

Determination of Optimal Protein Contents for a Protein Restriction Diet in Type 2 Diabetic Patients with Microalbuminuria

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NARITA, T., KOSHIMURA, J., MEGURO, H., KITAZATO, H., FUJITA, H. and ITO, S. *Determination of Optimal Protein Contents for a Protein Restriction Diet in Type 2 Diabetic Patients with Microalbuminuria.* Tohoku J. Exp. Med., 2001, **193** (1), 45-55 — To establish the method by which the optimal dietary protein content for type 2 diabetic patients with nephropathy could be determined, dietary protein content was reduced in gradated steps and renal function was evaluated at the completion of each diet. Eight type 2 diabetic patients with microalbuminuria were examined in this study. Renal function, urinary albumin excretion rate (AER) and urinary excretion rates of prostaglandins were evaluated at the completion of each of three consecutive one-week dietary periods where the protein content was 1.2, 0.8 and 0.6 g · kg Body Weight (BW)⁻¹ · day⁻¹ on the first, second and third week, respectively. Filtration fraction (FF), AER and urinary excretion rates of prostaglandin E₂ and 6-keto-prostaglandin F_{1α} significantly decreased in response to reduced dietary protein content from 1.2 to 0.8 g · kg BW⁻¹ · day⁻¹. No additional decreases in FF, AER and urinary excretion rates of these two prostaglandins were obtained after the 0.6 g · kg BW⁻¹ · day⁻¹ low protein diet period. The method evaluating renal hemodynamics at the completion of several consecutive one-week dietary periods was confirmed to be useful to determine the optimal protein contents in type 2 diabetic patients with nephropathy. The result showed that the optimal protein content in type 2 diabetic patients with microalbuminuria was 0.8 g · kg BW⁻¹ · day⁻¹ and protein restriction of less than 0.8 g · kg BW⁻¹ · day⁻¹ was not necessary for patients with this stage of diabetic nephropathy. A part of reasons in which FF decreased after reduced protein content in diet may be due to decreased prostaglandins production in the kidneys. ——— optimal dietary protein content; type 2 diabetes mellitus; microalbuminuria; diabetic nephropathy; prostaglandin

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Several studies (The Diabetic Control and Complications Trial Research Group 1995; Ohkubo et al. 1995; UK Prospective Diabetes Study Group 1998) definitively proved that tight glycaemic control could reduce the risk of onset and progression of nephropathy in patients with both type 1 and type 2 diabetes. Systemic blood pressure elevation accelerates the progression of diabetic nephropathy in patients with both type 1 diabetes (Mogensen 1982) and type 2 diabetes (Yokoyama et al. 1997). Effective antihypertensive treatment reduces albuminuria and the rate of decline in glomerular filtration rate (GFR) in this condition (Parving et al. 1995; Mogensen 1999).

In contrast to the effects of aggressive glycaemic and blood pressure control on the onset and the progression of nephropathy, use of a low protein diet (LPD) appears to require further study, since some investigators have supported a restriction or limitation of protein intake to prevent or delay development of diabetic renal disease (Cohen et al. 1987; Zeller et al. 1991) whereas others have not (Nyberg et al. 1987; Jameel et al. 1992). The exact reason for this discrepancy remains unclear. However, one possibility is that the optimal dietary protein content may differ from patient to patient, given that the renal function of patients with diabetic nephropathy may differ from patient to patient. This idea led us to consider that it will be necessary to establish the method by which optimal protein contents for protein restriction diet may be determined in each of patients who have different renal functions. Furthermore, it will be desirable to determine optimal protein contents in diet for a short time, since patients must continue LPD for long time until either beneficial effect or invalid effect of LPD on the progression of their nephropathy becomes clear if long time trial of protein restrictive diet may be carried out.

