PROTEIN RESTRICTION IN CHILDREN WITH CHRONIC RENAL FAILURE

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PROTEIN RESTRICTION IN CHILDREN WITH CHRONIC RENAL FAILURE

Een eiwitbeperkt dieet bij kinderen met chronische nierinsufficiëntie

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR AAN DE ERASMUS UNIVERSITEIT VAN ROTTERDAM OP GEZAG VAN DE RECTOR MAGNIFICUS PROF. DR. P.W.C. AKKERMANS
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Voor Boetsie, PappaHan en Frits

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Chapter 1

Introduction

1.1 Prologue

The progressive nature of renal functional impairment has been recognized for a long time (Mitch 1976, Rutherford 1977). Once glomerular filtration rate has decreased to 25 ml/min/1.73m^2 progression to end stage renal disease is inevitable and independent of the primary renal disease (Leumann 1978, Arbus 1981, Warshaw 1982, Warshaw 1985, Claris-Appiani 1986, Fine 1991, Norwick 1991).

Based on the assumption that renal functional deterioration is related to renal work-load, Addis suggested in 1948 to decrease protein intake in patients with chronic renal failure (Addis 1917, Addis 1948). His aim was not to reduce uremic symptoms (fatigue, thirst, stunted growth, itching) but rather to prevent an increase in the "workload" of surviving nephrons of diseased kidneys in order to prevent further loss of renal function. This concept was supported by the early finding in rats that renal mass increases with long-term feeding of protein (MacKay 1928).

This interesting idea lead to widespread advocation of protein restricted diets for children with chronic renal failure. Notwithstanding theoretical ideas and evidence from animal studies there are no prospective randomized controlled studies in children.

1.2 Hyperfiltration theory and other theories

In 1982 Brenner advanced a hypothesis to account for the progressive deterioration in kidney function seen in chronic renal failure: the hyperfiltration theory, figure 1 (Brenner 1982). Hyperfiltration is defined as a raised transcapillary pressure and flow. This can be measured by micropuncture of individual nephrons. His hypothesis was supported by numerous animal studies documenting premature death from renal failure in partially nephrectomized rats (Kleinknecht 1979, Brenner 1982, El Nahas 1983, Brenner 1985, Provoost 1989, Provoost 1990). Ultrastructurally, fusion of the epithelial cell foot processes followed by focal glomerular basement membrane denudation and progressive mesangial expansion occurs and ultimately leads to glomerular sclerosis and death of the glomerulus (Shimamura 1975).

The underlying mechanisms that result in glomerular sclerosis are poorly understood.

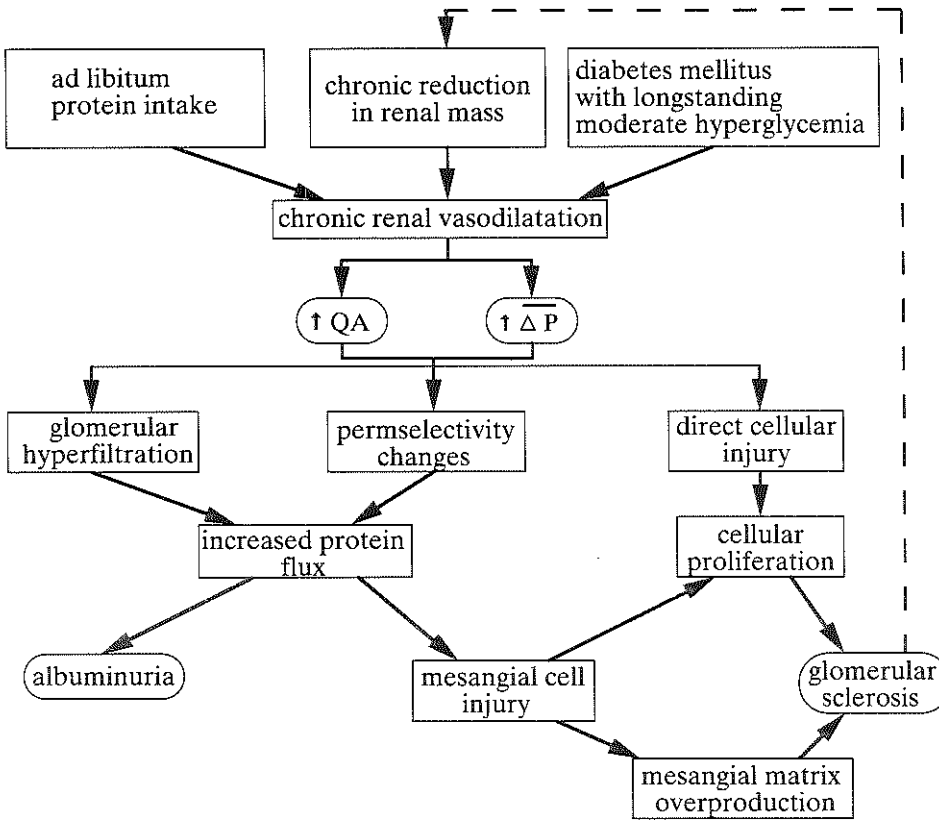

Figure 1. The role of sustained increments in glomerular pressures (Δp) and flows (QA) in the initiation and progression of glomerular sclerosis. Reproduced with permission from Brenner BM, Meyer TW and Hostetter TH, N Engl J Med 1982; 11: 652-659.

Suggested are not only hemodynamical changes in the glomerulus but also increased glomerular metabolism (Fogo 1990), local hypercoagulobility (Klahr 1986), mesangial macromolecular deposition (Grond 1985) and hyperlipedemia (Fogo 1990). In a model proposed by Fogo and Ichikawa (Fogo 1990), all the factors above, either individually or in concert with others, stimulate the production/release of glomerular growth promoters, which in turn produce glomerular hypertrophy and mesangial matrix production and accumulation causing glomerular sclerosis, figure 2. Numerous glomerular growth promoting factors have been suggested, including platelet-derived growth factor (Schultz 1988), interleukin 1 (Lovett 1983), angiotensine II (Homma 1990),

epidermal growth factor (Tsivitse 1987) and endothelin (Simonson 1989).
Furthermore Nath suggests that the loss of renal mass evokes increased oxygen consumption in surviving nephrons, thereby increasing the oxidant stress in the remnant nephrons (Nath 1987, Nath 1988).

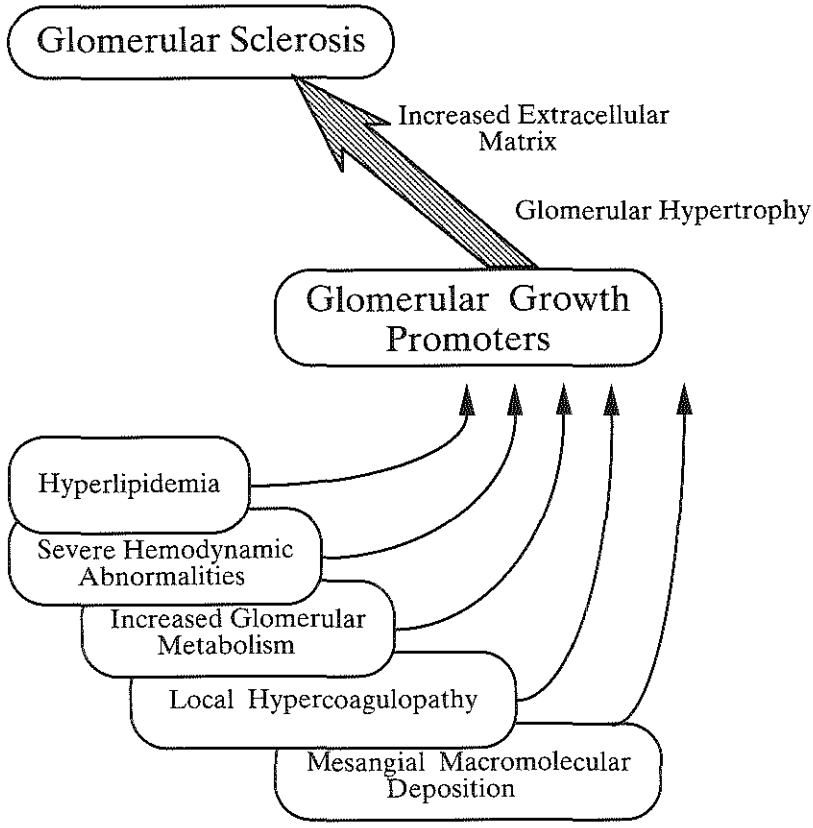

Figure 2. Speculation on the relationship between glomerular growth promoters and other potentially pathogenic intermediary mechanisms for glomerular sclerosis. Currently available experimental observations in renal and non-renal tissues suggest the possibility that these and other mechanisms may affect the kidney through glomerular growth promoters, which have capacity to induce both glomerular hypertrophy and mesangial matrix accumulation, leading to the typical glomerular sclerotic lesion. Reproduced with permission from Fogo A and Ichikawa I. Glomerular growth promotes the common channel to glomerular sclerosis. J of Nephrology 1990; 3: 213.

1.3 Evidence from animal studies

1.3.1 Short term effects of protein intake

Ingestion of individual protein meals has an acute and transient effect on the renal perfusion and filtration rates. In harbour seals renal blood flow and glomerular filtration rates rise by as much as 150 % above baseline after a meal of fish (Hiatt 1942). Increases of renal blood flow and glomerular filtration rate as much as 40 - 100 % have been observed in dogs fed single meals of meat (Shannon 1932, Reinhardt 1975, O'Connor 1976). The promptness and completeness of the kidney's response to protein feeding reaches its extreme in the vampire bat, which can consume half its weight in blood during a single meal. Rates of water and total solute excretion in these animals rise by several hundred per cent within two or three hours of eating (McFarland 1969).

1.3.2 Long term effects of protein intake

Many studies show a beneficial effect of a protein restricted diet (12 %) as compared to a high protein diet (36 %) on the survival and the progression of renal failure in subtotally nephrectomized rats (Kleinknecht 1979, Brenner 1982, El Nahas 1983, Brenner 1985, Provoost 1989, Provoost 1990). Figure 3 shows that rats after unilateral nephrectomy progress faster to end stage renal disease and death compared to sham operated two kidney rats, and that progression of renal failure can be attenuated by a low protein diet (Provoost 1989). The development of renal failure is generally preceded by proteinuria (Brenner 1982, Provoost 1990).

Such protein restricted diet is well tolerated and does not affect the growth of these rats. However when partially nephrectomized rats had been fed an extremely low protein diet (6 %) growth of these rats was found to be retarded (Salusky 1981, Hostetter 1986). The beneficial effect of a protein restricted diet on renal function could not be found in other species. For example in dogs with renal failure induced by subtotal nephrectomy no difference was found between a group fed a protein restricted diet versus a control group fed a normal protein diet after four years of follow-up. A group of dogs fed a high protein diet did however have a lower glomerular filtration rate (Robertson 1986).

1.4 Evidence from human studies

1.4.1 The effect of protein intake on healthy subjects

It is assumed that changes in renal function induced by protein intake reflect evolutionary adaptation of the kidney to meet the excretory needs of carnivores whose protein intake was not constant. Only in the past 5.000 to 10.000 years have agriculture and herding allowed a more continuous pattern of food ingestion. Daily intake for adults in many Western countries now averages approximately 3000 kcal and 100 g of protein.

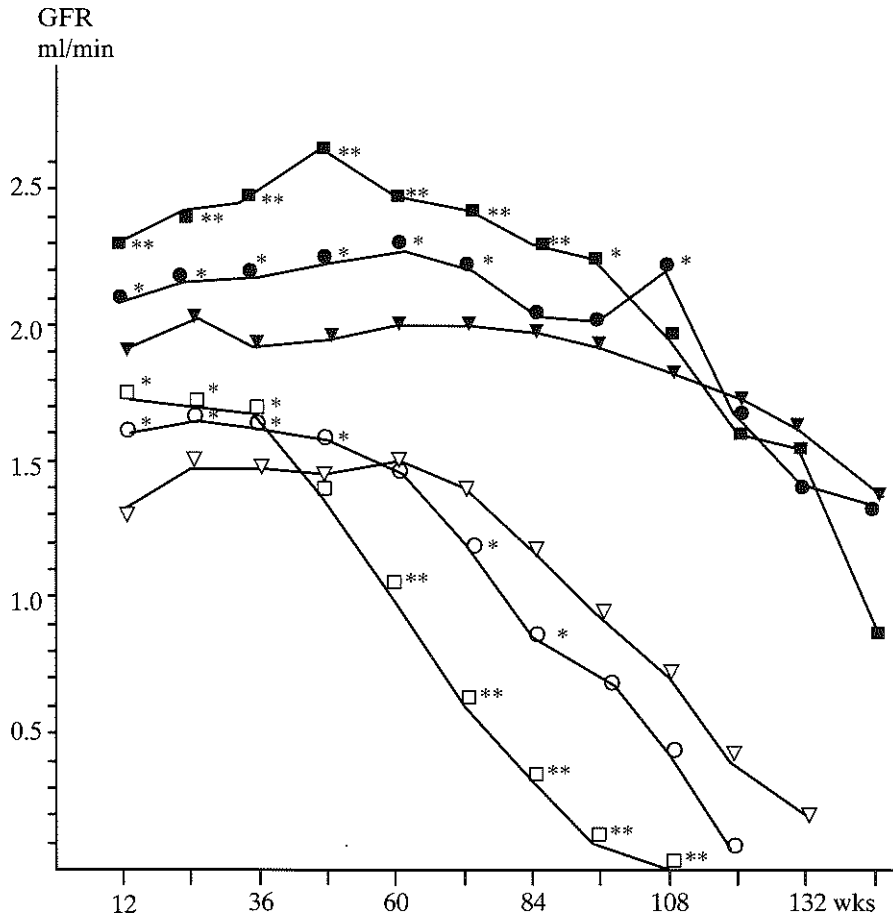

Figure 3.
*Lifelong changes in glomerular filtration rates of normal rats: ■ on a high protein diet (36 %), ● normal protein diet (24 %), ▼ low protein diet (12 %); unilateral nephrectomized rats □ on a high protein diet (36 %), ○ normal protein diet (24 %), ▽ low protein diet (12 %). All values for unilateral nephrectomized rats were significantly different from those of normal rats on the same diet ($p < 0.05$). * $p < 0.05$ versus normal rats or unilateral nephrectomized rats on a low protein diet. ** $p < 0.05$ versus normal rats or unilateral nephrectomized rats on a low protein and a normal protein diet. Reproduced with permission from Provoost AP, de Keijzer MH, Molenaar JC, J Lab Clin Med 1989; 114: 22.*

Unlimited intake of protein rich food, now generally regarded as normal, may be responsible for dramatic differences in the demand on renal function between modern human beings and their prehistoric predecessors who hunted and scavenged for meat. After ingestion of a large meal vasodilator mechanisms associated with protein feeding act in concert with diet-induced extracellular fluid volume expansion to increase total renal bloodflow and glomerular filtration rates. This results in rapid excretion of water, electrolytes and nitrogenous wastes. In patients receiving long term parenteral hyperalimentation striking increases in kidney mass have been reported (Cochran 1979). Likewise the glomerular filtration rate is 50 % higher in patients during a 12 hour infusion of amino acid rich solutions than during the following 12 hour amino acid free interval (Klein 1980).
It has been suggested that the augmented (several times a day, every day of the week) intrarenal pressures and flows associated with ad libitum feeding may contribute to the age associated glomerular sclerosis observed in human beings (Kaplan 1975). Age-related studies of renal function in healthy human beings indicate that renal blood flow and glomerular filtration rates decline progressively after the third decade; values in the eighth decade are only one half to two thirds of those measured in young adults (Davies 1950, Rowe 1976). This "physiological" decline of renal function during life is reflected morphologically by the presence of sclerosis of 10 to 30 per cent of the total glomerular population between the fourth and eight decades of life (Kaplan 1975). Baudoin studied 121 adults after unilateral nephrectomy in childhood. His data suggest that the decline in renal function and the increase in bloodpressure and urinary albumin excretion appeared at a faster rate in men over 30 years of age rate than reported for men of similar age and two kidneys (Baudoin 1992).

1.4.2 Protein restricted diets in patients with chronic renal failure.
In the eighties several studies claimed a favourable effect of protein restriction on the development of renal function of adults and children with chronic renal failure. Unfortunately these studies either had no control group with an unrestricted diet or were not randomized (Barsotti 1981, Maschio 1982, Alvestrand 1983, Alvestrand 1983, Barsotti 1983, Gretz 1983, Mitch 1984, Giovanetti 1985, Acchiardo 1986, El Nahas 1986, Lucas 1986, Oldrizzi 1989, Jureidini 1990, Gretz 1991).
Bergström demonstrated that progression of chronic renal failure could also be retarded with more frequent clinical follow-ups and better bloodpressure control without the prescription of a protein restricted diet (Bergström 1986).
Recently four controlled and randomized studies on the effect of a protein restricted diet on renal function of adults were published. The first randomized controlled study

was reported by Rosman (Rosman 1984). He studied 149 adults with chronic renal failure for at least 18 months and concluded that a low protein diet (0.4-0.6 g/kg/day) could slow down progression of renal disease. However, this conclusion was later withdrawn since after a follow up period of four years there was no clear difference in the decline in creatinine clearance between both groups (Rosman 1989). Locatelli organized a prospective multicentre trial; 456 adult patients with chronic renal failure were randomly assigned either to a low protein diet (0.6 g/kg/day), or a "normal" controlled diet (1g/kg/day). He found no support for the hypothesis that protein restriction retards the progression of chronic renal failure (Locatelli 1991). Likewise Williams found no significant difference in creatinine clearance after a follow-up period of 19 months in 95 adults with chronic renal failure between three groups with different protein intakes (Williams 1991). Ihle did find that dietary protein restriction is effective in slowing the rate of progression of chronic renal failure (Ihle 1989). His study included 64 patients with serum creatinine concentrations ranging from 350 μmol/l to 1000 μmol/l. The patients were randomly assigned either to an unrestricted diet or to an isocaloric protein-restricted diet (0.4 g protein/kg/day). After 18 months significantly more patients developed end-stage renal failure in the group who followed the unrestricted diet compared to patients who followed the protein restricted diet ($p<0.05$). The mean glomerular filtration rate, as measured by the clearance of Cr EDTA, also fell significantly in the unrestricted diet group ($p<0.01$) whereas there was no significant fall in the protein restricted group. However of the patients in the protein restricted group, weight, transferrin and total lymphocyte count were significantly lower at the end of the study, each parameter indicating malnutrition. A large American multicentre randomized trial is still in progress (Klahr 1989).

