005 *Metabolism*, Vol 00, No 00 (x), 1987: pp 000-000 000

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013 *Supported in part by a Grant-in-aid for the Encouragement of*
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020 *0026-0495/87/*

023 Effects of Chronic Renal Failure on the Regulation of Pyruvate Kinase

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025 Akio Ando, Yoshimasa Orita, and Takenobu Kamada

026 **The effects of chronic renal failure on the enzyme activity of pyruvate kinase and the mRNA level of this enzyme were**
027 **studied in 7 out of 8 nephrectomized rats. The mRNA level was measured by RNA-DNA dot blot hybridization, using cloned**
028 **pyruvate kinase cDNA as hybridized probe. Neither the activity of M_1-type pyruvate kinase nor the level of this enzyme in**
029 **rat gastrocnemius muscle was affected by chronic renal failure, whereas L-type pyruvate kinase enzyme activity in uremic**
030 **rat liver was lower than that in control at both fasted and refed states. The levels of L-type pyruvate kinase mRNA were not**
031 **different between two groups at the fasted state. Induction of L-type pyruvate kinase mRNA after high carbohydrate diet**
032 **refeeding was suppressed proportionally to the severity of chronic renal failure, which was expressed by the serum**
033 **creatinine concentrations ($r = -.876$, $P < .005$). These results indicate that the suppression of L-type pyruvate kinase**
034 **activity in uremia was partly reflected by the decreased accumulation of this enzyme mRNA. There was a significantly**
035 **negative correlation between L-type pyruvate kinase mRNA levels and plasma glucagon/insulin ratios ($r = -.719$, $P < .05$).**
036 **Hyperglucagonemia in uremia might play a major role in this suppression.**

038 CHRONIC RENAL FAILURE results in a variety of
039 metabolic derangements that perturb glucose homeo-
040 stasis.[1,2] The mechanisms contributing to these changes are
041 unclear, but seem to involve a combination of humoral and
042 cellular factors. These include disorders of one or more key
043 glycolytic enzymes. For example, an inhibition in the skeletal
044 muscle glycolytic enzyme, phosphofructokinase, has been
045 found in uremic rats.[3] It is also known that some degree of
046 insulin resistance and hyperglucagonemia in uremia contrib-
047 ute to glucose intolerance.[2]
048 Pyruvate kinase (EC 2.7.1.40) is one of key enzymes of
049 glucose metabolism. Four types of pyruvate kinase have been
050 isolated from rat tissues.[4,5] M_1-type pyruvate kinase is a
051 major form of skeletal muscle, and liver has L-type as a major
052 form. L-type pyruvate kinase operates as a regulator of
053 antagonistic pathways of carbohydrate metabolism, glycoly-
054 sis, and gluconeogenesis. Precisely, L-type pyruvate kinase
055 functions at pyruvate-phosphenolepyruvate crossroads of the
056 two pathways. In addition, there is approximately ten times
057 more pyruvate kinase activity than phosphoenolpyruvate
058 carboxykinase activity in rat liver,[5] suggesting that L-type
059 isozyme must be appreciably inhibited for net gluconeogene-
060 sis to occur. L-type pyruvate kinase is controlled by dietary
061 and hormonal stimuli, while that of M_1-type is not influenced
062 by these stimuli.[4-6] However, the effect of chronic renal
063 failure on the regulation of pyruvate kinase synthesis has not
064 been estimated.
065 In this study, we measured the activities of pyruvate
066 kinase and the levels of translatable mRNA of this enzyme in
067 skeletal muscle and liver from uremic rats. Our results
068 indicate that hyperglucagonemia in uremia may inhibit the

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072 accumulation of L-type pyruvate kinase mRNA, resulting
073 partly in the suppression of this isozyme activity.

074 ## MATERIALS AND METHODS

075 ### *Materials*

076 [α-^{32}P]dCTP was purchased from Amersham International
077 (Buckinghamshire, England), and nitrocellurose filters (BA85) were
078 from Schleicher & Schuell (Dassel, West Germany). DNA poly-
079 merase I was obtained from Bethesda Research Laboratories (Rock-
080 ville, MD), and DNase I from Takara Shuzo (Kyoto, Japan).
081 Formalin-fixed Staphylococcus aureus was obtained from Zymed
082 Laboratories (San Francisco), and x-ray film was from Eastman
083 Kodak Co (Rochester, NY). Other chemicals were of highest purity
084 available from commercial sources.

085 ### *Animals*

086 Male Sprague-Dawley rats, weighing 120 to 130 g, were nephrec-
087 tomized in two stage. Initially, three fourths of the left kidney was
088 resected, and 2 weeks later the left kidney was removed. At each
089 stage care was taken to leave the adrenal glands intact. Sham
090 operation was performed on a control group of rats. The rats were
091 fed standerd laboratory chow (Oriental Yeast Co., Japan) and free
092 access to water. After two months, the rats were placed singly in
093 metabolic cages, and maintained on 15 g of special chow containing
094 24% casein for a week, given twice a day: 10 g (9 to 11 AM) and 5 g (6
095 to 8 PM). Then, the rats were left on 18 hours starvation. Half were
096 killed immediately (fasted state), and the rest were fed with high
097 carbohydrate, containing 50% glucose and 12% casein, for 24 hours
098 and then killed (refed state). Body weight of uremic rats was
099 significantly lower than that of controls. Serum creatinine concen-
100 tration and blood urea nitrogen (BUN) of uremic rats were signifi-
T1 101 cantly higher than those of control rats (Table 1). Plasma HCO_3
102 concentration of uremic rats (17.4 ± 2.9 mmol/L) was significantly
103 lower than that of control rats (21.4 ± 2.9 mmol/L) ($P < .001$).

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104 ### *Isolation of Total Cellular RNA and Dot Blot*
105 ### *Hybridization of RNA*

106 Total cellular RNA was extracted from muscle and liver by the
107 phenol/chloroform procedure, as described elsewhere.[7] The M_1-type
108 and L-type pyruvate kinase mRNAs were quantitated by dot blot
109 hybridization, as reported previously.[7] Briefly, total RNA was
110 denatured by heating at 60 °C for 15 minutes in a 7% solution of
111 formaldehyde and followed by rapid cooling. Denatured RNA was
112 spotted onto a nitrocellulose filter that had been prepared with 20 ×
113 SSC (SSC = 0.15 mol/L NaCl, 15 mmol/L sodium citrate). The
114 filter which retained denatured RNA was baked at 80 °C for two
115 hours, and then prehybridized for more than four hours at 50 °C in
116 50% formamide, 5 × SSC, 50 mmol/L sodium phosphate (pH 6.5),
117 0.1% Ficoll, 0.1% polyvinylpyrrolidine, 0.1% bovine serum albumin,
118 0.1% sodium dodecyl sulfate (SDS), and sonicated, denatured
119 salmon testis DNA (250 μg/mL). The hybridization was carried out
120 for 48 hours at 50 °C in the same solution plus ^{32}P-labeled M_1- or
121 L-type enzyme cDNA. M_2-type pyruvate kinase cDNA used in this
122 study cross-hybridizes to M_1-type isozyme mRNA under high
123 stringency conditions as reported.[6] The nitrocellulose filter was then
124 washed twice with 0.1 × SSC containing 0.1% SDS at room
125 temperature, and three times at 55 °C. RNA spots were trimmed
126 and counted in a liquid scintillation counter. Values were corrected
127 for the background by subtracting the radioactivity of contiguous
128 areas of the same filter not containing bound RNA.

129 ### *Northern Analysis of RNA*

130 Electrophoresis of RNA under denatured condition was carried
131 out by the method of Lehrach et al.[8] The RNA was transferred to a
132 nitrocellulose filter. The filter was hybridized with ^{32}P-labeled
133 cDNA and subjected to autoradiography.[6]

134 ### *Nick Translation*

135 Cloned M_2- or L-type pyruvate kinase cDNA (pM$_2$PK-33[6] or
136 pLPK-1[7]) was purified on gel by the method of Dretzen et al and

84

140 nick-translated with [α-^{32}P]dCTP (800 Ci/mmol), to a specific
141 activity of 0.5 to 1.5 × 10^8 cpm/μg.

142 *Other Assays*

143 L-type pyruvate kinase activity was assayed by the 2,4-dinitrophe-
144 nylhydrazone method in the presence of excess anti-(M_1-type pyru-
145 vate kinase) antiboby to neutralize the M_2-type isozyme activity as
146 described by Tanaka et al[10] except that 1 mmol/L fructose 1,6-
147 bisphosphate was added. One unit of enzyme is expressed as the
148 amount of enzyme that catalyzes the formation of 1 μmol of
149 pyruvate/min at 37 °C. Protein was estimated by the biuret meth-
150 od[11] with bovine serum albumin as a standard. Plasma glucagon was
151 measured by a double-antibody radioimmunoassay method using
152 specific antibody for pancreatic glucagon, OAL-123 (Otsuka Assay
153 Laboratories, Tokushima, Japan). Plasma insulin was measured by
154 radioimmunoassay.
155 Values in the table and figures are given as mean ± SD. A
156 two-tailed *t*-test was used in the statistical evaluation of data and *P*
157 values <.05 were taken to indicate statistically significant differ-
158 ences.