In order to find out such a suitable method,

renal hemodynamics and urinary albumin excretion rates at each of the completion of three consecutive one-week dietary periods, where the content of dietary protein was 1.2, 0.8 and 0.6 g · kg BW⁻¹ · day⁻¹ on the first, second and third week, respectively, were measured in 8 microalbuminuric type 2 diabetic patients. The evaluation was carried out according to the following idea: If restriction of protein content in diet is effective as LPD for individual patients, either filtration fraction (FF) or GFR in these patients must be decreased after one-week dietary period.

Furthermore, some investigators reported contributory roles of vasodilatory prostaglandins on glomerular hyperfiltration in type 1 diabetic patients (Viberti et al. 1989) and reduced urinary excretions of vasodilatory prostaglandins after LPD in patients with renal failure (Rosenberg et al. 1987). Therefore, urinary excretions of prostaglandins were also measured at the end of each of three consecutive dietary periods in order to examine the causal relationship between urinary excretions of prostaglandins and changes in renal function in response to LPD.

MATERIALS AND METHODS

Subjects

Eight in-patients aged 49–71 (mean 61.3 years old) with type 2 diabetic patients were recruited for this study. Entry criteria included normal arterial blood pressure (BP < 140/90 mmHg, measured using a standard clinical sphygmomanometer with Korotkoff phase V as the diastolic values), microalbuminuria (urinary albumin excretion rate [AER] was confirmed as 15–200 µg/min after hospitalization) and no evidence of illness other than diabetes mellitus. Urinary tract infection was excluded by normal urine sediment. All patients were fully informed prior to their

consent.

Study design

After admission to our hospital, they were placed on a standard diabetic diet which contained approximately $1.2 \text{ g} \cdot \text{kg BW}^{-1} \cdot \text{day}^{-1}$ of protein and $30 \text{ kcal} \cdot \text{kg BW}^{-1} \cdot \text{day}^{-1}$ of total energy intake composed of 60% carbohydrates, 24% fat and 16% protein (normal protein diet [NPD]). Ideal BW was calculated as $(\text{height [m]})^2 \times 22$ (kg).

Once the plasma glucose levels had been stabilized, the patients were placed on two consecutive one-week LPD periods as shown in Fig. 1. Throughout this two-week period, a total energy intake similar to that of the preceding NPD was prescribed. The patients received a diet containing $0.8 \text{ g} \cdot \text{kg BW}^{-1} \cdot \text{day}^{-1}$ of protein in the first week and $0.6 \text{ g} \cdot \text{kg BW}^{-1} \cdot \text{day}^{-1}$ of protein in the second week. In first and second LPD periods, caloric intake consisted of 65% carbohydrates, 24% fat and 11% protein, and 67% carbohydrates, 25% fat and 8% protein, respectively. On the last day of each dietary period, a 24-hour urine collection was performed and urinary concentrations of urea nitrogen, albumin, prostaglandin E2 (PGE2) and 6-keto-prostaglandin F1 α (6-keto-PGF1 α) were measured. The urinary urea nitrogen (UUN) for each person was used to estimate the protein intake using the following

formulae: $\text{UUN} + \text{non-urea nitrogen (NUN)} = \text{IN}$, $\text{IN} \times 6.25 = \text{protein intake (g/day)}$ where IN is nitrogen intake and NUN is taken to be $31 \text{ mg} \cdot \text{kg BW}^{-1} \cdot \text{day}^{-1}$ (Isaksson 1980; Maroni et al. 1985).

Assessment of renal hemodynamics

On the first day of these two low protein dietary periods and the day after these two dietary periods had been completed, the renal hemodynamics including GFR and renal plasma flow (RPF) were estimated in the morning after an overnight fast as the renal clearance of thiosulphate (Thios, Banyu Pharmaceutical Co., Ltd., Tokyo) and the renal clearance of *p*-aminohippurate (PAH, Daiichi Pharmaceutical Co., Ltd., Tokyo) (Brun 1950, 1951). A steady state of water diuresis was induced by oral water loading. A teflon cannula was inserted into an antecubital vein of each arm for infusion and blood sampling. After injection of priming doses of Thios and PAH, a sustaining dose of continuous injection was started to maintain constant plasma concentration of 15–25 mg/100 ml and 2–4 mg/100 ml, respectively. After 1-hour equilibration period, the patients were required to void completely and to collect 1-hour exact timed urine. Blood samples were drawn at the midpoint of the 1-hour urine collection period. Then plasma and urinary concentrations of Thios and PAH were mea-

