-analysis of the effects of dietary protein restriction on the rate of decline in renal function. Am J Kidney Dis 31:954–961
7. Pedrini MT, Levey AS, Lau J, Chalmers TC, Wang PH (1996) The effect of dietary protein restriction on the progression of diabetic and nondiabetic renal diseases: a meta-analysis. Ann Int Med 124:627–632
8. Association AD (2008) Nutritional recommendations and interventions for diabetes. A position statement of the American Diabetes Association. Diabetes Care 30(Suppl 1):S61–S78
9. Johnson DW (2006) Dietary protein restriction as a treatment for slowing chronic kidney disease progression: the case against. Nephrology (Carlton) 11:58–62
10. Robertson L, Waugh N, Robertson A (2007) Protein restriction for diabetic renal disease. Cochrane Database Syst Rev (4): Art. no. CD002181. doi:10.1002/14651858.
11. Klahr S, Levey AS, Beck GJ et al (1994) The effects of dietary protein restriction and blood-pressure control on the progression of chronic renal disease. Modification of diet in renal disease study group. N Engl J Med 330:877–884
12. Levey AS, Greene T, Beck GJ et al (1999) Dietary protein restriction and the progression of chronic renal disease: what have all of the results of the MDRD study shown? Modification of Diet in Renal Disease Study group. J Am Soc Nephrol 10:2426–2439
13. Levey AS, Greene T, Sarnak MJ et al (2006) Effect of dietary protein restriction on the progression of kidney disease: long-term follow-up of the Modification of Diet in Renal Disease (MDRD) Study. Am J Kidney Dis 48:879–888
14. The resources council of the science and technology agency of Japan (1983) Standard tables of food composition in Japan, 4th edn. Printing Bureau, Ministry of Finance, Tokyo
15. Maroni BJ, Steinman TI, Mitch WE (1985) A method for estimating nitrogen intake of patients with chronic renal failure. Kidney Int 27:58–65
16. Imai E, Horio M, Nitta K et al (2007) Estimation of glomerular filtration rate by the MDRD study equation modified for Japanese patients with chronic kidney disease. Clin Exp Nephrol 11:41–50
17. Ware JE Jr, Sherbourne CD (1992) The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Med Care 30:473–483
18. Pijls LT, de Vries H, Donker AJ, van Eijk JT (1999) The effect of protein restriction on albuminuria in patients with type 2 diabetes mellitus: a randomized trial. Nephrol Dial Transpl 14:1445–1453
19. Hansen HP, Christensen PK, Tauber-Lassen E, Klausen A, Jensen BR, Parving HH (1999) Low protein diet and kidney function in insulin dependent diabetic patients with diabetic nephropathy. Kidney Int 55:621–628
20. Remuzzi G, Schieppati A, Ruggenenti P (2002) Clinical practice. Nephropathy in patients with type 2 diabetes. N Engl J Med 346:1145–1151
21. Menon V, Kopple JD, Wang X et al (2009) Effect of a very low-protein diet on outcomes: long-term follow-up of the Modification of Diet in Renal Disease (MDRD) Study. Am J Kidney Dis 53:208–217
22. Mogensen CE (1999) Microalbuminuria, blood pressure and diabetic renal disease: origin and development of ideas. Diabetologia 42:263–285
23. Simonson DC (1988) Etiology and prevalence of hypertension in diabetic patients. Diabetes Care 11:821–827
24. Grossman E, Messerli FH (2008) Hypertension and diabetes. Adv Cardiol 45:82–106
25. Ritz E, Dikow R (2006) Hypertension and antihypertensive treatment of diabetic nephropathy. Nat Clin Pract Nephrol 2:562–567
26. KDOQI (2007) KDOQI clinical practice guidelines and clinical practice recommendations for diabetes and chronic kidney disease. Am J Kidney Dis 49(Suppl 2):S1–S179
27. Khan NA, Hemmelgarn B, Herman RJ et al (2008) The 2008 Canadian hypertension education program recommendations for the management of hypertension: part 2 - therapy. Can J Cardiol 24:465–475
28. Brenner BM, Meyer TW, Hostetter TH (1982) Dietary protein intake and the progressive nature of kidney disease: the role of hemodynamically mediated glomerular injury in the pathogenesis of progressive glomerular sclerosis in aging, renal ablation, and intrinsic renal disease. N Engl J Med 307:652–659
29. Hostetter TH, Meyer TW, Rennke HG, Brenner BM (1986) Chronic effects of dietary protein in the rat with intact and reduced renal mass. Kidney Int 30:509–517
30. Lewis EJ, Hunsicker LG, Clarke WR et al (2001) Collaborative Study Group: renoprotective effect of the angiotensin-receptor antagonist irbesartan in patients with nephropathy due to type 2 diabetes. N Engl J Med 345:851–860
31. Brenner BM, Cooper ME, de Zeeuw D et al (2001) Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. N Engl J Med 345:861–869
32. Gæde P, Vedel P, Larsen N, Jensen GVH, Parving H-H, Pedersen O (2003) Multifactorial intervention and cardiovascular disease in patients with type 2 diabetes. N Engl J Med 348:383–393
33. Gæde P, Lund-Andersen H, Parving HH, Pedersen O (2008) Effect of a multifactorial intervention on mortality in type 2 diabetes. N Engl J Med 358:580–591
34. Bakris GL, Williams M, Dworkin L et al (2000) Preserving renal function in adults with hypertension and diabetes: a consensus approach. National kidney foundation hypertension and diabetes executive committees working group. Am J Kidney Dis 36:646–661
35. Kimmel PL (2006) Update in nephrology and hypertension. Ann Int Med 144:281–285
36. Parving HH, Persson F, Lewis JB, Lewis EJ, Hollenberg NK; AVOID Study Investigators (2008) Aliskiren combined with losartan in type 2 diabetes and nephropathy. N Engl J Med 358:2433–2446

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Diabetic Nephropathy Remission and Regression Team Trial in Japan (DNETT-Japan): Rationale and study design

Kenichi Shikata [a], *Masakazu Haneda* [b], *Daisuke Koya* [c], *Yoshiki Suzuki* [d], *Yasuhiko Tomino* [e], *Kenichi Yamada* [f], *Shiro Maeda* [g], *Norito Kawakami* [h], *Takashi Uzu* [i], *Motonobu Nishimura* [j], *Chikage Sato* [a], *Daisuke Ogawa* [a], *Hirofumi Makino* [a,*]