No prospective randomized studies on the effect of a protein restricted diet on renal function in children are currently available.

Our study presented here is the first long term prospective randomized study in children (Kist-van Holthe 1989, Kist-van Holthe 1990, Kist-van Holthe 1992, Kist-van Holthe 1993) and is part of an ongoing European multicentre study (Wingen 1991, Wingen 1992).

In addition to the risk of malnutrition, dietary restriction in children potentially carries the extra risk of retarding statural growth. This is even more so in children with chronic renal failure who are already at risk for growth retardation (Betts 1974). In the children with congenital diseases like obstructive uropathy, reflux nephropathy and renal dysplasia, growth is often compromised not only by a reduced glomerular filtration rate but also by abnormal electrolyte and water homeostasis and metabolic acidosis as a result of renal tubular dysfunction. It has been concluded, based upon retrospective data

that children with a glomerular filtration rate < 25 ml/min/1.73m^2 are especially at risk for growth retardation (Chantler 1973, Betts 1974, Chantler 1980, Kleinknecht 1983, Rees 1989, Ridgden 1990). Recent studies show that therapy with biosynthetic growth hormone gives an impressive improvement of height velocity in growth retarded prepubertal children with chronic renal failure (Koch 1989, Tönshoff 1990, Hokken-Koelega 1991). However, long term results of the effect of growth hormone on adult height and renal function are not yet available.

1.5 Aims of the study

1.5.1 General aim

The main aim of the study was to investigate the effect of a protein restricted diet on the development of renal function and growth of children with chronic renal failure.

1.5.2 Specific aims

1. What is the correlation between protein intake derived from a prospective dietary diary and 24 hour urinary urea excretion ? (Chapter 3)
2. Can Dutch children with chronic renal failure comply with a protein restricted diet ? (Chapter 4)
3. Can a protein restricted diet slow down or halt progression of chronic renal failure in children ? (Chapter 4)
4. Does a protein restricted diet have an effect on growth of children with chronic renal failure ? (Chapter 4)
5. Is supplementation of a protein restricted diet with essential amino acids necessary? (Chapters 5 and 6)
6. Does a protein restricted diet have an effect on plasma amino acids and / or intracellular amino acids of children with chronic renal failure ? (Chapters 5 and 6)
7. What is the effect of a protein restricted diet on fat intake and serum lipids of children with chronic renal failure ? (Chapter 7)

Chapter 2

General outline of the study

Patients

To be eligible for the study the children (age 2 - 18 years) had to be under treatment for chronic renal failure (glomerular filtration rate (GFR) 15 - 60 ml/min/1.73m^2) for at least six months. Excluded from the study were children with cystinosis, oxalosis, lupus erythematodes, severe hypertension (blood pressure exceeding 110 % of the 97 th percentile according to André) (André 1978) and other severe diseases and children on corticosteroid, growth hormone or erythropoietin therapy. Progressive renal failure was defined as a significant decrease in GFR with respect to the last six to twelve months before the study.

Fifty-six children with chronic renal failure (glomerular filtration rate 15-60 ml/min/ 1.73m^2) from five different University hospitals (Academisch Medisch Centrum, Amsterdam; Academisch Ziekenhuis Groningen; Radboud Ziekenhuis, Nijmegen; Sophia Kinderziekenhuis Rotterdam; Universitaire Instelling Antwerpen) participated in the study. Table 1 shows the characteristics of the patients.

	m/f	age	diagnosis	CrCl	heightSDS	weight %	p/s	pr/c
1	m	17.3	reflux	39	-2.35		p	pr
2	m	12.9	obstr urop	21	-1.34	94	p	c
3	m	4.1	reflux	42	-0.57	83	s	c
4	f	6.5	atn	38	-0.86	91	s	c
5	m	17.7	reflux	35	-3.01		p	pr
6	m	3.7	dysplasia	19	-2.27	96	s	c
7	f	6.7	reflux	60	-2.96	101	s	pr
8	m	12.4	atn	53	-4.07	98	s	pr
9	m	17.3	reflux	15	-2.62		p	c
10	m	16.5	reflux	63	-0.26	87	s	c

11	m	8.9	reflux	59	0.83	101	s	pr
12	m	6	obstr urop	20	-3.30	90	p	c
13	m	17.2	Prune Belly	58	0.6	113	s	pr
14	f	5.9	dysplasia	23	-3.04	85	p	pr
15	m	9.7	reflux	24	-0.06	115	s	c
16	m	6.5	mesang cap	32	-0.57	122	p	c
17	m	13.4	obstr urop	47	0.09	110	s	pr
18	m	4.3	foc loc scl	17	-4.51	108	p	c
19	f	13.6	interst n	48	-1.02	78	s	pr
20	m	10	dysplasia	37	-0.67	122	s	pr
21	m	2.3	dysplasia	40	-1.64	93	s	c
22	f	4.8	atn	21	-0.47	88	s	c
23	f	9	mesang cap	21	1.92	97	p	pr
24	f	2.9	polycystic	26	-1.91	94	p	pr
25	f	11.2	reflux	51	-1.31	107	s	c
26	m	12.9	reflux	57	-1.39	87	s	pr
27	m	13.5	obstr urop	32	-0.97	99	p	c
28	f	3.3	Bartter	28	-5.47	79	s	pr
29	m	8.4	dysplasia	51	-3.33	111	s	c
30	m	5.4	dysplasia	46	-0.59	107	s	pr
31	m	15.2	nephronopht	22	-2.52	118	s	c
32	m	14.5	hus	32	-1.39	89	p	c
33	f	10.7	ivc neurobl	42	-0.41	84	s	c
34	m	4.4	atn	30	-1.68	100	s	pr
35	m	3.4	obstr urop	26	-0.96	96	s	pr
36	m	6.3	nephronopht	49	-1.21	95	s	c
37	f	11.2	hus	18	-0.33	91	p	pr
38	m	12.7	Fanconi	44	-1.64	112	p	pr
39	m	10.9	obstr urop	57	1.52	116	p	pr
40	m	5.9	reflux	59	-1.98	88	s	c
41	m	5.4	dysplasia	50	-0.16	98	s	pr
42	m	10.4	obstr urop	57	-0.14	119	s	c
43	m	13.2	Bartter	30	-0.84	90	p	c
44	m	7.7	Prune Belly	59	-0.90	94	p	c
45	m	17.1	Prune Belly	31	-0.18	77	p	pr
46	m	10.1	obstr urop	51	-1.01	93	s	c
47	f	14.6	mesang cap	49	-0.41	105	s	c

48	f	9.4	hus	44	-0.99	111	p	c
49	m	5.5	dysplasia	61	-1.71	86	s	pr
50	m	16.5	Alport	41	-2.12		p	pr
51	m	13.7	obstr urop	37	-2.68		p	c
52	m	3.8	obstr urop	43	-2.14	83	s	c
53	f	10.3	oligo m n	25	-1.23	107	s	pr
54	f	10.8	reflux	50	1.17	86	s	pr
55	m	2.5	Prune Belly	34	-3.69	86	s	c
56	m	9.5	obstr urop	51	-0.92	155	p	pr

Table 1. Characteristics of the patients at the time of the randomization in a protein restricted and a control group. m/f: male/female; age in years; diagnosis: obstr urop: obstructive uropathy; atn: acute tubulus necrosis; mesang cap: mesangial capillary nephritis; foc loc scl: focal local sclerosis; interst n: interstitial nephritis; polycystic: polycystic kidney disease; nephronopht: nephronophtisis; hus: hemolytic uremic syndrome; ivc neurobl: intravascular coagulation and unilateral nephrectomy for neuroblastoma; Fanconi: idiopatic Fanconi syndrome; oligo m n: oligomeganephronia; CrCl: calculated creatinine clearance (40 x length cm /serum creatinine μmol/l) (van Collenburg 1980, Schwartz 1987); heightSDS: height standard deviation score for healthy Dutch children (Roede 1985); weight %: weight for height percentage; p/s: progressive/ stable chronic renal failure; pr/c: protein restricted diet/control group.

In order to minimize interobserver variations one single paediatrician and one dietician visited the patients in the different centres every three months throughout the course of the study. We regard this as an essential aspect of the study.

Randomization

After an observation period of three months the children were randomly assigned to a protein restricted group (n = 27) or a control group (n = 29) after stratification for renal disease, progression of renal failure and treatment centre. Both groups were well balanced with respect to various baseline characteristics, table 2.

characteristics of the patients	protein restricted group	control group
number of patients	27	29
age (years)	10.2 (4.7)	9.3 (4.5)
gender (boys, girls)	18 / 9	23 / 6
progressive RF	11	10
weight for height (%)	100 (17)	97 (11)
height standard deviation	-1.16 (1.70)	-1.57 (1.17)
systolic BP (mmHg)	110 (12)	110 (10)
diastolic BP (mmHg)	70 (8)	72 (7)
calculated creatinine clearance (ml/min/1.73m^2)	42 (14)	38 (14)

Table 2. Patient characteristics. Data given are numbers of patients or means (SD). progressive RF: progressive RF renal failure; systolic BP: systolic blood pressure; diastolic BP: diastolic blood pressure.

Diet

The children in the protein restricted group were advised to reduce their protein intake to the safe levels of the World Health Organization which vary according to age and gender between 0.8 and 1.1 g/kg/day (WHO 1985). The children in the control group were advised to eat at least 1.5 to 2.0 times the safe levels of protein intake, according to age, which equals the normal protein intake for healthy Dutch children, table 3 (Ministries of Health and Agriculture 1988). For all children the target caloric intake was at least 100 % of the energy requirement advised by the World Health Organization, table 4 (WHO 1985). Supplementation of calories was preferably advised as (polyunsaturated) fat (for example: whole fat products instead of 'light' products, more vegetable margarine on a sandwich, toasted bread can take more margarine, baked potatoes with mayonnaise, add unwhipped cream or butter / margarine to food).

The dietician advised the parents and children of the protein restricted group as well as the control group on their food intake. The children in the protein restricted group received a multivitamin preparation to prevent vitamin and mineral deficiencies. Some children were advised to take extra vitamin B6 and calcium on the basis of the recommended Dutch daily dosages (Voedingsraad 1989).

age	protein restricted group g/kg/day	control group g/kg/day	healthy children g/kg/day
4 - 6 years	1.0	2.0	2.4
7 - 9 years	1.0	2.0	2.3
10 - 15 years f	0.9	1.4	1.5
m	1.0	1.5	1.9
16 - 21 years f	0.8	1.2	1.3
m	0.9	1.4	1.4

Table 3. Protein intake as advised for the protein restricted group (safe levels WHO 1985) and the control group (1.5 - 2.0 x safe levels) and protein intake of healthy Dutch children (Ministries of Health and Agriculture). f: female; m: male.

age	healthy children kcal/kg/day	World Health Organization kcal/kg/day
1 - 3 years	107	103
4 - 6 years	90	93
7 - 9 years	75	80
10 - 15 years f	50	50
m	63	60
16 - 21 years f	42	40
m	46	46

Table 4. Caloric intake of healthy Dutch children (Ministries of Health and Agriculture) and as advised by the World Health Organization (WHO 1985). f: female; m: male.

Dietary assessment

The children and their parents were asked to provide a prospective dietary diary once every three months which covered two days during the week and one day in the weekend. The dietician then discussed the dietary diary with parents and children to be sure that nothing was omitted. Protein and caloric intakes as well as that of other food components (percentage protein of animal origin, vitamins and minerals) were calculated using the Dutch Nutrition Index (Nederlands voedingsstoffen bestand 1987). If protein and/or caloric intake calculated from the dietary diary of a child did not correspond to the prescribed quantity, the parents and children were reinstructed so that the intake could be adjusted.

Growth and nutritional assessment

Every three months weight, height, upper arm circumference and triceps skinfold thickness were measured by the same observer. Weight is expressed as weight for height percentage and height as height standard deviation score for healthy Dutch children (Roede 1985). For the children who were put on renal replacement therapy or died during the study, the last weight, height, upper arm circumference and triceps skinfold thickness were carried forward. Serum albumin and transferrin were measured every three months as parameters of protein malnutrition (Blumenkrantz 1980).

Chapter 3

Comparison of protein intake from the dietary diary with 24-hour urinary urea excretion in children with chronic renal failure

3.1 Introduction

As protein restriction may slow down or even halt progression of chronic renal failure (Brenner 1982) it is relevant to be able to assess dietary compliance. Nitrogen balance is a good but time consuming and difficult method to assess dietary compliance of children with chronic renal failure on a protein restricted diet. As it requires admission to a hospital it is also expensive and not very popular with children and their parents. Moreover it does not reflect compliance with a diet at home. It is well known that urinary urea excretion can be used to calculate protein intake of adults with chronic renal failure admitted to a hospital (Grodstein 1979, Blumenkrantz 1980, Maroni 1985, Mitch 1985). Urinary urea excretion reflects approximately 70 % of nitrogen loss. The remaining nitrogen loss is said to be hardly or not dependant on nitrogen intake, suggesting that changes in nitrogen intake are well reflected by changes in urinary urea excretion (Maroni 1985, Mitch 1985). There are however no comparable studies in children. We wanted to know whether protein intake of children with chronic renal failure can reliably be estimated in the outpatient clinic from dietary diaries and urinary urea excretion.

3.2 Patients and methods

Fifty four children with chronic renal failure were asked to collect three 24-hour urine collections at home. The urine collections were made with an interval of a month during three months. The urine was collected in acidified plastic bottles to prevent degradation of urea by urease producing bacteria. When the children woke up at for example 7 o'clock they emptied their bladder in the toilet. From then the children collected the urine in the bottle until the next day at seven o'clock, when they emptied their bladder in the bottle.

A dietician especially assigned to the study instructed parents and children how to keep a three day prospective dietary diary. The dietary diary consisted of two days during the week and one day in the weekend. The urine was collected in the same period. We asked the children to keep to their regular dietary habits, in order to rule out the possible effect of compliance with a prescribed diet. This study was conducted in the observation phase of the main study which will be described in chapter four, before randomization of the children in a protein restricted group and a control group. The dietician talked the dietary diary over with parents and children at the outpatient clinic to be sure that nothing was omitted. She then calculated the mean protein intake using the Dutch nutrition index (Nederlands Voedingsstoffen Bestand 1987). Of the fifty-four children only thirty-seven succeeded in collecting 24-hour urine, the remaining children were either incontinent for urine or had great trouble collecting 24-hour urine. The age of these children (26 boys and 11 girls) varied from 3 years and 4 months to 16 years and 11 months, the mean age was 10 years and 5 months. Five children had a glomerulopathy, 25 had either reflux nephropathy, obstructive uropathy and/or renal dysplasia. Seven children had miscellaneous diagnoses. Calculated creatinine clearance (40 x length cm/ creatinine µmol/l) (van Collenburg 1980, Schwartz 1987) varied from 18 to 60 ml/min/ 1.73m^2, mean 42 ml/min/1.73m^2. In order to assess the accuracy of the urine collections the same children were asked to collect five 24-hour urines with an interval of three months. Creatinine excretion was calculated for the 30 children who where able to collect five 24-hour urines. Urinary urea excretion was measured enzymatically as first described by Talke and Schubert (1965) using an automatic chemical analyzer.
We determined the relation between protein intake calculated from the dietary diary and protein intake derived from 24-hour urinary urea excretion. We also calculated the difference between protein intake estimated from the dietary diary (PI) and urinary urea excretion (UE). This difference (PI-UE) represents nitrogen retention for growth and non urea nitrogen lost in urine (ammonia, creatinine, uric acid), faeces, sweat and cell loss and changes in the urea pool (blood urea x total body water), figure 1. Dietary compliance will also have an influence on PI-UE. For example "bad" compliance with a protein restricted diet will give an erroneous low value for PI-UE as these children eat more than they admit to in their dietary diary and as a consequence will excrete more urea than calculated from the protein intake of that diary.

Statistics

The relation between urinary urea excretion and protein intake was investigated by linear least-squares regression. In this analysis only data of the last measurement was used when patients had more than one observation. Variations in urinary urea excretion

between measurements on different occasions (within patients) when protein intake differed less than 10 %, were assessed using analysis of variance by calculation of components of variance. Five percent was considered the limit of statistical significance.

3.3 Results

Five patients had one, thirty patients had two and two patients had three successful urine collections in the same period as a diet diary. Protein intake calculated from the diet records varied from 0.83 to 2.99 g/kg/day (0.13-0.48 gN/kg/day), mean 1.62 g (0.26 gN)/ kg/day. Urinary urea excretion varied from 2.9 to 13.3 mmol/kg/day (0.08-0.38 gN/kg/ day) mean 6.9 mmol (0.20 gN) /kg/day. Two children had proteinuria of 2 - 2.5 g/day, the other children had no significant proteinuria (<1g/day). The coefficient of variation for creatinine excretion of thirty children, for each child calculated from five 24-hour urine collections, was 14 %. The correlation coefficient between protein intake and urinary urea excretion per day is 0.58 ($p < 0.001$) (figure 2, upper panel). The correlation coefficient between protein intake / kg body weight and urea excretion / kg body weight is 0.42 ($p < 0.01$) (figure 2, lower panel). Using multiple regression no relation was found between urea excretion / kg and age or body weight. Besides the large variation in urinary urea excretion between patients (figure 2), there was also a large variation within patients. In 14 patients selected on a within-patient variation of protein intake of less than 10 % the coefficient of variation for urinary urea excretion was 41 %!