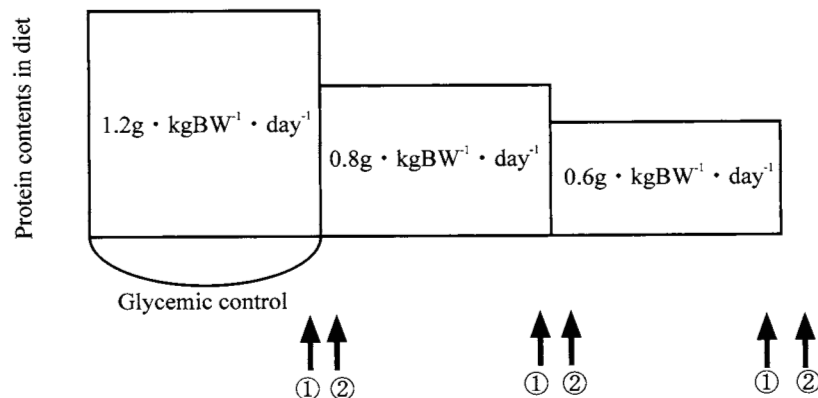


Fig. 1. Experimental setup of three consecutive one-week diet periods. ①, 24-hour urine collection; ②, renal hemodynamic examination.

sured to calculate the renal clearance of Thios and PAH, respectively. FF was calculated as the ratio of the renal clearance of Thios (C-Thios) to the renal clearance of PAH (C-PAH). GFR and RPF were corrected for body surface area and were expressed as /1.73 m². Body surface area was calculated from height and body weight.

Laboratory methods

Albumin levels in urine and serum were measured by radioimmunoassay using a double antibody technique (Brodows et al. 1986) in our laboratory. Intra- and interassay coefficients of variation for this method were 5 and 12%, respectively. Urinary urea nitrogen was determined by urease-UV method. HbA_{1c} was measured by high performance liquid chromatography method using an automated analyzer (HLC 723GHbVA_{1c} 2.2 Tosoh, Tokyo). Thios and PAH concentrations in plasma and urine were measured according to standard methods (Brun 1950, 1951). Measurement of two kinds of prostaglandins (PGE₂ and 6 keto-PGF₁α) in urine samples was carried out by radioimmunoassay using a commercial kit (Du Pont-New England Nuclear, Boston, MA, USA) according to the technique described by Kawano et al. (1987).

All the samples of urine and serum of the subjects were stored at -80°C until measurement.

Statistical analysis

Values are expressed as mean values ± s.d.

or median with a range. To test for differences in repeated values in each individual, the repeated Friedman test were used. After multiple comparisons revealed a significant difference, statistically significant differences between two repeated values in each individual were calculated using the Wilcoxon signed-ranks test. If statistically significant differences between two repeated values in each individual were not observed by the Wilcoxon signed-ranks test (non-parametric), those were re-evaluated by the Student's *t*-test for paired values (parametric).

RESULTS

Table 1 shows the clinical characteristics of 8 type 2 diabetic patients. One patient had simple retinopathy while the remaining 7 patients, who had previously received photocoagulation therapy, had proliferative retinopathy. Glycemic control (indicated as mean of 3 premeal blood glucose levels), blood pressure levels and serum albumin levels on the last day of all 3 dietary periods revealed no differences. Estimated protein intake levels were comparable to the prescribed dietary protein contents (Table 2).