DNETT-Japan Study Group

[a] *Department of Medicine and Clinical Science, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan*
[b] *Department of Medicine, Division of Metabolism and Biosystemic Science, Asahikawa Medical College, Asahikawa, Japan*
[c] *Division of Endocrinology and Metabolism, Department of Internal Medicine, Kanazawa Medical University, Ishikawa, Japan*
[d] *Health Administration Center, Niigata University, Niigata, Japan*
[e] *Division of Nephrology, Department of Internal Medicine, Juntendo University School of Medicine, Tokyo, Japan*
[f] *Metabolic Diseases Clinic, Chiba, Japan*
[g] *Laboratory for Endocrinology and Metabolism, RIKEN Center for Genomic Medicine, Yokohama, Japan*
[h] *Department of Mental Health, Graduate School of Medicine, University of Tokyo, Tokyo, Japan*
[i] *Department of Medicine, Shiga University of Medical Science, Otsu, Shiga, Japan*
[j] *Department of Internal Medicine, Japan National Hospital Organization Chiba-East National Hospital, Chiba, Japan*

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ABSTRACT

The prevalence of end-stage renal disease (ESRD) is uprising in the paralleled with the increase of chronic kidney disease (CKD) patients. Diabetic nephropathy (DN) is the most important underlying disease of CKD and a leading cause of ESRD in Japan. Intensified multifactorial intervention in patients with type 2 diabetes with microalbuminuria slows the progression to nephropathy, and progression of retinopathy and autonomic neuropathy. However, further studies are needed to establish the effect of intensified multifactorial treatment on DN with overt proteinuria. In this trial, doctors and co-medicals collaborate to treat the DN patients to prevent the deterioration of DN by multifactorial intensive therapy. Diabetic Nephropathy Remission and Regression Team Trial in Japan (DNETT-Japan) is an open, randomized controlled trial to evaluate the efficacy of renal protection of multifactorial intensive therapy in type 2 diabetes patients with overt proteinuria (urinary albumin-to-creatinine ratio ≥300 mg/g creatinine). The study has a targeted enrollment of 600 Japanese patients, and divided into two protocols by renal insufficiency (protocol A: serum creatinine: <1.2 mg/dl in male and <1.0 mg/dl in female, and protocol B: serum creatinine: 1.2–2.5 mg/dl in male and 1.0–2.5 mg/dl in female). The patients were allocated standard treatment or intensive multifactorial treatment. Intensive treatment was a stepwise implementation of behavior modification, pharmacological therapy targeting hyperglycaemia, hypertension, dyslipidaemia, and proteinuria. The primary outcome is the

* *Corresponding author at:* Department of Medicine and Clinical Science, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikata-cho, Kita-ku, Okayama 700-8558, Japan. Tel.: +81 86 235 7235; fax: +81 86 222 5214.
E-mail address: makino@md.okayama-u.ac.jp (H. Makino).
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proteinuria in protocol A and the composite endpoint of time to the first occurrence of doubling of serum creatinine, ESRD (the need for chronic dialysis, or renal transplantation) or death in protocol B. The follow-up period is 5 years and the study ends in 2014.

1. Introduction

Diabetic nephropathy (DN), one of the major complications of diabetes mellitus, is the leading cause of end-stage renal disease (ESRD) and about 43% of the patients newly introduced to chronic dialysis therapy are due to DN in Japan [1]. More than 275,000 HD are under chronic dialysis, and the number of the patients newly introduced to chronic dialysis is still increasing [1]. In line with the growing concerns about escalating medical costs and the increased risk of cardiovascular disease caused by chronic kidney disease (CKD) [2], the effective intervention in the development of DN and the progression of ESRD has been urgently required.

It is critical to manage blood glucose, hypertension and proteinuria aggressively to interrupt the development of DN [3]. In recent studies, strict blood glucose and blood pressure control using renin-angiotensin system (RAS) inhibitors has been confirmed to reduce the progression of DN. Although it is not sufficient to reduce the increasing numbers of ESRD patients, Steno 2 trial conducted in type 2 diabetic patients with microalbuminuria has shown that multifactorial approach can slow the progression of microvascular complications including DN [4,5]. This trial showed that the progression of DN is modifiable by multifactorial intervention. Furthermore, it is reported that strict management of hyperglycemia and hypertension with RAS inhibitors can lead to the remission and regression of DN [6]. However, these studies were performed at a single institution and the scale of the studies is relatively small, and so far, there is no evidence that intensive multifactorial therapy can inhibit the progression of DN with overt proteinuria.

In order to examine the effect of intensive multifactorial intervention on the inhibition of progression of DN with overt proteinuria, we are conducting a large-scale clinical trial named DNETT-Japan (Diabetic Nephropathy Remission and Regression Team Trial in Japan). This trial is a randomized, open labeled, multi-centered study to investigate whether intensive multifactorial intervention that includes changes in behavior and pharmacological therapy can conduct the remission and regression of DN in type 2 diabetic patients with overt proteinuria compared with a standard treatment.

2. Methods

2.1. Patients

The DNETT-Japan is a multi-center study currently underway in Japan (Clinical Trials gov number, NCT00253786). Japanese patients with type 2 DN met the inclusion and exclusion criteria have been shown in Table 1 and have been enrolled. The trial is being conducted under the Helsinki Declaration, and was approved by the Institutional Review Board at each trial site. All participants have been fully informed by the investigators and gave their written informed consent.

2.2. Study design

This clinical trial is a randomized, open labeled, multi-center study (Fig. 1). During the 2-month screening period (already completed), patients were assessed for inclusion and exclusion criteria for eligibility for entering this study. The active treatment period is 5 years. Patients are randomly assigned standard treatment or intensive multifactorial treatment with behavior modification and stepwise introduction of pharmacological therapy (Table 2).

