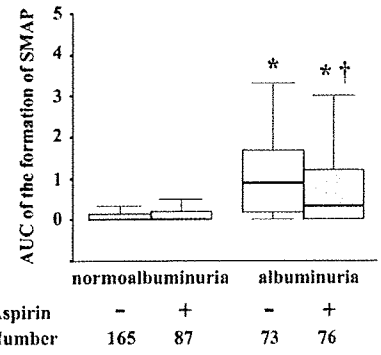


Figure 2—*Box-and-whisker plots of SMAP assessed by AUC in diabetic patients with normoalbuminuria and albuminuria, treated with or without aspirin. In these plots, lines within the boxes represent median values; the upper and lower lines of the boxes represent the 25th and 75th percentiles, respectively; and the upper and lower bars outside the boxes represent the 90th and 10th percentiles, respectively. Kruskal-Wallis test:* $P < 0.001$, *$P < 0.001$ *vs. normoalbuminuric patients treated with and without aspirin,* †$P = 0.01$ *vs. albuminuric patients without aspirin (Mann-Whitney U test).*

moalbuminuria showed no such difference according to aspirin intake (0.00 [0.00–0.14] vs. 0.00 [0.00–0.21]) (Fig. 2). In addition, the strong correlation between the degree of SMAP and the levels of urinary AER as continuous variables was investigated in the patients without aspirin intake ($\gamma = 0.53$, $P < 0.001$), whereas the weak association between them was found in those with aspirin intake ($\gamma = 0.27$, $P < 0.001$).

Correlation between platelet microaggregation and GPIIb/IIIa and P-selectin expression

To further evaluate the active state of SMAP, we quantitated the expression levels of active GPIIb/IIIa and P-selectin on the platelets. The expression levels of active GPIIb/IIIa were higher in diabetic patients with SMAP than in those without SMAP (29.9% [interquartile range 19.3–42.2] vs. 15.5% [11.7–21.9]). Similarly, the expression levels of P-selectin were higher in diabetic patients with detectable SMAP than those without it (8.1% [5.1–16.0] vs. 4.4% [3.2–5.6]). The expression levels of both markers correlated significantly with the degree of SMAP (Spearman $\gamma = 0.59$ for active GPIIb/IIIa and $\gamma = 0.55$ for P-selectin).

Finally, we investigated the relationship between the expression levels of these surface markers and urinary AER in the subgroups based on aspirin intake. In patients not taking aspirin, the expression level of each surface marker correlated significantly with urinary AER (Spearman $\gamma = 0.60$ for active GPIIb/IIIa and $\gamma = 0.57$ for P-selectin) (Fig. 3*A* and *C*). Similar correlations were also observed in patients taking aspirin, albeit with a slightly weaker coefficient of correlation (Spearman $\gamma = 0.51$ for active GPIIb/IIIa and $\gamma = 0.34$ for P-selectin, Fig. 3*B* and *D*).

CONCLUSIONS— The present study provided new evidence that both increased AER and baPWV are independent factors associated with the abnormal formation of SMAP in type 2 diabetic patients. Furthermore, the patients with SMAP showed an irreversible pattern of platelet microaggregation by ADP, and the degree of SMAP correlated with the enhanced expression of active GP IIb/IIIa and P-selectin, indicating that SMAP is pathophysiologically active. These early-activated platelet profiles were signifi-

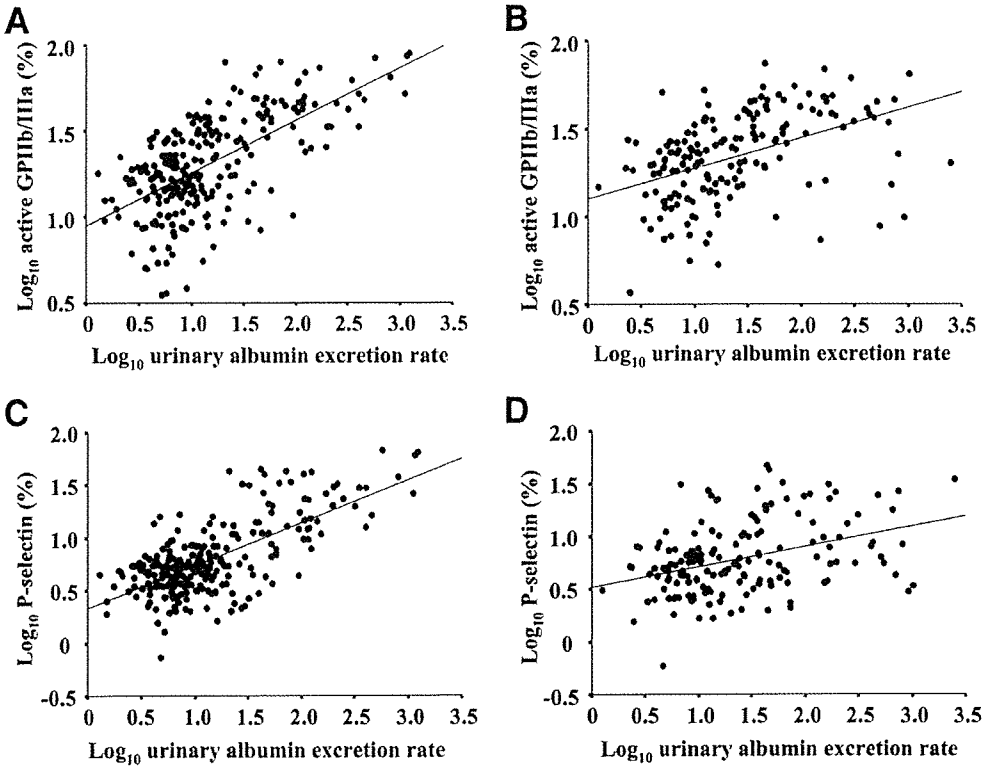


Figure 3—*Correlation between urinary albumin excretion rate and expression of platelet surface markers, active GPIIb/IIIa, and P-selectin. The patients were divided into two subgroups based on aspirin intake: for active GPIIb/IIIa, those not taking aspirin (A)* ($\gamma = 0.60$; $P < 0.001$) *and those taking aspirin (B)* ($\gamma = 0.51$; $P < 0.001$), *and for P-selectin, those not taking aspirin (C)* ($\gamma = 0.57$; $P < 0.001$) *and those taking aspirin (D)* ($\gamma = 0.34$; $P < 0.001$). *Log-transformed values were plotted on each figure.*

cantly inhibited in albuminuric patients with aspirin intake, although the effect was incomplete.

Assessment of inappropriate platelet activation is one way to risk-stratify patients who are at high risk of atherosclerosis and atherothrombosis. The microaggregates produced in the early phase of platelet activation are now considered to potentially aggravate thrombus formation (7). Under normal conditions, these platelet microaggregates dissolve within a few minutes, as shown in this study (Fig. 1*D*). In the present study, 53% of type 2 diabetic patients showed SMAP and abnormal irreversibility of the microaggregation after low-dose ADP. This high proportion of diabetic patients with abnormal platelet hyperaggregability is in agreement with the result from a cohort studied previously (12). The SMAP formation was observed under a low-shear stress that mimicked the state of the arterial bloodstream without the stimulation of exogenous agonists. In addition, the degree of SMAP was associated with enhanced expression of active GPIIb/IIIa and P-selectin. Thus, the formation of SMAP is considered to show the abnormal activated state of platelets in diabetic patients, which is enhanced according to the increase of albuminuria.

In the present study, SMAP occurrence was associated with both albuminuria and baPWV. In agreement with these results, other investigators reported that high levels of platelet microaggregation were associated with adverse outcomes in patients with cardiovascular disease (16) and the ankle-brachial index in patients with peripheral arterial disease (17). In addition, diabetic patients with SMAP showed overexpression of platelet surface markers, active GPIIb/IIIa, and P-selectin. Such upregulation of these molecules has also been associated with the development of atherogenesis (18). Taken together, the early platelet activation in type 2 diabetic patients may be a risk factor for cardiovascular disease.