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RESULTS

160 *Effects of Chronic Renal Failure on M_1-Type Pyruvate*
161 *Kinase Activity and M_1-Type Pyruvate Kinase mRNA*
162 *Level in Rat Gastrocnemius Muscle*

163 M_1-type pyruvate kinase activity of gastrocnemius muscle
164 in rat with chronic renal failure was not different from that in
F1 165 control rat (Fig 1). Between fasted and refed state we could
166 not observe any difference in M_1-type enzyme activity either.
F2 167 Figure 2 shows dot blot analysis of M_1-type pyruvate kinase
168 mRNA in gastrocnemius muscle using M_1-type pyruvate
169 kinase cDNA as a probe. The levels of hybridizable mRNA
170 of M_1-type pyruvate kinase in gastrocnemius muscle revealed
171 no difference between uremic and control rats at fasted or
172 refed state. RNase is considered to be increased in serum and
173 tissues of rat with chronic renal failure.[12,13] Since dot blot
174 analysis could not identify degradated mRNA, we employed
175 Northern blot analysis to determine the size distribution of
176 RNA molecules containing M_1-type isozyme mRNA. As
F3 177 shown in Fig 3, M_1-type pyruvate kinase mRNA isolated
178 from rat muscle with chronic renal failure was intact. Thus,
179 the size of M_1-type pyruvate kinase mRNA in chronic renal
180 failure was identical in control.

181 *Effect of Chronic Renal Failure on L-Type Pyruvate Kinase*
182 *Activity and L-Type Enzyme mRNA Level in Rat Liver*

183 The L-type pyruvate kinase activity of liver in rat with
184 chronic renal failure was significantly decreased compared
185 with that in control, at fasted and refed state ($P < .05$ and
F4 186 $P < .01$, respectively) (Fig 4). One simple explanation for
187 this result is the presence of inhibitor, which may cause an
188 alteration in the enzyme activity without any change in the
189 amount of enzyme protein. This possibility was tested by
190 quantitative immunoprecipitation with L-type pyruvate
191 kinase antibody and protein A from *Staphylococcus aureus*,
F5 192 as reported elsewhere.[15] Figure 5 shows that liver extracts
193 obtained from uremic and control rats at fasted and refed
194 state showed the same equivalence point. Thus, the alter-
195 ations in L-type pyruvate kinase activity described above
196 reflect the changes in the amount of immunoreactive enzyme
197 protein. The level of L-type pyruvate kinase mRNA was
198 measured by dot blot hybridization assay. It is clear from Fig
F6 199 6 that the levels of L-type isozyme mRNA between control
200 and uremic rats were not different from each other at fasted
201 state. When refed with high-carbohydrate diet, L-type iso-
202 zyme mRNA levels of control rats were increased 33-fold
203 (2,706 ± 519 cpm/10 μg total RNA) compared to fasted

state (79 ± 20 cpm/10 μg total RNA), while those of uremic rats were increased 21-fold (1,871 ± 667 cpm/10 μg total RNA) compared to fasted state (83 ± 17 cpm/10 μg total RNA). There was significant negative correlation between serum creatinine concentrations and L-type isozyme mRNA levels when refed with high carbohydrate ($r = -.876$, $P < .005$) (Fig 7). These results suggest that the induction of L-type pyruvate kinase mRNA was decreased proportional to the severity of chronic renal failure. Figure 8 shows Northern analysis of L-type pyruvate kinase mRNA when refed with high carbohydrate. L-Type pyruvate kinase mRNAs isolated from rat liver with chronic renal failure and from control were identical in size, while were different in quantity.

L-type pyruvate kinase enzyme activity is stimulated by insulin and suppressed by glucagon and these hormones are considered to regulate the synthesis of L-type isozyme at the pretranslational level.[14] Plasma insulin levels of fasted state were 18.7 ± 4.7 μU/mL in chronic renal failure and 16.7 ± 2.6 μU/mL in control. Plasma insulin level was increased to about 60 μU/mL in both groups after high carbohydrate diet refeeding. There was no correlation between plasma insulin levels and L-type isozyme mRNA levels after high-carbohydrate diet refeeding (data not shown). Plasma glucagon levels of uremic rat at fasted state (103.7 ± 44.8 pg/mL) and at refed state (94.2 ± 46.3 pg/mL) were significantly higher than those of control (35.2 ± 15.5 pg/mL) and (30.4 ± 11.7 pg/mL), respectively. We observed a significantly negative correlation between plasma glucagon levels and L-type pyruvate kinase mRNA levels ($n = 9$, $r = -.886$, $P < .005$). As shown in Fig 9, plasma glucagon/insulin ratios were negatively correlated with L-type isozyme mRNA levels ($r = -.719$, $P < .05$).

DISCUSSION

Type-M_1 pyruvate kinase distributes in specially differentiated tissues, such as skeletal muscle, heart, and brain, and operates as a key enzyme in glycolysis to supply energy for these tissues.[4,5] It is well-known that the activity of type-M_1 pyruvate kinase is independent of acute hormonal regulation, such as insulin, glucagon, and cathecholamines. Moreover, M_1-type isozyme is not changed in chronic adaptation, such as starvation, high-carbohydrate diet, or diabetes mellitus.[4,5] However, the activity of M_1-type isozyme in chronic renal failure has not been studied yet. We demonstrate here that the activity of M_1-type pyruvate kinase or the level of this isozyme mRNA were not changed in uremia. (Figs 1–3). Thus, the activity and the synthesis of M_1-type pyruvate kinase remain to be intact even in the uremia.

In contrast to M_1-type pyruvate kinase, L-type isozyme exists in liver, kidney, and intestine, which have the ability of gluconeogenesis, and operate in the regulation of glycolysis and glyconeogenesis.[5] L-Type pyruvate kinase activity is markedly altered by acute hormonal stimuli and chronic adaptation. For example, a high carbohydrate diet increases the enzyme activity, while starvation decreases it.[11] As shown in Fig 4, the activities of L-type pyruvate kinase in uremia were significantly lower than those in control at both fasted and refed state. Moreover, the activities at refed state were higher than those at the fasted state in both control and uremic group. These changes in enzyme activity cannot result from phosphorylation or dephosphorylation of L-type isozyme, since the enzyme activity was assayed in the presence of fructose 1,6-bisphosphate.[15] However, some other modification of the enzyme could cause alteration in the enzyme activity without any change in the amount of enzyme protein. This possibility was tested by quantitative

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immunoprecipitation with fixed *Staphylococcus aureus* (Fig 5). The quantitative immunotitration reveals that the titration curves in control and uremia were superimposable. Thus, the alterations in the L-type isozyme activity described above reflect changes in the amount of immunoreactive enzyme protein. In other words, the effect of any inhibitors, such as uremic toxins, on the enzyme activity is negligible.

Since L-type isozyme activity is dependent on the amount of enzyme, the decrease in L-type isozyme activities could be primarily explained by changes in the level of translatable mRNA of this isozyme. As shown in Fig 6, we cannot find any significant difference in L-type isozyme mRNA level between control and uremia. However, mRNA at refed state in uremia has a tendency to decrease. More precisely, the accumulation of L-type isozyme mRNA after refeeding was decreased proportionally to the severity of chronic renal failure expressed by serum creatinine level (Fig 7). The discrepancy between L-type isozyme activity and mRNA level (Fig 4 and 6) could be explained as follows. There is a time lag between the induction of L-type isozyme mRNA and the consequent synthesis of the enzyme protein. The maximal protein synthesis needs 24 to 100 hours from its mRNA accumulation.[16] Moreover, the life span of mRNA is only a few hours, while that of the enzyme protein is considered to be 50 to 75 hours.[16] When the pool size of L-type isozyme is considerably large compared to the newly synthesized L-type isozyme, the difference in the enzyme activities at fasted state could still reflect the results at refed state. The translational efficiency of L-type isozyme mRNA remains to be elucidated, which is essential for further discussion. Lastly, the degradation rate of L-type isozyme in uremia may be accelerated.

Metabolic acidosis, which is commonly observed in uremia, may affect L-type pyruvate kinase activity. This is supported by the evidence that metabolic acidosis markedly increases phosphoenolpyruvate carboxykinase activity in the kidney.[17] However, there has been no report concerning the effect of metabolic acidosis on L-type pyruvate kinase activity, which should be of considerable interest from the pathophysiologic point of view.

Insulin may be the primary regulator of L-type isozyme, which increases the rate of transcription of L-type isozyme gene by stimulating synthesis of some unknown protein.[14] Thus, this hormonal disorder may affect the expression of L-type pyruvate kinase gene. In this study, we found that plasma insulin levels in control and uemic rats were much the same. After high-carbohydrate refeeding plasma insulin levels increased to the same extent in both groups. These findings may be limited to our uremic models in which we employed Sprague-Dawley rats younger than four-months-old. Klahr et al observed the same results (personal communication). The changes in L-type isozyme mRNA level in uremia (Fig 6) cannot be explained by the alternations of plasma insulin level, as far as we used the uremic model as described above.