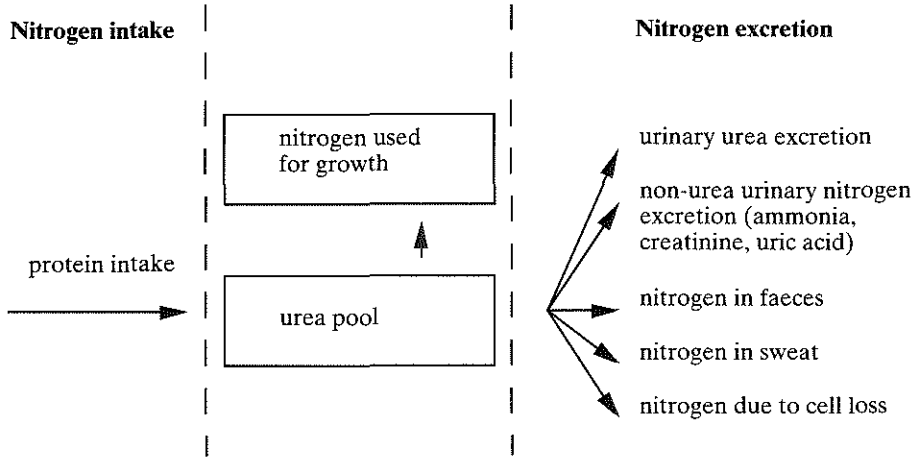

Figure 1. Nitrogen intake and excretion

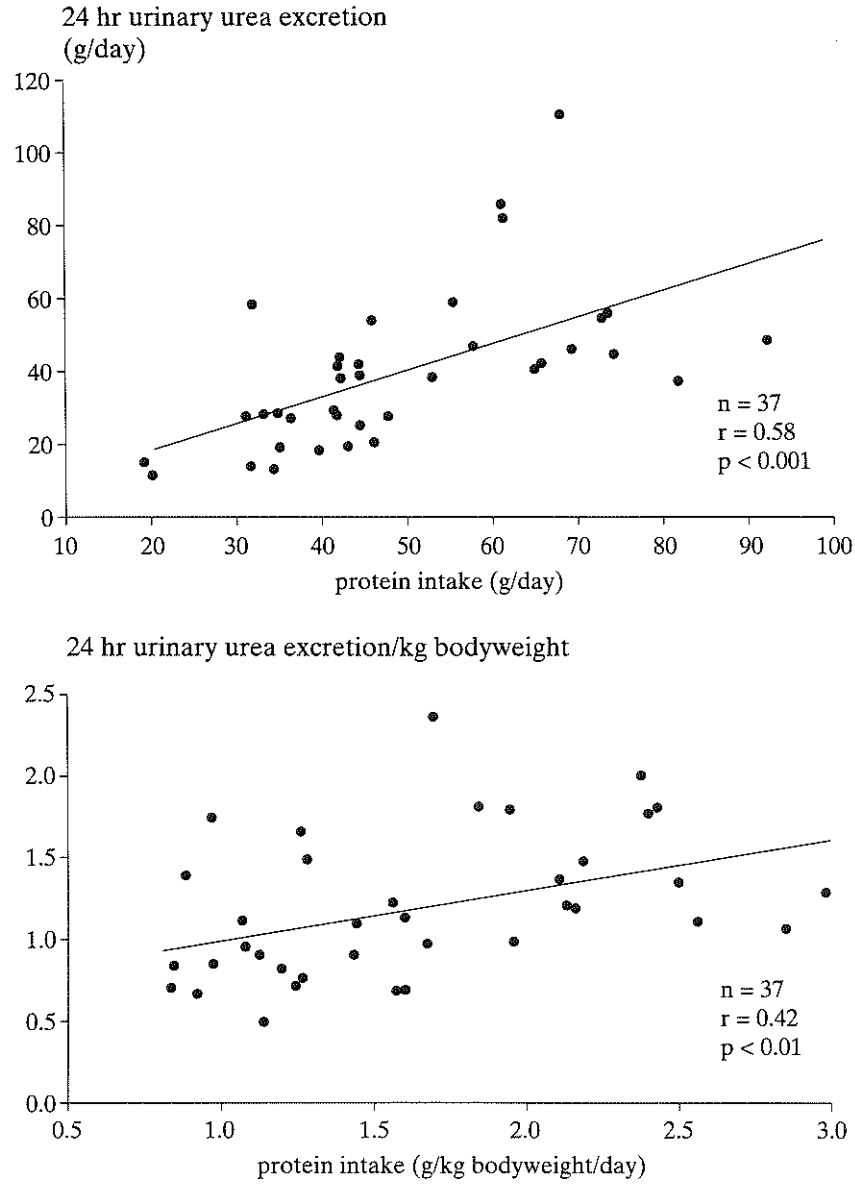

Figure 2. Upper panel: Urinary urea excretion versus protein intake per day. Lower panel: Urinary urea excretion versus protein intake per kg bodyweight per day. Urinary urea excretion (mmol) x 0.18 = g "protein".

An increase in protein intake was associated with an increase of PI-UE ($r = 0.73$, $p < 0.001$). The children who consumed a comparable amount of protein as in adult studies (0.8 - 1.2 g/kg/day) had a mean calculated PI-UE of 21 mgN/kg/day. When protein intake exceeded 1.2 g/kg/day PI-UE increased substantially. We found no relation between PI-UE and glomerular filtration rate, nor between PI-UE and age.

3.4 Discussion

The correlation we found between protein intake and urinary urea excretion per day ($r = 0.58$) (figure 2, upper panel) is disappointingly low. Ziegler (1977) performed 1148 nitrogen balance studies in hospital in 123 healthy children 1 to 11 years old. He found a considerably better correlation in a hospital setting between nitrogen intake and urinary nitrogen excretion per day ($r = 0.76$) than the results found in our outpatient study. The better correlation found by Ziegler can be partly explained by the slight difference between studying nitrogen excretion and urea excretion, and by studying children in the hospital setting compared to the outpatient setting. Coles (1989) found a correlation similar to ours ($r = 0.6$, $p < 0.001$) between protein intake and 24-hour urinary nitrogen measurements in an outpatient study of 39 adults with chronic renal failure.

Can we explain the low correlation between protein intake calculated from a dietary diary and protein intake derived from 24-hour urinary urea excretion in the outpatient setting ?

Various factors may be responsible: inaccuracies of the dietary diary, errors in collecting the 24-hour urine, non urea nitrogen excretion (nitrogen excretion in urine other than as urea, nitrogen lost in faeces, sweat and cell loss), changes in the urea pool, nitrogen used for growth and non compliance with a diet (figure 1).

1. Inaccuracies of the dietary diary.

Errors in estimating protein intake from a dietary diary even though they were written prospectively and were each time thoroughly talked over with the children and their parents by the dietician are potentially large. During this study a systematic flaw might be that children who eat more tend to overestimate their intake more than children who eat relatively small amounts of protein. Furthermore children on a free diet, as these children were, tend to have day to day fluctuations in protein intake that are reflected in day to day fluctuations in urinary urea excretion. Mean protein intake calculated from a three day diet diary does not reflect this variation, but will give a more reliable average protein intake.

2. Errors in the collecting the 24-hour urine collection.

Major errors in urine collection are ruled out by a coefficient of variation within patients

of 24-hour creatinine excretion of 14 %, which is acceptable.

3. Non urea nitrogen excretion.

Non urea urinary nitrogen excretion in urine (ammonia, creatinine, uric acid) and nitrogen lost in faeces, sweat and cell loss are relatively small amounts. Ammonia is formed in proportion to net acid excretion which is dependent on protein intake (Chantler 1987). In adult studies non urea nitrogen excretion varies from 23 to 34 mg/kg/day (Cottini 1973, Kopple 1973, Mitch 1977, Maroni 1985). Non urea urinary nitrogen - with the exception of ammonia - and nitrogen lost in faeces do not correlate with dietary intake, nor with glomerular filtration rate (Kopple 1973, Maroni 1985, Mitch 1985).

4. Changes in the urea pool of the body.

The children had a stable renal function. Changes in the urea pool, although they are not negligible, can not be held responsible for the discrepancy between protein intake estimated from a dietary diary and protein intake derived from 24-hour urinary urea excretion. For example in a child of 25 kg serum urea was found to be 2 mmol/l higher compared to the previous day. This is a change in the urea pool of: 2 mmol x 0.6 (total bodywater) x 25 (bodyweight) = 30 mmol urea. The body needs 5.4 g of protein to make 30 mmol of urea (30 mmol x 0.18 = 5.4 g protein). This child must have eaten 5.4 g of protein more than the previous day. 5.4 g protein intake for this child is 0.2 g protein/kg/day = 0.03 gN/kg/day. This amount of protein is not negligible, but cannot explain the low correlation between protein intake and 24-hour urinary urea excretion.

5. Protein used for growth.

Only a very small part of daily protein intake is used for growth ±0.01 gN/kg/day = 0.06 g protein/kg/day (Diem 1975). However the day to day variation might be bigger due to changes in catabolism and anabolism. This cannot explain a systematic flaw but might cause a wide range of individual observations.

6. Non compliance with a diet.

The children were on a free diet and were asked to continue their regular dietary habits. Therefore non compliance with a prescribed diet does not play a role in this study.

We found that an increase in protein intake is associated with an increase in PI-UE. This is in disagreement with studies performed in adults with chronic renal failure where no correlation is found between protein intake and non urea nitrogen loss. The children in our study who consumed a comparable amount of protein as in adult studies (0.8 - 1.2 g/kg/day) have a mean calculated PI-UE of 21 mgN/kg/day. This is similar to the non urea nitrogen loss found in adult studies of 23 - 34 mgN/ kg/day (Kopple 1973, Mitch 1977, Maroni 1985, Coles 1989). However when in our study protein intake exceeded 1.2 g/kg/day the calculated PI - UE increased to values much higher than those found in

adults. This may be caused by an overestimation of protein intake in the dietary diary of children who eat relatively large amounts of protein. In addition it might also be caused by urinary ammonia excretion which is dependent on protein intake but was not measured in this study (Chantler 1987).
We found no relation between PI - UE and glomerular filtration rate, nor between PI - UE and age. This is in agreement with studies performed in adults (Maroni 1985).

Conclusion
As the children were on a free diet and an enormous effort was made to ensure a correct prospective dietary diary, the dietary diary was considered to be a reliable method for assessing protein intake. Because major errors in urine collection were ruled out, urea excretion is therefore more likely to be the factor causing the inaccuracies.
Although it is common practice to use 24-hour urinary urea excretion for the assessment of compliance with a protein restricted diet, we cannot confirm its value for individual patients on a free diet in the outpatient setting.

Chapter 4

Effect of a protein restricted diet on renal function and growth of children with chronic renal failure

4.1 Introduction

Hyperfiltration, the adaptive response to the loss of functioning kidney mass, is thought to be detrimental to long term kidney function because of the development of glomerulosclerosis (Hostetter 1981). It is postulated that protein restriction can decrease hyperfiltration and slow down progression of chronic renal failure (Brenner 1982). Studies have shown a beneficial effect of a protein restricted diet on renal function in laboratory rats with chronic renal failure (Kleinknecht 1979, Okuda 1987, Provoost 1990). However, the growth of some rats on a very low protein diet was impaired (Salusky 1981).

It is still unknown whether protein restriction can slow down progression of chronic renal failure in man. Although numerous retrospective and prospective but not randomized controlled studies have shown that dietary protein restriction is effective in slowing down the rate of progression of chronic renal failure in adults (Maschio 1982, Alvestrand 1983, Barsotti 1983, Gretz 1983, Mitch 1984, Acchiardo 1986, Lucas 1986), only four prospective controlled studies have been performed (Ihle 1989, Rosman 1989, Locatelli 1991, Williams 1991). One of these studies (Ihle 1989) could and three others (Rosman 1989, Locatelli 1991, Williams 1991) could not confirm this conclusion. For children with chronic renal failure one prospective, but not controlled, study has demonstrated a favourable effect of a protein restricted diet on renal function (Jureidini 1990). A prospective randomized European multicentre study in children is now in progress (Wingen 1991, Wingen 1992). In addition, the potential benefit of protein restriction for children has to be balanced against the risk of retarded growth. We present the results of a three-year follow-up of a prospective randomized study of the effect of a protein restricted diet on renal function and growth in children with chronic renal failure. This study is part of the European multicentre study (Wingen 1991, Wingen 1992).

4.2 Patients and methods

Fifty-six children with chronic renal failure (glomerular filtration rate 15-63 ml/min/ 1.73m^2) participated in the study.

The characteristics of the patients are given in table 1 and 2 of chapter 2. The diagnosis of the patients are shown in table 1.

glomerulopathy	9	hemolytic uremic syndrome	3
		mesangiocapillary glomerulonephritis	3
		focal local sclerosis	1
		Alport syndrome	1
		interstitial nephritis	1
uropathy/dysplasia	36	reflux nephropathy	11
		obstructive uropathy	12
		renal dysplasia	10
		Prune Belly syndrome	3
miscellaneous	11	acute tubulus necrosis	4
		Bartter syndrome	2
		nephronophthisis	2
		idiopatic Fanconi syndrome	1
		polycystic kidneys	1
		intravascular coagulation and unilateral nephrectomy for neuroblastoma	1

Table 1. Diagnosis of the children with chronic renal failure.

Dietary compliance

Protein intake calculated from the dietary diaries and the serum urea/creatinine ratio were measured every three months. Twenty-four hour urinary urea excretion is not a

reliable method for assessment of nitrogen intake as is described by Coles and in chapter three of this thesis (Coles 1989, Kist-van Holthe tot Echten 1992).

Renal function

Once every three months renal function was assessed by means of the endogenous creatinine clearance and the calculated creatinine clearance (40 x length in cm / plasma creatinine µmol/l) (van Collenburg 1980, Schwartz 1987). Endogenous creatinine clearance was computed from 24-hour urine collections and plasma creatinine. For the children who were put on renal replacement therapy or died during the study, the endogenous creatinine clearance and the calculated creatinine clearance were counted as 0 ml/min/1.73m^2 for the remainder of the study. Endogenous creatinine clearance and calculated creatinine clearance are relatively easy noninvasive procedures for assessment of renal function in children and could therefore be determined at regular three-month intervals. Because it is a more invasive procedure, determination of the inulin clearance was only performed after two and three years. This provided validation of the endogenous and calculated creatinine clearances.

Inulin clearance was measured after a light standardized breakfast in well hydrated patients, by administration of a continuous inulin infusion and timed collection of urine and plasma samples during a four hour period.

Microalbuminuria

Microalbuminuria was measured every three months in 24-hour urine collections using a radioimmunoassay method. Microalbuminuria is defined as a 24-hour albumin excretion of more than 30 mg/day on at least two occasions.

Renal osteodystrophy

Before randomization and after one, two and three years a X-ray of the left hand and wrist was made to detect renal osteodystrophy. The children were considered to have either a normal bone structure and mineral bone content, or renal osteodystrophy. Every three months serum calcium, phosphate, alkaline phosphatase and plasma parathormone were measured. The children received dihydroxycholecalciferol or 1,25 vitamin D when necessary for renal osteodystrophy and for prevention of renal osteodystrophy when the GFR decreased below 40 ml/min/1.73m^2.

Miscellaneous parameters

Systolic and diastolic blood pressure, haemoglobin and serum uric acid and bicarbonate levels were measured every three months. Hypertension (a diastolic blood pressure

exceeding the 97th percentile according to André) was treated with diuretics and if necessary combined with vasodilators and / or angiotensine converting enzyme inhibitors. Bicarbonate was supplied when serum bicarbonate levels decreased below 22 mmol/l.

Statistics

Differences in the means of various parameters between the two groups were assessed for statistical significance using the Mann-Whitney test. In addition, repeated measurements analysis of variance (BMDP, Module 5V) was used to compare changes in the course of time in calculated creatinine clearance, endogenous creatinine clearance, (micro)albuminuria, weight with respect to height, height standard deviation score, upper arm circumference and triceps skinfold thickness. The decrease in calculated creatinine clearance for individual patients was characterized by the individual slope of the least squares regression line for calculated creatinine clearance versus time after randomization. P-values given are two-sided; five percent was considered the limit of significance. Data is the means ± standard deviation, unless otherwise indicated. Microalbuminuria was analyzed after logarithmic transformation to approximate normal distributions.

4.3 Results

One child from the control group with mesangiocapillary glomerulonephritis received a course of high-dose corticosteroids because of a rapidly progressive phase of his disease three months after randomization. This child was considered nonevaluable and was excluded from further analysis. None of the other 55 children dropped out of the study during the first two years. In the last year of the study one child from the control group and seven children from the protein restricted group dropped out of the study. Four of these children received growth hormone therapy which was an exclusion criterium and four children could not comply with the protein restricted diet any longer.

Renal function

In the protein restricted group four children reached endstage renal disease requiring renal replacement therapy 10, 23, 31 and 35 months after randomization respectively. In the control group one child died of endstage renal disease three months after randomization and one received a renal transplant after 21 months and two children were started on dialysis both 28 months after randomization. Creatinine clearances could be calculated for 42 (75 %) of the 55 patients; for the remaining children urine could not be collected because of incontinence or social problems. There was no

significant difference between the experimental and the control group in either calculated creatinine clearance or endogenous creatinine clearance during the three-year follow-up period (figures 1a and 2). In both groups the mean calculated creatinine clearance slowly declined: -2.9 (±1.0) ml/min/1.73m^2 / year for the protein restricted group and -2.1 (±0.8) ml/min/1.73m^2 / year for the control group. Figure 1b shows the decrease in calculated creatinine clearance / year for individual patients. Inulin clearance was measured after two years in 45 patients (six refused or could not supply timed urine collections, three were on renal replacement therapy and one patient had died) and after three years in 33 patients. The mean inulin clearance after two years was 38 (± 16) ml/min/1.73m^2 for the protein restricted group, which did not differ significantly from 36 (±15) ml/min/1.73m^2 for the control group; and after three years 36 (± 15) ml/min/1.73m^2 for the protein restricted group which likewise did not differ significantly from 32 (±14) ml/min/1.73m^2 for the control group. Calculated creatinine clearance and endogenous creatinine clearance correlated significantly with inulin clearance: r = 0.85 and r = 0.78, respectively.

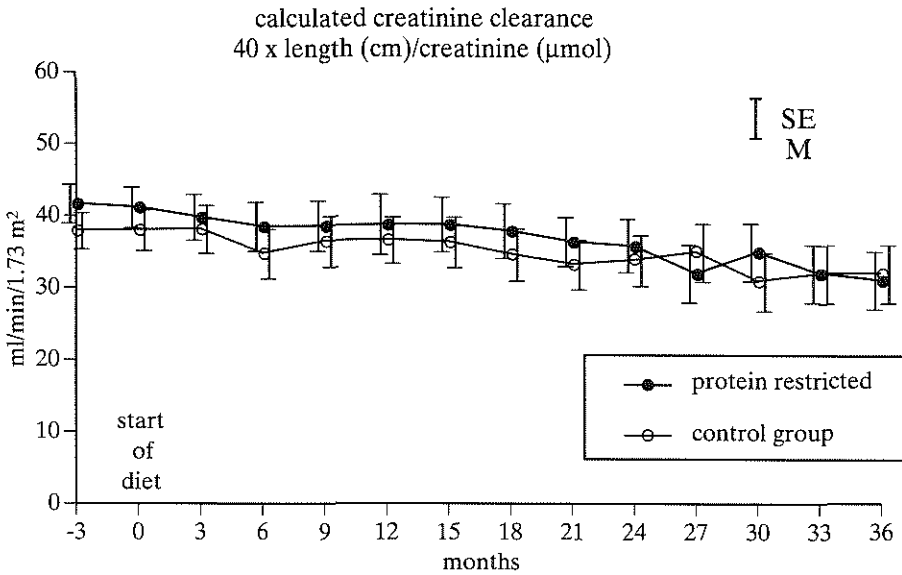

Figure 1a. Calculated creatinine clearance (ml/min/1.73m^2) assessed three months before to thirty-six months after randomization.