A recent guideline of the American Diabetes Association recommended prophylactic use of antithrombotic agents, with low-dose aspirin, for diabetic subjects, especially those with albuminuria (19). However, Di Minno and Violi (20) indicated that aspirin alone is insufficient to prevent thrombosis in diabetic patients with angiopathy. In the present study, SMAP formation was inhibited in albuminuric patients taking aspirin compared with those not taking it. However, SMAP formation was still associated with albuminuria, even in patients taking aspirin, suggesting that the inhibitory effect of aspirin may be insufficient in diabetic patients with albuminuria. Further follow-up and intervention studies are required to investigate whether the incomplete inhibition of early-activated platelet profiles is a cardiovascular risk.

Several plausible mechanisms underlying the cardiorenal interaction have been proposed. Deckert et al. (21) proposed in the Steno hypothesis that excess leakage of albumin into urine reflects widespread vascular (endothelial) damage. This endothelial dysfunction is considered a common feature of cardiorenal interactions (22); it leads to platelet activation, adhesion, and subsequent platelet aggregate formation. Platelets are also a rich source of chemokines and cytokines, released within seconds of platelet activation (23). Thus, the formation of SMAP in diabetic patients with albuminuria might either cause or reflect systemic vascular endothelial dysfunction. An alternative explanation is that any pathophysiological alteration in a diabetic kidney could directly affect platelet activation. Glomerular hypertension, which induces mechanical shear stress (24), and excess renal production of type IV collagen in the kidney (25), a powerful activator of platelets, might induce activation of platelets that circulate into the kidney.

The present study had some limitations. It was not possible to ascertain whether the early-activated platelet profile was a cause or consequence of the increased urinary albumin excretion because of the nature of the cross-sectional study. The present study also could not address whether the incidence of future cardiovascular disease is higher in patients with SMAP and whether the inhibitory effect of aspirin on the degree of SMAP would be sufficient to prevent the development of cardiovascular disease.

In summary, the majority of type 2 diabetic patients with albuminuria showed an altered profile of early platelet activation including SMAP events. Given the growing concern over cardiovascular consequences in type 2 diabetic patients, further follow-up and intervention studies are needed to establish whether the inhibition of SMAP is a therapeutic target to prevent cardiovascular complications in type 2 diabetic patients.

Acknowledgments— This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

No potential conflicts of interest relevant to this article were reported.

We acknowledge Mayumi Yamanaka for the data management.

References

1. de Zeeuw D, Raz I. Albuminuria: a great risk marker, but an underestimated target in diabetes. Diabetes Care 2008;31(Suppl. 2): S190–S193
2. Adler AI, Stevens RJ, Manley SE, Bilous RW, Cull CA, Holman RR, UKPDS Group: Development and progression of nephropathy in type 2 diabetes: the United Kingdom Prospective Diabetes Study (UKPDS 64). Kidney Int 2003;63: 225–232
3. de Zeeuw D, Remuzzi G, Parving HH, Keane WF, Zhang Z, Shahinfar S, Snapinn S, Cooper ME, Mitch WE, Brenner BM. Albuminuria, a therapeutic target for cardiovascular protection in type 2 diabetic patients with nephropathy. Circulation 2004;110:921–927
4. Araki S, Haneda H, Koya D, Hidaka H, Sugimoto T, Isono M, Isshiki K, Chin-Kanasaki M, Uzu T, Kashiwagi A. Reduction of microalbuminuria as an integrated indicator for renal and cardiovascular risk reduction in patients with type 2 diabetes mellitus. Diabetes 2007;56:1727–1730
5. Colwell JA, Nesto RW. The platelet in diabetes: focus on prevention of ischemic events. Diabetes Care 2003;26:2181–2188
6. Ferroni P, Basili S, Falco A, Davì G. Platelet activation in type 2 diabetes mellitus. J Thromb Haemost 2004;2:1282–1291
7. Ruggeri ZM. Platelets in atherothrombosis. Nat Med 2002;8:1227–1234
8. Cerbone AM, Macarone-Palmieri N, Saldalamacchia G, Coppola A, Di Minno G, Rivellese AA. Diabetes, vascular complications and antiplatelet therapy: open problems. Acta Diabetol. In press
9. Born GV, Hume M. Effects of the numbers and sizes of platelet aggregates on the optical density of plasma. Nature 1967;215: 1027–1029
10. Cardinal DC, Flower RJ. The study of platelet aggregation in whole blood. Br J Pharmacol 1979;66:94–95
11. Ozaki Y, Satoh K, Yatomi Y, Yamamoto T, Shirasawa Y, Kume S. Detection of platelet aggregates with a particle counting method using light scattering. Anal Biochem 1994;218:284–294
12. Matsuno H, Tokuda H, Ishisaki A, Zhou Y, Kitajima Y, Kozawa O. P2Y12 receptors play a significant role in the development of platelet microaggregation in patients

with diabetes. J Clin Endocrinol Metab 2005;90:920–927
13. Matsuno H, Kozawa O, Nagashima S, Kanamaru M, Uematsu T. Comparative antiplatelet effects of aspirin, vapiprost and GR144053, a GPIIb/IIIa antagonist, with a special reference to the role of platelet microaggregates. Br J Pharmacol 1999; 127:1129–1134
14. Abrams CS, Ellison N, Budzynski AZ, Shattil SJ. Direct detection of activated platelets and platelet-derived microparticles in humans. Blood 1990;75:128–138
15. Fijnheer R, Modderman PW, Veldman H, Ouwehand WH, Nieuwenhuis HK, Roos D, de Korte D. Detection of platelet activation with monoclonal antibodies and flow cytometry: changes during platelet storage. Transfusion 1990;30:20–25
16. Miyamoto S, Kawano H, Sakamoto T, Soejima H, Kajiwara I, Shimomura H, Kojima S, Hokamaki J, Sugiyama S, Hirai N, Yoshimura M, Ozaki Y, Ogawa H. Formation of platelet microaggregates correlates with adverse clinical outcome in patients with coronary artery disease. Thromb Haemost 2003;89:681–686
17. Kudoh T, Sakamoto T, Miyamoto S, Matsui K, Kojima S, Sugiyama S, Yoshimura M, Ozaki Y, Ogawa H. Relation between platelet microaggregates and ankle brachial index in patients with peripheral arterial disease. Thromb Res 2006;117: 263–269
18. Gawaz M, Langer H, May AE. Platelets in inflammation and atherogenesis. J Clin Invest 2005;115:3378–3384
19. American Diabetes Association. Standards of medical care in diabetes—2009. Diabetes Care 2009;32(Suppl. 1):S13–S61
20. Di Minno G, Violi F. Aspirin resistance and diabetic angiopathy: back to the future. Thromb Res 2004;113:97–99
21. Deckert T, Feldt-Rasmussen B, Borch-Johnsen K, Jensen T, Kofoed-Enevoldsen A. Albuminuria reflects widespread vascular damage: the Steno hypothesis. Diabetologia 1989;32:219–226
22. Satchell SC, Tooke JE. What is the mechanism of microalbuminuria in diabetes: a role for the glomerular endothelium? Diabetologia 2008;51:714–725
23. Gleissner CA, von Hundelshausen P, Ley K. Platelet chemokines in vascular disease. Arterioscler Thromb Vasc Biol 2008; 28:1920–1927
24. Kikkawa R, Koya D, Haneda M. Progression of diabetic nephropathy. Am J Kidney Dis 2003;41(Suppl. 1):S19–S21
25. Haneda M, Kikkawa R, Horide N, Togawa M, Koya D, Kajiwara N, Ooshima A, Shigeta Y. Glucose enhances type IV collagen production in cultured rat glomerular mesangial cells. Diabetologia 1991; 34:198–200

Diabetologia (2009) 52:2037–2045
DOI 10.1007/s00125-009-1467-8

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

Received: 24 December 2008 / Accepted: 15 June 2009 / Published online: 4 August 2009

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.