On the other hand, glucagon inhibits accumulation of L-type isozyme mRNA caused by insulin at the level of transcription and caused by fructose at posttranscriptional step.[14] In uremia glucagon levels are markedly elevated because the kidney plays a major role in glucagon metabolism. It is demonstrated that of the various subunit of glucagon, plasma levels of inactive component (MW 9,000) are greatly elevated, whereas levels of the active component (MW 3,500) are mildly but significantly elevated.[18] We found that plasma glucagon level in uremic rats was significantly higher than that in control. We cannot identify

344 active/inactive components of glucagon since immunoreac-
345 tive glucagon measured by the radioimmunoassay included
346 both active and inactive component. However, the elevated
347 plasma glucagon level probably plays a role in accumulation
348 of L-type pyruvate kinase mRNA. This is supported by
349 evidence that both plasma glucagon levels and glucagon/
350 insulin ratios were negatively correlated with L-type isozyme
351 mRNA levels (Fig 9). Elevated plasma glucagon may sup-
352 press accumulation of L-type pyruvate kinase mRNA at two
353 steps. The first step of suppression occurred at transcrip-
354 tional level. We have observed that glucagon inhibited
355 accumulation of L-type pyruvate kinase mRNA caused by
356 insulin.[8] Vaulont et al[19] also reported that glucagon blocks
357 the transcription of the genes encoding L-type pyruvate
358 kinase. The second step is posttranscriptional level. Glucagon
359 inhibits accumulation of the L-type pyruvate kinase mRNA
360 caused by fructose, which acts as stabilizer of the nuclear
361 L-type isozyme mRNA precursors.[8] Thus, the imbalance of
362 insulin and glucagon levels observed in our uremic models
363 may affect accumulation of L-pyruvate kinase mRNA and
364 consequent synthesis of enzyme protein.
365 Hyperglucagonemia in uremia could also induce phos-
366 phorylation of L-type pyruvate kinase by cAMP-dependent
367 protein kinase. The phosphdylated enzyme is more sensitive
368 to proteolytic enzyme than the intact enzyme,[20] which may
369 explain our results.

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436 **Fig 1.** Activities of M_1-type pyruvate kinase of rat gastro-
437 cnemius muscle. *Uremic (U) and control (C) rats were fasted for 18*
438 hours or refed a high carbohydrate diet for 24 hours. Values are
439 means ± SD.

441 **Fig 2.** Effect of chronic renal failure on the levels of M_1-type
442 pyruvate kinase mRNA isolated from rat gastrocnemius muscle.
443 Total RNA was isolated from the muscle of uremic (U) and control
444 (C) rats fasted for 18 hours or refed a high-carbohydrate diet for
445 24 hours. Samples of 4 μg of each RNA were spotted onto the
446 same nitrocellulose filter in duplicate and analyzed by dot blot
447 hybridization using a nick-translated cDNA isolated from pM_2PK-
448 33.[7] Values are means ± SD. Abbreviation: M_1-PK, M_1-type pyru-
449 vate kinase.

451 **Fig 3.** Northern analysis of total cellular RNA isolated from
452 gastrocnemius muscle of rats. Total cellular RNA were isolated
453 *from the gastrocnemius muscle of uremic and control rats* at the
454 fasted and refed state. Five micrograms of total cellural RNA were
455 separated by electrophoresis on 0.8% agarose gel containing 2.2
456 mol/L formaldehyde. The RNA was then transferred to a nitrocel-
457 lulose filter and hybridized ^{32}P-labeled M_2-type pyruvate kinase
458 cDNA. Abbreviation: KB, kilobases.

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460 **Fig 4.** Activities of L-type pyruvate kinase of rat liver. Uremic
461 (U) and control (C) rats were fasted for 18 hours or refed a high
462 carbohydrate diet for 24 hours. Values are means ± SD.

464 **Fig 5.** Immunotitration with antibody of L-type pyruvate
465 kinase in liver extracts. Various amounts of liver extract were
466 *incubated at 20° C for 15 minutes with 250 mU of anti-*(L-type
467 pyruvate kinase) immunoglobulin and an excess (650 mU) of
468 anti-(M_1-type pyruvate kinase) immunoglobulin to neutralize the
469 *M_2-type activity completely. After addition of 25 μL of 10%*
470 *Staphylococcus aureus,* the mixtures were incubated further for
471 ten minutes at 2,000 × g. The resultant supernatants were
472 assayed for pyruvate kinase activity by 2,4 dinitrophenylhydrazone
473 method, except that anti-(M_1-type isozyme) antibody was omitted.
474 Control rats fasted for 18 hours (○) or refed high carbohydrate diet
475 for 24 hours (△), or uremic rats fasted (●) or refed (▲).

477 **Fig 6.** Levels of L-type pyruvate kinase mRNA in rat liver. Total
478 RNA was isolated from the liver of uremic (U) and control (C) rats
479 fasted for 18 hours or refed a high-carbohydrate diet for 24 hours.
480 Samples of 10 μg of each RNA were spotted onto the same
481 nitrocellulose filter in duplicate and analyzed cDNA insert from
482 *pLPK-1.6 Values are mean ± SD. Abbreviation:* L-PK, L-type
483 pyruvate kinase.

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485 **Fig 7.** *Relationship between serum creatinine* concentrations
486 and levels of L-type pyruvate kinase mRNA of rats after refed a
487 high-carbohydrate diet. RNA was isolated from liver of uremic (●)
488 and control (○) rats. Abbreviation: L-PK, L-type pyruvate kinase.

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490 **Fig 8.** Northern analysis of total cellural RNA isolated from
491 liver of rats. Total cellural RNA were isolated from the liver of
492 uremic and control rats after refed a high-carbohydrate diet. Five
493 micrograms of total cellular RNA were separated by electrophore-
494 sis ~~on 0.8% agarose gel containing~~ 2.2 mol/L formaldehyde. The

498 RNA was then transferred to a nitrocellulose filter and hybridized
499 with ^{32}P-labeled L-type pyruvate kinase cDNA probe. Abbreviation:
500 KB, kilobases.

502 *Fig 9. Relationship between plasma insulin levels and levels of*
503 L-type pyruvate kinase mRNA. RNA was isolated from liver of
504 uremic (●) and control (○) rats after high carbohydrate diet refed.
505 Abbreviation: L-PK, L-type pyruvate kinase.

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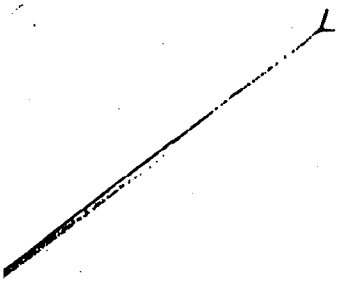

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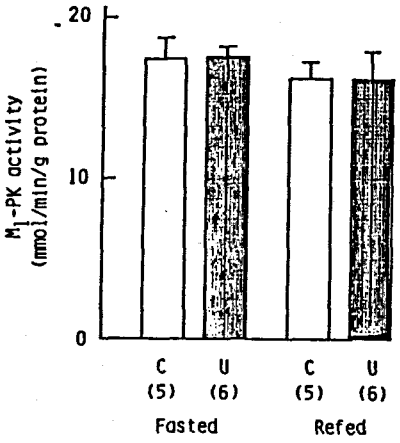
005 **Table 1. Body Weight, Serum Creatinine, and BUN**

006		n	Body Weight (g)	s-Cr (mg/dL)	BUN (mg/dL)
013	Fasted				
018	Control	5	379 ± 7	0.7 ± 0.1	13 ± 3
023	Uremic	6	337 ± 34*	1.8 ± 0.4*	57 ± 13*
028	Refed				
033	Control	5	405 ± 10	0.7 ± 0.1	8 ± 3
038	Uremic	6	346 ± 35*	2.3 ± 1.0*	67 ± 32*

043 Values are given as mean ± SD.
044 *$P < .01$ compared with respective control.

047 4145 430 28160 1MM:META307J09.98



JOB NAME: Metabolism ISSUE March 87
AUTHOR Imai

JOB NAME: META
JOB #: 7360-307-7 FOLDER #: H1/11
AUTHOR: IMAI FIG #: 1
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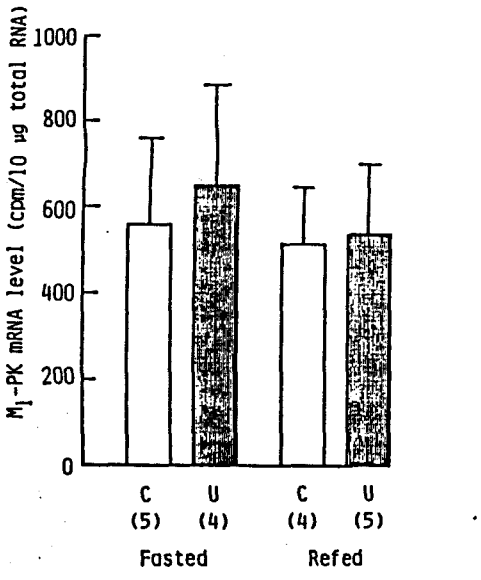