The study is a randomized, open, parallel trial. Patients are randomly assigned standard treatment or intensive multifactorial intervention with behavior modification and stepwise introduction of pharmacological therapy (Table 2). The stepwise approach was chosen to maximize compliance to the protocol. Standard treatment is performed according to the guideline of the Japanese Diabetes Society, Japanese Society of Hypertension, and Japanese Atherosclerosis Society. Patients in the intensive group are treated by a project team (doctor, nurse, dietician and pharmacologist) at each institution. The aim of dietary intervention is a total intake of protein less than 0.8 g/kg/day, and intake of sodium less than 5 g/day, and total daily energy intake less than 30 kcal/kg/day. Hemoglobin A1c (HbA1c) values should be below 5.8% on diet alone, and if patients are unable to maintain HbA1c < 5.8%, oral hypoglycemic agents or insulin is started. The target blood pressure

Table 1 – Eligibility criteria.

Inclusion criteria
(1) Patients with type 2 diabetes
(2) Urinary albumin-to-creatinine ratio: ≥300 mg/g creatinine twice in the first morning urine sample
(3) Serum creatinine level: ≤2.5 mg/dl
(4) Patients aged 20–75 years

Exclusion criteria
(1) Type 1 diabetes
(2) Hereditary diabetes or secondary diabetes
(3) Non-diabetic nephropathy
(4) Familial hypercholesterolemia
(5) Secondary hypertension
(6) Unstable angina pectoris or history of myocardial infarction/stroke within 6 months prior to consent acquisition
(7) Malignant tumor or life threatening disease
(8) History of angioedema
(9) Patients undergoing LDL apheresis
(10) Billiary system obstruction or severe liver injury
(11) Liver dysfunction
(12) Allergy for ACE-Is, ARBs or HMG-CoA reductase inhibitors
(13) Pregnant or nursing patients
(14) Others: patients who are not suitable for this trial

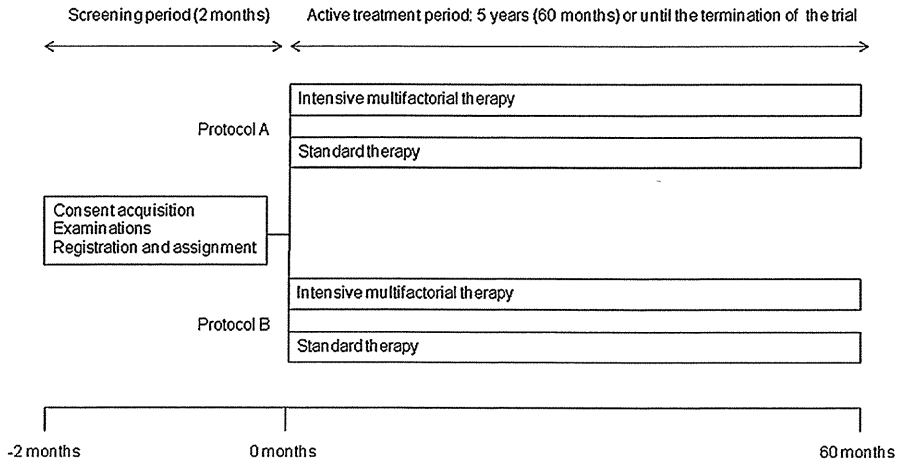

Fig. 1 – Overview of the design of DNETT-Japan.

should be <125 mmHg systolic blood pressure and <75 mmHg diastolic blood pressure (seated blood pressure) in intensive therapy group using angiotensin receptor blockers (ARBs) or angiotensin-converting enzyme inhibitors (ACE-Is) for the management of hypertension. If the target blood pressure of less than 125/75 mmHg is not achieved, both ARBs and ACE-Is are used concomitantly. Even if the target blood pressure levels are not achieved in a patient with ARBs and ACE-Is, long acting calcium channel blockers are used. HMG-CoA reductase should be used for the reduction of LDL-cholesterol levels less than 100 mg/dl. All patients who smoke and their spouses are invited to smoking cessation courses. All patients received multivitamin supplement daily.

All patients will visit the clinic at every 3 months throughout the study duration. At each visit, blood pressure will be measured and clinical samples collected for the measurement of the urinary protein-to-creatinine ratio, and the levels of serum creatinine and serum potassium. Glomerular filtration rate (GFR) is estimated using the following modified MDRD formula for Japanese participants: GFR (ml min^{-1} 1.73 m^{-2}) = 194 × [serum creatinine (μmol/l)]$^{-1.094}$ × [age (years)]$^{-0.287}$ × (0.739 if female) [7]. All randomized patients including those discontinued from the study for any reason other than death will be followed up to collect information on primary and secondary endpoints until termination of study.

2.3. *Study endpoints*

The primary and secondary endpoints are shown in Table 3. The primary endpoint is a proteinuria in protocol A, and a composite endpoint of the time to first occurrence of doubling of serum creatinine, ESRD, or death in protocol B. ESRD is defined as the need for chronic dialysis or renal transplantation. The secondary endpoints are GFR, cardiovascular event, progression of retinopathy, urinary albumin/creatinine ratio,

Table 2 – Treatment goals and interventions for standard and intensive multifactorial groups.

	Intensive multifactorial	Standard
Blood glucose	HbA1c < 5.8%	HbA1c < 6.5%
Blood pressure	SBP < 125 mmHg DBP < 75 mmHg	SBP < 130 mmHg DBP < 80 mmHg
Lipid profile	T-cho < 180 mg/dl LDL-cho < 100 mg/dl HDL-cho >40 mg/dl	T-cho < 200 mg/dl LDL-cho < 120 mg/dl HDL-cho >40 mg/dl
Dietary intervention	TDEI < 30 kcal/kg/day Sodium < 5 g/day Protein < 0.8 g/kg/day	TDEI 25-30 kcal/kg/day Sodium < 6 g/day Protein < 1.0 g/kg/day
Pharmacological intervention	ACE-Is or ARBs HMG-CoA reductase inhibitors Muitivitamins	No restrictions (continuing prior therapy)
Instruction by co-medicals	Taking medicines Smoking cessation Nutrition care	No restrictions (continuing prior therapy)

SBP, systolic blood pressure; DBP, diastolic blood pressure; T-cho, total cholesterol; LDL-cho, LDL-cholesterol; HDL-cho, HDL-cholesterol; TDEI, total daily energy intake.

Table 3 – Primary and secondary endpoints.