Clinical trial registration: ClinicalTrials.gov NCT 00448526
Funding: Research grant from the Ministry of Health, Labour and Welfare of Japan

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

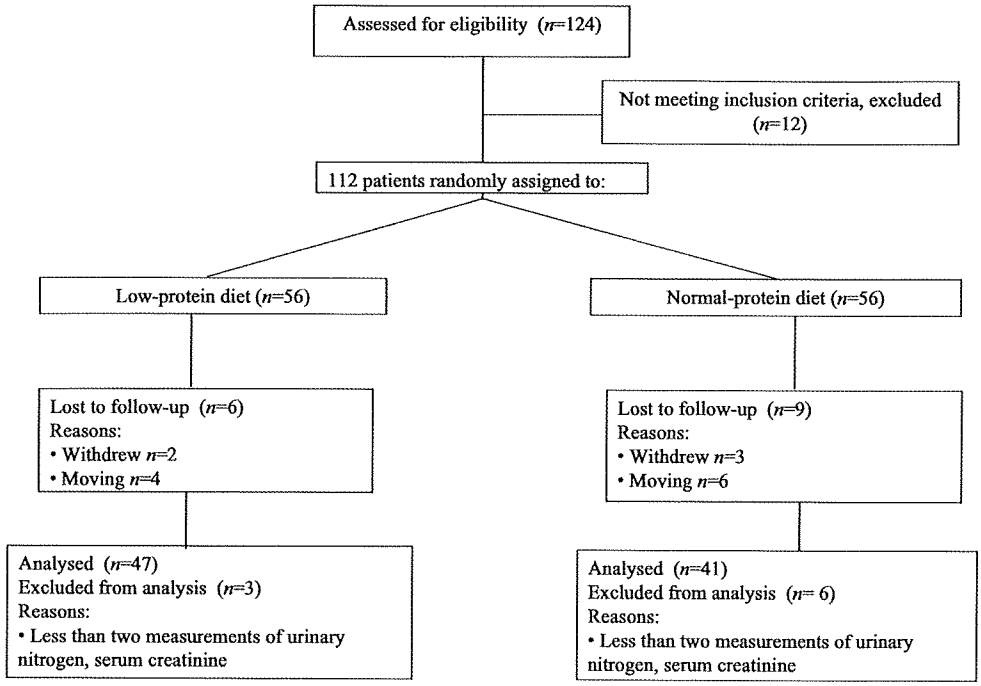


Fig. 1 Design of the trial. Fifteen patients were lost during follow-up because they moved away or withdrew informed consent within 1 year of follow-up. Nine patients were excluded from analysis because they had less than two measurements of urinary nitrogen excretion and serum creatinine

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)

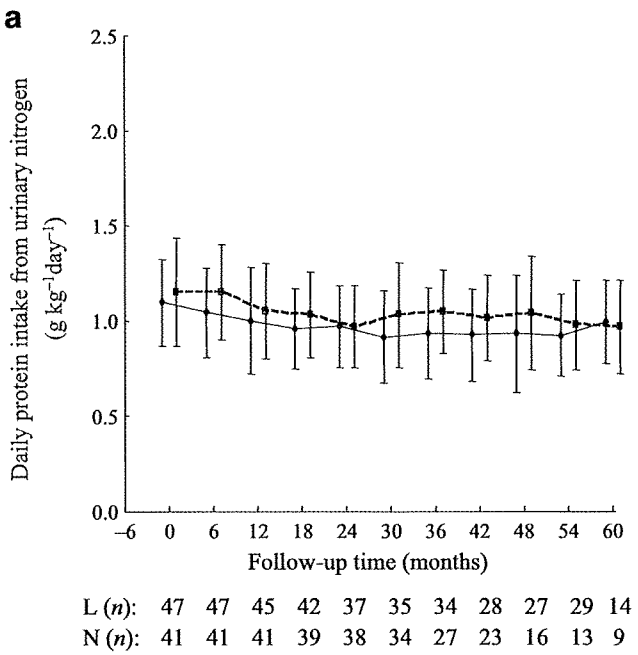
not significant (p=0.14) (Fig. 2a). This was in contrast to the significant difference between the two groups based on food record (p<0.0001) (Fig. 2b).

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



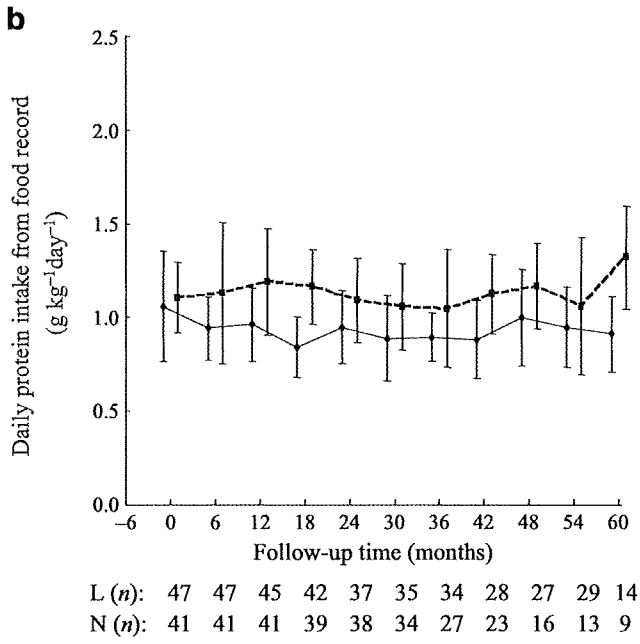


Fig. 2 Dietary protein intake in the low-protein diet (continuous lines) and normal-protein diet (dashed lines) groups estimated (**a**) from urinary nitrogen excretion and (**b**) from 3 day food record during the study. L (*n*), low-protein diet group (*n* participants); N (*n*), normal-protein diet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

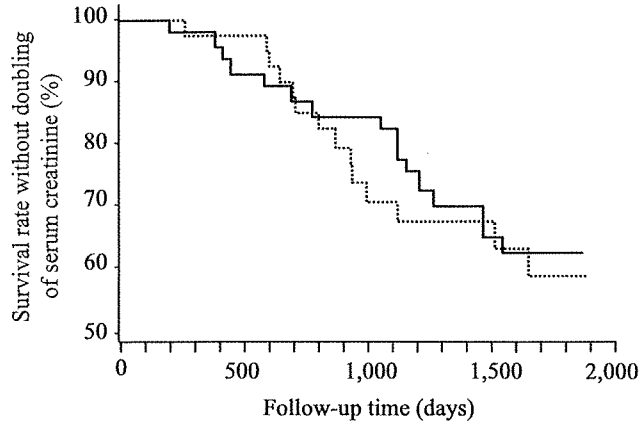


Fig. 3 Kaplan–Meier estimates of the primary endpoints from the study entry to time of doubling of baseline serum creatinine. The time to doubling was similar in both low-protein diet (continuous line) and normal-protein diet (dashed line). p=0.66 by logrank test

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

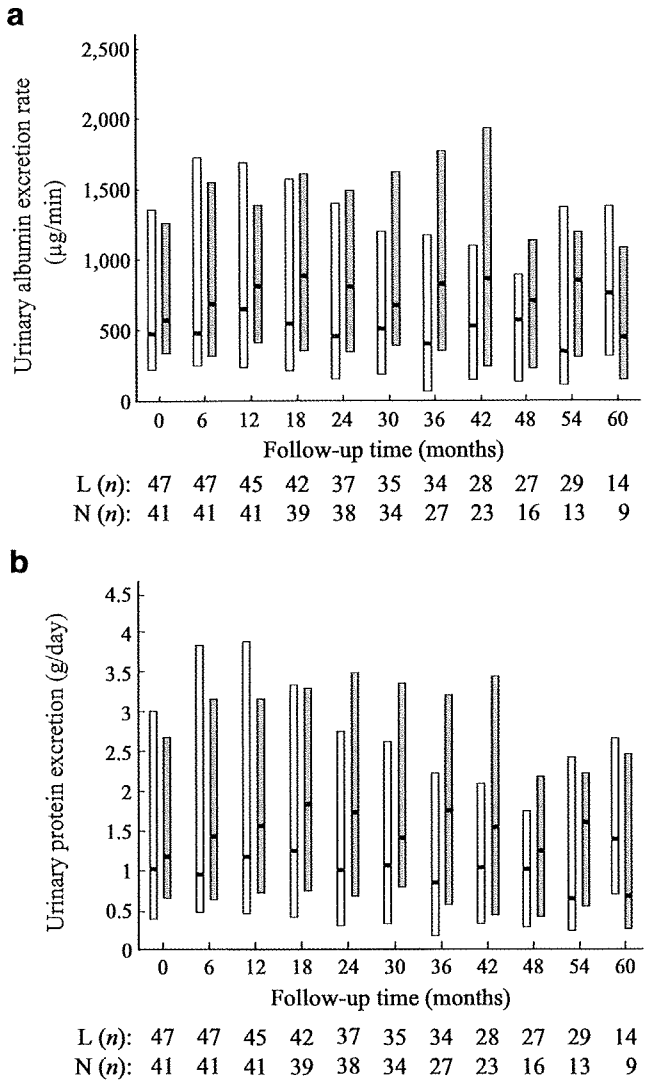


Fig. 4 The effect of low-protein diet (white columns) and normal protein intake (grey columns) on albuminuria (**a**) and proteinuria (**b**). Boxes indicate 25th and 75th percentiles of albuminuria or proteinuria. Horizontal lines indicate median. L (*n*), low-protein diet group (*n* participants);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