JOB NAME: Metabolism ISSUE March 87
AUTHOR Imai

JOB NAME: META
JOB #: 7360-307-7 FOLDER #: H1/11
AUTHOR: IMAI FIG #: 2
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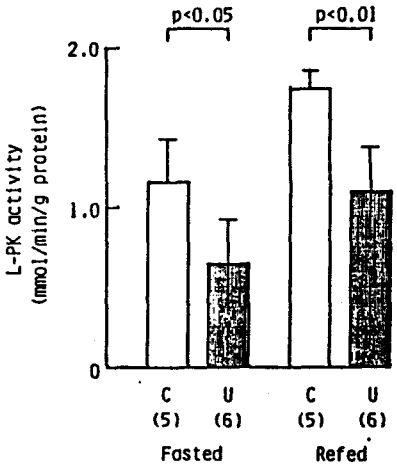

JOB NAME: Metabolism ISSUE March 87
AUTHOR Imai

JOB NAME: META
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AUTHOR: IMAI FIG #: 4
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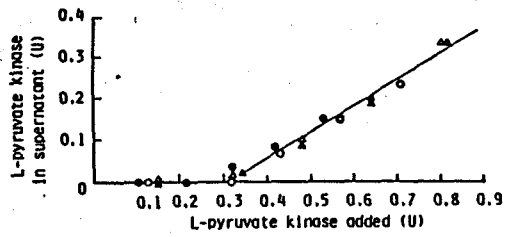

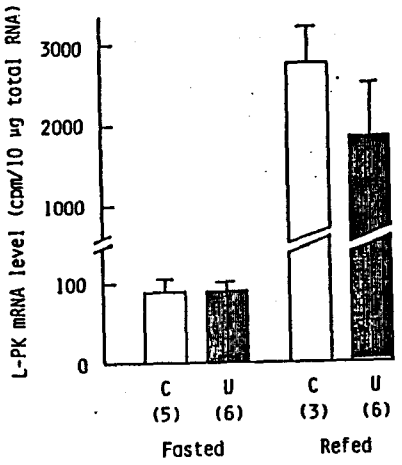

JOB NAME Metabolism ISSUE March 87
AUTHOR Imai

JOB NAME: META
JOB #: 7360-307-7 FOLDER #: H1/11
AUTHOR: IMAI FIG #: 5
56.5 % FINAL SIZE: 19 , 0 X 8 , 9

JOB NAME Metabolism ISSUE March 87
AUTHOR Imai

JOB NAME: META
JOB #: 7360-307-7 FOLDER #: H1/11
AUTHOR: IMAI FIG #: 6
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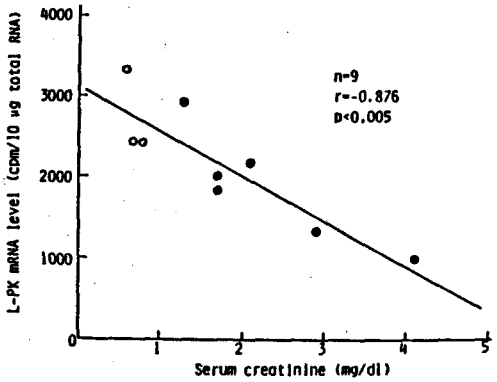

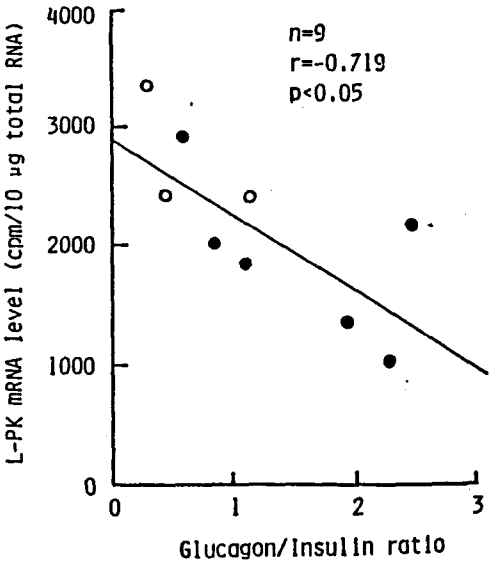

JOB NAME Metabolism ISSUE March 87
AUTHOR Imai

JOB NAME: META
JOB #: 7360-307-7
AUTHOR: IMAI FIG #: 7
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FOLDER #: H1/11

JOB NAME Metabolism ISSUE March 87
AUTHOR Imai

JOB NAME: META
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AUTHOR: IMAI FIG #: 9
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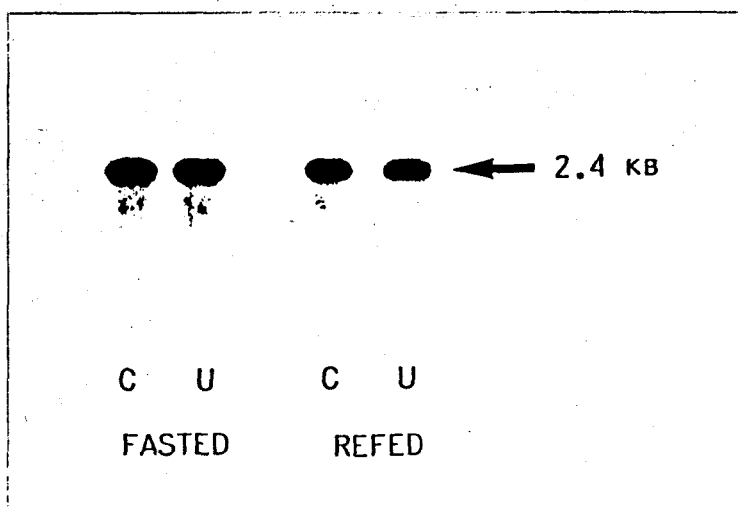

Fig 3

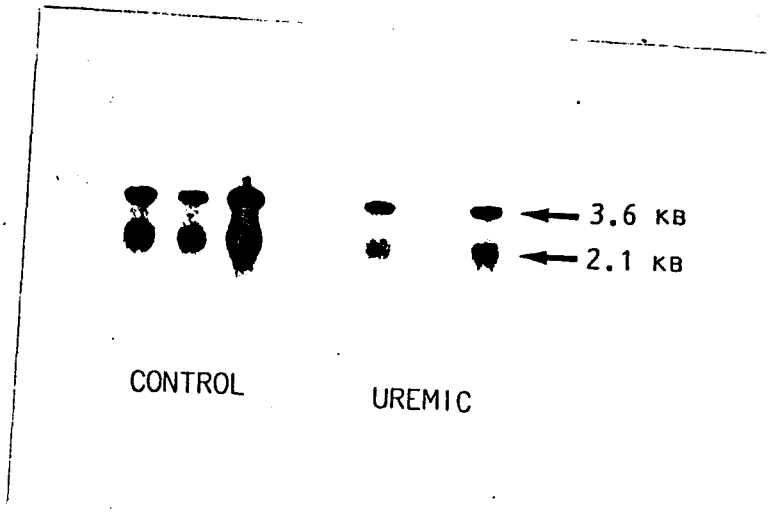

Fig 8

参考論文

Effects of a low protein diet and essential amino acid supplementation therapy on the progression of chronic renal failure

ENYU IMAI*, AKIO ANDO**, HIROSHI MIKAMI*, MASAMITSU FUJII***, AKIRA OKADA*, ATSUSHI YAMAUCHI*, YASUKO NAKAJIMA****, NAOHIKO UEDA*, YOSHIMASA ORITA*, and TAKENOBU KAMADA*

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Reprinted from

Japanese Journal of Nephrology Vol. 28, No.3, 1986

Effects of a low protein diet and essential amino acid supplementation therapy on the progression of chronic renal failure

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Key words: chronic renal failure, low protein diet, essential amino acid, serum creatinine

Abstract

The effects of a low protein diet and essential amino acid supplementation therapy on the rate of progression of chronic renal failure caused by chronic glomerulonephritis were examined retrospectively in 31 patients. A comparison of the rates of progression of renal failure in the low protein diet group with those in the non-low protein diet group was made using the slopes of regression lines between the reciprocal values of the serum creatinine and time. The mean slope of -5.4×10^{-3} dl/mg/month in the low protein diet group was significantly different from that of -9.9×10^{-3} dl/mg/month in the non-low protein diet group. In addition, the influence of the low protein diet therapy on the progression of chronic renal failure was examined in 4 patients who started on the low protein diet therapy in mid-course of the follow-up period. Three of them revealed significantly smaller values of serum creatinine during the low protein diet therapy than those predicted by extrapolation of their respective rates of progression before treatment. The effect of essential amino acid supplementation therapy on the rate of progression of chronic renal failure was studied in 2 patients who changed from a low protein diet to essential amino acid supplementation therapy. The creatinine concentrations under the essential amino acid supplementation therapy increased at a significantly slower rate than predicted during the low protein diet alone. These results suggest that the low protein diet therapy may have slowed the progression of chronic renal failure, and that the essential amino acid supplementation therapy could be more effective for retardation of the progress of chronic renal failure than the low protein diet alone.