FF and AER significantly decreased from 26 ± 3.8 to 22 ± 1.6% ($p < 0.05$, mean ± s.d.) and 43.5 (23.0-161.2) to 33.5 (16.2-120.9) μg/min ($p < 0.05$, median [range]), respectively, in response to reduced dietary protein content from 1.2 to 0.8 g · kg BW⁻¹ · day⁻¹. Both the Wilcoxon signed-ranks test and the Student's

TABLE 1. Clinical characteristics of 8 microalbuminuric diabetic patients

Age (years)	61.3 ± 6.4
Gender (male/female)	(6/2)
Body mass index (kg/m ²)	22.7 ± 3.7
Known duration (years)	12.7 ± 6.7
Hemoglobin Alc (%)	8.7 ± 2.2
Antidiabetic treatment (diet/oral agent/insulin)	(1/2/5)
Retinopathy (nil/simple/proliferative)	(0/1/7)

Data are expressed as mean ± s.d.

TABLE 2. Effect of low protein diet (LPD) on GFR (thiosulphate clearance), RPF (para-aminohippurate clearance), FF (filtration fraction), AER (urinary albumin excretion rate), protein intake estimated from urinary urea excretion, blood pressure, glycaemic control and serum albumin level at the end of each of 3 diet periods

	NPD	0.8 g • kgBW ⁻¹ • day ⁻¹ LPD	0.6 g • kgBW ⁻¹ • day ⁻¹ LPD	p (Friedman test)
GFR (ml • min ⁻¹ • [1.73 m ²] ⁻¹)	102.8 ± 47.0	92.1 ± 33.3	86.1 ± 23.3	N.S.
RPF (ml • min ⁻¹ • [1.73 m ²] ⁻¹)	409.8 ± 220.2	418.9 ± 165.1	388.6 ± 116.9	N.S.
FF (%)	26 ± 3.8	22 ± 1.6 ^a	22 ± 1.4 ^a	0.0347
AER (μg/min)	43.5 (23.0-161.2)	33.5 (16.2-120.9) ^a	28.8 (12.7-104.3) ^a	0.0076
Estimated protein intake (g/kgBW)	1.1 ± 0.24	0.80 ± 0.12 ^a	0.68 ± 0.12 ^{a,b}	0.0003
Systolic blood pressure (mmHg)	117.4 ± 17.3	120.0 ± 16.9	115.8 ± 12.0	N.S.
Diastolic blood pressure (mmHg)	71.0 ± 8.1	70.1 ± 9.0	67.5 ± 5.7	N.S.
Serum albumin (g/liter)	41 ± 4.3	41 ± 4.3	41 ± 3.5	N.S.
Mean of pre-meal plasma glucose (mmol/liter)	8.6 ± 2.4	8.3 ± 2.9	8.6 ± 2.2	N.S.

Data are expressed as mean ± s.d. except for AER which is median (range).

^ap < 0.05 when compared with values of normal protein diet (NPD) period using Wilcoxon signed-rank test.

^bp < 0.05 when compared with values of 0.8 g • kgBW⁻¹ • day⁻¹ LPD period using Wilcoxon signed-ranks test.