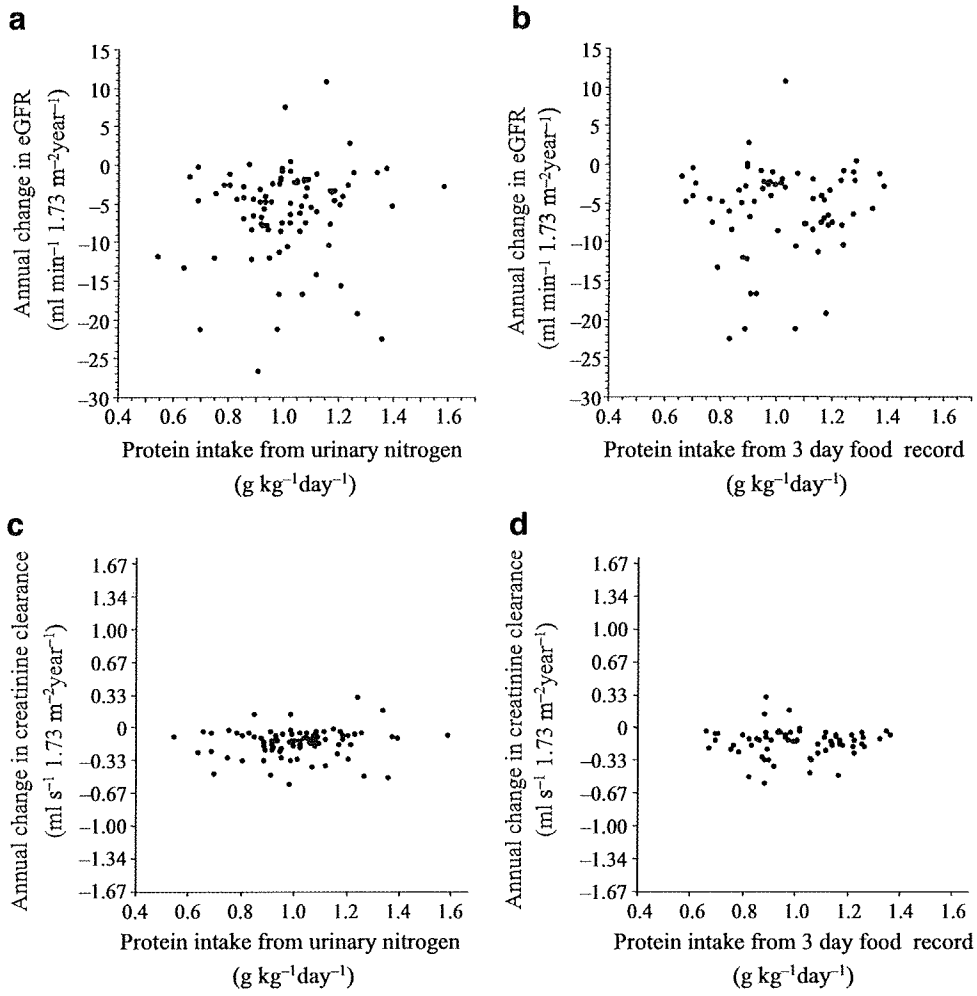


Fig. 5 Correlation between achieved protein intake estimated (**a**) from urinary nitrogen excretion and the annual change in eGFR, and (**b**) from 3 day food record and the annual change in eGFR. **c** Correlation between achieved protein intake, estimated from urinary nitrogen excretion and (**d**) 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

Acknowledgements We thank the patients who participated in the study. We also thank the independent data and safety monitoring board for their support and helpful discussions: T. Nagasawa, S. Koshikawa, O. Sakai, Y. Shigeta, Y. Ohashi and S. Nakano. This study was supported by a research grant from the Ministry of Health, Labour and Welfare of Japan (to R. Kikkawa and H. Makino).

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 Diasease (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

20 糖尿病性腎症

近年，透析療法導入症例の原疾患のうち糖尿病性腎症の占める割合が急増してきており，医学的・社会的に重要な課題となっている．2008年末の日本透析医学会の全国集計でも，慢性透析療法に新規に導入された症例のうち，糖尿病性腎症が43.2%(16,126例)と第1位を占めている(http：//docs.jsdt.or.jp/overview/index.html)．さらに，腎症から透析導入された患者の生命予後は，5年生存率が約50%と慢性糸球体腎炎と比較してきわめて不良である．

糖尿病性腎症からの末期腎不全が増加の一途を辿っている第一の原因は，糖尿病患者数の増加である．厚生労働省が発表した平成18年国民健康・栄養調査結果では，糖尿病の該当者と予備群の数は，前回調査から4年間で250万人増加し，計1,870万人と推計された．第二の原因は，糖尿病患者の約半数が医療機関をいったん受診してもその後の受診を中断する，あるいは最初から受診していないことである．その他の原因として，受診していても的確に糖尿病性腎症の診断が行われていない，あるいは治療目標の達成がきわめて困難であることもあげられる(**表Ⅲ-110**)．

また，新たな疾患概念としての慢性腎臓病(CKD)(166頁)の主たる原疾患は糖尿病性腎症である．前述した透析導入に至るのみならず，心血管疾患の発症や死亡リスクが高いことも明らかにされてきた．つまり，糖尿病であればすでに慢性腎臓病のハイリスク群であることを認識して，糖尿病性腎症に加え心血管疾患に対する戦略も講じなければならない．

このような背景から，基礎的には糖尿病性腎症の発症・進展機序の解明による抜本的治療法の開発が，臨床的にはエビデンスに基づいた糖尿病性腎症に対する診断と治療の確立が切に望まれる．

表Ⅲ-110　糖尿病性腎症が増加の一途を辿っている要因

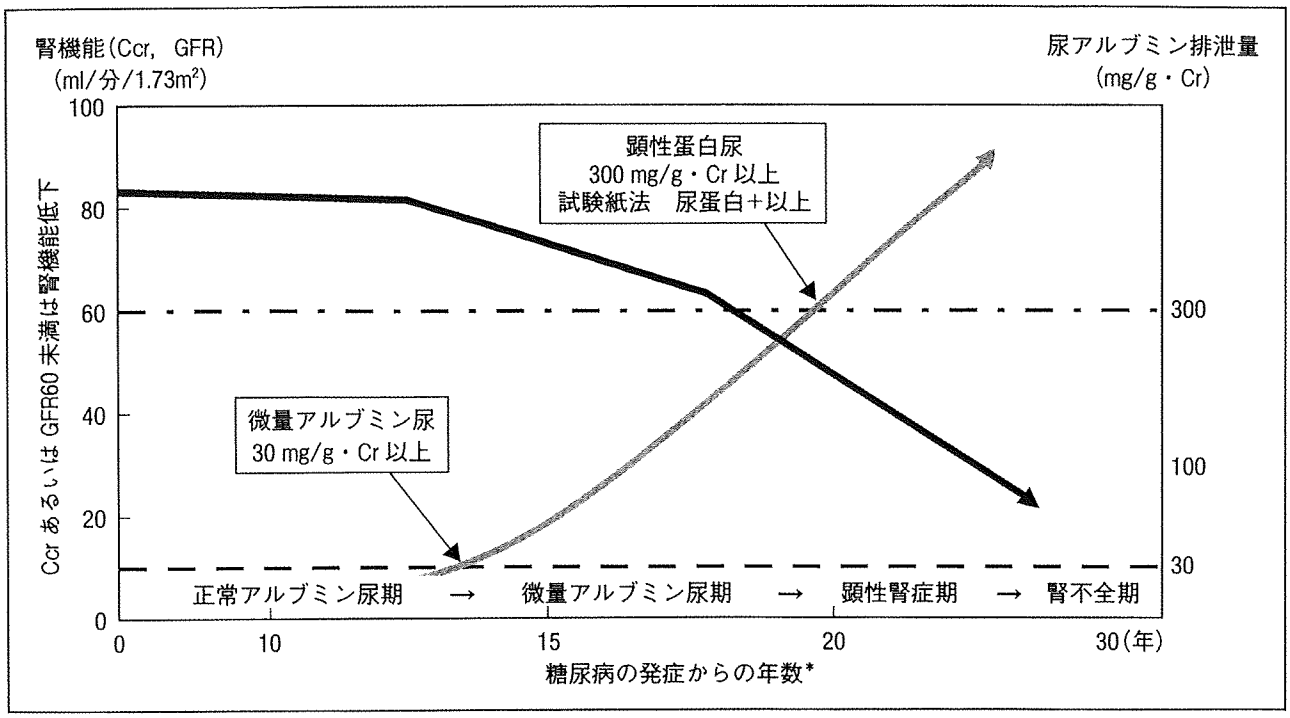
- 糖尿病患者数の増加
- 糖尿病患者および予備軍の受診中断あるいは未受診
- 腎症の的確な診断不足と血糖，血圧，脂質管理目標の未達成