Introduction

In most patients with chronic renal failure, the decline in renal function seems to occur at a constant rate. Their reciprocal values of serum creatinine concentration decrease linearly with time as renal failure progresses [1]. The slope of the regression line between the reciprocal of the serum creatinine concentration and time indicates the rate of progression of chronic renal failure: the slope could be changed by treatment [2, 3]. Observation of the reciprocal value of the serum creatinine makes it possible to estimate the rate of progression of chronic renal failure and the effects of therapy.

The protective effects of a low protein diet on the course of chronic renal failure have been con-

Received October 15, 1985

firmed in animal models [4. 5], and several groups have observed that restriction of dietary protein and/or phosphorus retarded the progress of chronic renal failure in humans [2, 3, 6–10]. A previous report [11], using the Markov process, demonstrated that essential amino acid supplementation therapy can retard the progress of chronic renal failure as compared to a low protein diet alone. In the present study, a retrospective analysis of patients with chronic renal failure was undertaken to determine how the progression of renal failure was retarded by a low protein diet or essential amino acid therapy, by contrasting the slope of the regression lines between the reciprocal values of the serum creatinine and time.

Methods

Patients

Two groups of patients with chronic renal failure caused by chronic glomerulonephritis were studied. The non-low protein diet group (protein intake above 0.7 g/kg/day) included 21 patients (15 males, 6 females) aged 23 to 76 (mean 45.9) years (**Table 1**). Their mean diastolic blood pressure was 91.1 (range 76.3 to 105.2) mmHg, and the mean duration of observation was 21.4 (range 8 to 45) months. The average daily urinary protein was 2.0 (0.5 to 3.5) g/day. The low protein diet group (protein intake less than 0.7 g/kg/day. The low protein diet group (protein intake less than 0.7 g/kg/day) included 14 patients (5 males, 9 females) aged 21 to 76 (mean 46.1) years (**Table 2**). Their mean diastolic blood pressure was 89.9 (range 76.0 to 110.1) mmHg, and the mean duration of observation was 21.3 (range 12 to 40) months. The average daily urinary protein was 1.6 (range 0.3 to 2.7) g/day. In mid-course of this follow-up period, 4 patients (Pt. 18, 19, 20 and 21) on the non-low protein diet thereapy started on the low protein diet and 2 patients (Pt. 25 and 26) changed from the low protein diet to essential amino acid supplementation therapy. Patients with hypocalcemia were given a supplementation

Table 1. Profile of chronic renal failure patients treated without a low protein diet

Pt. No.	Age	Sex	Initial s-Cr (mg/dl)	Duration (months)	Slope (dl/mg month)	MDBP (mmHg)	MUP (g/day)	MTNI (mg/kg/day)
1	45	M	2.8	23	-0.0075	80.8	1.9	133
2	70	M	2.4	38	-0.0089	83.7	0.9	132
3	23	F	2.5	17	-0.0178	87.1	3.4	137
4	38	M	2.2	8	-0.0386	104.7	3.5	114
5	27	M	3.6	13	-0.0165	89.2	3.5	113
6	62	F	5.5	23	-0.0044	98.8	1.9	156
7	36	M	2.7	21	-0.0063	88.7	2.7	122
8	33	F	3.1	10	-0.0130	82.6	1;0	173
9	66	M	5.2	21	-0.0047	94.5	0.9	133
10	39	F	3.4	36	-0.0012	91.6	0.5	139
11	48	M	2.7	33	-0.0053	89.4	2.2	154
12	67	M	3.1	24	-0.0066	88.7	0.9	145
13	43	M	2.8	45	-0.0060	96.1	2.3	146
14	40	M	5.7	26	-0.0036	94.2	3.1	142
15	34	M	4.2	18	-0.0070	95.1	2.0	134
16	31	F	3.0	15	-0.0167	92.9	0.9	159
17	71	M	5.2	14	-0.0081	76.3	1.1	143
18	41	M	3.0	26	-0.0057	97.3	2.7	123
19	42	F	2.8	16	-0.0094	97.1	2.2	133
20	51	M	3.5	9	-0.0104	105.2	3.5	200
21	75	M	3.3	14	-0.0092	80.0	0.8	151
Mean	46.8		3.5	21.4	-0.0099	91.1	2.0	142
±SD	± 15.6		± 1.0	± 9.9	±0.0079	±7.7	±1.0	±20

Abbreviations: MDBP, mean diastolic blood pressure; MUP, mean urinary protein; MTNI, mean total nitrogen intake.

Table 2. Profile of chronic renal failure patients treated with a low protein diet

Pt. No.	EAA	Age	Sex	Initial s-Cr (mg/dl)	Duration (months)	Slope (dl/mg month)	MDBP (mmHg)	MUP (g/day)	MTNI (mg/kg/day)
18	-	44	M	4.9	26	-0.0001	99.0	2.3	112
19	+	45	F	5.7	27	-0.0045	98.7	2.3	100
20	+	52	M	5.1	10	-0.0104	99.3	2.5	104
21	+	76	M	4.1	12	-0.0074	76.0	1.6	86
22	-	61	F	5.5	16	-0.0050	82.8	2.7	89
22	-	21	F	2.4	31	-0.0081	73.8	0.9	79
24	-	40	F	3.5	35	-0.0052	108.0	0.9	107
25	+/-	42	F	3.3	27	-0.0035	86.2	0.5	112
26	+/-	49	F	4.7	40	-0.0017	80.0	1.5	93
27	+	21	M	6.6	14	-0.0067	82.4	1.5	79
28	+	42	M	5.5	12	-0.0079	110.1	1.9	76
29	+	58	F	4.6	12	-0.0031	83.1	0.3	95
30	+	45	F	5.0	14	-0.0074	101.0	1.2	107
31	+	54	F	5.9	15	-0.0047	78.6	1.6	102
Mean ±SD		46.4 ±14.4		4.8** ±1.1	21.3 ±9.7	-0.0054* ±0.0027	89.9 ±12.3	1.6 ±0.7	96*** ±12

Abbreviations: EAA, essential amino acid therapy; MDBP, mean diastolic blood pressure; MUP, mean urinary protein; MTNT, mean total nitrogen intake. *$p<0.05$, **$p<0.01$, ***$p<0.001$, compared to chronic failure patients treated without a low protein diet.

of calcium lactate (1.5–3.0 g/day) and/or lα-OH vitamin D_3 (0.25-0.5 μg/day), and those with hyperphosphatemia were given aluminium hydroxide orally.

Diet control

The prescription for protein intake in the low protein group was 0.5-0.6 g/kg/day, and that in the non-low protein group was 0.8–1.0 kg/day. We placed both groups on the high calorie diet (more than 35 kcal/kg/day). The management of essential amino acid therapy was as reported previously [12]. Briefly, the low protein diet was supplemented with 8 essential amino acids plus histidine (Amiyu-G® 7.5 g/day), which further restricted the protein intake corresponding to the nitrogen intake from the amino acids. The dietary intake of the patients was reviewed once a month by a dietician and assessed at interviews as well as by dietary questionnaires. Using Maroni's method [13], the dietary protein intake was estimated on the basis of the urea nitrogen appearance.

Assessment of progression of chronic renal failure

The serum creatinine concentration was determined 6 or more times over an observation periode of more than 8 months in each case. We measured BUN, serum albumin, daily urinary excretion of creatinine and protein. The reciprocal value of the serum creatinine was plotted against time in each patient, and the data were analyzed by least-squares linear regression. All patients revealed significant regression ($p < 0.01$). Evaluation of the progression rate of chronic renal failure as a group was made from the slopes of these regression lines. For statistical comparisons of the slopes in these groups of patients, Wilcoxon's test was used.

In patients whose dietary management was changed, the effect of the low protein diet alone or essential amino acid thereapy on the progression of chronic renal failure was estimated from the change in the reciprocal of the serum creatinine concentration after the therapy. We compared the observed values of the reciprocal of the serum creatinine after the therapy with the predicted values obtained by extrapolation of

the line obtained during pretherapy. The paired t-test was employed for statistical analysis.

Results

1) Comparison between groups (Table 1 and 2; Fig. 1)

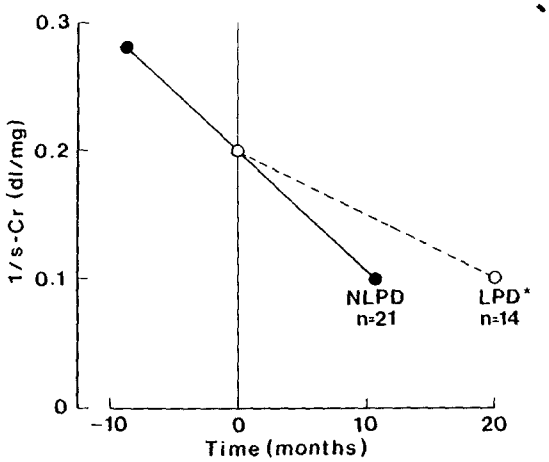

Fig. 1. Changes in slope of the reciprocal of the serum creatinine against time in patients with chronic renal failure who were treated with LPD (o- - -o: -5.4x10-3 dl/mg/month) and with NLPD (●———●: slope, -9.9 x 10^{-3} dl/mg/month). The asterisk denotes $p<0.05$ for a comparison of the slopes between LPD and NLPD. LPD, low protein diet; NLPD, non-low protein diet.