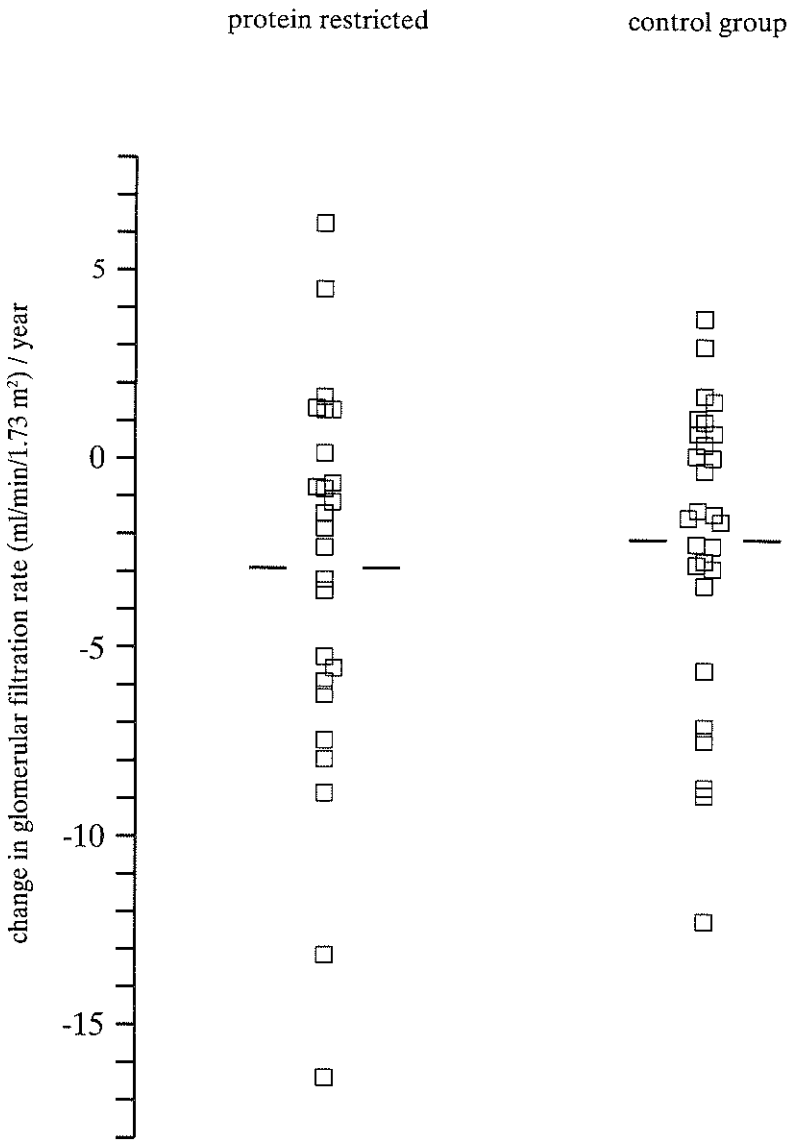

Figure 1b. Change in calculated creatinine clearance (ml/min/1.73m² / year) for individual patients of the protein restricted versus the control group. Bars denote median values.

Microalbuminuria

Twenty-four hour microalbuminuria was measured five to thirteen times (average 9.8 times) in 42 patients. The remaining children could not collect 24-hour urine because of incontinence or social problems. Nine children from the protein restricted group and six children from the control group developed (micro)albuminuria during the study. None of the children from both groups who had (micro)albuminuria at the beginning of the study had a 24-hour albumin excretion of less than 30 mg/day at the end of the study.

Growth and nutritional assessment

There was no significant difference between the control group and the protein restricted group in either weight with respect to height (figure 3) or delta height standard deviation score (figure 4). Weight with respect to height increased in both groups during the study: mean 2.1 (± 0.5) % / year for the protein restricted group which did not significantly differ from the mean 1.0 (± 0.8) % / year for the control group. The height standard deviation score did not change significantly during the three-year follow-up period. Upper arm circumference (figure 5) and triceps skinfold thickness (figure 6) did not differ significantly between the protein restricted group and the control group, the same applies for serum albumin (figure 7) and transferrin (figure 8).

Dietary assessment

During the three-year follow-up period, the mean protein intake of the children in the protein restricted group, calculated from the prospective dietary diaries and expressed as a percentage of the prescribed diet was approximately one hundred percent (figure 9). The mean protein intake of the children in the control group always exceeded 100 % of the advised amount, which indicates that protein intake for this group was at least 1.5-2.0 times the safe levels for protein intake which approximates the normal protein intake of Dutch children (Ministries of Health and Agriculture). The percentage protein intake of animal origin for children in the protein restricted group was significantly lower compared to the control group (figure 10). Caloric intake of all children, except one in the control group, was adequate (> 80 % of the advised amount) and there was no significant difference between the children in the protein restricted group and those of the control group (figure 11).

The serum urea / creatinine ratio was significantly lower during the entire study period for the protein restricted group with respect to the control group (figure 12).

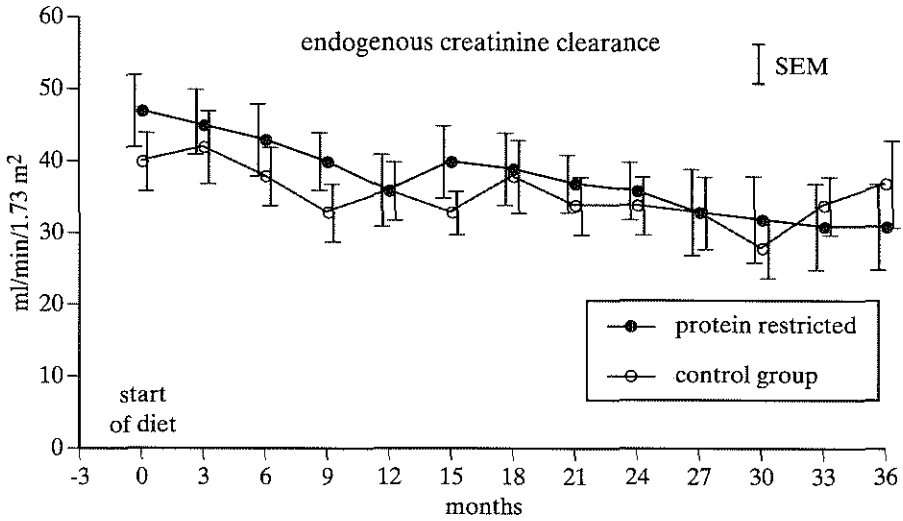

Figure 2. Endogenous creatinine clearance (ml/min/1.73m²/year) assesed from the start of the diet to thirty-six months after randomization.

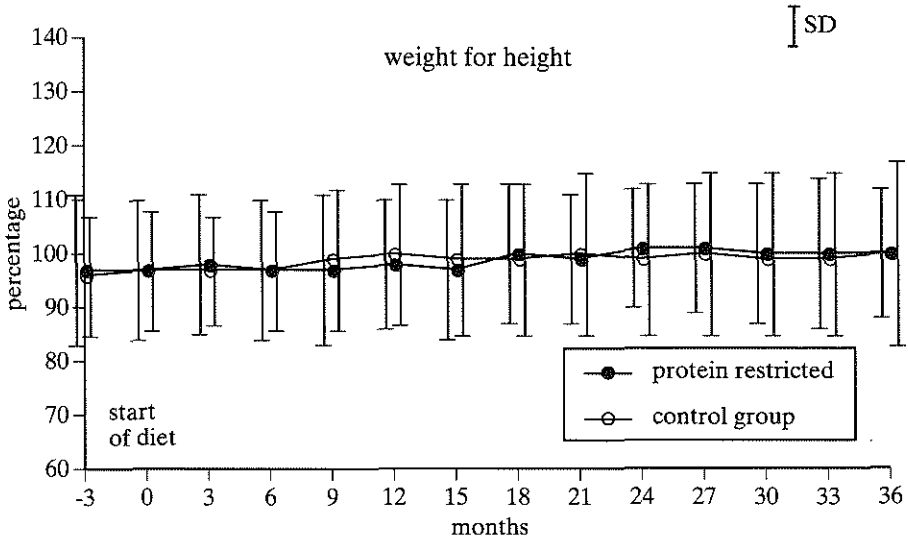

Figure 3. Weight with respect to height (%) assessed three months before to thirty-six months after randomization.

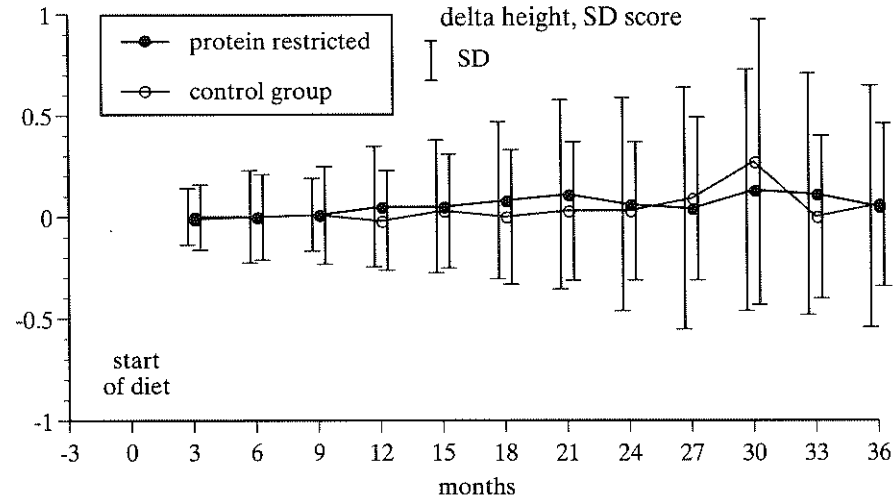

Figure 4. Delta height standard deviation assessed three months before to thirty-six months after randomization.

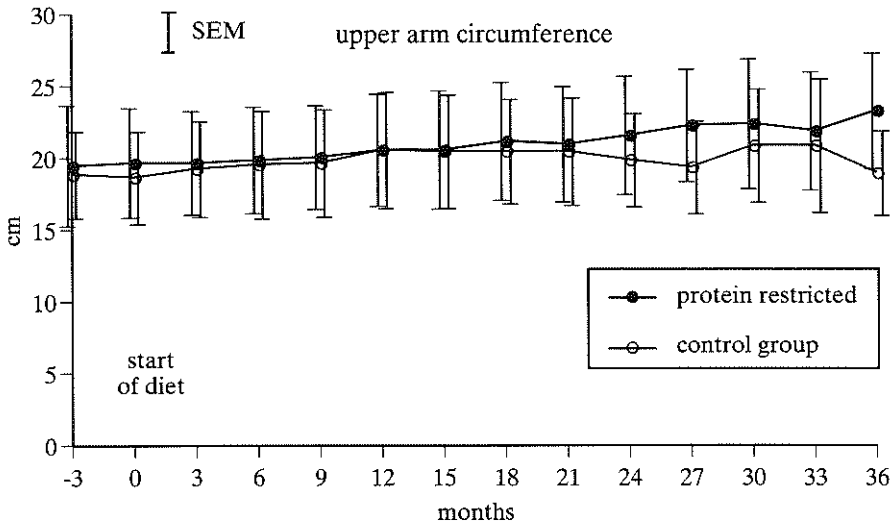

Figure 5. Upper arm circumference (cm) assessed three months before to thirty-six months after randomization.

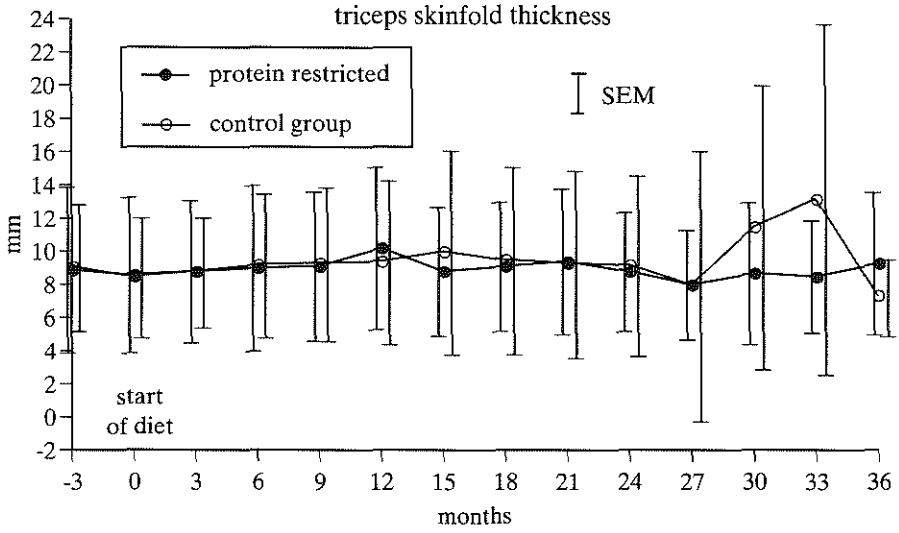

Figure 6. Triceps skinfold thickness (mm) assessed three months before to thirty-six months after randomization.

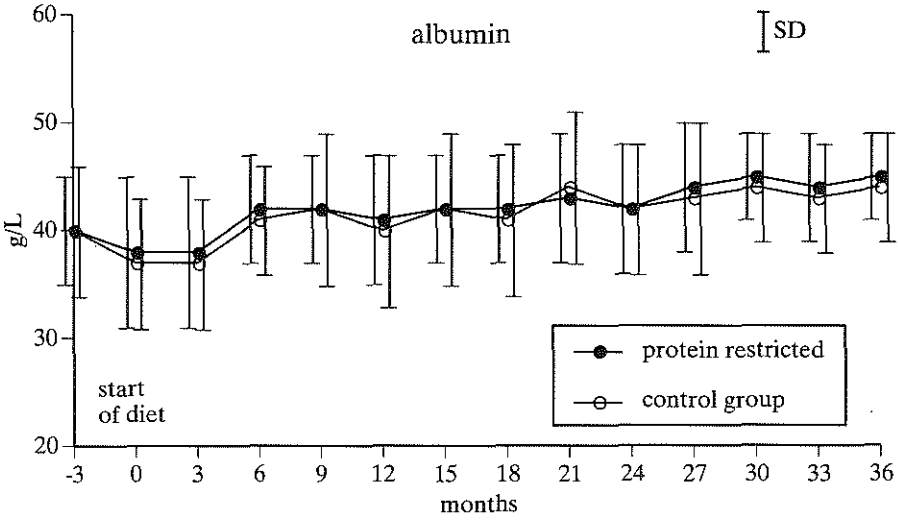

Figure 7. Serum albumin (g/l) assessed three months before to thirty-six months after randomization.

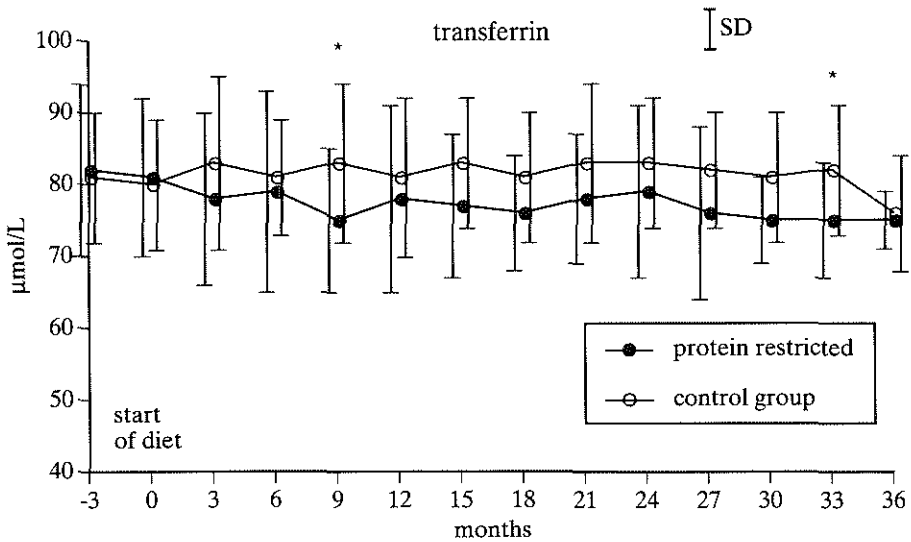

*Figure 8. Serum transferrin (µmol/l) assessed three months before to thirty-six months after randomization. * $p < 0.05$ = statistical difference between the protein restricted group and the control group.*

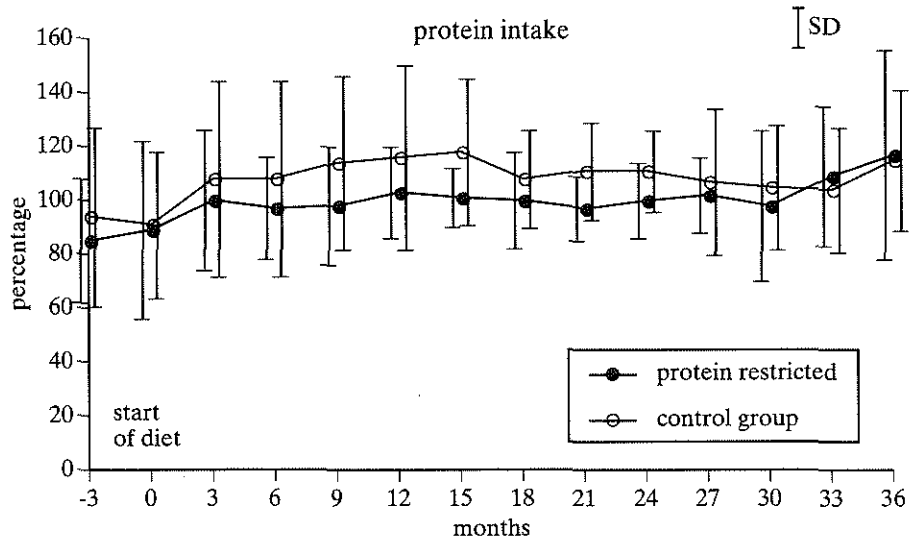

Figure 9. Protein intake (percentage of the advised protein intake) assessed three months before to thirty-six months after randomization.