Protocol A
- Primary outcomes
  - Urinary protein/creatinine ratio (in the first morning urine sample)
- Secondary outcomes
  - (1) GFR
  - (2) Cardiovascular event
  - (3) Progression of retinopathy
  - (4) Urinary albumin/creatinine ratio
  - (5) Proteinuria (24 h collection sample)

Protocol B
- Primary outcomes
  - Composite endpoint of time to first occurrence of
    - (1) Doubling of serum creatinine
    - (2) Need for chronic dialysis or renal transplantation
    - (3) Death
- Secondary outcomes
  - (1) GFR
  - (2) Cardiovascular event
  - (3) Progression of retinopathy
  - (4) Urinary albumin/creatinine ratio
  - (5) Urinary protein/creatinine ratio

and proteinuria in protocol A and GFR, cardiovascular event, progression of retinopathy, urinary albumin/creatinine ratio, and protein/creatinine ratio in protocol B.

2.4. *Statistical analysis*

The primary efficacy analysis set will be the full analysis set (FAS). The FAS will include all patients satisfying the following conditions: (1) fulfilled all entry criteria; (2) assigned randomly; (3) were followed up with intensive or standard treatment; (4) were evaluated at least once after randomization. The secondary efficacy analysis set will be per protocol set (PPS). The PPS will consist of patients included in the FAS who had no major protocol violations.

The Cox regression model will be used to estimate the hazard ratios with 95% confidence intervals in the renal composite event rate, the cerebro/cardiovascular composite event rate, and the event rate for each renal, cerebro- or cardiovascular event separately. The covariates included in the model will be determined based on the results of blind data review before the study is unblinded. The candidate covariates are gender, age, ACE-I treatment, baseline urinary albumin:-creatinine ratio and baseline serum creatinine level. The cumulative event rate for each defined event will be estimated by the Kaplan–Meier's method for each treatment group. The linear mixed effect model, including study drugs, measurement times and other covariates selected after the blind data review, will be used for comparing the trend in the percent change in proteinuria, and the trend in the reciprocal of the serum creatinine level between treatment groups. Similar analyses for each endpoint will also be applied for the subgroup of each prognostic factor.

Adverse events will be summarized for each treatment group. The cumulative occurrence rate of all adverse events and drug-related adverse events in each treatment group will be estimated by the Kaplan–Meier's method, and the log-rank test will be used to compare two groups. The summary statistics, such as the mean, median and standard deviation for the quantified laboratory test values, will be calculated at each measurement point, and scatter plots of each of the test values for pre- and post-treatment will be presented. Contingency tables showing the number of patients and the percentage of patients within each category pre- and post-treatment will also be presented for the categorical test values.

3. Discussion

The purpose of DNETT-Japan is to investigate that intensive multifactorial treatment may attenuate the progression of DN in patients with type 2 diabetes and overt proteinuria in the Japanese populations. DN is a leading cause of ESRD in Japan, and the HD patients are still increasing based on the epidemic of type 2 diabetic patients. DN is also the most popular CKD, and recently it is well recognized that CKD is a high risk factor for cardiovascular disease (CVD) and stroke. In consideration for the rising burden of ESRD and CVD, there is a need to establish the treatment for DN in Japanese diabetic patients.

Strict control of blood glucose and blood pressure is principal in the treatment of DN. Intensive glucose control had a beneficial effect on aggregate diabetes-related endpoints and significantly reduced the rate of progression from normoalbuminuria to microalbuminuria in the United Kingdom Prospective Diabetes Study [8]. However, there is no significant reduction in the risk of progression of DN; intensive blood glucose control alone seems insufficient to treat diabetic patients with overt proteinuria. Moreover, recent study reported that intensive glucose lowering therapy increased the mortality and did not reduce the cardiovascular events in type 2 diabetic patients [9]. Although intensive insulin therapy had the effect on progression to proteinuria [10], there is no evidence that strict control of blood glucose solely prevents the progression of DN with overt proteinuria.

In order to interrupt the development of DN, it is critical to manage not only blood glucose, but also hypertension. ACE-Is or ARBs are recommended as first-line drugs in the treatment of hypertension according to the American Diabetes Association (ADA) Position Statement [11] and the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7) [12]. Both classes of drugs reduced the risks of onset of DN, increase in proteinuria and progression to ESRD [13]. ARBs are recommended especially for DN in type 2 diabetes, based on the evidence of large-scale randomized controlled trials in DN [14–17]. In recent years, large-scale clinical trials conducted in type 2 diabetic patients with microalbuminuria, such as the INNOVATION of telmisartan [14] and the IRMA-2 of irbesartan [15], have shown that angiotensin II receptor blockers (ARBs) can prevent the progression of microalbuminuria to overt proteinuria. The clinical trials of DN with proteinuria, such as the RENAAL study of losartan [16], the IDNT study of irbesartan [17], have demonstrated that the treatment with ARBs can significantly reduce the risk of doubling of the serum creatinine level, dialysis, renal transplantation, and death.

When the protocol for DNETT-Japan was designed in 2005, the recommended target blood pressure according to the

hypertension treatment guidelines in Japan (JSH 2004) was <125/75 mmHg. If blood pressure is above 125/75 mmHg, we recommend to use both ACE-I and ARB in this trial. A combination therapy with an ACE-I and ARB has been suggested to exert stronger anti-proteinuric effects than either agent used alone [3], and this combination effect of an ACE-I and ARB on proteinuria has been examined in DN patients [18]. We will evaluate the effect of strong inhibition of rennin-angiotensin system in addition to the tight control of blood pressure on the progression of DN.

Intensified multifactorial intervention improved the progression of DN and the mortality in patients with microalbuminuria in Steno 2 study [4,5,19]. This study pointed out that multifactorial approach, not only the treatment for hyperglycemia and hypertension but also dyslipidaemia and other pharmacological therapy using vitamins and aspirin, is beneficial for the progression from microalbuminuria to overt proteinuria. However, thus far, there is no evidence that intensive multifactorial therapy can reduce the progression of DN with overt proteinuria. Thus, we designed this trial to clarify the effect of intensive multifactorial intervention on remission and regression of DN, and to establish the treatment of DN by medical team with doctors and co-medicals.