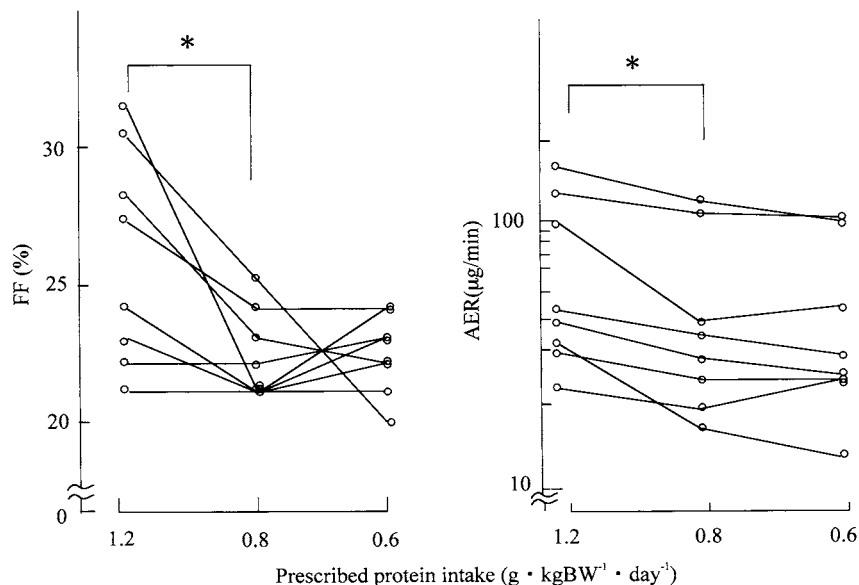


Fig. 2. Changes in filtration fraction (FF) and AER in response to normal and two graded LPD therapies in type 2 diabetic patients with microalbuminuria. Significant decrease in FF and AER were found after $0.8 \text{ g} \cdot \text{kg BW}^{-1} \cdot \text{day}^{-1}$ of LPD and were shown by $*p < 0.05$. No further decrease in FF and AER were seen after $0.6 \text{ g} \cdot \text{kg BW}^{-1} \cdot \text{day}^{-1}$ of LPD. AER is expressed as logarithmically transformed scale.

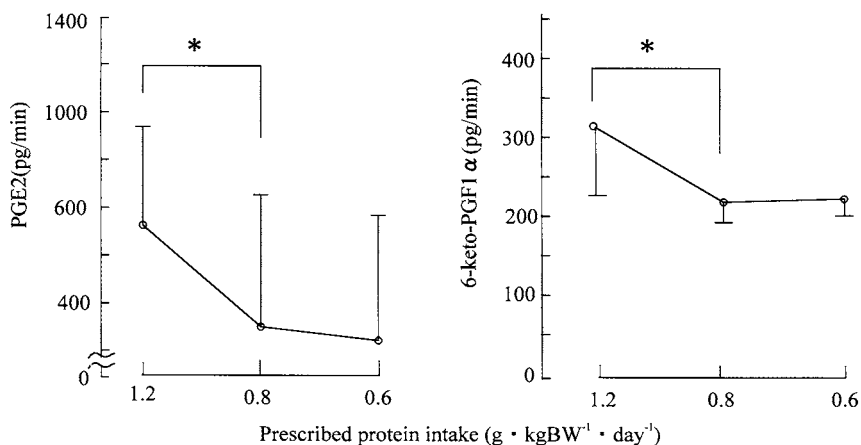


Fig. 3. Changes in urinary prostaglandins excretion rates in response to normal and two graded LPD therapies in type 2 diabetic patients with microalbuminuria. Significant decrease in urinary prostaglandins excretions were seen after $0.8 \text{ g} \cdot \text{kg BW}^{-1} \cdot \text{day}^{-1}$ of LPD and were shown by $*p < 0.05$. No further decrease in urinary prostaglandins excretions were demonstrated after $0.6 \text{ g} \cdot \text{kg BW}^{-1} \cdot \text{day}^{-1}$ of LPD. Data are indicated as mean \pm s.d.. Vertical bars indicate s.d.. PGE₂, urinary excretion rate of prostaglandin E₂; 6-keto-PGF₁ α , urinary excretion rate of 6-keto-prostaglandin F₁ α .

t-test for paired values showed no statistically significant decrease in FF or AER after the $0.6 \text{ g} \cdot \text{kg BW}^{-1} \cdot \text{day}^{-1}$ LPD period (Fig. 2 and Table 2). A tendency for a decrease in GFR at the end of the $0.8 \text{ g} \cdot \text{kg BW}^{-1} \cdot \text{day}^{-1}$ LPD was obtained, but it was not significant by either the

Wilcoxon signed-ranks test or the Student's *t*-test for paired values as shown in Table 2. Significant decreases in urinary excretion rates of PGE₂ and 6-keto-PGF₁ α (from 556.6 ± 427.5 to 365.8 ± 269.9 pg/min [$p < 0.05$, mean \pm s.d.] and from 334.8 ± 90.8 to 246.0 ± 57.2 pg/

min [$p < 0.05$, mean \pm s.d.], respectively), were found at the end of the $0.8 \text{ g} \cdot \text{kg BW}^{-1} \cdot \text{day}^{-1}$ LPD by the Wilcoxon signed-ranks test. No further decreases in them were demonstrated at the end of the $0.6 \text{ g} \cdot \text{kg BW}^{-1} \cdot \text{day}^{-1}$ LPD (PGE₂, 339.8 ± 259.7 pg/min; 6-keto-PGF_{1 α} , 251.3 ± 57.9 pg/min) as shown in Fig. 3.