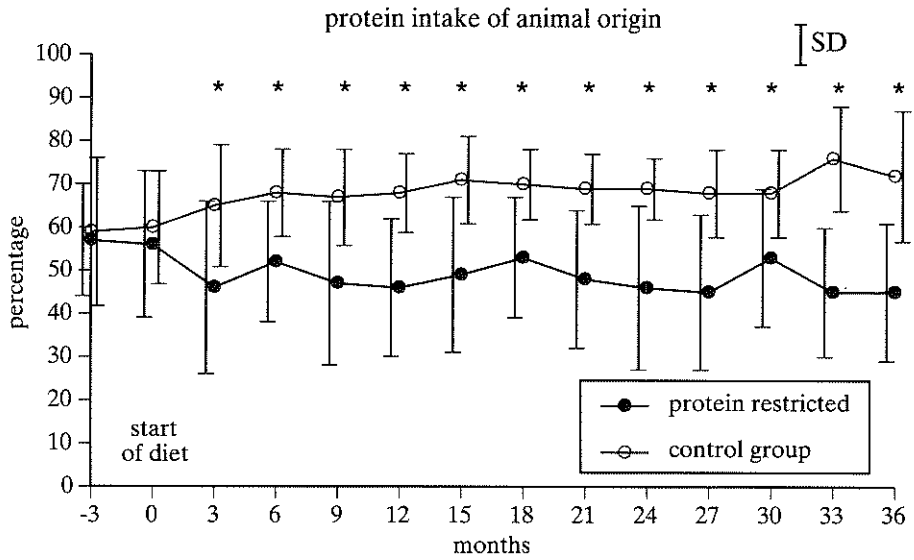

*Figure 10. Protein intake of animal origin (percentage of total protein intake) assessed three months before to thirty-six months after randomization. * $p < 0.05$ = statistical difference between the protein restricted group and the control group.*

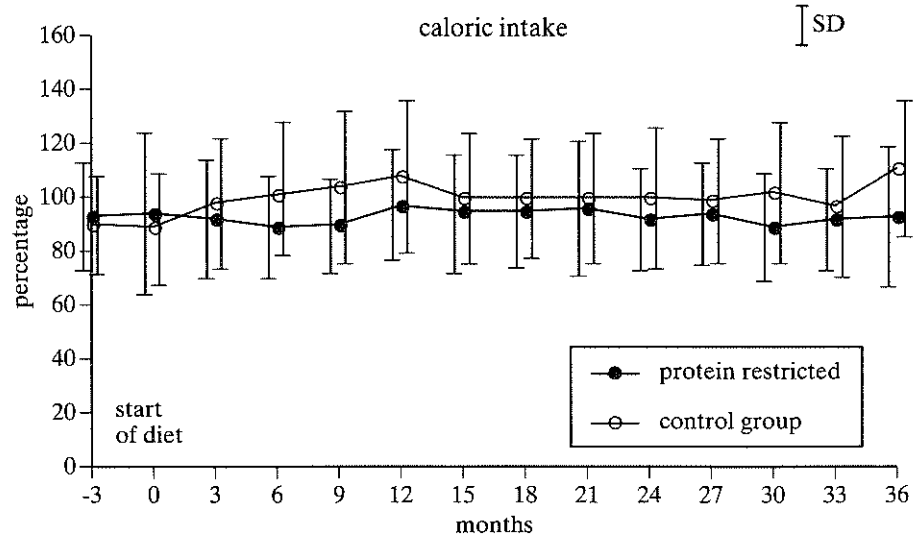

Figure 11. Caloric intake (percentage of the advised caloric intake) assessed three months before to thirty-six months after randomization.

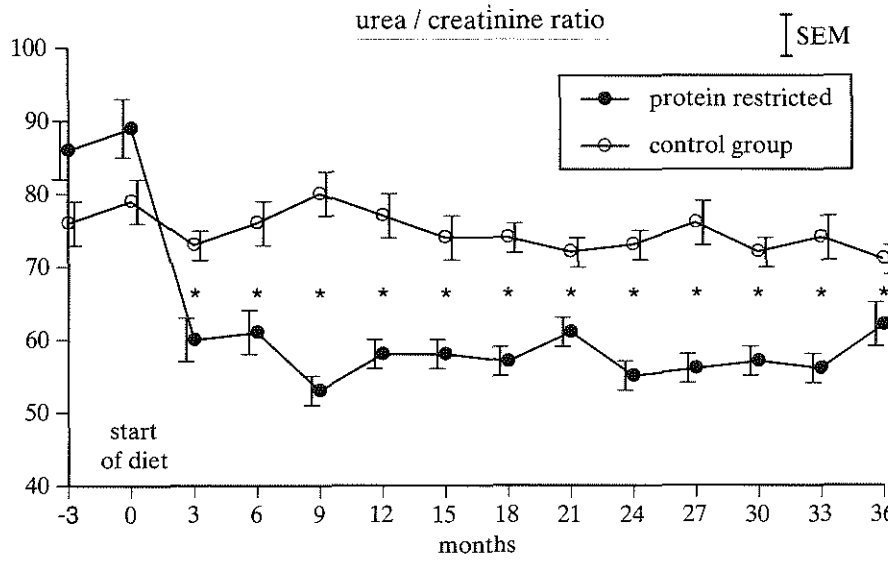

*Figure 12. Serum urea / creatinine ratio assessed three months before to thirty-six months after randomization. * $p < 0.05$ = statistical difference between the protein restricted group and the control group.*

As expected from our previous finding (Kist-van Holthe tot Echten 1992, chapter three) and as is described by Coles (Coles 1989) that 24 hour urinary urea excretion does not correlate well with protein intake derived from a prospective dietary diary, there was no significant difference between the 24 hour urinary urea excretion from the protein restricted group as compared to the control group.

Renal osteodystrophy

We found no significant difference between the protein restricted group and the control group with respect to the occurrence of renal osteodystrophy as assessed by a X-ray of the hand and wrist of the patients, table 2. Likewise did serum calcium and phosphate and plasma parathormone not differ significantly between both groups. There was no change in the prescription of dihydroxycholecalciferol or 1,25 vitamin D in both groups during the three year follow-up period. The children in the control group were prescribed ±150 % of the amount of calciumcarbonate of the protein restricted group, to keep serum phosphate in the low-normal range.

	normal bone		renal osteodystrophy	
	control / group	protein restricted group	control / group	protein restricted group
randomization	18 / 13		9 / 14	
one year	20 / 15		8 / 11	
two years	19 / 16		8 / 10	
three years	14 / 9		9 / 6	

Table 2. Results of the X-ray of hand and wrist, before randomization and after one, two and three years.

Miscellaneous parameters

There was no significant difference between the protein restricted and the control group during the three-year follow-up period in systolic or diastolic blood pressure or the haemoglobin and bicarbonate levels. Neither was there a difference between both groups in the prescription of sodium bicarbonate. Uric acid before randomization was significantly higher in the protein restricted group: 0.41 (± 0.10) mmol/l compared to 0.37 (± 0.07) mmol/l for the control group. This difference did not change during the three-year follow-up period.

4.4 Discussion

Although it is common practice to advise a protein restricted diet for children with chronic renal failure, this study cannot support the hypothesis that protein restriction can preserve renal function at least during an observation period of three years. Although eight children had to stop with the study during the third year, the results in the last year of the study resemble those found in the preceding two years. The results of our study are in accordance with those reported by Rosman (1989), Locatelli (1991) and Williams (1991), who found no beneficial effect of a protein restricted diet in larger randomized studies of adults (248 patients (Rosman 1989), 456 patients (Locatelli 1991) and 95 patients (Williams 1991)) with a degree of chronic renal failure similar to that in our study. They restricted protein intake to 0.4-0.6 g/kg/day. Only Ihle (1989) found a favourable effect of a protein restricted diet (0.4 g/kg/day) in a randomized study of 65

adult patients with severe progressive chronic renal failure (serum creatinine 350 - 1000 µmol/l). But in his study the patients with a protein restricted diet lost significantly more weight and had significantly lower levels of serum transferrin and a significantly lower total lymphocyte count, each parameter indicating malnutrition, compared to the control group. Possibly the protein restriction prescribed in our study was not severe enough to protect against haemodynamically mediated glomerular injury when there is a significant reduction in functioning nephrons. However, since children with chronic renal failure are already at risk for growth retardation (Chantler 1973, Betts 1974, Chantler 1980, Kleinknecht 1983, Rees 1989, Ridgden 1990), caution is essential when a protein restricted diet is recommended. The protein restriction followed in our study was combined with an adequate caloric intake and did not cause growth impairment. It does not appear advisable to reduce protein intake below the safe levels recommended by the World Health Organization for protein intake for children in the process of growth, but prospective controlled studies are not available (Hellerstein 1987, Mehls 1989, Raymond 1990). Although the children and their parents were extensively coached and supported during the entire study, diet compliance may not always have been optimal but was in our opinion the best to be achieved. The protein restricted group as a whole had significantly lower serum urea / creatinine ratios compared to the control group.

In order to keep the serum phosphate in the low-normal range the children in the control group needed more (150 %) calciumcarbonate than the children in the protein restricted group.

Creatinine clearances can be assessed at regular intervals but rely on adequate urine collection; in contrast a calculated creatinine clearance only requires a plasma creatinine determination and the height of the patient, and there is a close correlation with creatinine clearance and inulin clearance. Although the clearance of inulin is the golden standard for estimation of the GFR we have focused on the calculated creatinine clearance and the endogenous creatinine clearance since this allowed us to perform multiple measurements at regular intervals for each patient. Moreover we did measure inulin clearances two and three years after randomization.

Supplementation of a protein restricted diet with essential amino (keto) acids is still controversial (Jones 1983, Giordano 1987). In view of the adequate growth and similar amino acid levels in plasma and granulocytes (chapters five and six) of children in the protein restricted and the control groups in our study, supplementation with essential amino acids does not seem necessary for this diet.

We conclude that the protein restricted diet used in this study has no obvious effect on renal function in children with chronic renal failure. One further important conclusion

can be drawn from the study. Long term protein restriction according to the safe levels of the World Health Organization does not negatively influence body growth of these children if adequate amounts of calories are supplied.

Chapter 5

Plasma amino acids in children with chronic renal failure

5.1 Introduction

An important element of our study on the effect of a protein restricted diet on renal function of children with chronic renal failure was to ensure that adequate growth was maintained. When prescribing the protein restricted diet, we wanted to know first if the children were provided with sufficient essential amino acids for growth. Secondly we were interested in the levels of amino acids in the plasma as a tentative measurement of essential amino acid metabolism.

It is well known that adults with chronic renal failure have abnormal plasma and intracellular amino acid levels. These abnormalities are attributed to an altered amino acid metabolism and malnutrition (Fürst 1978, Tizianello 1980, Young 1982, Takala 1983, Young 1985, Garibotto 1987). The consequences of these amino acid abnormalities are until now unclear. Cellular malnutrition in children with chronic renal failure may contribute to their poor growth (Tizianello 1985, Metcoff 1989). As a consequence the question arises wether supplementation of a protein restricted diet with essential amino acids is necessary (Broyer 1983).

Although some data on plasma amino acids in children with chronic renal failure are available, the number of patients studied is small (Broyer 1980, Jones 1983, Canepa 1992).

In the present study we report essential amino acid intakes and plasma amino acid levels and the relation to renal function of fifty children.

5.2 Patients and methods

Fifty children with chronic renal failure who participated in a prospective randomized multicentre study on the effect of a protein restricted diet on renal function and growth were studied. All patients were in a stable condition without concurrent illnesses.

Table 1 shows the characteristics of the patients in the protein restricted (n = 25) and the control group (n = 25).

characteristics of the patients	protein restricted group	control group
number of patients	25	25
age (years)	11.0 (4.9)	10.6 (4.2)
gender (boys, girls)	17 / 8	19 / 6
diagnosis (1/2/3)*	3 / 17 / 5	3 / 16 / 6
weight for height (%)	98 (13)	99 (13)
height standard deviation	-1.26 (1.68)	-1.30 (0.90)
calculated creatinine clearance (ml/min/1.73m^2)	40 (16)	39 (16)

Table 1. Characteristics of the protein restricted and control group at the time of bloodsampling for amino acids expressed as mean values (standard deviation) or numbers of patients. *Diagnosis 1: glomerulopathy, 2: reflux nephropathy, obstructive uropathy and/or dysplasia, 3: miscellaneous.

Intake of essential amino acids was derived from Souci-Fachmann-Kraut (1986) and compared to minimal daily requirements of amino acids for children (WHO 1985). The intake of the nutrients was defined as the mean intake per day calculated from the three day dietary diary.

Six to fifteen months after randomization into the protein restricted and the control group, blood was drawn for amino acid analysis after an overnight fast. Plasma amino acids were measured by liquid column chromatography on a LKBα+ (Pharmacia, Sweden) amino acid analyzer. The plasma amino acids are expressed as μmol/ml and are given as the median and their range. The results were compared to fasting plasma amino acid values of fifteen healthy children (seven girls and eight boys) with a similar age distribution: the mean age was 9 years (sd 3.3 years).

Statistics

Differences in mean intake of essential amino acids between the protein restricted

group and the control group, and differences in the median levels of plasma amino acids between the protein restricted group, the control group and the healthy children were evaluated by the Mann - Whitney test. The correlations between the amino acid levels in plasma and the calculated creatinine clearance were computed using regression analysis after logarithmic transformation of the amino acid levels as the latter had skewed distributions. Five percent was considered the limit of significance.

5.3 Results

Mean (standard deviation) protein intake according to the dietary diaries of the protein restricted group was 0.94 (0.13) g/kg/day corresponding with 98 % of the advised amount of protein. Mean (standard deviation) protein intake of the control group was 1.98 (0.54) g/kg/day which is 113 % of the advised protein intake. The percentage protein of animal origin was significantly higher in the control group (69 %) as opposed to the protein restricted group (50 %). Caloric intake of both groups was adequate : 92 % of energy requirement for the protein restricted group and 105 % for the control group, and did not differ significantly. Although the intake of all essential amino acids was significantly lower in the protein restricted group compared to the control group (figure 1), all mean amino acid intakes of the protein restricted group were well above 100 % of minimal daily requirement (WHO 1985). Mean intake of methionine / cystine was lowest of all amino acid intakes, but was still 142 % of the minimal daily requirement. Table 2 shows plasma amino acid levels of the children in the protein restricted group, the control group and the healthy children. No amino acids were significantly lower in the protein restricted group compared to the control group, whereas three plasma amino acids showed significantly higher median levels in the protein restricted group opposed to the control group: serine, glycine and arginine. The protein restricted group had significantly lower values for plasma valine, serine, asparagine and tyrosine, while glycine, citrulline and arginine were significantly higher compared to the healthy children. Likewise did the control group have a significantly lower plasma serine, asparagine and tyrosine, while citrulline was significantly higher compared to the healthy children. One amino acid showed a significant but weak correlation with the calculated creatinine clearance: citrulline ($r = -0.52$, $p=0.002$).

essential amino acids	protein restricted group		control group		p 1	healthy children		p 2	p 3
	median	range	median	range		median	range		
leucine	0.08	0.04-0.16	0.10	0.05-0.21	ns	0.09	0.06-0.12	ns	ns
isoleucine	0.04	0.02-0.22	0.05	0.02-0.11	ns	0.05	0.03-0.06	ns	ns
valine	0.16	0.02-0.22	0.16	0.01-0.32	ns	0.02	0.14-0.26	<0.05	ns
phenylalanine	0.05	0.03-0.11	0.04	0.03-0.09	ns	0.05	0.03-0.07	ns	ns
methionine	0.02	0.01-0.08	0.02	0.01-0.04	ns	0.02	0.00-0.03	ns	ns
threonine	0.08	0.05-0.16	0.09	0.06-0.17	ns	0.10	0.07-0.15	ns	ns
lysine	0.16	0.06-0.27	0.13	0.08-0.25	ns	0.14	0.09-0.15	ns	ns
histidine	0.07	0.03-0.12	0.07	0.04-0.10	ns	0.06	0.04-0.13	ns	ns
non essential amino acids									
serine	0.09	0.06-0.18	0.08	0.03-0.14	<0.01	0.12	0.08-0.19	<0.001	<0.001
asparagine	0.05	0.02-0.10	0.06	0.04-0.11	ns	0.08	0.05-0.11	<0.001	<0.001
glutamate	0.05	0.01-0.69	0.06	0.02-0.60	ns	0.06	0.01-0.17	ns	ns
glutamine	0.55	0.39-0.97	0.54	0.32-0.63	ns	0.53	0.30-0.58	ns	ns
glycine	0.36	0.20-0.67	0.28	0.14-0.56	<0.0	0.24	0.14-0.43	<0.001	ns
alanine	0.32	0.21-0.64	0.30	0.12-0.57	ns	0.28	0.18-0.56	ns	ns
citrulline	0.06	0.01-0.10	0.06	0.03-0.08	ns	0.03	0.01-0.04	<0.001	<0.001
tyrosine	0.04	0.02-0.06	0.04	0.02-0.10	ns	0.06	0.04-0.08	<0.001	<0.001
ornithine	0.07	0.03-0.10	0.05	0.03-0.11	ns	0.06	0.03-0.10	ns	ns
arginine	0.08	0.06-0.12	0.07	0.03-0.10	<0.05	0.06	0.02-0.10	=0.01	ns

Table 2. Amino acid levels in plasma (μmol/ml) of the children in the protein restricted group, the control group and the healthy children. p values are given if there is a significant difference of the median amino acid levels in plasma between children in the protein restricted group and the control group and of both groups compared to the healthy children. p 1:protein restricted group versus control group; p 2: protein restricted group versus healthy children; p 3: control group versus healthy children; ns: not significant.