In conclusion, DNETT-Japan aims to investigate the efficacy of intensive multifactorial therapy in Japanese type 2 diabetic patients with DN. Results from this trial are expected to provide further evidence regarding the treatment strategy in patients with overt proteinuria.

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Conflict of interest

There are no conflicts of interest.

REFERENCES

[1] Japanese Society for Dialysis Therapy, Condition as of December 31, 2007, in: An Overview of Regular Dialysis Treatment in Japan, Japanese Society for Dialysis Therapy, 2008, pp. 2–22 (in Japanese).
[2] M.J. Sarnak, A.S. Levey, A.C. Schoolwerth, J. Coresh, B. Culleton, L.L. Hamm, et al., Kidney disease as a risk factor for development of cardiovascular disease: a statement from the American Heart Association Councils on Kidney in Cardiovascular Disease, High Blood Pressure Research, Clinical Cardiology, and Epidemiology and Prevention, Circulation 108 (2003) 2154–2169.
[3] W.A. Wilmer, B.H. Rovin, C.J. Hebert, S.V. Rao, K. Kumor, L.A. Hebert, Management of glomerular proteinuria: a commentary, J. Am. Soc. Nephrol. 14 (2003) 3217–3232.
[4] P. Gaede, P. Vedel, H.H. Parving, O. Pedersen, Intensified multifactorial intervention in patients with type 2 diabetes mellitus and microalbuminuria: the Steno type 2 randomised study, Lancet 353 (1999) 617–622.
[5] P. Gaede, P. Vedel, N. Larsen, G.V. Jensen, H.H. Parving, O. Pedersen, Multifactorial intervention and cardiovascular disease in patients with type 2 diabetes, N. Engl. J. Med. 348 (2003) 383–393.
[6] S. Araki, M. Haneda, T. Sugimoto, M. Isono, K. Isshiki, A. Kashiwagi, et al., Factors associated with frequent remission of microalbuminuria in patients with type 2 diabetes, Diabetes 54 (2005) 2983–2987.
[7] S. Matsuo, E. Imai, M. Horio, Y. Yasuda, K. Tomita, K. Nitta, et al., Revised equations for estimated GFR from serum creatinine in Japan, Am. J. Kidney Dis. 53 (2009) 982–992.
[8] UK Prospective Diabetes Study (UKPDS) Group, Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment, risk of complications in patients with type 2 diabetes, (UKPDS, 33), Lancet 352 (1998) 837–853.
[9] H.C. Gerstein, M.E. Miller, R.P. Byington, D.C. Goff Jr., J.T. Bigger, J.B. Buse, et al., Effects of intensive glucose lowering in type 2 diabetes, N. Engl. J. Med. 358 (2008) 2545–2559.
[10] Y. Ohkubo, H. Kishikawa, E. Araki, T. Miyata, S. Isami, S. Motoyoshi, et al., Intensive insulin therapy prevents the progression of diabetic microvascular complications in Japanese patients with non-insulin-dependent diabetes mellitus: a randomized prospective 6-year study, Diabetes Res. Clin. Pract. 28 (1995) 103–117.
[11] American Diabetes Association, Diabetic nephropathy: diagnosis, prevention, and treatment, Diabetes Care 25 (2002) 85S–89S.
[12] A.V. Chobanian, G.L. Bakris, H.R. Black, W.C. Cushman, L.A. Green, J.L. Izzo Jr., et al., The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report, JAMA 289 (2003) 2560–2572.
[13] G.F. Strippoli, M. Craig, J.J. Deeks, F.P. Schena, J.C. Craig, Effects of angiotensin converting enzyme inhibitors and angiotensin II receptor antagonists on mortality and renal outcomes in diabetic nephropathy: systematic review, BMJ 329 (2004) 828.
[14] H. Makino, M. Haneda, T. Babazono, T. Moriya, S. Ito, Y. Iwamoto, et al., Prevention of transition from incipient to overt nephropathy with telmisartan in patients with type 2 diabetes, Diabetes Care 30 (2007) 1577–1578.
[15] H.H. Parving, H. Lehnert, J. Brochner-Mortensen, R. Gomis, S. Andersen, P. Arner, The effect of irbesartan on the development of diabetic nephropathy in patients with type 2 diabetes, N. Engl. J. Med. 345 (2001) 870–878.
[16] B.M. Brenner, M.E. Cooper, D. de Zeeuw, W.F. Keane, W.E. Mitch, H.H. Parving, et al., Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy, N. Engl. J. Med. 345 (2001) 861–869.
[17] E.J. Lewis, L.G. Hunsicker, W.R. Clarke, T. Berl, M.A. Pohl, J.B. Lewis, et al., Renoprotective effect of the angiotensin-receptor antagonist irbesartan in patients with nephropathy due to type 2 diabetes, N. Engl. J. Med. 345 (2001) 851–860.
[18] C.E. Mogensen, S. Neldam, I. Tikkanen, S. Oren, R. Viskoper, R.W. Watts, et al., Randomised controlled trial of dual blockade of renin-angiotensin system in patients with hypertension, microalbuminuria, and non-insulin dependent diabetes: the candesartan and lisinopril microalbuminuria (CALM) study, BMJ 321 (2000) 1440–1444.
[19] P. Gaede, H. Lund-Andersen, H.H. Parving, O. Pedersen, Effect of a multifactorial intervention on mortality in type 2 diabetes, N. Engl. J. Med. 358 (2008) 580–591.

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ORIGINAL ARTICLE

Design and methods of a strategic outcome study for chronic kidney disease: Frontier of Renal Outcome Modifications in Japan

Kunihiro Yamagata · Hirofumi Makino · Tadao Akizawa · Kunitoshi Iseki · Sadayoshi Itoh · Kenjiro Kimura · Daisuke Koya · Ichiei Narita · Tetsuya Mitarai · Masanobu Miyazaki · Yoshiharu Tsubakihara · Tsuyoshi Watanabe · Takashi Wada · Osamu Sakai · Advisory Committee for FROM-J

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Abstract
Background The continuous increase in the number of people requiring dialysis is a major clinical and socioeconomical issue in Japan and other countries. This study was designed to encourage chronic kidney disease (CKD) patients to consult a physician, enhance cooperation between nephrologists and general practices, and prevent the progression of kidney disease.