During the follow-up period averaging 21 months, the mean slope obtained in the regression analysis of the reciprocal creatinine against time was -5.4×10^{-3} dl/mg/month in the low protain diet group (mean nitrogen intake 96 mgN/kg/day) and -9.9×10^{-3} dl/mg/month in the non-low protein diet group (mean nitrogen intake 142 mgN/kg/day). The difference between the two groups was statistically significant ($p < 0.05$). If started on the low protein diet therapy at a level of serum creatinine of 4.7 mg/dl, patients treated with the low protein diet had 9.1 more months before they reached a level of serum creatinine of 10 mg/dl, referred to as "renal death" (**Fig. 1**).

2) Effects of low protein dietary therapy compared to non-low protein diet in individual case (Table 3; Fig. 2)

Pt. 18 started on the low protein diet alone at the point of 6.0 mg/dl serum creatinine. The slope of the regression line increased from -5.7×10^{-3} dl/mg/month to -0.1×10^{-3} dl/mg/month after the therapy and the reciprocal value of the serum creatinine during the therapy was significantly higher ($P<0.001$) than that predicated from the patient's prior rate of progression. Pt. 19, 20, and 21 started on the essential amino acid supplementation therapy at a level of serum creatinine of 4.9, 5.2, and 5.8 mg/dl, respectively. The slope of the re-

Table 3. Effects of a low protein diet and essential amino acid therapy on the progress of chronic renal failure

Pt. No.	EAA	Slope before treatment (10^3xdl/mg/month)	s-Cr at start of treatment (mg/dl)	Slope after treatment (10^3xdl/mg/month)	Last s-Cr (mg/dl)	p value#	Outcome
18	-	-5.7	5.5	-0.1	6.8	<0.001	treatment continued
19	+	-9.4	4.9	-4.5	11.5	<0.001	hemodialysis
20	+	-10.4	5.2	-10.4	12.8	ns	hemodialysis
21	+	-9.2	5.8	-7.4	9.1	<0.001	hemodialysis

#p values, calculated using Student's t-test, show that the reciprocal of the serum creatinine concentration during treatment was lower or higher than that predicted from the previous rate of progression.
Abbreviations: EAA, essential amino acid therapy; ns, not significant.

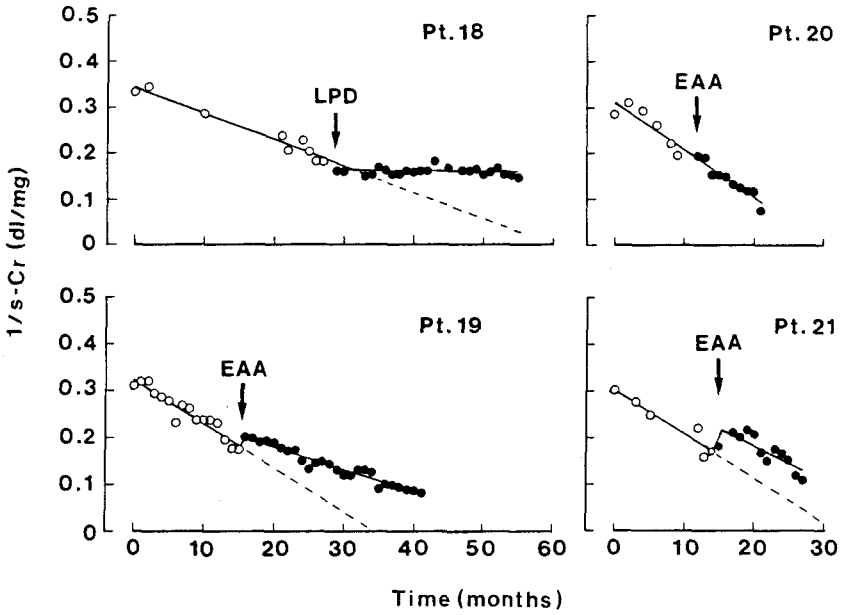

Fig. 2. Effects of a low protein diet on the progression of chronic renal failure. The reciprocal values of the creatinine concentration before treatment are represented by open circles (○) and those during the low protein diet therapy by solid circles (●). The regression line which fitted the pretherapy values is shown as a solid line and its extrapolation during therapy is indicated by the broken line.

gression line in Pt. 19 increased from -9.4 x 10^{-3} to -4.5 x 10^{-3} dl/mg/month, and that in Pt. 21 from -9.2 x 10^{-3} to -7.4 x 10^{-3} dl/mg/month. The reciprocal values of the serum creatine in Pt. 19 and 21 after therapy were significantly higher than those predicted on the basis of the pretreatment data ($p<0.001$). However, in Pt. 20, the essential amino acid supplementation therapy exerted no effect on the progression of chronic renal failure. In 3 of the patients (Pt. 18, 19 and 21), the serum concentrations of uric acid during the low protein diet were significantly decreased compared to those in the non-low protein diet. The CaxP products of these patients were not changed significantly.

3) Effects of essential amino acid supplementation therapy compared to low protein diet alone in individual cases (Table 4; Fig. 3)

Pt. 25 and 26 changed from the low protein diet alone to the essential amino acid therapy at a level of serum creatinine of 6.0 and 5.2 mg/dl, respectively. Their average total nitrogen intake was unchanged after therapy (Pt. 25, 111 to 112 mgN/kg/day, Pt. 26, 86 to 89 mgN/kg/day) and their blood pressure was unchanged. In Pt. 25, the slope of the regression line increased from -9.7 x 10^{-3} to -4.1 x 10^{-3} dl/mg/month, and the reciprocal value of the serum creatinine during the essential amino acid supplementation therapy was significantly higher ($p<0.001$) than that predicated from the patient's prior rate of progression. In Pt. 26, the slope increased form -4.7 x 10^{-3} to -0.8 x 10^{-3} dl/mg/month, and the reciprocal value of the serum creatinine during the essential amino acid supplementation thereapy was significantly higher than that predicted from the low protein diet alon ($P<0.001$). In these patients, the body weight and serum albumin concentration before and after therapy demonstrated only slight changes. In Pt. 25, the serum CaxP products and uric acid were significantly decreased after the essential amino acid supplementation therapy, whereas Pt. 26 showed no change.

Table 4. Effects of essential amino acid therapy on the progression of chronic renal failure in patients treated with a low protein diet

Pt. No.	MTNI pre (mgN/kg/day)	MTNI post (mgN/kg/day)	Slope during LPD (10^3xdl/mg/month)	s-Cr at start of EAA (mg/dl)	Slope after EAA (10^3 xdl/mg/month)	Last s-Cr (mg/dl)	p value#	Outcome
25	111	112	-9.7	5.2	-4.1	10.5	<0.001	hemodialysis
26	86	89	-4.7	7.0	-0.8	6.6	<0.001	treatment continued

values, calculated using Student's t-test, show the probability that the reciprocal of the serum creatinine concentration during essential amino acid therapy was lower or higher than that predicted from the previous rate of progression.
Abbreviations: MTNI, mean total nitrogen intake; EAA, essential amino acid therapy; LPD, lower protein diet.

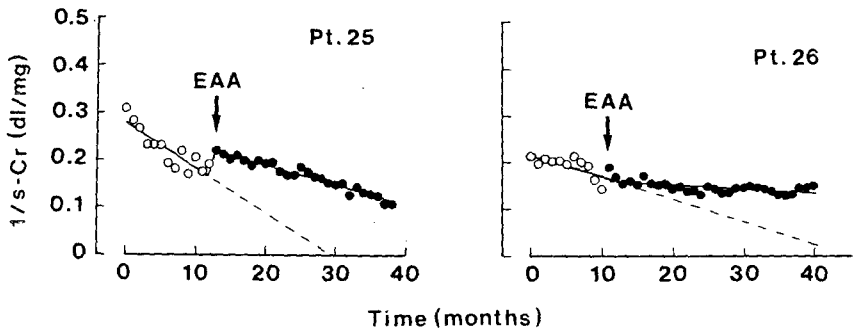

Fig. 3. Effect of essential amino acid thereapy on the progression of chronic renal failure in patients treated with a low protein diet. The reciprocal values of the serum creatinine before treatment are represented by open circles (○) and those during the essential amino acid therapy by solid circles (●). The regression line which fitted the pretherapy values is shown as a solid line and its extrapolation during is indicated by the broken line.

Discussion

Essential amino acid thereapy is advantageous for patients with chronic renal failure because of improvement of nitrogen metabolism and nutritional state as well as elimination of uremic symptoms. Giordano [14], Bergström [15] and our research group [12] have found that essenal amino acid supplementation therapy showes better improvement of nitrogen balance and aminograms in the plasma than a low protein diet alone. Furthermore, it has been demonstrated that the serum concentration and the urinary excretion of methylguanidine and guanidinosuccinic acid, assumed to be uremic toxins, were remarkably diminished during treatment with essential amino acid supplementation therapy compared to a low protein diet alone [12]. Reduction of the serum methylguanidine and guanidinosuccinic acid appears to contribute to the maintenance of patients with chronic renal failure in a favorable state.