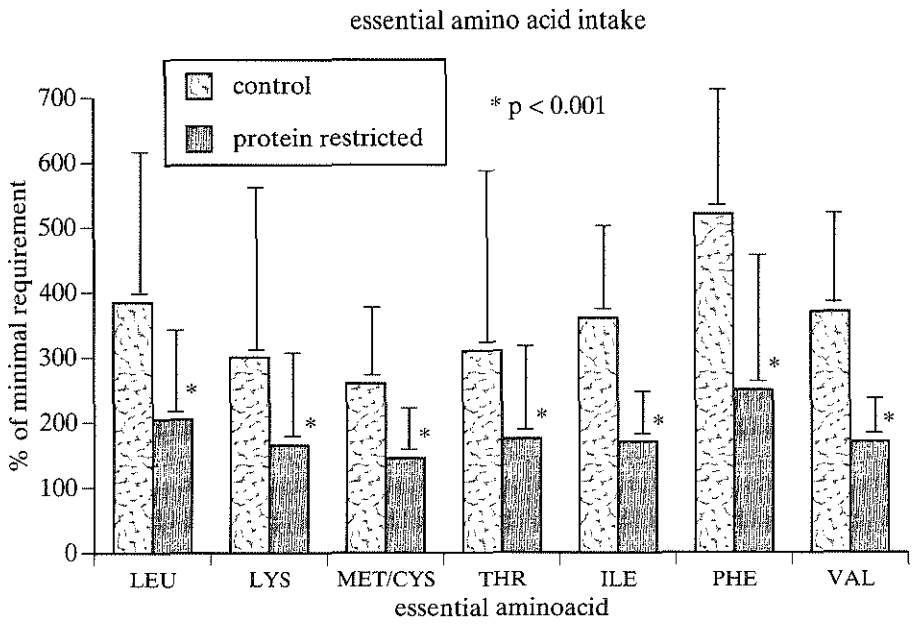

Figure 1.
*Intake of essential amino acids according to the prospective dietary diaries and expressed as a percentage of the minimal daily requirement (WHO 1985). *p<0.001; I standard deviation.*

5.4 Discussion

As growth is of utmost importance for children, a protein restricted diet given in order to try to preserve renal function must never lead to diminished growth. Although the protein restricted group consumed significantly less essential amino acids compared to the control group, the mean level of each essential amino acid was well above the minimal daily requirement (WHO 1985). As the children who were allocated to the protein restricted group were prescribed the safe levels for protein intake as designed by the World Health Organization, we were not surprised to find that the essential amino acids were adequately supplied in the diet.

Published values for plasma amino acids of children with chronic renal failure are scarce and concern only small numbers (Broyer 1980, Jones 1983, Canepa 1992). The results of our study indicate that there is no important difference in essential amino acids in plasma between the protein restricted and the control group. There were however three non essential amino acids (serine, glycine and asparagine) with a significantly higher

plasma level in the protein restricted group compared to the control group. Various alterations of plasma amino acids in chronic renal failure have been described. Plasma leucine, isoleucine and valine are often found to be low. Leucine, isoleucine and valine play a central role as precursors for synthesis of proteins, fatty acids, metabolic fuel, regulators of protein turnover and insulin release (Abidi 1978). They are highly interrelated probably because they have a mutual dependency on protein intake and catabolism of muscle protein and are degraded principally in muscle by a single transaminase (Young 1982). Low tyrosine has also been a "constant" finding in renal failure and can be attributed to decreased activity of phenylalanine hydroxylase in the kidney caused by renal impairment (Young 1982). This also explains the low tyrosine / phenylalanine ratio often found in chronic renal failure (Fürst 1978, Broyer 1980, Jones 1983, Rosman 1990). We confirmed the low tyrosine / phenylalanine ratio in children. Broyer (Broyer 1980) studied 20 children with chronic renal failure (creatinine clearance 5 - 70 ml/min/1.73m^2). A diet (allowing 100 % of the protein recommended daily allowance for statural age) was prescribed if creatinine clearance fell below 20 ml/min/1.73m^2. He found significantly lower values for plasma leucine, isoleucine, valine, phenylalanine, threonine and histidine compared to healthy children. Jones (Jones 1983) studied seven children with endstage renal disease (creatinine clearance 6 - 13 ml/min/1.73m^2). The children consumed 110 % of the minimum requirement for protein for height age, 20 % of this being given as essential amino acids. He found significantly lower plasma amino acid levels of leucine, valine, threonine and lysine. Canepa (Canepa 1992) studied fifteen children with endstage renal disease (creatinine clearance < 5 ml/min/1.73m^2). The children were prescribed a protein restricted diet containing 75 % of the recommended daily allowance for protein intake. He found significantly lower values for plasma leucine, isoleucine and valine compared to healthy children. Rosman (Rosman 1990) performed a prospective randomized study in adults with chronic renal failure (creatinine clearance 10 - 60 ml/min/1.73m^2). The 109 patients were randomly allocated to a protein restricted group (0.4-0.6 g/kg/day) or a control group (no protein restriction). He found significantly lower values for plasma phenylalanine, tyrosine, threonine and serine, whereas plasma alanine was significantly higher in the protein restricted group compared to the control group.

We found that of the essential plasma amino acids, only valine was significantly lower in the protein restricted group compared to the healthy children. Of the non essential amino acids some were significantly higher and some were lower.

Table 3 shows the results of our study compared to other studies (Broyer 1980, Jones 1983, Rosman 1990, Canepa 1992).

	this study	Broyer	Jones	Canepa	Rosman
essential amino acid					
leucine	=	<	<	<	<
isoleucine	=	<	=	<	<
valine	=/<	<	<	<	<
phenylalanine	=	<	=	=	=
threonine	=	<	<	=	<
methionine	=	=	=	=	=
lysine	=	=	<	=	<
histidine	=	>	=	=	=
non essential amino acid					
serine	<	<	<	<	<
asparagine	<	=	na	=	na
glutamate	=	=	na	=	na
glutamine	=	=/>	na	=	na
glycine	>/=	=/<	=	=	>
alanine	=	<	=	=	>
tyrosine	<	<	<	<	<
cystine	na	=	=	na	na
ornithine	=	=/>	=	=	>
arginine	>/=	=/>	=	=	<
citrulline	>	>	>	>	na
tyrosine/ phenylalanine	<	<	<	<	<
number of patients	50	20	7	15	109
age (years)	3-18	2-14	5-12	1-15	adults
protein intake (g/kg/day)	0.8-2.5	0 .8-1.5	1.1	1.1	0.4-0.6
creatinine clearance (ml/min/1.73m^2)	9-67	5-70	6-13	<5	10-60

Table 3.
Studies of plasma amino acids of patients with chronic renal failure compared to healthy children or adults. The plasma amino acid level of the patients with chronic renal failure is significantly lower than (<), does not differ significantly from (=) or is significantly higher (>) than that of the healthy children or adults; na: not available.(Broyer 1980, Jones 1983, Canepa 1992, Rosman 1990).

The essential amino acids in plasma are generally found to be lower for children and adults with chronic renal failure compared to healthy subjects. Except for valine we did not find such a difference. Many of the plasma amino acid abnormalities in uremia bear similarities to the findings in malnutrition and it has been suggested that they are attributable to dietary inadequacy. In our study the children were not malnourished, this is confirmed by a mean weight for height percentile of 98 % in the protein restricted group and 99 % in the control group and might explain why we did not find significant differences between the children with chronic renal failure and the healthy children. Young (1982) found in 17 adult patients all receiving intermittent hemodialysis that plasma valine, isoleucine, leucine, threonine, asparagine, weight and arm muscle circumference were interrelated and reflected malnutrition whereas body fat correlated with caloric intake, and histidine and serine with protein intake. Rosman found no difference when comparing plasma amino acid concentrations in two subdivisions of a large number of adults with chronic renal failure (n = 109) who had more (n=25) or less (n=84) than two kilograms weight loss over a median period of 42 months (Rosman 1990).

There are indications that plasma amino acids are of limited value for assessing the total pool of the free amino acids (Fürst 1978). We therefore studied amino acids in granulocytes as a model for intracellular amino acids as described by Metcoff (1989). We report on amino acids in granulocytes in chapter 6.

In conclusion the protein restricted diet used in this study which corresponds to the safe level of protein intake for healthy children also seems to be safe for children with chronic renal failure. Supplementation of this protein restricted diet (0.8-1.1 g/kg/day) with essential amino acids can therefore not be supported by this study.

Chapter 6

Amino acids in granulocytes of children with chronic renal failure

6.1 Introduction

It is well known that children and adults with chronic renal failure may have abnormal plasma and intracellular amino acid levels (Counahan 1976, Delaporte 1978, Fürst 1978, Broyer 1980, Jones 1983, Metcoff 1989, Canepa 1989, Canepa 1991, Canepa 1992). The plasma concentrations do not always reflect the intracellular concentrations. The abnormalities are attributed to an altered amino acid metabolism and malnutrition. The consequences of these amino acid abnormalities are still unclear. Cellular malnutrition in children with chronic renal failure may contribute to their poor growth. Information about extra- and intracellular amino acid composition may lead to a better understanding of the amino acid metabolism and its possible relation to growth of children with chronic renal failure. The question arises whether supplementation with some essential amino acids is necessary. Data of only a small number of patients on amino acids isolated from muscle tissue in children with chronic renal failure are available as it requires the invasive procedure of a muscle biopsy (Delaporte 1978, Broyer 1980, Metcoff 1989, Canepa 1992). However, the isolation of granulocytes, as described by Metcoff, requires only 10 ml of blood and is a suitable cell model to elucidate alterations of intracellular metabolism in human subjects with a variety of conditions (Metcoff 1983, Metcoff 1989). We studied twenty-four children with chronic renal failure who participated in a ongoing prospective randomized trial evaluating the effect of a protein restricted diet on renal function and growth. In the present report we describe the amino acid levels in the granulocytes and in plasma of children with chronic renal failure and compare them to the values found in a group of fifteen healthy children of a similar age. Furthermore we studied the relation between the amino acid levels in granulocytes and in plasma and protein intake, renal function and growth of the children during the last six months prior to blood sampling.

6.2 Patients and methods

Twenty-four children, from the University Hospital Nijmegen and Sophia Children's Hospital Rotterdam, with chronic renal failure who participated in a prospective randomized study on the effect of a protein restricted diet on renal function and growth were studied. All patients were in a stable condition without concurrent illnesses. Table 1 shows the characteristics of the patients with chronic renal failure.

characteristics of the patients	protein restricted group	control group
number of patients	10	14
age (years)	12.7 (5.2)	10.5 (4.1)
gender (boys,girls)	7 / 3	10 / 4
diagnosis (1,2,3)*	2 / 7 / 1	0 / 10 / 4
weight for height (%)	108 (25)	100 (14)
height standard deviation	-1.58 (1.1)	-1.12 (1.1)
calculated creatinine clearance (ml/min/1.72m^2)	37.3 (15.9)	36.1 (14.2)

Table 1. Characteristics of the patients at time of bloodsampling for amino acids in granulocytes and in plasma. Data given are numbers of patients or means (standard deviation). * Diagnosis 1: glomerulopathy, 2: obstructive uropathy and / or renal dysplasia, 3: miscellaneous.

The children were for at least eighteen months on a protein restricted diet or a diet without restrictions. Intake of essential amino acids was derived from Souci-Fachmann-Kraut (1986) and compared to the minimal daily requirements of amino acids for children (WHO 1985).

The blood was drawn for granulocyte isolation after an overnight fast. Weight, height, upper arm circumference and triceps skinfold of the children were measured by the same observer six months prior to and on the day blood was drawn for amino acid analysis. As we were especially interested in the growth of the children during the last

six months, the change (Δ) in weight for height was computed as weight for height at the time of blood sampling minus weight for height six months prior to blood sampling. The same procedure was used to assess Δ height standard deviation score, Δ upper arm circumference and Δ triceps skinfold. Granulocytes were isolated using a dextran - ficoll - hypaque sedimentation procedure as described by Metcoff (1983). The leucocyte suspensions contained 83 - 100 % granulocytes (mean 96 %) with a mean viability of 94 % (range 86-99 %). Amino acids were measured by liquid column chromatography on a LKBα+ (Pharmacia, Sweden) amino acid analyzer. Amino acids in the granulocytes are expressed as μmol/g protein, using albumin as a standard (Lowry method) as the protein content of cells of children with chronic renal failure on a well balanced diet does not differ from healthy children (Lowry 1951, Canepa 1992). The plasma amino acids are expressed as μmol/ml, both are given as the median and their range. The levels of amino acids in granulocytes and plasma of the children with chronic renal failure were compared to those of fifteen healthy children (seven girls and eight boys) with a similar age distribution: the mean age was 9 years (sd 3.3 years).

Statistics

Differences in mean intake of essential amino acids between the protein restricted and the control group, and differences in the median levels of amino acids in granulocytes and plasma between the protein restricted group and the control group versus the healthy children were evaluated by the Mann - Whitney test. The correlations between amino acid levels in granulocytes and plasma on one hand, and protein intake, glomerular filtration rate, Δ weight for height percentage, Δ height standard deviation score, Δ upper arm circumference and Δ triceps skinfold on the other hand were computed using regression analysis after logarithmic transformation of amino acid levels (or the level + 1 in case of occasional values of zero) as the latter had skewed distributions. Five percent was considered the limit of significance.

6.3 Results

Mean (sd) protein intake of the protein restricted group was 1.1 (0.4) g / kg / day corresponding with 113 % of the safe levels for protein intake. Mean (sd) protein intake of the group with no dietary restriction was 2.0 (0.6) g / kg / day which is 205 % of the safe levels for protein intake. Although caloric intake of both groups was adequate: 91 % of the energy requirement for the protein restricted group and 110 % for the control group, it did differ significantly ($p < 0.05$). All mean amino acid intakes of the protein restricted group were well above 100 % of minimal daily requirement. The intake of all essential amino acids was significantly lower in the protein restricted group

compared to the control group (figure 1). Mean intake of valine was lowest of all amino acid intakes in the protein restricted group, but was still 153 % (sd 74 %). There was no significant correlation between the intake of any of the essential amino acids on one hand with the amino acid levels in the granulocytes or with the amino acid levels in the plasma on the other hand.

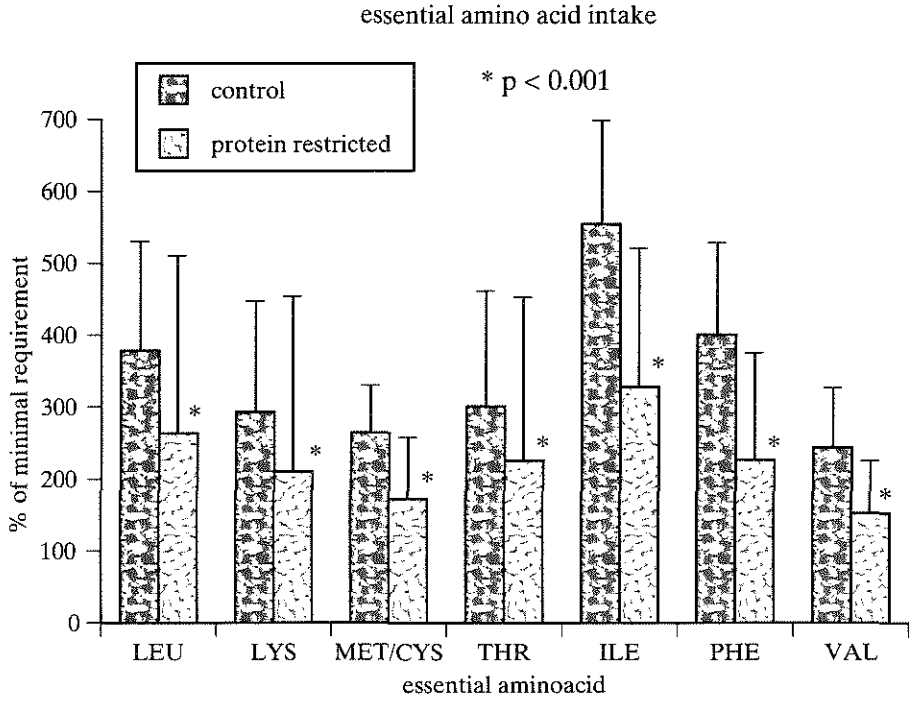

*Figure 1. Mean intake of essential amino acids of the protein restricted and the control group, as a percentage of the minimal daily requirement. * p < 0.05; 1 standard deviation*

Table 2 shows the amino acids levels in the granulocytes for the protein restricted group, the control group and the healthy children. We found a wide range of amino acid levels in the granulocytes of the children with chronic renal failure as well as of the healthy children. There was no significant difference between the protein restricted group and the control group. However both groups of children with chronic renal failure (the

protein restricted and the control group) had significantly lower median intracellular values for leucine, isoleucine, phenylalanine, methionine, lysine, histidine, tyrosine and arginine compared to healthy children.
Table 3 shows the amino acid levels in plasma of the children in the protein restricted group, the control group and the healthy children. In comparing the protein restricted group with the control group it appeared that in plasma only glycine was significantly higher ($p < 0.05$) in the protein restricted group. Such a single positive outcome is likely to be a false positive finding in view of the multitude of parameters tested. The children with the protein restricted diet had significantly higher median plasma levels of glycine and citrulline and significantly lower median levels of serine and asparagine compared to healthy children. The children from the control group had significantly higher median plasma values for citrulline and arginine, while only serine was significantly lower compared to healthy children.
Four amino acids (phenylalanine, asparagine, glycine and ornithine) had a significant negative correlation between its level in the granulocyte and in plasma. We found a significant correlation between glomerular filtration rate and plasma valine ($r = 0.52$, $p = 0.01$) and arginine ($r = 0.41$, $p < 0.05$). We found a significant correlation between seven amino acids isolated from the granulocytes and five plasma amino acids with the change (Δ) in weight for height in the preceding six months. In addition there was a significant correlation of four plasma amino acids with Δ height standard deviation score (table 4). The amino acids from the granulocytes had positive correlations, while the plasma amino acids with the exception of one had negative correlations (table 4). Two plasma amino acids had a negative correlation with Δ upper arm circumference, while there were no significant correlations with any of the intracellular amino acids. With respect to Δ triceps skinfold four amino acids from the granulocytes had a negative correlation (table 4).