Methods Subjects comprise CKD patients aged between 40 and 74 years consulting a general physician, and patients in CKD stage 3 with proteinuria and diabetes or hypertension. This trial is a stratified open cluster-randomized study with two intervention groups: group A (weak intervention) and group B (strong intervention). We have recruited 49 local medical associations (clusters) in 15 different prefectures, which were classified into four

K. Yamagata (✉)
Department of Nephrology, Institute of Clinical Medicine, Graduate School of Comprehensive Human Sciences, University of Tsukuba, 1-1-1, Ten-oudai, Tsukuba, Ibaraki 305-8575, Japan
e-mail: k-yamaga@md.tsukuba.ac.jp

H. Makino
Department of Medicine and Clinical Science, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan

T. Akizawa
Division of Nephrology, Department of Medicine, Showa University School of Medicine, Tokyo, Japan

K. Iseki
Dialysis Unit, University Hospital of The Ryukyus, Okinawa, Japan

S. Itoh
Division of Nephrology, Endocrinology and Vascular Medicine, Tohoku University Graduate School of Medicine, Miyagi, Japan

K. Kimura
Department of Nephrology and Hypertension, St. Marianna University School of Medicine, Kanagawa, Japan

D. Koya
Division of Endocrinology and Metabolism, Kanazawa Medical University, Ishikawa, Japan

I. Narita
Division of Clinical Nephrology and Rheumatology, Niigata University Graduate School of Medical and Dental Science, Niigata, Japan

T. Mitarai
Division of Nephrology and Hypertension, Saitama Medical Center, Saitama Medical School, Saitama, Japan

M. Miyazaki
Department of Internal Medicine, Miyazaki Clinic, Miyazaki, Japan

Y. Tsubakihara
Department of Nephrology, Osaka General Medical Center, Osaka, Japan

T. Watanabe
Department of Internal Medicine III, Fukushima Medical University School of Medicine, Fukushima, Japan

T. Wada
Department of Laboratory Medicine, Institute of Medical, Pharmaceutical and Health Sciences, Faculty of Medicine, Kanazawa University, Ishikawa, Japan

O. Sakai
Japan Kidney Foundation, Tokyo, Japan

regions (strata) based on the level of increase rate of dialysis patients. The patients in group A clusters were instructed initially to undergo treatment in accordance with the current CKD treatment guide, whereas patients in group B clusters were not only instructed in the same fashion but also received support from an information technology (IT)-based system designed to help achieve the goals of CKD treatment, consultation support centers, and consultations by dietitians visiting the local general practice offices. We assessed the rates of continued consultation, collaboration between general practitioners and nephrologists, and progression of CKD (as expressed by CKD stage).

Conclusion Through this study, filling the evidence-practice gap by facilitating effective communication and supporting general physicians and nephrologists, we will establish a CKD care system and decrease the number of advanced-stage CKD patients.

Keywords Chronic kidney disease · Evidence-practice gap · Cluster-randomized study · Educational intervention · Cooperation between nephrologists and general physicians

Introduction

The number of dialysis patients is continually increasing, with consequent rises in medical costs for the treatment of end-stage kidney disease (ESKD) patients becoming a socioeconomical concern worldwide. In fact, there are 2,153.2 dialysis patients per million of population in Japan [1]. Chronic dialysis treatment not only reduces the quality of life (QOL) of patients [2, 3] but also places considerable financial strain on society, with annual medical costs of five to six million yen per dialysis patient, or total expenses of one trillion yen. Moreover, it is estimated that there are more than ten million chronic kidney disease (CKD) patients in Japan [4]. Previous studies suggested that CKD is one of the most important risk factors for cardiovascular disease, among known risk factors of diabetes, hypertension, hyperlipidemia, obesity, smoking, and lifestyle-related disease [5–8]. Therefore, early detection and control of CKD are also important in terms of preventing cardiovascular complications and deaths.

The definition of CKD first appeared in the Kidney Disease Outcome Quality Initiative (KDOQI) Guidelines issued by the National Kidney Foundation (NKF) in 2002 [9], and was revised by Kidney Disease: Improving Global Outcomes (KDIGO) in 2005 [10]. Since then, the definition of CKD and renal function assessment methods are being accepted worldwide. CKD is defined as kidney damage or glomerular filtration rate (GFR) <60 ml/min/1.73 m^2 for 3 months or more, irrespective of cause. The concept of CKD comprehensively addresses a wide range of kidney patients, including ESKD and transplant patients. It is important to establish appropriate, consistent, and specific treatment and prevention-based care systems according to the progression of kidney disease. The Ministry of Health, Labor, and Welfare organized a study group to design strategic outcome studies and discuss the following research subjects: prevention of diabetes, prevention of suicide and depression (2005), cancer prevention, and AIDS/HIV prevention (2006), which have been started. Following these studies, a strategic study to improve the progression of CKD was planned based on these social and scientific demands to reduce new patients with initiation of renal replacement therapy due to ESKD, termed the Frontier of Renal Outcome Modifications in Japan (FROM-J).

Diabetic nephropathy, nephrosclerosis due to hypertension, and chronic glomerulonephritis are three major primary renal diseases in ESKD, not only in Japan but also in Western countries [1]. In Japan, the proportion of new ESKD patients due to chronic glomerulonephritis has recently been decreasing, while that of diabetic nephropathy is rapidly increasing. If this trend continues, in 5 years, patients undergoing dialysis due to diabetic nephropathy will account for 50.82% of the total whereas those with chronic glomerulonephritis will account for 19.54%. In other words, the primary renal disease in half of dialysis patients will be diabetic nephropathy, and the number of dialysis patients with chronic glomerulonephritis will decrease by 17%. The decreasing trend in chronic glomerulonephritis is due to annual urinalysis screening programs established by the Japanese government [11]. Also, more attention should be paid to preventing deterioration of renal function in patients with diabetic nephropathy and nephrosclerosis.