定義

糖尿病性腎症は，糖尿病に特異的な細小血管障害である．慢性的な高血糖により，病理学的にはメサンギウム領域の拡大，糸球体および尿細管基底膜の肥厚，糸球体硬化症，細動脈硬化症，尿細管・間質の線維化を生じる．臨床的には，アルブミン尿の増加(微量アルブミン尿)，蛋白尿，高血圧，浮腫，腎機能低下といった特徴的な臨床的徴候を示す病態である(**図Ⅲ-155**)．しかし，近年のCKDの概念から，アルブミン尿の増加がない，あるいは蛋白尿陰性であるが腎機能低下を伴う糖尿病症例が，特に2型糖尿病に多いことも明らかにされてきた(**図Ⅲ-156**)．つまり，糖尿病性腎症の大半は**図Ⅲ-155**に示すような典型的な病期を辿るが，非典型例としてアルブミン尿や蛋白尿を伴わない腎機能低下例があることも念頭に置かなければならない．

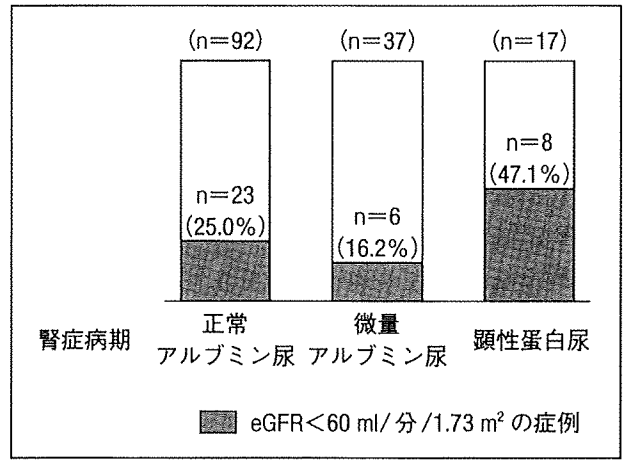
病因

高血糖が糖尿病性腎症の重要な成因であることは，Diabetes Control and Complications Trial (DCCT)，UK Prospective Diabetes Study



図Ⅲ-155 2型糖尿病性腎症の病期と特徴的な臨床的徴候
*2型糖尿病では発症時期が不明であることが多く，糖尿病と診断された時点から腎症の診断が必要.

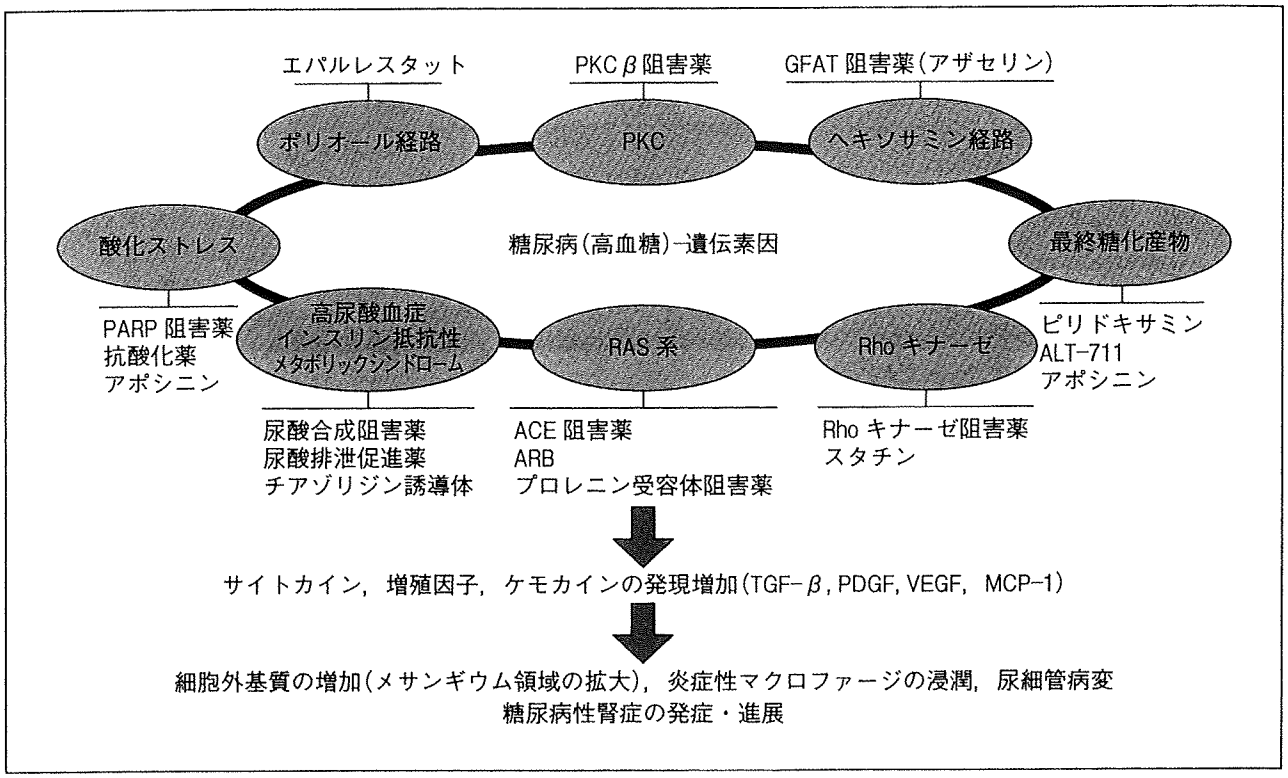
(UKPDS)，およびKumamoto Study，そして2008年発表されたADVANCEの結果より明らかにされている[1-4]．実際，膵移植後の10年にわたる正常な血糖管理によって，1型糖尿病性腎症の組織学的病変が改善しうることが示されている[5]．したがって，高血糖により惹起される腎あるいは腎構成細胞における生化学的異常が，糖尿病性腎症の発症・進展に重要な役割を演じていると推察できる．現在，高血糖により生じる，①ポリオール経路の亢進[6]，②ジアシルグリセロールdiacylglycerol(DAG)-プロテインキナーゼC protein kinase C(PKC)活性化[7]，③ヘキソサミン経路の亢進[8]，④酸化ストレス[9]，⑤最終糖化産物advanced glycation end products(AGEs)の蓄積およびAGEs受容体the receptor for AGEs(RAGE)の過剰[10]など生化学的異常が，それぞれ単独，あるいは相互に作用し腎臓に機能的および組織学的異常を惹起すると考えられている(図Ⅲ-157)．



図Ⅲ-156 2型糖尿病患者のアルブミン排泄量と推算GFR
金沢医科大学に通院中の外来2型糖尿病患者におけるアルブミン尿による病期分類と推算GFR値．正常アルブミン尿期および微量アルブミン尿期においても，GFR<60 ml/分/1.73 m²の症例が予想以上に多い．