6.4 Discussion

There are firm indications that plasma amino acids are of limited value for assessing the total pool of amino acids as they are known to mirror recent exogenous intake, as well as uptake, synthesis and export by cells (Fürst 1978, Metcoff 1989).
Granulocytes are interesting because the relatively short half-life and rapid turnover (from differentiation in the bone marrow, through extrusion into the blood and survival in the circulation about seven days) implies that activities in their metabolic pathways, e.g. protein synthesis or energy production, reflect the contemporaneous availability of nutrient substrates in their microenvironment.

essential aminoacids	protein restricted group	control group	p 1	healthy children	p 2	p 3
	median range	median range		median range		
leucine	6.57 1.30-74.67	6.52 1.19-44.26	ns	35.71 7.38-126.7	<0.05	=0.001
isoleucine	2.70 0.37-37.33	2.97 0.61-22.13	ns	17.14 4.42-55.25	<0.05	<0.001
valine	4.55 0.74-34.67	5.56 0.76-22.95	ns	11.11 0.78-73.28	ns	ns
phenylalanine	2.86 0.41-32.00	5.14 0.01-33.61	ns	9.62 3.69-60.77	<0.05	<0.05
methionine	2.19 0.01-21.33	2.42 0.01-12.29	ns	10.71 2.72-88.89	<0.05	<0.01
threonine	4.73 1.66-26.67	3.33 1.21-18.85	ns	9.62 0.75-68.97	ns	ns
lysine	7.71 0.78-58.67	5.23 1.52-22.42	ns	22.22 3.76-127.6	<0.05	=0.001
histidine	0.23 0.01-16.00	0.45 0.01-14.75	ns	1.22 0.01-17.24	ns	<0.05
non essential aminoacids						
serine	5.87 1.85-34.67	4.83 1.91-23.77	ns	9.06 1.10-81.03	ns	ns
asparagine	1.67 0.01-10.67	1.13 0.01- 5.74	ns	2.87 0.01-43.10	ns	ns
glutamate	25.48 0.01-34.66	17.13 6.90-71.31	ns	14.85 2.18-86.21	ns	ns
glutamine	5.72 3.69-21.19	5.04 0.01-15.57	ns	8.67 0.01-43.38	ns	ns
proline	0.28 0.01-10.66	2.24 0.01-12.64	ns	na		
glycine	12.11 5.33-32.00	7.80 4.60-37.70	ns	11.11 0.95-78.45	ns	ns
alanine	10.05 2.47-61.33	8.22 2.43-42.62	ns	16.24 1.79-146.6	ns	ns
citrulline	0.42 0.01- 2.67	0.36 0.01- 2.46	ns	na		
tyrosine	3.25 0.93-24.00	2.17 0.30-22.13	ns	9.58 2.33-43.97	<0.05	<0.01
ornithine	2.19 0.56- 5.33	2.44 0.80- 7.38	ns	2.22 0.01-17.24	ns	ns
arginine	4.08 0.60-32.00	2.89 0.46-13.93	ns	11.39 3.57-60.34	<0.01	<0.01

Table 2. Amino acid levels in granulocytes (µmol/g protein) for the protein restricted group, the control group and the healthy children. p values are given if there is a significant difference between the median amino acid level in the granulocytes between children in the protein restricted group and the control group, and of both groups compared to the healthy children. p1 protein restricted group versus control group; p2 protein restricted group versus healthy children; p3: control group versus healthy children; na: not available; ns: not significant.

essential aminoacids	protein restricted group		control group		p 1	healthy children		p 2	p 3
	median	range	median	range		median	range		
leucine	0.09	0.07-0.15	0.09	0.05-0.25	ns	0.09	0.06-0.12	ns	ns
isoleucine	0.05	0.04-0.10	0.05	0.03-0.11	ns	0.05	0.03-0.06	ns	ns
valine	0.17	0.12-0.23	0.16	0.09-0.33	ns	0.20	0.14-0.26	ns	ns
phenylalanine	0.05	0.04-0.09	0.05	0.03-0.09	ns	0.05	0.03-0.07	ns	ns
methionine	0.02	0.01-0.03	0.02	0.01-0.05	ns	0.02	0.01-0.03	ns	ns
threonine	0.10	0.06-0.13	0.09	0.06-0.20	ns	0.10	0.07-0.15	ns	ns
lysine	0.13	0.10-0.16	0.13	0.10-0.27	ns	0.14	0.09-0.15	ns	ns
histidine	0.06	0.05-0.09	0.07	0.04-0.10	ns	0.06	0.04-0.13	ns	ns
non essential amino acids									
serine	0.09	0.07-0.17	0.08	0.02-0.14	ns	0.12	0.08-0.19	<0.05	<0.001
asparagine	0.07	0.05-0.09	0.06	0.04-0.13	ns	0.08	0.05-0.11	<0.05	ns
glutamate	0.07	0.02-0.19	0.06	0.02-0.18	ns	0.06	0.01-0.17	ns	ns
glutamine	0.52	0.30-0.66	0.49	0.24-0.66	ns	0.53	0.30-0.58	ns	ns
proline	0.25	0.20-0.52	0.28	0.12-0.51	ns		na		
glycine	0.32	0.17-0.69	0.27	0.16-0.38	<0.05	0.24	0.14-0.43	<0.05	ns
alanine	0.33	0.17-0.82	0.31	0.18-0.53	ns	0.28	0.18-0.56	ns	ns
citrulline	0.06	0.04-0.10	0.06	0.02-0.10	ns	0.03	0.01-0.04	<0.001	<0.001
tyrosine	0.04	0.03-0.09	0.05	0.03-0.13	ns	0.06	0.04-0.08	ns	ns
ornithine	0.05	0.04-0.10	0.05	0.03-0.07	ns	0.06	0.03-0.15	ns	ns
arginine	0.07	0.07-0.10	0.08	0.03-0.12	ns	0.06	0.02-0.10	ns	<0.05

Table 3. Amino acid levels in plasma (μmol/ml) for the protein restricted group, the control group and the healthy children. p values are given if there is a significant difference between the median amino acid level in the granulocytes between children in the protein restricted group and the control group, and of both groups compared to the healthy children. p1 protein restricted group versus control group; p2 protein restricted group versus healthy children; p3: control group versus healthy children; na: not available; ns: not significant.

parameter	granulocyte amino acid	p	r	plasma amino acid	p	r
Δ weight for height	leucine	<0.05	0.48	phenylalanine	<0.01	-0.57
	isoleucine	<0.05	0.48	proline	<0.05	-0.59
	valine	<0.01	0.55	glycine	<0.05	-0.47
	methionine	<0.05	0.47	alanine	<0.05	-0.50
	lysine	<0.05	0.47	ornithine	<0.05	-0.45
	ornithine	<0.05	0.48			
	arginine	<0.05	0.44			
Δ height SDS				glutamate	<0.001	0.66
				glutamine	<0.05	-0.52
				citrulline	<0.01	-0.55
				arginine	<0.05	-0.48
Δ upper arm circum				phenylalanine	<0.05	-0.43
				ornithine	<0.05	-0.51
Δ triceps skinfold	histidine	<0.05	-0.45			
	asparagine	<0.05	-0.45			
	glycine	<0.05	-0.50			
	citrulline	<0.05	-0.52			

Table 4. Correlations between - amino acids in granulocytes and in plasma - and the change in (Δ) weight for height, height standard deviation score, upper arm circumfe rence, triceps skinfold thickness of the children with chronic renal failure. Of 19 amino acids tested in each group only significant correlations are presented. p: level of significance; r: correlation coefficient; height SDS: height standard deviation score.

Furthermore the impaired host immunity associated with uremia is mainly caused by functional and metabolic disturbances of the granulocytes. These abnormalities include increased intracellular calcium concentration caused by elevated blood levels of parathyroid hormone, iron overload, zinc deficiency and undefined and well-characterized circulating plasma factors (Haag-Weber 1993).
Contrary to a muscle biopsy, isolation of granulocytes is non invasive and therefore an elegant method to obtain intracellular levels of amino acids. Published values of amino

acids in the granulocytes in children with renal failure are scarce and concern only ten patients on hemodialysis and eight on CAPD (continuous ambulatory peritoneal dialysis) (Canepa 1989, Canepa 1991). There is no data on patients with chronic renal failure. Although exact comparison of the results is not possible due to different laboratory techniques and ways of expressing the data, the trend is the same: most essential amino acids in the granulocytes (leucine, isoleucine, valine, phenylalanine, methionine, lysine) are significantly lower compared to healthy subjects.

Amino acids measured in muscle cells of children with chronic renal failure are expressed per 100 g wet tissue or per liter intracellular water which also makes comparison difficult (Delaporte 1978, Broyer 1980, Canepa 1992). Broyer found decreased levels in muscle cells of valine and alanine and increased levels of glutamine in twenty children with chronic renal failure compared to healthy children (Broyer 1980). Delaporte found that muscle pools of essential and non essential amino acids were increased in eight children with chronic renal failure (Delaporte 1978). Canepa found lower levels of valine and isoleucine in muscle cells of fifteen children with endstage renal disease compared to healthy children, these results are comparable to our findings in granulocytes. The reason why the intracellular branched-chain amino acids (leucine, isoleucine and valine) are low in uremia is still unclear. These amino acids are mainly metabolized in muscle by deamination to their ketoanalogues. This is followed by oxidation which is mediated via a common branched-chain keto acid dehydrogenase complex which is rate limiting for branched-chain amino acid degradation. In normal and uremic rats acidosis appears to enhance protein degradation in muscle, an effect which is thought to be due to stimulation of branched-chain amino acid decarboxilation and thereby to cause depletion of the branched-chain amino acids (May 1986, May 1987). The children in our study with chronic renal failure had no metabolic acidosis.

Some of the plasma and intracellular amino acid abnormalities in uraemia bear similarities to the findings in malnutrition and it has been suggested that they are in part attributable to dietary inadequacy. The children in our study were not malnourished; this is confirmed by the adequate caloric intake and a mean weight for height of 102 %. Although the protein restricted group in our study consumed significantly less essential amino acids compared to the control group, the mean intake of each essential amino acid was well above the minimal daily requirement. The results of our study indicate that there is no important difference in intracellular and plasma amino acids between children on a well balanced protein restricted diet and children with a normal protein intake. Supplementation of this protein restricted diet (0.8-1.1 g / kg / day) with essential amino acids can therefore not be supported by this study.

The median levels of eight of the amino acids isolated from granulocytes were found to be significantly lower in children from the protein restricted and the control group compared to healthy children. This might suggest cellular malnutrition in children with chronic renal failure.

We found a correlation between the change in weight for height in the preceding six months in seven out of nineteen intracellular amino acids and five plasma amino acids. The intracellular amino acids had a positive correlation while the plasma amino acids had a negative correlation suggesting that relatively high intracellular amino acid levels and relatively low extracellular levels may contribute to better growth of these children.

The explanation of the low intracellular amino acid concentration in granulocytes from patients with renal failure, is uncertain. Similar results were reported by Metcoff in children on CAPD (Metcoff 1989). A reduced protein synthesis was demonstrated in the granulocytes of these children. An altered transport of amino acids into the granulocytes and intracellular metabolism of these amino acids in the granulocytes resulting in an abnormal pattern of intracellular protein, both could contribute to the lowered intracellular amino acid concentration. These hypotheses require further study.

Our results are not in agreement with the study of Broyer, who found that a poor growth rate was generally, except for valine, associated with higher muscle amino acids (Broyer 1980).

The protein restricted diet used in this study which corresponds with the safe level for protein intake for healthy children also seems to be safe for children with chronic renal failure because there was no effect of protein restriction on intracellular and plasma amino acids.

Chapter 7

Protein restriction affects fat intake and serum lipids in children with chronic renal failure

7.1 Introduction

Hyperlipidemia is frequently observed in adult (Brunzell 1977, Bagdade 1978) and pediatric patients (Penninsi 1976, Querfield 1988) with end stage renal disease and has been proposed as one of the main risk factors associated with the high prevalence of cardiovascular complications in adult patients on maintenance dialysis (Bagdade 1978). Conditions associated with high levels of low density lipoprotein cholesterol (LDL cholesterol) are associated with increased atherosclerosis (Council of the Cardiovascular Diseases in the Young and the Nutrition Committee 1986). In addition there is an inverse relationship between circulating high density lipoprotein (HDL) cholesterol and coronary heart disease, suggesting that the type of transporting lipoproteins, rather than the concentrations of the circulating lipids determines the rate of development of atherosclerosis (Mitch 1985). Finally diseases characterized by increased plasma triglyceride levels also seem to have a higher incidence of atherosclerosis (Council of the Cardiovascular Diseases and the Nutrition Committee 1986).

Patients with chronic renal failure are often prescribed a protein restricted diet in order to preserve renal function (Brenner 1982). We studied the baseline fat intake of children with chronic renal failure and the effect of the institution of a protein restricted diet on the fat intake and the resulting plasma lipid levels.

7.2 Patients and methods

Forty-eight children with chronic renal failure who participated in a prospective randomized study on the effect of a protein restricted diet on renal function and growth were studied. Age at the first blood sampling for the present study varied from two years and six months to eighteen years and eight months (mean age was ten years and eight months). There were thirty-six boys and twelve girls. Mean (standard deviation)

calculated creatinine clearance (40 x length (cm) / creatinine (μmol/l)) was 39 (16) ml/min/1.73m^2, range 9 - 69 ml/min/1.73m^2. The diagnoses were: glomerulonephritis 6; obstructive uropathy, reflux nephropathy and or renal dysplasia 31; miscellaneous 11. Table 1 shows the characteristics of the protein restricted and the control group.

patient characteristics	protein restricted group	control group
number of patients	23	25
age (years)	10.9 (5.0)	10.4 (4.4)
gender (boys, girls)	17 , 6	19 , 6
diagnosis (1, 2, 3)*	3, 16, 4	3, 16, 6
weight for height (%)	101 (16)	98 (13)
height standard deviation	-1.28 (1.70)	-1.31 (1.00)
calculated creatinine clearance (ml/min/1.73m^2)	40 (17)	37 (16)

Table 1. Characteristics at the time of first blood sampling of the protein restricted and control group expressed as mean values (standard deviation). * Diagnosis 1: glomerulopathy, 2: reflux nephropathy, obstructive uropathy and/or dysplasia, 3: miscellaneous.

Fat and cholesterol intake and the polyunsaturated fat / saturated fat ratio (p/s ratio) were calculated using the Dutch Nutrition Index (Dutch Nutrition Index 1987).
Blood was drawn in the fasting state for serum lipids on two occasions. The first time was at a mean of 11.4 months (range 3-15 months) after randomization, and the second time was twelve months later. Serum total cholesterol was measured with an enzymatic reagent kit (Boehringer Mannheim) calibrated with a human calibrator serum to ensure accurate results (Kattermann 1984). The HDL fraction was isolated by precipitating apo-B-containing lipoproteins with a 4 % tungstate solution with Mg^{2+} ions present, followed by centrifugation. HDL cholesterol was then quantitated in the supernatant liquid (Warnick 1985). The laboratory is standardized in the CDC/NHBLI lipid standardization program. Triglycerides were measured with an enzymatic reagent kit (first measurement: Boehringer Mannheim, second measurement: Technicon). Both

assays were calibrated with the same human calibrator serum (Stein 1989). VLDL cholesterol and LDL cholesterol were calculated with the Friedewald equation (Friedewald 1972). The values obtained in our patients were compared to values of healthy Dutch children for serum cholesterol (3.6 - 6.3 mmol/l) and HDL cholesterol (0.7 - 2.0 mmol/l) (van Stiphout 1985). Serum triglycerides, VLDL and LDL cholesterol concentrations were compared to values for healthy adults in the absence of reference values for children: 0.3 - 1.6 mmol/l, 0.13 - 0.48 mmol/l and < 3.5 mmol/l respectively (adult reference ranges measured in 100 healthy blooddonors with the same laboratory methods). This comparison was valid because of the standardized laboratory methodology (Myers 1989).

Statistics

Differences in mean levels between groups were assessed for statistical significance using the Mann-Whitney test. Wilcoxon 's test was used to evaluate differences between the first and second measurement within the groups. Five percent was considered the limit of significance.

7.3 Results

According to the dietary diaries mean (standard deviation) protein intake in the protein restricted group was 0.94 (0.13) g/kg/day corresponding to 98 % of the advised amount of protein. Mean (sd) protein intake in the control group was 1.98 (0.54) g/kg/day which is 113 % of the advised protein intake. The caloric intake of the protein restricted group was 97 (24) % of energy requirement as advised by the World Health Organization and 106 (27) % for the control group, and did not differ significantly. The intake of carbohydrates expressed as percentage of caloric intake did not differ significantly between the protein restricted and the control group: 59 (9.8) % for the protein restricted group and 52 (8.3) % for the control group. Fat intake expressed as percentage of total caloric intake did not differ significantly between the protein restricted and the control group, and resembles the fat intake as advised for Dutch children (maximal 30-35%) (Ministries of Health and Agriculture 1988) (figure 1 and table 2). Although the cholesterol intake (mg/MJ) was significantly lower in the protein restricted group opposed to the control group, it was within advised limits (figure 1 and table 2). The p/s ratio was significantly higher in the protein restricted group compared to the control group on both occasions (table 2). The recommended p/s ratio of 0.5 - 1 is only attained by the protein restricted group. The children in the protein restricted group have a significantly higher p/s ratio at the second measurement compared to the first assessment, one year earlier.

	sample	control group	protein restricted group	recommended[1]	significance of difference between groups
diet					
calorie, % of	1	106 (27)	97 (24)	100	ns
recommended by WHO	2	105 (18)	95(21)		ns
carbohydrate, % of caloric	1	52 (8)	59 (10)	55	ns
intake	2	53 (8)	53 (7)		ns
fat,% of caloric intake	1	37 (7)	35(7)	30-35	ns
	2	37 (7)	38 (6)		ns
cholesterol, mg/MJ	1	25 (8)	17 (6)	<33	<0.001
	2	24 (8)	15 (5)		<0.001
p/s ratio	1	0.36 (0.19)	0.51 (0.20)	0.5-1.0	=0.02
	2	0.35 (0.12)	0.78* (0.46)		<0.01
serum					
cholesterol, mmol/l	1	4.52 (0.60)	4.48 (0.95)	3.6-6.3	ns
	2	4.77* (0.81)	4.51 (0.70)		ns
triglycerides, mmol/l	1	1.38 (0.88)	1.62 (0.76)	0.3-1.6	ns
	2	1.37 (0.73)	1.71 (1.12)		ns
HDL cholesterol, mmol/l	1	1.25 (0.39)	1.20 (0.55)	0.7-2.0	ns
	2	1.20 (0.35)	1.09 (0.36)		ns
LDL cholesterol, mmol/l	1	2.63 (0.54)	2.66 (0.79)	<3.5	ns
	2	2.93* (0.60)	2.61 (0.70)		ns
VLDL cholesterol, mmol/l	1	0.62 (0.39)	0.73 (0.34)	0.13-0.48	ns
	2	0.62 (0.33)	0.77 (0.50)		ns

Table 2. Means (SD) of food intake and of serum lipids. * $p<0.05$, second value is higher than the first. Sample 1 = first measurement; sample 2 = second measurement.
[1] Recommended food intake for Dutch children, serum values of healthy Dutch children and adults.