DISCUSSION

The present result showed that FF and AER in type 2 diabetic patients with microalbuminuria decreased after the second of three dietary periods, where the protein content was $0.8 \text{ g} \cdot \text{kg BW}^{-1} \cdot \text{day}^{-1}$ for one week and that the decreased FF and AER did not change further after the third dietary period, where the protein content was $0.6 \text{ g} \cdot \text{kg BW}^{-1} \cdot \text{day}^{-1}$ for one week. FF has been reported to be parallel variations of intraglomerular pressure in salt sensitive hypertensive patients (Isshiki et al. 1987; Bigazzi et al. 1994). Furthermore, as to clinical significance of changes in AER and renal hemodynamics after treatment in patients with impaired renal function or diabetic patients, it has been reported that a decrease in fractional albumin excretion during antihypertensive treatment predicted a later attenuated rate of fall in GFR in diabetic nephropathy (Rossing et al. 1994) and that initial fall in GFR or GFR together with FF in response to antihypertensive therapy (Bjöck et al. 1992; Apperlod et al. 1997) or LPD therapy (Levey et al. 1996; Hansen et al. 1999) may predict the long-term stability of renal function. The present finding together with these previous reports suggested that the optimal protein contents in LPD is $0.8 \text{ g} \cdot \text{kg BW}^{-1} \cdot \text{day}^{-1}$ for type 2 diabetic patients with microalbuminuria and that protein restriction of $0.6 \text{ g} \cdot \text{kg BW}^{-1} \cdot \text{day}^{-1}$ is not necessary for type 2 diabetic patients with this stage of nephropathy. This result supported the recommendation that a protein intake of $0.8 \text{ g} \cdot \text{kg BW}^{-1} \cdot \text{day}^{-1}$ is sufficiently restrictive for individuals with evidence of

diabetic nephropathy (Franz et al. 1994). Furthermore, decreased AER is comparable to the results of previous reports which evaluated the effects of a short term LPD on hemodynamics and AER in hyperfiltering Type 1 diabetic patients (Rudberg et al. 1988). Thus, the present method, where protein contents in diet were by steps decreased every one week and changes in renal hemodynamics were measured after each diet, seems to be useful to determine the optimal protein contents in diabetic patients with different stages of nephropathy, even though protein contents used in the test meal seems to need further decrease in patients with the more advanced nephropathy.

Kupin et al. (1987) studied effect on renal function of change from high to moderate protein intake in type 1 diabetic patients after each of two consecutive dietary periods of 1 week. They found that renal hemodynamics were able to be changed by the one-week period of each diet with remarkably different protein contents. Based on their finding, three consecutive dietary periods of one week were selected in the present study. The present result supported their finding. However, further studies would be necessary to examine how minimum days does it take for renal hemodynamics to be changed by LPD and to examine whether fall in FF obtained after LPD for one week leads to the long term beneficial effect on the progression of nephropathy.