It has been reported previously that the expected progression to end-stage renal failure, calculated by the Markov process, was considerably slower in patients with essential amino acid supplementation therapy than in those receiving a low protein diet alone [11]. The Markov process based on relatively short-term observations might be useful for evaluating the progression of the disease. We can calculate the probability of progression of chronic renal failure in a mass number of patients by the Markov process. As reported by Mitch et al. [1],

analysis of the reciprocal serum creatinine and time can accurately determine the rate of progression of chronic renal failure. Especially, in individual cases, the analysis of this relationship relies on the predictability of the course of the chronic renal failure and can help to evaluate the effects of therapy.

Recently, hemodynamic renal injury, also referred to as glomerular hyperfiltration and hyperpressure, and the deposition of several substances in the renal parenchyma have been found to be involved in the mechanism of progression of renal disease. Hostetter et al. [16] showed in rats with experimental renal failure that the increase in glomerular capillary blood flow and pressure caused by a reduction of the renal mass leads to glomerular damage; however, these changes can be ameliorated by restriction of dietary protein. If the remnant nephrons are injured hemodynamically, the load of amino acids, such as glycine, arginine, and alanine, which increase the glomerular filtration rate, may advance the renal injury. Johannsen et al. [17] and Tank et al. [18] demonstrated in anesthesized dogs that intravenous administration of glycine and alanine produced an increase in glomerular filtration rate and renal blood flow, respectively. Gollaher et al. [19] found that intravenous arginine infusion caused a significant increase in glomerular filtration rate in normal rats. The effects of a low protein diet and essential amino acid supplementation therapy may be based patially on decreasing the load of these amino acids. However, the amino acid concentrations in fasting plasma from the patients showed little change between before and after low protein diet therapy. It remains to be determined therefore whether the decreased load of these amino acids affects the hemodynamics of the glomeruli or not.

In addition, a low protein diet and essential amino acid supplementation therapy may contribute to prevention of the decline of renal function, reducing or removing deposits of harmful substances such as phosphate, uric acid, etc. Ivels et al. [20], employing a remnant kidney model in rats, reported that the calcification of the parenchyma produced by the altered phosphorous metabolism present in the uremic state incites an inflammatory and fibrotic reaction leading to destruction of the remnant kidney. Urate is another possible substance which may precipitate in the kidney with reduced function. Chronic hyperuricemia is well known to impair renal function, and it is quite likely that severe hyperuricemia due to renal failure exerts the same effect [21]. The finding that the serum CaxP products and uric acid levels were significantly reduced in some of our patients treated with a low protein diet and essential amino acid supplementation therapy appears to support this hypothesis.

It has been demonstrated retrospectively in the present study that a low protein diet and essential amino acid supplementation therapy retarded the progression of chronic renal failure caused by glomerulonephritis, and that the essential amino acid therapy was more effective in such retardation than the low protein diet alone.

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Reprint from

clinical nephrology

An International Journal

Dustri-Verlag Dr. Karl Feistle
München-Deisenhofen

Clinical Nephrology, Vol. 25 No. 4 – 1986 (pp. 181–185)

Plasma concentration and peritoneal clearance of oxalate in patients on continuous ambulatory peritoneal dialysis (CAPD)

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Abstract. Accumulation of oxalate, resulting in high plasma levels, is a common finding in end-stage renal disease. We investigated plasma concentration and peritoneal clearance of oxalate in 14 patients on continuous ambulatory peritoneal dialysis. The plasma oxalate levels in these patients (30.2 ± 11.2 μmol/l) were as high as those in hemodialysis patients before dialysis (31.9 ± 11.1 μmol/l). There was a significant correlation between plasma oxalate and urea nitrogen appearance (UNA). Dietary protein seems to be an important oxalate source in these patients, because the UNA reflects protein intake in stable patients. The mean peritoneal oxalate clearance was 6.64 ± 1.56 l/day, close to the creatinine clearance. These results suggest that the plasma oxalate levels in CAPD patients may be sufficiently high to induce calcium oxalate deposition, and that methods of increasing oxalate removal and reducing oxalate burden are necessary for CAPD patients.

Key words: CAPD – oxalate – urea nitrogen appearance – peritoneal clearance

Introduction

The secondary oxalosis of renal failure has been recognized for more than 20 years, but the details of its prevalence and clinical features have only recently been addressed. There is evidence that the incidence and severity of oxalate deposits are related to the duration of renal failure or to the duration of hemodialysis therapy [Fayemi et al. 1979, Salyer and Keren 1973]. The most frequently involved sites of calcium oxalate deposition include the kidney, the myocardium, and the blood vessels, although deposits have also been noted in various other organs. Tissue accumulation is often asymptomatic, but recent reports suggest that patients with renal failure may develop clinically apparent diseases related to organ deposits of calcium oxalate cystals [Hoffman et al. 1982, op de Hoek et al. 1980, Salyer and Hutchins 1974].

Received April 24, 1985, in revised form September 12, 1985.
Reprint requests to Dr. A. Yamauchi.

Oren et al. [1984] demonstrated that CAPD patients had an unusually high incidence of calcium oxalate kidney stone formation and urine ionic-oxalate concentrations in these patients were significantly higher than in normal subjects. This report suggested secondary oxalosis also occurred in CAPD patients. However, plasma concentration and peritoneal clearance of oxalate have not been reported in peritoneal dialysis except in three patients with primary hyperoxaluria [Watts et al. 1984, Zarembski et al. 1969].

The present study examines the plasma oxalate levels and peritoneal oxalate clearances in patients on continuous ambulatory peritoneal dialysis.

Patients and methods

This study included 14 patients (7 males, 7 females) undergoing CAPD. The duration of dialysis prior to the study varied from 3 months to 36 months of peritoneal dialysis, with an average of 17.4 months. Their mean age was 41.7 years (13–75 years). Before the initiation of CAPD, 5 of the 14 patients had been

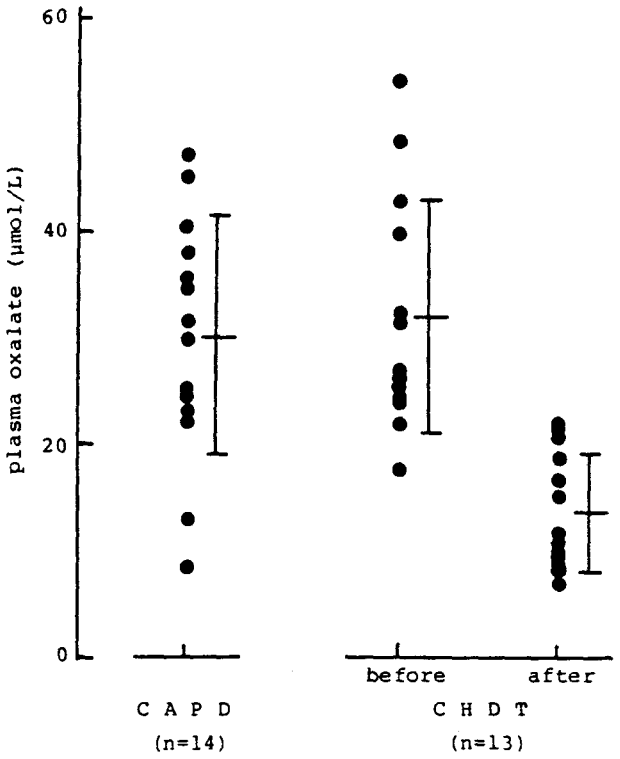

Fig. 1 Plasma oxalate levels in patients on CAPD and CHDT (mean ± SD).

on hemodialysis (mean 18.6 months). In 9 patients, CAPD was the first treatment of end-stage renal disease. All patients were in stable nutritional status, and did not experience any infections or other major complications.

A surgical inserted Tenckhoff catheter and 2 liters of commercially available dialysis solution (Dianeal®) were used. This solution contains: Na 132 mEq/l, Cl 102 mEq/l, Mg 1.5 mEq/l, Ca 3.5 mEq/l, lactate 35 mEq/l, and dextrose 1.5 or 4.25 g%.

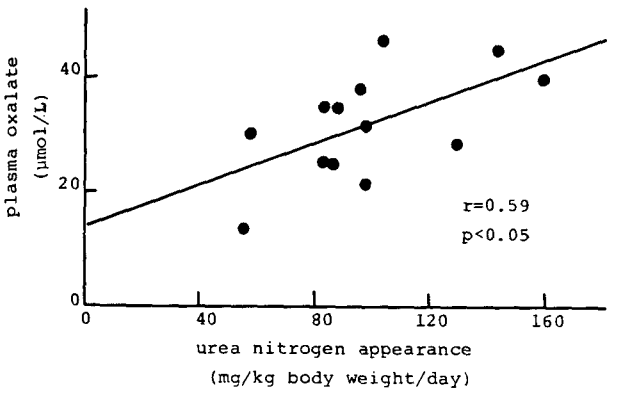

Fig. 2 Correlation between plasma oxalate and urea nitrogen appearance in CAPD patients (n = 13).

Five of the 14 patients exchanged dialysis solution three times per day (dwell time 6–11 hours), and the others four times per day (dwell time 3–8 hours).

Dialysate levels of urea, creatinine, urate, and oxalate were determined in all samples obtained from the three or four exchanges a day. Plasma samples were obtained at the end of the clearance study.