Although diabetic nephropathy is the primary underlying disease in dialysis patients in many developed countries, it has been showing a decreasing trend in some regions and countries, including Denmark. In Denmark, after a steady increase from 52 in 1990 to 183 in 2002, the number of dialysis patients with diabetic nephropathy decreased by 15%, to 155–156 patients per million people [12]. This indicates that aggressive management of both blood pressure and glucose, administration of renin angiotensin system (RAS) inhibitors, and advice on lifestyle can reduce ESKD with diabetic nephropathy by more than 15%. According to the 2002 diabetes survey conducted by the Ministry of Health, Labor, and Welfare of Japan, only 33.3% of patients in Japan had controlled their HbA1c to less than 6.5%, and these interventions are expected to achieve marked effects. Furthermore, although 50.2% of males and 38.3% of females aged 40 years or

older in Ibaraki Prefecture showed hypertension, only 41.9% and 49.2% of them, respectively, were receiving antihypertensive treatment [13], and blood pressure was not adequately controlled in about 50% of those who were receiving treatment [14]. Appropriate interventions are assumed to bring about noticeable effects in Japan, in which RAS inhibitors have not been used effectively as antihypertensive therapy, although a slight increase has occurred in recent years [15].

Recently, the CKD Clinical Practice Guide for future treatment methods was developed by the Japanese Society of Nephrology [16], describing the treatment target for every CKD stage. Although all items of the treatment method were supported by clinical evidence, there were no prospective studies showing the effect of practices such as the CKD Clinical Practice Guide targets on renal and cardiovascular outcomes in sufficient number of CKD patients.

In this strategic CKD study, a prospective stratified cluster-randomized trial to examine the effectiveness of a care system designed to prevent progression of CKD through collaboration between nephrologists and general physicians was selected. One of the goals of the study is a 15% reduction in the estimated number of new dialysis patients in 5 years by increasing the rates of compliance with the CKD Clinical Practice Guide. The study also aims to encourage CKD patients to see their family physician, consult a nephrologist, and receive nutritional and lifestyle advice, while discussing health care measures to reduce the number of new dialysis patients.

Hypotheses of study

The study hypothesis encompasses the following four core issues:

1. Clinical practice in accordance with the Japanese CKD Clinical Practice Guide will improve the prognosis of CKD patients and reduce the speed of renal function deterioration.
2. Education-based interventions for CKD patients by registered dietitians and other co-medicals will help achieve strict CKD treatment goals in accordance with the Japanese CKD Clinical Practice Guide.
3. Collaboration concerning clinical practices among general physicians, nephrologists, and co-medicals will reduce the gap between clinical practice and evidence-based care measures, and improve the rate of continued consultation and prognosis in CKD patients.
4. These active interventions to improve CKD treatment will achieve the desired effects in terms of medico-economics.

Subjects and methods

Study organization and duration

Since the increase in the rate of dialysis patients varies from region to region in Japan [17], we divided the country into four regions (Fig. 1) as strata, so that they would

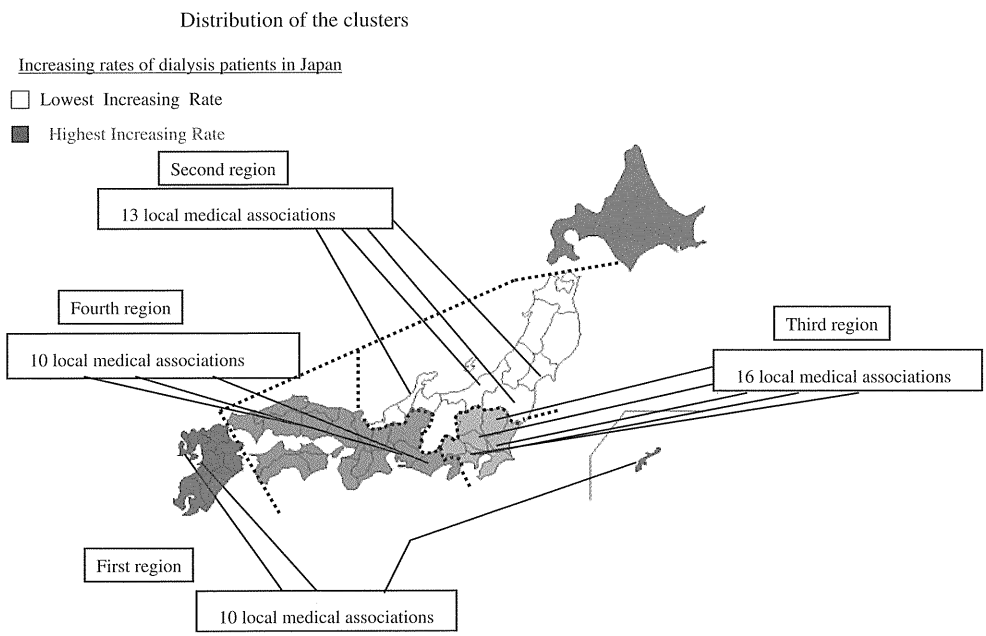

Fig. 1 Distribution of the clusters. We have recruited 49 local medical associations (clusters) in 15 different prefectures, which were classified into four regions (strata) based on the level of increase in the rate of dialysis patients [17]

include at least one managing facility and two or more clusters. The primary intervention study duration is from October 2008 to March 2012.

Rationale for setting the number of patients

This project aims to examine whether or not intervention can reduce the incidence of dialysis patients by 15% over the next 5 years. Regarding the calculation, we estimated the annual decrease in GFR as 0.59 ml/min/year (standard deviation (SD) 0.04 ml/min/year), based on changes in renal function among healthy Japanese people who underwent health checkups [17, 18] and the rate of renal deterioration in patients in CKD stage 3 with diabetes or hypertension [mean serum creatinine = 1.69 mg/dl (SD = 0.57 mg/dl), annual decrease rate = 5.93 ml/min/year (SD 4.321 ml/min/year), $n = 569$] [18, 19]. The required study size was calculated as 2,038 when the unknown intracluster correlation coefficient was assumed to be 0.5. We determined the required number as 2,264 for groups A and B, assuming that 10% would withdraw. We applied the simple number of 2,500 (1,250 for each group) as the target number of patients to perform this study.

Eligible patients

Each registered general physician obtained written informed consent for the study from eligible patients. They were formerly registered after the data center verified their eligibility. Inclusion criterion were: (1) age between 40 and 74 years; (2) in CKD stage 1, 2, 4, or 5; (3) in CKD stage 3 with proteinuria (ratio of urinary protein/urinary creatinine $\geq$0.3, or proteinuria $\geq$1+) and diabetes or hypertension.