1 ポリオール経路の亢進

ポリオール経路は解糖経路の側副路であり，グルコースはアルドース還元酵素aldose reductase (AR)によりソルビトールに，ソルビトールはソルビトール脱水素酵素sorbitol dehydrogenase (SDH)によりフルクトースに変換される(図Ⅲ-158)．ポリオール経路の亢進は，ソルビトール



図Ⅲ-157　糖尿病性腎症の病因と新たな対策

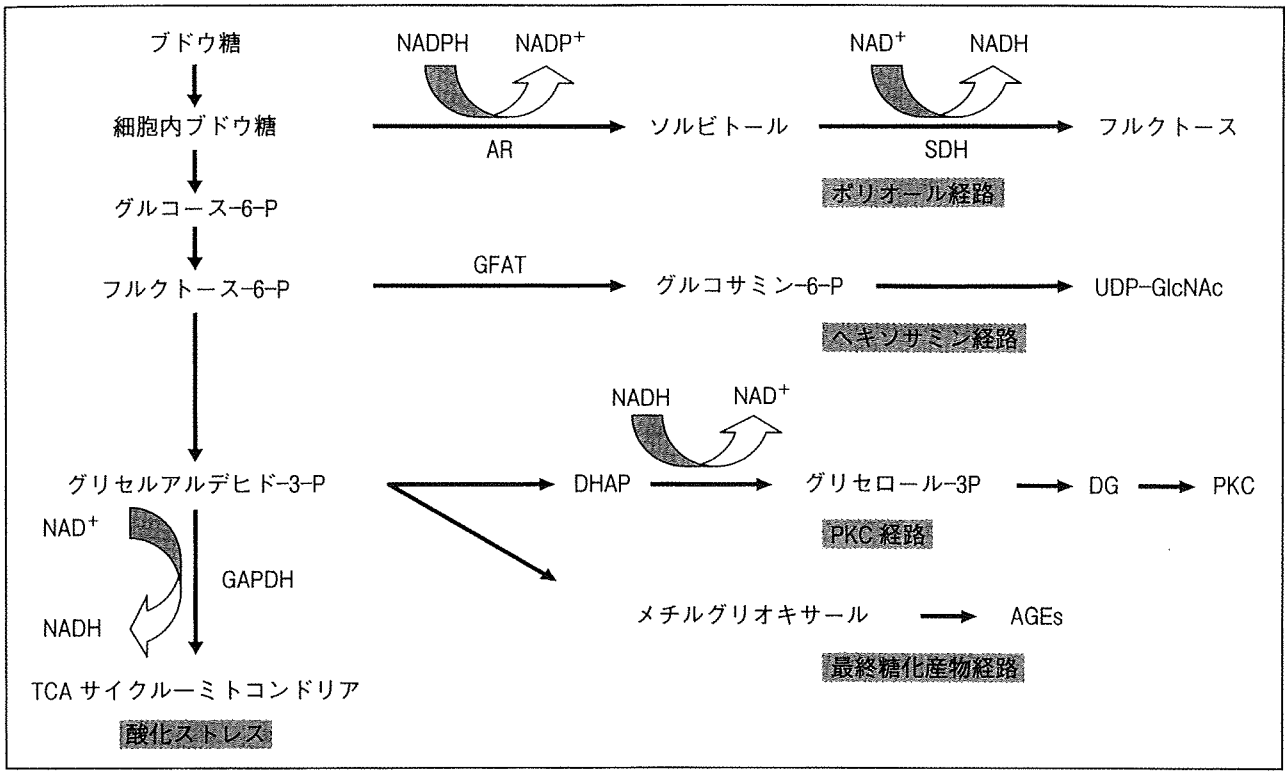
蓄積による細胞内浸透圧の上昇，NADPHの低下，フルクトースによるAGEsの蓄積，NADH/NAD^+比の上昇による細胞内偽虚血状態pseudohypoxiaを介して，腎機能を障害する．また，ポリオール経路の亢進によるNADH/NAD^+比の上昇は，後述する*de novo* DG産生の増加とPKC活性化を生じやすくする．ポリオール経路亢進の糖尿病神経障害に対する意義はアルドース還元酵素阻害薬aldose reductase inhibitor(ARI)を用いて実証されているが，糖尿病性腎症に関して有効であるとの一定した見解はいまだ得られていない．しかし，Hottaらの報告により，エパルレスタット投与群において非投与群と比較して，微量アルブミン尿期から進展した顕性腎症期に至る悪化症例が減少し，一方，正常アルブミン尿に改善した症例が増加していたことが示されている(**図Ⅲ-157**)[11]．

2 ヘキソサミン経路の亢進

ヘキソサミン合成経路は，フルクトース-6リン酸fructose-6-phosphate(F6P)がグルタミン：フルクトース-6リン酸アミドトランスフェラーゼglutamine：fructose-6-phosphate amidotransferase(GFAT)によりグルコサミン-6リン酸glucosamine-6-phosphate(GlcN6P)に変換された後，UDP-N-アセチルグルコサミン-6リン酸UDP-N-acetylglucosamine(UDP-GlcNAc)となる(**図Ⅲ-158**)．高血糖によるヘキソサミン経路の亢進は細胞外基質産生作用を有するtransforming growth factor-β(TGF-β)遺伝子発現を増強するが，GFAT阻害薬によりその異常が改善される(**図Ⅲ-157**)[12]．この遺伝子発現の分子機構に，転写因子SP-1のセリン・スレオニン残基の酵素的糖化(O-linked glycation)が関与していることが明らかにされた[13]．最近，Yangらによりインスリンシグナリングに関わる細胞内分子の酵素的糖化がインスリン抵抗性の分子基盤であることも明らかにされ，メタボリックシンドロームの病態に本経路の亢進が関わっている可能性も示唆されている[14]．

3 protein kinase C(PKC)の活性化

高血糖による*de novo* DG合成の亢進によりPKCが活性化される[6]．糖尿病性腎症に対する意



図Ⅲ-158 高血糖により惹起される細胞内代謝異常〔文献 10)より改変〕

義は，PKC 阻害薬を用いた研究成果によって検証されてきた(**図Ⅲ-157**)．特に，経口投与可能である PKCβ 阻害薬は，糖尿病実験動物にみられる糸球体過剰濾過，アルブミン尿のみならず，メサンギウム領域の拡大も改善する[15,16]．また，高血圧を呈する糖尿病実験動物においても，PKCβ 阻害薬によって糸球体病変と尿細管間質の炎症細胞浸潤と線維化が改善されることが示された[17,18]．その後，Tuttle らによって PKCβ 阻害薬の投与がヒト糖尿病性腎症に対して有効であったとの報告がなされた．すでにレニン・アンジオテンシン(RA)系阻害薬にて治療を受け，尿アルブミン/クレアチニン(Cr)比が 200～2,000 mg/g・Cr の病期の進展した 2 型糖尿病患者に対して 1 年間の PKCβ 阻害薬投与が行われた．プラセボ投与群では，観察開始時と比較して，アルブミン尿の増加と腎機能の顕著な低下が試験終了時にみられたが，PKCβ 阻害薬投与群ではアルブミン尿が−24% と有意に減少するとともに腎機能低下速度も軽減されていることが示された[19]．

4 酸化ストレス

糖尿病性腎症をはじめとする糖尿病合併症の一成因として，酸化ストレスの亢進が以前から注目されており，抗酸化薬によって糖尿病実験動物の腎障害が改善されることが示されている．実際，細胞内 oxidant 測定用蛍光プローブである dichlorofluorescin(DCFH)を用いると，糖尿病誘発ラットから単離した糸球体における酸化ストレスの存在が正常ラット腎糸球体に比し増強しており，それら酸化ストレス亢進が抗酸化薬であるビタミン E によって改善されることも示されている[20]．酸化ストレスはグリケーション(糖化)，グルコース自己酸化，ポリオール経路の亢進，PKC 活性化，NOS アンカップリング，ミトコンドリア機能異常，プロスタグランジン代謝異常，キサンチン酸化酵素 xanthine oxidase，NADP(H) oxidase，および後に述べる AGEs の産生過程および AGEs-RAGE などを介した活性酸素種の産生経路と，抗酸化酵素であるグルタチオンペルオキシダーゼ glutathione peroxidase，カタラーゼ catalase，Cu, Zn-SOD, Mn-SOD の機能低下，つまり活性