In stable CAPD patients, urea nitrogen appearance (UNA) can be determined as the sum of the urine and dialysate urea nitrogen [Blumenkranz and Schmidt 1981]. UNA was calculated as the sum of dialysate urea nitrogen per day in 13 stable patients whose residual kidney function was almost zero (urine volume <50 ml/day).

Peritoneal clearances (l/day) of these solutes were calculated as ratio of the sum of dialysate solute per day to plasma concentration.

Plasma and peritoneal dialysate levels of oxalate were determined by spectrophotometric method described by Kohlbecker and Butz [1981]. In 5 normal volunteers plasma oxalate levels were all below 5 μmol/l.

Plasma oxalate concentrations were also assayed in 13 patients on chronic hemodialysis treatment (CHDT). Hemodialyses were performed 3 times a week, 5 to 6 hours each time.

None of the patients were receiving supplementary ascorbate nor suffering from primary hyperoxaluria.

Results

Figure 1 shows the plasma oxalate levels in patients on CAPD and CHDT. In CAPD patients the plasma oxalate levels ranged from 8.6 to 47.3 μmol/l (n = 14, mean 30.2 ± 11.2 μmol/l). In hemodialysis patients, predialysis plasma oxalate levels ranged between 17.8 and 54.3 μmol/l (n = 13, mean 31.9 ± 11.1 μmol/l) and were reduced after dialysis to 43% of predialysis values ($p < 0.001$), ranging from 6.8 to 21.4 μmol/l (mean 13.6 ± 5.5 μmol/l). The plasma oxalate levels in CAPD patients were not significantly different from those in CHDT patients before dialysis. There was no correlation between plasma oxalate and plasma creatinine, urea, or urate, in either CAPD patients or CHDT patients before dialysis.

Figure 2 shows the relationship between plasma oxalate and UNA (mg/kg body weight/day): there was a significant correlation ($r = 0.59$, $p < 0.05$). UNA was also correlated with plasma urea nitrogen ($r = 0.83$, $p < 0.01$), but not with plasma creatinine and urate.

Table 1 shows the peritoneal clearances of urea, oxalate, creatinine and urate in CAPD patients with three or four daily exchanges. The clearance values for

each solute with four daily exchanges were always significantly higher than those with three daily exchanges. The relative ordering of clearance values for these solutes was nearly the same for both three and four daily exchange groups: the clearance values for urea were significantly higher than those for the other solutes, and the clearance values for oxalate were close to those for creatinine, while these tended to be higher, but not significantly so, than those for urate. The plasma level of oxalate was not correlated with its clearance value.

Table 1 Peritoneal clearances of urea, oxalate, creatinine, and urate. (mean ± SD)

	Three daily exchanges (n = 5)	Four daily exchanges (n = 9)	All patients (n = 14)
Curea	7.06 ± 0.68	8.93 ± 1.42[a]	8.26 ± 1.50
Cox	5.39 ± 0.66[e]	7.33 ± 1.50[ad]	6.64 ± 1.56[d]
Ccr	5.49 ± 0.70[e]	7.08 ± 0.83[be]	6.51 ± 1.09[e]
Curate	4.87 ± 0.50[f]	6.87 ± 0.86[cf]	6.15 ± 1.23[f]

Curea = urea clearance, Cox = oxalate clearance, Ccr = creatinine clearance, Curate = urate clearance (l/day); [a] $p < 0.05$ vs. three daily exchanges, [b] $p < 0.01$ vs three daily exchanges, [c] $p < 0.001$ vs. three daily exchanges, [d] $p < 0.05$ vs. Curea, [e] $p < 0.01$ vs. Curea, [f] $p < 0.001$ vs. Curea.

Discussion

In normal subjects, most urinary oxalate is derived from endogenous sources because oxalate absorption from the gastrointestinal tract is quite small [Williams 1978]. The two major sources of oxalate are glyoxylate and ascorbate.

The most important precursor of oxalate is glyoxylate, and the main precursor of glyoxylate seems to be glycine. Other amino acids such as serine and hydroxyproline are also discussed as precursors of oxalate in man. Ribaya and Gershoff [1982] described that the urine of rats fed diets supplemented with 3% glycine and 5.2% hydroxyproline contained unexpectedly high amounts of endogenously formed oxalate. Robertson et al. [1979] reported that a dietary increase of 34 g/day of animal protein significantly increased urinary oxalate in man.

We found high plasma levels of oxalate in CAPD patients , although no patient was treated with ascorbate. Further, there was a significant correlation between the plasma oxalate and the UNA. Because UNA reflects protein intake in stable patients [Blumenkranz and Schmidt 1981], dietary protein seems to be one of the most important sources of oxalate in CAPD patients without supplementary ascorbate.

Ascorbate is an established oxalate precursor and may account for about 30% of daily urinary oxalate excretion in normal subjects [Baker et al. 1966]. Ascorbate in large doses (>5 g/day) has been reported to increase urinary excretion of oxalate [Briggs et al. 1973, Schmidt et al. 1981]. Recently, Pru et al. [1985] reported that serum oxalate levels before hemodialysis showed a good correlation to the levels of ascorbate in hemodialysis patients supplemented with ascorbate.

It is known that there are several other precursors of oxalate. For example, some cases of acute renal failure secondary to hyperoxaluria and resultant intrarenal oxalate deposition have been reported following the use of methoxyflurane and ethylene glycol [Frascino et al. 1970, Parry and Wallach 1974]. Secondary oxalosis has been observed in a patient given large intravenous doses of xylitol [Ludwig et al. 1984]. Recently, Ribaya-Mercado and Gershoff [1984] established that the intake of galactose resulted in greater excretion of endogenously formed oxalate in rats. It is probable that an excessive intake of these precursors increases plasma oxalate and induces severe oxalate deposition in uremic patients.

It is well known that pyridoxine deficiency leads to hyperoxaluria in both human and experimental animals [Gershoff 1964, Ribaya and Gershoff 1982, Ribaya-Mercado and Gershoff 1984]. Blumberg et al. [1983] claimed that the plasma and erythrocyte vitamin B6 levels in some patients on CAPD were lower than normal. The administration of vitamin B6 may be effective against hyperoxalemia in these patients [Balcke et al. 1982].

Peritoneal clearance studies have been reported in only three patients with primary hyperoxaluria. Zarembski et al. [1969] described the peritoneal clearance of oxalate averaged 5 to 6 ml/min in a patient with primary hyperoxaluria (dwell time 30 min). Watts et al. [1984] reported that the oxalate clearance values ranged from 4.8 to 5.9 ml/min in two patients on CAPD with four cycles of 1 or 2 liters of dialysis fluid daily.

We found that oxalate clearances in CAPD patients without primary hyperoxaluria were almost equal to those with primary hyperoxaluria. Oxalate clearances were close to creatinine clearances. It is well known that peritoneal permeability decreases as the solute molecular weight increases. Because oxalate is not protein-bound and is freely dialyzable [Williams 1978], it is reasonable that the oxalate clearance was closer to the creatinine (MW 113) clearance rather than the urea (MW 60) or urate (MW 168) clearance, considering its molecular weight (MW 90).

As dialysate flow rate increases, peritoneal clearances of these small solutes increase rapidly in CAPD [Robson et al. 1978]. In the present study, the

mean clearances of oxalate, urea, creatinine and urate with four daily exchanges were 125–140% of those with three daily exchanges. There seems to be a linear increase of clearance with flow rates for these solutes, although patient characteristics influence clearances [Rubin et al. 1981]. Increasing dialysate flow rate is very effective for oxalate removal in CAPD.

The secondary oxalosis of renal failure is believed to be the results of chronic elevation of the plasma concentration of oxalate [Thompson and Weiman 1984], but it is not known with certainty if there is a critical plasma concentration at which precipitation and tissue deposition occurs. Constable et al. [1979] have demonstrated that in chronic renal failure without primary hyperoxaluria, the concentration of plasma oxalate may be sufficiently high to induce calcium oxalate deposition. Recently, Worcester et al. [1985] suggested that uremic serum, even after hemodialysis, was supersaturated with respect to calcium oxalate. We found high plasma levels of oxalate in CAPD patients, although no patient received supplementary ascorbate. Therefore, CAPD patients also are likely to develope severe organ deposits of calcium oxalate crystals.

It is possible that oxalate deposits in patients with end-stage renal disease are an important contributing factor to morbidity and mortality. Cardiovascular complications in particular have caused the largest proportion of deaths in CHDT and CAPD patients [Broyer et al. 1982, Wu et al. 1983]. Oxalate deposition in the heart and the blood vessel walls may contribute to these complications. It is known that CAPD is not as efficient as hemodialysis in terms of small-solute clearances per week, although CAPD is more efficient for larger solutes such as vitamin B12 and inulin [Popovich et al. 1978]. For CAPD patients, methods of increasing oxalate removal (e. g., increase of dialysate exchange rate, augmentation of blood flow, enhancement of peritoneal permeability) and reducing oxalate burden (e. g., low intake of oxalate precursors, vitamin B6 supplementation) seem to be necessary, in view of the results that plasma levels of oxalate in these patients were as high as those in CHDT patients before dialysis.

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