Dialysis patients and those who did not consent were excluded from this study.

Assignment and randomization

This trial is a stratified open cluster-randomized study with two intervention groups: group A (weak intervention) and group B (strong intervention). We have recruited 49 local medical associations (clusters) in 15 different prefectures, which were classified into four regions (strata) based on the level of increase in the rate of dialysis patients (Fig. 1). Each local medical association recruited 10–58 general physicians by whom patients in this study has been treated. Local medical associations are randomized when the enrolment period is completed.

Intervention methods

Patients in group A clusters are instructed initially to undergo treatment in accordance with the current CKD treatment guide only, whereas patients in group B clusters are not only instructed in the same fashion but also receive consultations by dietitians visiting the local general practice offices. In addition, the data center closely monitors the treatment status and provides the group B general practice office with comments on the data.

Goals for the treatment of chronic kidney disease (groups A and B)

Participants in the study, or patients, will receive treatment according to the CKD Clinical Practice Guide [16]. Table 1 shows a summary of targets for CKD treatment applied to all patients. In patients with CKD, lifestyle modifications to avoid obesity and stop smoking are necessary. Strict blood pressure control (less than 130/80 mmHg), strict blood sugar control (HbA1c <6.5%), and low-density lipoprotein (LDL)-cholesterol control (LDL-C <120 mg/dl) are shown as targets for CKD treatment. The standards for referral from general physicians to nephrologists are as follows: (1) ratio of urinary protein/urinary creatinine $\geq$0.5, or proteinuria $\geq$1+; (2) estimated GFR (eGFR) <50 ml/min/1.73 m^2; (3) both proteinuria and hematuria positive ($\geq$1+); and (4) when family physicians judge that patients should consult a nephrologist. Estimated GFRs in this study are calculated using the following formula:

$$\mathrm{eGFR(ml/min/1.73\ m^2)} = 194 \times \mathrm{Age}^{-0.287} \times \mathrm{Cre}^{-1.094} (\times 0.739 \text{ in the case of women}).$$

Monitoring of treatment status by the data center (only group B)

The data center closely monitors the treatment status and provides the group B general practice office with comments on the data. In addition, the data center will provide information on the patients scheduled to visit the office, examinations, and treatment that patients should undergo on their next visit, patients who did not visit hospitals as scheduled, those who are going to receive lifestyle/dietary advice, and those who meet the conditions for referral to nephrologists. The center also monitors patients and their schedules: the next consultation date, required examinations, details of treatment and care provided, and advice on lifestyle and nutrition. The centers will contact patients by mail, telephone, or email a week before the consultation day and encourage those who have not consulted a physician for over 2 months to receive care, trying to prevent their withdrawal from treatment. To facilitate referrals to nephrologists, the centers send a list of patients who meet the criteria for referral to the physicians and clinical research coordinators (CRCs).

Table 1 CKD practice guide target in this study

CKD stages	Lifestyle	Diet	Blood pressure	Blood sugar	Lipid metabolism	Hemoglobin
Stage 1	Smoking cessation BMI <25 kg/m^2	Sodium chloride <6 g/day for hypertensives	<130/80 mmHg	HbA1c <6.5%	LDL-C <120 mg/dl	
Stage 2	Smoking cessation BMI <25 kg/m^2	Sodium chloride <6 g/day for hypertensives	<130/80 mmHg	HbA1c <6.5%	LDL-C <120 mg/dl	
Stage 3	Smoking cessation BMI <25 kg/m^2	Sodium chloride <6 g/day for hypertensives DPI: 0.6-0.8 g/kg/day	<130/80 mmHg	HbA1c <6.5%	LDL-C <120 mg/dl	Hb 10–12 g/dl
Stage 4	Smoking cessation BMI <25 kg/m^2	Sodium chloride <6 g/day for hypertensives DPI: 0.6–0.8 g/kg/day Potassium restriction	<130/80 mmHg	HbA1c <6.5%	LDL-C <120 mg/dl	Hb 10–12 g/dl
Stage 5	Smoking cessation BMI <25 kg/m^2	Sodium chloride <6 g/day for hypertensives DPI: 0.6–0.8 g/kg/day Potassium restriction	<130/80 mmHg	HbA1c <6.5%	LDL-C <120 mg/dl	Hb 10–12 g/dl
Others			<125/75 mmHg If proteinuria >1 g/day			

BMI body mass index, *DPI* dietary protein intake

Nutrition and lifestyle improvement (only group B)

Registered dietitians provide support according to the instructions and advice from family physicians. They help patients achieve their CKD treatment goals, explaining to patients about examination results, achievements in CKD care, and their implications. Registered dietitians receive training so that they will be able to provide integrated and consistent advice.

Data collection

At each consultation, physicians will measure patients' blood pressure, and check their blood pressure conditions at home. Examinations or surveys will be performed every 6 months regarding body weight, abdominal circumference, smoking status, fasting serum creatinine, blood urea nitrogen (BUN), potassium, hemoglobin (Hb), high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC), triglyceride (TG), uric acid, total protein, albumin, fasting blood glucose, HbA1c (only in the case of diabetes), urinary creatinine levels, amount of urinary proteins, eGFR, number of patients referred by nephrologists, number of new dialysis patients, and incidence of cardiovascular events.

Parameters for assessment

Primary parameters for assessment are: (1) the rate of continuous clinic visits of CKD patients, (2) the proportion of patients under cotreatment between general physicians and nephrologists, and (3) annual changes in CKD stage.

Secondary parameters are: (1) the proportion of adherence to the complete CKD treatment guide, (2) the rate of achievement of blood pressure goals, (3) the number of subjects with 50% reduction in urinary protein, (4) the number of subjects with a doubling of serum creatinine or 50% reduction in eGFR, (5) yearly changes in the number of patients starting renal replacement therapy, and (6) the incidence of cardiovascular events.

Statistical analysis

Statistical analyses will be performed using an intent-to-treat approach. Differences in primary endpoints between intervention groups are described by their 95% confidence intervals. The declining velocity of eGFR is tested by analysis of variance, using the efficacy of interventions as fixed effects and cluster effects as random effects. We employ a generalized linear model with age, gender, complications, and previous GFR as covariates where appropriate. The significance level on both sides in hypothesis testing is set at 0.05.