Rudberg et al. (1988) studied the effects on renal function of 10 days of a diet of $0.9 \text{ g} \cdot \text{kg BW}^{-1} \cdot \text{day}^{-1}$ of dietary protein using a cross-over design. They found that GFR, FF and AER were reduced for patients with hyperfiltration in response to reduced dietary protein. Their report suggests that protein restriction may ameliorate the intraglomerular hypertension in humans, as the same manner in the case of diabetic animals (Brenner et al. 1982). The exact mechanism by which excess of chronic protein intake may cause the intrag-

lomerular hypertension still remains unclear. In the present study, urinary prostaglandin excretions decreased after the second dietary period and no further decrease in them were found after the third dietary period. The phenomenon was in parallel with decrease in FF. The same result was reported as reduced urinary excretions of vasodilatory prostaglandins after LPD in patients with renal failure (Rosenberg et al. 1987). Reversely, glomerular hyperfiltration state in type 1 diabetic patients were accompanied by increased urinary excretions of vasodilatory prostaglandins in humans (Viberti et al. 1989). Furthermore, it was reported that administration of prostaglandin inhibitors restored myogenic vasoconstriction of afferent arteriole in diabetic rats (Hayashi et al. 1992) and attenuated the increase in GFR following protein meal ingestion in human (Krishna et al. 1988), and that treatment of vasodilatory prostaglandin E1 analog misoprostol ameliorated ibuprofen (prostaglandin synthesis blocker) induced reduction in GFR in human (Bakris et al. 1995). Taken these results into consideration all together, it seems likely that a part of mechanisms by which FF was decreased in humans after LPD in the present study may be explained by reduced renal prostaglandins production, which may induce vasoconstriction of the afferent arteriole and the succeeding result as a decrease in FF.

The degree of patient's compliance in terms of protein intake was examined according to the formula reported by Maroni et al. (1985). Protein intake estimated using urinary urea excretion was approximately equivalent to the prescribed protein intake. This result appears to confirm the validity of the present finding.

It must be emphasized in the present study that not 0.6 but 0.8 g · kg BW⁻¹ · day⁻¹ LPD is confirmed to be the optimal protein content in protein restrictive diets for microalbuminuric type 2 diabetic patients since the degree of decrease in FF and AER did not differ significantly between 0.8 and 0.6 g · kg BW⁻¹ ·

day⁻¹ LPD. However, this hypothesis should be modified if long term follow-up studies definitively prove that 0.6 but not 0.8 g · kg BW⁻¹ · day⁻¹ LPD is optimal. In view of the nutritional problems, as evidence of protein undernutrition has been reported to be detected with protein intake of 0.6 g · kg BW⁻¹ · day⁻¹ LPD (Brodsky et al. 1992), administration of 0.6 g · kg BW⁻¹ · day⁻¹ to type 2 diabetic patients with microalbuminuria for a long time may be harmful from a nutritional point of view. In one recent report, which evaluated the long-term effect of an intensive treatment (LPD, strict glycemic control and anti-hypertensive treatment with angiotensin converting enzyme inhibitor) for type 1 diabetic nephropathy (early stage, AER range of 73 ~ 500 μg/min), patients had well adhered to 0.8 g · kg BW⁻¹ · day⁻¹ LPD containing 32 ± 9 kcal kg BW⁻¹ · day⁻¹ for three years with maintaining nutritionally safe conditions (Manto et al. 1995). In their report, a rise in GFR after the intensive treatment was obtained. The caloric contents of LPD of 30 kcal kg BW⁻¹ · day⁻¹ in our present study was similar to that of their report. Taken with these results together, 0.8 g · kg BW⁻¹ · day⁻¹ LPD containing 30 kcal kg BW⁻¹ · day⁻¹ may be optimal for microalbuminuric diabetic patients from both aspects of the nutrition and the renal hemodynamic effect.

It has been reported that LPD may induce the reduced TGF-β production in the kidney (Okuda et al. 1991; Eddy 1994), in addition to renal hemodynamic changes. TGF-β was reported to play an important role in the onset of diabetic nephropathy (Yamamoto et al. 1991; Nakamura et al. 1993). The present method which was evaluated only by changes in renal hemodynamics and AER may have a shortcoming, since non-hemodynamic effects (Heidland et al. 1995) of LPD including reduced TGF-β production was not taken into consideration in the present study. However, considering that the optimal protein contents in

LPD without adverse effects were selected within a short time for every patient with different stage of nephropathy, the present method would be worth attempt.

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