酸素種の消去機能の低下によって生じる[21]．中でも，ミトコンドリアにおいて過剰に産生される活性酸素の抑制によって，ヘキソサミン経路の亢進，PKC活性化，AGEs形成亢進が抑制されることから，細胞内代謝異常とミトコンドリア起因の酸化ストレスが相互に連鎖しているとの新たな合併症仮説がBrownleeらによって示され注目されている[9]．実際，uncoupling protein-1(UCP-1)やMn-SODを過剰発現することによって，高ブドウ糖による酸化ストレスが消失し，ポリオール経路，ヘキソサミン経路，PKC経路の活性化が改善されることが，内皮細胞を用いた実験において示されている．また，ミトコンドリア起因の酸化ストレスによるPoly(ADP-ribose)polymerase(PARP)活性化によって解糖系の酵素であるglyceraldehydes-3 phosphate dehydrogenase(GAPDH)が抑制され，結果として前述した経路が活性化されることも示されている(**図Ⅲ-158**)．その後，PARP阻害薬がそれら経路を抑制する有効な手段であり，*db/db*マウスの尿アルブミンとメサンギウム領域の拡大が同薬により改善されることも示されている(**図Ⅲ-157**)[22]．

その他，酸化ストレス産生に関わるNADPH oxidase活性の亢進がPKCや後述するAGEsによる腎症の発症に関わっていることも示されている．実際，PKC阻害薬あるいはNADPH oxidase阻害化合物であるアポシニンによって，アルブミン尿と糸球体硬化が改善されることが報告されている[23,24]．

5 advanced glycation end products (AGEs)

ブドウ糖に比べて反応性の高いメチルグリオキサールなど解糖系からのアルデヒドがAGEs形成に関わっている(**図Ⅲ-158**)．AGEs化された蛋白質の分解の低下やAGEsによる細胞機能の変異，あるいは酸化ストレスを生じることにより，腎症の発症・進展に関連していると考えられている[25]．実際，AGEs合成阻害薬であるアミノグアニジン，ピリドキサミンによって，糖尿病モデル動物の尿アルブミンの増加やメサンギウム領域の拡大が改善されると報告されている(**図Ⅲ-157**)．最近，AGEsを分解するcross-link breaker(ALT-711)によって，AGEs分解物の尿中への排泄増加とともに腎組織の改善がみられると報告されている(**図Ⅲ-157**)[26]．

さらに，AGEsはその受容体の一つであるRAGEを介して腎症を含めた血管障害を惹起することも，RAGEトランスジェニックマウスや可溶性RAGEによる治療介入により示されている[27]．

6 腹部肥満(メタボリックシンドローム)

肥満に伴う腎障害の存在はすでに知られており，実際に，高脂肪食負荷による腹部肥満に伴い，代謝異常(血糖および血圧上昇，脂質異常)と腎障害が生じる[28]．これら肥満に伴う腎障害は腎内脂質代謝の変異により生じており，それら異常がインスリン抵抗性の解除により改善されることも示されている．したがって，すでに臨床応用されているチアゾリジン誘導体を用いたインスリン抵抗性解除が糖尿病性腎症の新たな治療戦略となる可能性も考えられる(**図Ⅲ-157**)．

また，Krolewskiらにより，臨床的に血清尿酸値の高値が腎機能低下と関連していることが報告された[29]．興味あることに，Nakagawaらも，フルクトース負荷によるメタボリックシンドローム病態(肥満，高血圧，耐糖能異常，脂質異常)と腎障害に高尿酸血症が関連していると報告しており[30]，今後，インスリン抵抗性状態にみられる高尿酸血症に対する治療戦略が(**図Ⅲ-157**)，糖尿病性腎症の対策として新たな展開を迎える可能性がある．

7 遺伝素因

同一家系内に糖尿病性腎症が集積することや，同等の血糖管理や血圧管理状態においても腎症を発症しやすかったり，一方で逆も報告されており，その解決を目指して感受性遺伝子に関するSNPsをマーカーとしたゲノムワイドなケースコントロール相関解析が行われてきた．しかし，いまだ明確に糖尿病性腎症感受性候補遺伝子としてコンセンサスが得られる遺伝子の同定には至っていない[31]．

表Ⅲ-111 糖尿病性腎症病期分類

病期	臨床的特徴		主な治療法
	尿蛋白(尿アルブミン)	GFR(Ccr)	
第1期(腎症前期)	正常	正常，時に高値	血糖コントロール
第2期(早期腎症)	微量アルブミン尿	正常，時に高値	厳格な血糖コントロール降圧治療
第3期-A(顕性腎症前期)	持続性蛋白尿	ほぼ正常	厳格な血糖コントロール降圧療法・蛋白制限食
第3期-B(顕性腎症後期)	持続性蛋白尿(1 g/日以上)	低下(60 ml/分以下)	厳格な降圧療法・蛋白制限食
第4期(腎不全期)	持続性蛋白尿	著明低下(sCr 上昇)	厳格な降圧療法・低蛋白食・透析療法導入
第5期(透析療法)	透析療法中	透析療法中	腎移植

〔糖尿病性腎症に関する合同委員会，改訂，糖尿病 44：623，2001(会報)〕

診断

糖尿病性腎症は，尿中アルブミン排泄量の増加(微量アルブミン尿)にて早期腎症を発症し，持続性蛋白尿の顕性腎症期，腎不全期へと至る経過をたどる(**図Ⅲ-155**)．基本的に尿中アルブミン排泄量および腎機能(Ccr)を測定して各病期を診断する(**表Ⅲ-111**)．最近では，推算糸球体濾過量(GFR)を指標としてステージ分類されるCKDの概念が普及しており，後述するように腎機能を推算GFR式から評価した新たな病期分類が提唱される可能性もある．

1 早期腎症の診断

微量アルブミン尿の存在により診断する．24時間尿を含め時間尿を用いる場合は，20～199 μg/分(30～299 mg/日)である．しかし，一般臨床において蓄尿は煩雑であり，来院時尿を含む随時尿を用い，同時に尿Cr濃度を測定し，その割り算から30～299 mg/g・Crを微量アルブミン尿とする．ただし，尿アルブミン排泄量は日差変動が大きく，日差変動の少ない早朝第一尿を用いるのが望ましい．腎機能は，この病期ではほぼ正常範囲にある．また，微量アルブミン尿を呈する症例では，他の腎疾患を鑑別したうえで，早期腎症の診断を行う．この際，一定期間(約5年)以上の糖尿病罹病期間を有すること，糖尿病網膜症や神経障害などの他の糖尿病性合併症が存在すること，高度の血尿を合併しないことなどが診断の参考になる(**表Ⅲ-112**)[32]．

表Ⅲ-112 糖尿病性腎症の早期診断基準

測定対象
　尿蛋白陰性か陽性(+1程度)の糖尿病患者
必須事項
　尿中アルブミン値(早朝あるいは来院時の随時尿)
　　30～299 mg/g・Cr　3回測定中2回以上
参考事項
　尿中アルブミン排泄率(時間尿)
　　30～299 mg/日または30～199 μg/分
　尿中Ⅳ型コラーゲン値
　　7～8 μg/g・Cr以上
　腎サイズ　腎肥大(エコー検査，CT検査など)

〔文献32)より〕

2 顕性腎症の診断

持続性蛋白尿(顕性蛋白尿)の出現で診断する．試験紙法で持続的に蛋白尿陽性となるが，正確には定量して診断を行う．尿アルブミンを定量する場合は，時間尿で200 μg/分(300 mg/日)以上，随時尿で300 mg/g・Cr以上，尿蛋白を定量する場合は24時間尿で500 mg/日以上，随時尿で500 mg/g・Cr以上が顕性蛋白尿に相当する．この顕性腎症期には，腎機能の低下している症例が多くみられるようになる．

3 腎不全の診断

他の腎疾患による慢性腎不全と同様であり，血清クレアチニン値2.0 mg/dl以上でCcr 30 ml/分以下が腎不全に相当する．

4 CKDを考慮した新たな腎症の病期分類

先に述べた腎機能による病期分類はCcr値により分別されている．しかし，Ccrによる腎機能評価は必ずしも正確でないとの指摘もあり，日本腎臓学会から2008年5月に日本人のGFRの推算式がプレス発表された[33]．今後，尿アルブミン・蛋白排泄量とともに腎機能として推算GFRを評価項目とした新たな腎症の病期分類が必要であろう．つまり，**表Ⅲ-111**に示したように，正常アルブミン尿期や早期腎症期において腎機能は正常か時に高値と記載されているが，推算GFRによって評価すると，これら病期においてもGFR＜60 ml/分/1.73 m^2の症例が約20%みられ，1年後においても同様の傾向であった(**図Ⅲ-156**)．今後，このように正常あるいは微量アルブミン尿期であり，かつ腎機能の低下した患者において，腎症病期が進展しやすく末期腎不全に至るリスクが高いのか，さらに，心血管疾患を合併し死亡するリスクが高いのかを前向き疫学研究により明らかにしていくことによって，わが国の新たな病期分類の構築が可能となるであろう．

治療

糖尿病性腎症の治療法に関しては，多くのランダム化比較試験の成績が発表されており，いわゆるエビデンスに基づく治療が可能となってきた．さらに，糖尿病性腎症の病期改善，すなわちremission(寛解)も生じうることもわかってきた．

1 血糖コントロール

血糖コントロールの重要性は多くのランダム化比較試験から明らかである[1-5]．したがって，現時点での糖尿病性腎症の発症・進展阻止を目指した治療としては，わが国から発信されたKumamoto Studyより得られたHbA_{1c}値6.5%未満を目標とする血糖コントロールが重要である．しかし，すでに生じている糸球体病変の治癒を目指すという目的では，長期間にわたる血糖値の正常化を目指す糖尿病治療が必要であろう．2008年6月に発表されたADVANCE試験によると，2型糖尿病患者における約5年間の厳格な血糖管理群(最終HbA_{1c}値6.5%)は，通常の血糖管理群(最終HbA_{1c}値7.3%)と比較して顕性腎症の発症が30%抑制されていることが再確認されている[4]．

2 血圧コントロール

糖尿病患者には高血圧の合併が多く，さらに糖尿病性腎症が発症するとその頻度は高くなる．また，高血圧が腎症をより進行させる因子であることもよく知られており，これらの悪循環を断つためには血圧コントロールは重要である．また，糖尿病性腎症の進行に関与すると提唱されている糸球体高血圧の是正にも血圧コントロールが有効である．2004年に発表され2009年に改訂された日本高血圧学会のガイドラインでは，糖尿病患者の降圧目標値は130/80 mmHg未満とされている[34]．尿蛋白が1 g/日以上の場合には，さらに低い125/75 mmHg未満が推奨されている．

3 RA系阻害薬

糖尿病性腎症に対するRA系阻害薬の効果に関する大規模なランダム化比較試験が行われた結果，アンジオテンシン変換酵素(ACE)阻害薬，アンジオテンシンⅡ受容体拮抗薬(ARB)ともに，正常から早期腎症の発症，早期腎症から顕性腎症への進行，顕性腎症における腎機能低下を有意に抑制することが示された[35-41]．これらの成績より，RA系阻害薬が，少なくとも高血圧を有する腎症例において現時点で第一選択薬である．しかし，RA系阻害薬を漫然と投与するだけでなく，投与後に治療が有効であるかどうかを評価することが重要であることを強調したい．つまり，治療後に，アルブミン尿あるいは蛋白尿，および腎機能(GFR)を指標として，それら推移を経時的に観察する必要がある．その結果，治療によってアルブミン尿あるいは蛋白尿やGFRの年間低下率が，治療前と比べて改善している時には，腎保護が期待できる．一方，改善傾向がみられない場合には，高K血症や急速な腎機能の悪化に留意して，保険適応内で適切な増量が望まれる．2008年6月に発表されたParvingらの報告によると，顕性腎症を呈した2型糖尿病患者において，経口レニン阻害薬でアリスキレンをARBであるロサルタンの最大投与量100 mgに追加投与すると，プラ

セボに比較してさらにアルブミン尿の減少効果がみられたことが示されている[42].

4 集約的治療

Steno Diabetes Centerでは，早期腎症である微量アルブミン尿を呈する2型糖尿病症例に，血糖・血圧・血清脂質の目標値を定めた集約的治療を行った研究成果を発表している．腎症を含む細小血管障害の進行を一次エンドポイントとした4年間の研究成果がまず報告され[43]，その後，大血管障害の進展を一次エンドポイントとし，細小血管障害の進展を二次エンドポイントとした約8年の検討結果が発表された(Steno-2 study)[44].

治療目標値は，HbA_{1c}値＜6.5%，血圧値＜130/80 mmHg，血清コレステロール＜175 mg/dl，血清中性脂肪＜150 mg/dlであり，集約的治療群では全例にACE阻害薬かARB，スタチン，アスピリンに加えて抗酸化薬が投与されている．その結果，集約的治療によって，大血管障害の進展のみならず腎症の進展も有意に抑制されることがわかった(リスク低下61%)．さらに，研究終了の5.3年後の成果も発表され，集約的治療を行っていた群では，通常治療群と比較して，死亡や心血管死のみならず，末期腎不全に至った症例も有意に抑制されていることが示された[45].

5 寛解導入

滋賀医科大学において，微量アルブミン尿を呈する2型糖尿病患者を対象として6年間の追跡調査をしたところ，顕性腎症期以上に進展した症例は28%にすぎなかったものの，正常アルブミン尿期へ病期の改善した，いわゆる寛解した症例は51%に，また尿アルブミン排泄量が50%以上に減少した症例も54%にみられた[46]．この寛解や50%アルブミン尿の低下に関連する因子は，①微量アルブミン尿発症が早期であること，②RA系阻害薬を服用していること，③HbA_{1c}(7%未満)および収縮期血圧(130 mmHg未満)が厳格に管理されていること，であるとわかった．さらに，寛解あるいはアルブミン尿が50%以上減少した症例では，その後の経過観察により，推算GFRの年間低下率が有意に抑制され，かつ末期腎不全を含む心血管イベントを発症するリスクが約60%低かった[47].

表Ⅲ-113 糖尿病性腎症の治療指針と評価

治療指針
・生活習慣の改善
減量，運動，過剰な蛋白摂取の制限・食塩・アルコール制限，禁煙
・厳格な血糖コントロール：HbA_{1c}値6.5%未満，少なくとも7%未満を目標とする
RA系阻害薬の使用
血圧値130/80 mmHg未満を目標に増量する
尿蛋白が1 g/日以上では125/75 mmHg未満
目標血圧値が達成できない場合：Ca拮抗薬，利尿薬を併用し，目標値達成を図る
・フィブラート薬およびスタチン系薬による脂質異常の管理
・蛋白質制限食，吸着炭(クレメジン)，エリスロポエチン
随時尿および血清クレアチニンを用いた治療の評価
評価ポイント：①アルブミン尿，蛋白尿の少なくとも30%の減少
②GFR年間低下率の抑制
上記の評価には定期的な下記の検査が必要
1. 1日尿あるいは随時尿を用いたアルブミン尿，蛋白尿の定量
2. 推算GFRの算出

6 管理のポイント

糖尿病性腎症に対する現行で行える診治療指針および評価を**表Ⅲ-113**にまとめた．脂質異常に対するスタチンやフィブラート薬による腎保護効果もエビデンスといえる段階ではないが報告されている．その他，進行した糖尿病性腎症に対しては，蛋白質制限食，吸着炭，エリスロポエチンの適応も病期に応じて行うことが望まれる．

透析療法導入数を減少させる，さらには糖尿病性腎症を発症している状況から寛解を導くためには，日常診療において定期的なアルブミン尿の検査を行い，微量アルブミン尿である早期腎症の診断と前述の治療法を駆使した集約的治療によって，地道に目標値を長期間にわたって達成しなければならない．

7 病因に基づいた新たな対策

プロレニン受容体の阻害，Rhoキナーゼ阻害薬やスタチンによって糖尿病性腎症が改善されることも報告されている[48,49]．したがって，高血糖により惹起される細胞内代謝異常の是正，腹部肥満