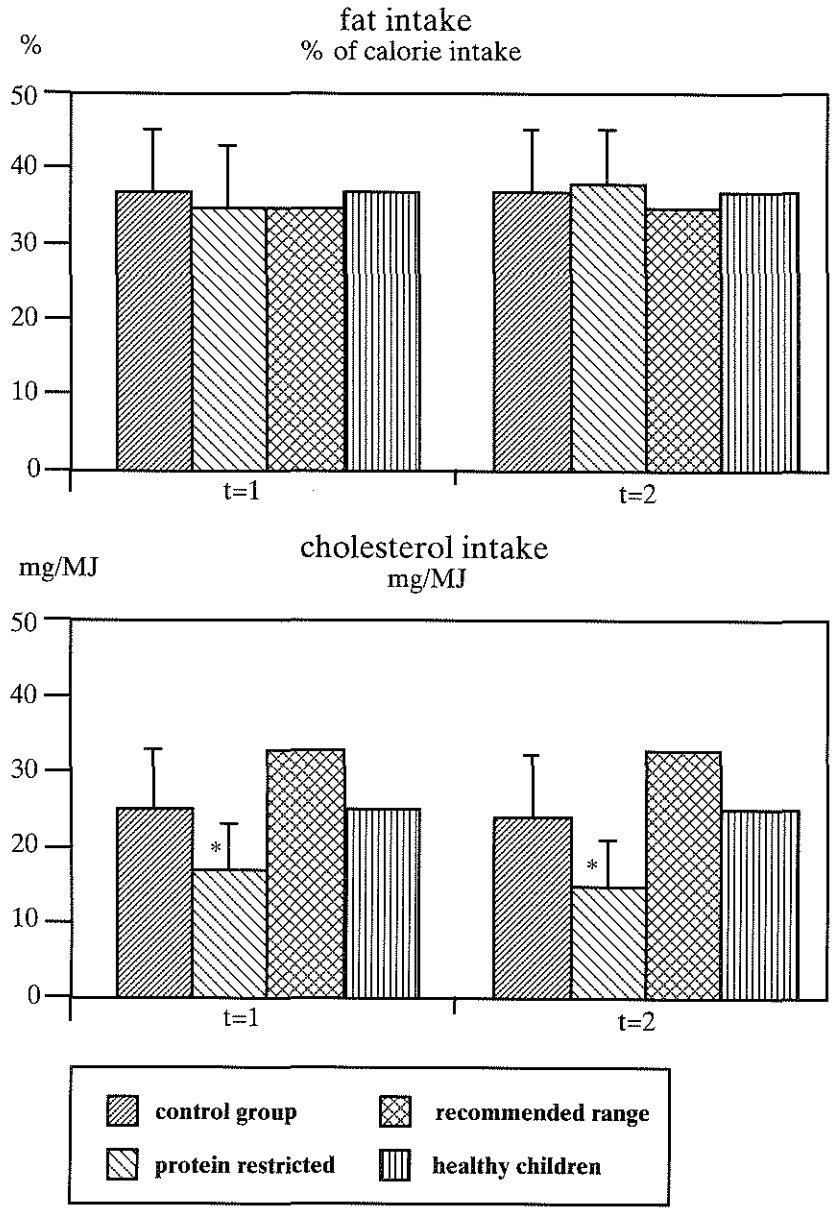

*Figure 1. Mean fat intake expressed as a percentage of caloric intake (upper panel) and cholesterol intake (lower panel) of the control group, the protein restricted group, recommended for healthy Dutch children and eaten by healthy Dutch children. t = 1 refers to the first measurement, t = 2 to the second measurement. Bars denote standard deviation. * $p < 0.05$ between groups.*

Mean serum cholesterol, HDL cholesterol and LDL cholesterol levels of the children with the protein restricted diet as well as the children with the unrestricted diet were not significantly different from the mean values of healthy Dutch children. One patient from the control group and three patients from the protein restricted group had values below the lower reference limit for serum cholesterol. None had higher values. All patients had normal values for HDL cholesterol except one in the control group (too low) and one in the protein restricted group (too high). One patient from the protein restricted and one from the control group had values above normal for LDL cholesterol. Significantly more children than the expected 5 % - from both the protein restricted and the control group - had serum triglycerides and VLDL cholesterol above reference limits for healthy adults.
Although serum triglycerides were higher in the protein restricted group compared to the control group, this difference did not reach significance.
During the observation period of 12 months there was a significant rise of serum cholesterol and LDL cholesterol values in the control group, this could not be found in the protein restricted group (table 2).

7.4 Discussion

As fat has a relatively high caloric content in a small volume, its prescription can be of advantage for children with a low caloric intake and a poor appetite, commonly seen in children with chronic renal failure. Futhermore in attempting to preserve renal function with a protein restricted diet, caloric intake is often boosted by the use of fat, but the consequence should not be an increased development of atherosclerosis and more cardiovascular complications.
Data from this study shows that cholesterol intake is significantly lower and the p/s ratio is significantly higher in the protein restricted group compared to the control group. This may result from the children in the protein restricted group eating less meat and milk, which are of animal origin and contain more cholesterol and saturated fat. Moreover, supplementation of the protein restricted diet mostly with polyunsaturated fat also had a favourable effect on the p/s ratio. On the other hand the mean values for cholesterol intake of the children from the control group are still within the advised limit for healthy Dutch children (Voedingsraad, Nederlandse voedings normen 1989). Serum cholesterol and LDL cholesterol concentrations do not differ significantly between both groups, but serum cholesterol as well as LDL cholesterol levels did rise significantly in the control group during the one year follow up period. Protein restriction had no effect on carbohydrate intake which may explain that no significant differences in fasting serum

triglycerides were found. Serum triglycerides were high in both groups, which may be caused by the decreased activity of lipoprotein lipase and a decreased triglyceride clearance in chronic renal failure (Mitch 1985).
The rise in one year of serum cholesterol and LDL cholesterol in children with chronic renal failure does suggest an unfavourable effect on fat metabolism of a diet without restrictions compared to a protein restricted diet. The results of our study suggest that children with chronic renal failure should keep to the fat and cholesterol intake as recommended by the Dutch Nutrition Council and try to attain the recommended p/s ratio for healthy children of 0.5 - 1.

Chapter 8

General discussion

Compliance of rats with a protein restricted diet is relatively easy because they live in cages. With children you have to rely on the trustworthiness of the parents and children in the adherence to the prescribed diet. Compliance with the protein restricted diet assessed from the dietary diaries and serum urea/creatinine ratio was good. Twenty-four hour urinary urea excretion turned out not to be a reliable method for assessment of protein intake according to the prospective dietary diaries. There are no other objective methods available for assessment of dietary compliance. Because the parents and children were extensively coached by one dietician and pediatrician who visited the children every three months throughout the study we are convinced that dietary compliance of the children allocated the protein restricted diet was the best to be achieved in the outpatient setting.

We conclude that the protein restricted diet used in this study has no apparent effect on renal function in children with chronic renal failure and that the protein restricted diet does not negatively influence growth of these children if adequate amounts of calories are supplied.

The results of our study are in accordance with three large studies performed in adults with a comparable degree of chronic renal failure (Rosman 1989, Locatelli 1991, Williams 1991). They found no support for the hypothesis that protein restriction retards the progression of chronic renal failure. Only Ihle observed a favourable effect of a severe protein restricted diet (0.4 g/kg/day) on renal function in 65 adults with progressive renal failure (Ihle 1989). However of the patients in the protein restricted group weight, serum transferrin and the total lymphocyte count were significantly lower after a follow-up period of 18 months, each parameter indicating malnutrition. For children the prescription of such a severely restricted diet, besides causing malnutrition, carries the extra risk of retarding growth, which would be intolerable. Our results are contributed to a large multicentre European study (200 children) which is in progress. The results after one year are not different from our results but are more difficult to interpret because of different culinary habits in different countries throughout Europe, while our study is performed in a homogenous population with one supervising

pediatrician and one dietician for all the patients. It would be of interest to analyze the effect of the low protein diet after stratification for underlying primary renal disease. The relatively small number of our patients did not allow us to do this but the analysis of the patients of the European multicentre study did not show such a difference, at least for the first year.

When the glomerular filtration rate has fallen below 60 ml/min/1.73m^2 the single nephron glomerular filtration rate has already reached its maximum. It is unlikely that any intervention can prevent or even halt the inexorable focal and segmental glomerulosclerosis that has been abundantly documented in rats and in a few human studies. The next step might be investigating the effect of a protein restricted diet in patients with a near normal glomerular filtration rate. However the disadvantages of keeping to such an unpleasant diet must balanced against the risk for such a patient to progress to end stage renal disease.

Supplementation of the protein restricted diet used in our study with essential amino acids does not seem necessary because the intake of essential amino acids was, although lower than in the control group, well above 100 % of the minimal daily requirement. Moreover no amino acid levels in plasma nor in granulocytes were significantly lower in the protein restricted group compared to the control group. The data from the amino acid levels in the granulocytes is interesting because in granulocytes extensive metabolism is possible in the mitochondria, lysosomes and peroxisomes and the function of the granulocytes is impaired in chronic renal failure. Comparing levels of amino acids of the children with chronic renal failure with those of healthy children we found significantly lower levels of most of the essential amino acids (leucine, isoleucine, phenylalanine, methionine, lysine and histidine). An altered transport of amino acids into the granulocytes and intracellular metabolism of these amino acids in the granulocytes resulting in an abnormal pattern of intracellular protein, both could contribute to the lowered intracellular amino acid concentration. These hypotheses require further study.

Cardiovascular complications of patients with chronic renal failure have been associated with hyperlipidemia present in many of these patients. Because caloric intake in children with chronic renal failure and a bad appetite is often boosted by fat we were afraid that the institution of a protein restricted diet might have an adverse effect on fat intake and serum lipid profiles. The reverse was true because the children in the protein restricted group tended to eat less protein of animal origin (meat, milk, eggs) as it contains a lot of protein in a small volume and they would still be hungry after a meal. If they ate more potatoes and bread they were less hungry. The consequence was a lower cholesterol intake in the protein restricted group. Moreover supplementation of the protein restricted diet mostly with polyunsaturated fat also had a favourable effect on

the polyunsaturated / saturated fat ratio. The significant rise of serum cholesterol and LDL cholesterol when using an unrestricted diet does suggest an unfavourable effect on fat metabolism compared to a protein restricted diet.
It might be of interest to study the effect on renal function of a more severely protein restricted diet than was used in our study, supplemented with essential amino acids. Such a diet will however be extremely difficult for children to keep to and might compromise the growth of these children.

Summary

Chapter one outlines the background and the hypothesis on which the thesis is based. Children with chronic renal failure are at risk of progressing to end stage renal disease. The initial rate of progression is dependent on the primary renal disease. Once glomerular filtration rate has fallen to 25 ml/min/1.73m^2 progression to end stage renal disease is inevitable and renal replacement therapy (renal transplantation, hemodialysis or peritoneal dialysis) will become necessary.
Studies in rats after the partial removal of one kidney and the complete removal of the other demonstrated that the elevated glomerular capillary pressure and flow that characterizes the adaptive response of hyperfiltration may be the cause of focal and segmental glomerulosclerosis in otherwise normal nephrons. Dietary protein restriction proved to attenuate glomerular injury and prolong the survival of rats with different forms of experimentally induced renal disease.
The aim of this study was to investigate if a protein restricted diet could slow down or even halt progression of chronic renal failure in children while growth should not be compromised.

Chapter two describes the general outline of the study.
Fifty-six children with chronic renal failure treated in five University Hospitals were randomly assigned to a protein restricted group or a control group. The children in the protein restricted group were advised to reduce their protein intake to the safe levels of the World Health Organization which vary according to age and gender between 0.8 and 1.1 g/kg/day. The children in the control group were advised to eat at least 1.5 to 2.0 times the safe level of protein intake, according to age, which equals the normal protein intake for healthy Dutch children. In order to minimize interobserver variations one single pediatrician and one dietician visited the patients every three months in the different centres throughout a three year period. We regard this as an essential aspect of the study. Compliance with the diet was assessed using prospective dietary diaries and measuring serum urea / creatinine ratio's every three months. Statural growth, weight for height, upper arm circumference and triceps skinfold were measured every three months in order to assess the growth of the children.
Renal function was measured by three methods. Endogenous creatinine clearances

were assessed at three month intervals but rely on adequate urine collection; in contrast a calculated creatinine clearance only requires a plasma creatinine determination and the height of the patient, and there is a close correlation with inulin clearance ($r = 0.85$). Although the clearance of inulin is the golden standard for estimation of the glomerular filtration rate we have focused on the calculated creatinine clearance and the endogenous creatinine clearance since this allowed us to perform multiple measurements at three month intervals for each patient. Inulin clearance was measured after two and after three years.

Chapter three deals with the validity of twenty-four hour urinary urea excretion in assessing protein intake of children with chronic renal failure.
There were no restrictions with respect to protein intake during the investigation period. The twenty-four hour urinary urea excretion correlated poorly with the protein intake estimated from the dietary diary ($r = 0.58$). Major errors in urine collection were ruled out by a coefficient of variation within patients of 24 hour creatinine excretion of 14 %, which is acceptable. Besides a large variation in urea excretion between patients, there was also a large variation within patients. In fourteen patients selected on a within-patient variation of protein intake of less than 10 % the coefficient of variation for urinary urea excretion was 41 %. We conclude that although it is common practice to assess compliance with a protein restricted diet in children with chronic renal failure with 24 hour urinary urea excretion, its value is questionable.

Chapter four reports the effect of a protein restricted diet on renal function and growth fifty-six children with chronic renal failure.
Compliance with the protein restricted diet, as indicated by the prospective dietary diaries and the serum urea / creatinine ratio, was good.
Statural growth and weight for height of the children from the protein restricted group did not differ from the control group, and was equal to that of healthy children. Upper arm circumference and triceps skinfold also were not influenced by the long term protein restriction. We found no significant difference between the protein restricted group and the control group with respect to the occurrence of renal osteodystrophy.
In both groups four children progressed to end stage renal disease during the course of the study. There was no significant difference in renal function between the protein restricted and the control group during the three year follow-up period. In both groups the mean calculated creatinine clearance slowly declined: -2.9 ($\pm$ 1.0) ml/min/1.73m^2 / year for the protein restricted group and -2.1 ($\pm$ 0.8) ml/min/1.73m^2 / year for the control group. There was no significant difference in the development of microalbuminuria

during the study; nor did the protein restricted diet induce a reduction in proteinuria. The study implicates that children with chronic renal failure do not benefit from a protein restricted diet.

Chapter five deals with plasma amino acids and their relation to protein intake of fifty children with chronic renal failure. The intake of each amino acid was well above the minimal daily requirement in the protein restricted group. No plasma amino acid levels were significantly lower and three amino acid levels (serine, glycine and arginine) were significantly higher in the protein restricted group compared to the control group. These results suggest that the protein restricted diet used in this study is safe. It is concluded that supplementation of this diet with essential amino acids can not be supported.

Chapter six describes the results of the amino acid levels in granulocytes and plasma of twenty-four children with chronic renal failure. The data were compared to the amino acid levels of fifteen healthy children. In addition we looked at the correlation between - the intracellular and plasma amino acid levels - and protein intake, renal function and growth. Granulocytes were isolated from 10 ml blood using a dextran - ficoll - hypaque procedure.
There was no effect of protein restriction on intracellular amino acids. However intracellular levels of leucine, isoleucine, phenylalanine, methionine, lysine, histidine, tyrosine and arginine were significantly lower in children with chronic renal failure compared to healthy children. No intracellular amino acids and only two plasma amino acids had a significant correlation with glomerular filtration rate. Seven intracellular amino acids had a positive correlation whereas and five plasma amino acids had a negative correlation with the change in weight for height in the last six months before blood was drawn for granulocyte isolation. The change in height standard deviation score in the preceding six months was significantly correlated with four plasma amino acids, but not with intracellular amino acid levels. The significantly lower intracellular levels and higher plasma levels of amino acids found in children with chronic renal failure compared to healthy children and the significant correlations found between both - intracellular and plasma amino acids - and the change in weight for height and height standard deviation may be related to a defect in the transport of amino acids over the cell membrane or intracellular metabolism causing cellular malnutrition and influencing growth in children with chronic renal failure.

Chapter seven deals with the effect of a protein restricted diet on fat intake and serum lipids.

We studied the effect of a protein restricted diet compared to an unrestricted diet on fat intake and serum lipid profiles of forty children with chronic renal failure. Although total fat intake did not change, we found a lower cholesterol intake and a higher polyunsaturated / saturated fat ratio in the patients with the protein restricted diet. This is probably caused by the restriction of animal protein which results in the replacement of animal fat by vegetable fat in the protein restricted group. Moreover we observed an increase of plasma cholesterol and low density lipoprotein cholesterol in patients with the unrestricted diet which was absent in the protein restricted group. This suggests a favourable effect of the institution of a protein restricted diet on lipid intake and plasma profile.

Chapter eight discusses the value of a protein restricted diet for children with chronic renal failure. We conclude that a protein restricted diet has no positive nor negative effect on renal function and growth of children with chronic renal failure.

Samenvatting

In *hoofdstuk één* worden de achtergronden en de hypothesen die in het onderzoek getoetst worden besproken. Kinderen met chronische nierinsufficiëntie lopen het risico terminale nierinsufficiëntie te krijgen. De mate van progressie van de nierinsufficiëntie is aanvankelijk afhankelijk van de oorspronkelijke nierziekte. Als de glomerulaire filtratie snelheid echter eenmaal lager dan 25 ml/min/1.73m^2 wordt, is progressie tot terminale nierinsufficiëntie onvermijdelijk en wordt uiteindelijk nierfunctie vervangende behandeling noodzakelijk (niertransplantatie, hemodialyse of peritoneaal dialyse). Onderzoek bij ratten toonde, na verwijdering van 4/5 deel van het functionerend nierweefsel, een verhoogde capillaire druk en "flow" in de glomeruli, die hyperfiltratie wordt genoemd. Na enige tijd ontstond bij deze ratten focale segmentale sclerose in tevoren gezonde glomeruli. Indien de ratten een eiwitbeperkt dieet kregen nam de nierinsufficiëntie langzamer toe en leefden ze langer dan ratten die normale voeding kregen.
Het doel van deze studie was te onderzoeken of een eiwitbeperkt dieet de progressie van nierinsufficiëntie bij kinderen met een chronische nierinsufficiëntie kon tegengaan, terwijl de groei van de kinderen niet gecompromitteerd mocht worden.

In *hoofdstuk twee* worden de patiënten en methoden van het onderzoek beschreven. Zesenvijftig kinderen met chronische nierinsufficiëntie, die in vijf academische ziekenhuizen werden behandeld, kregen door het lot een eiwitbeperkt dieet of een dieet zonder restricties toegewezen. De kinderen in de eiwitbeperkte groep werden geadviseerd hun eiwit inname te reduceren tot de "safe levels" van de Wereld Gezondheid Organisatie. Dit komt, afhankelijk van de leeftijd en het geslacht van de kinderen, overeen met 0.8 - 1.1 g eiwit / kg / dag. De kinderen in de controle groep werden geacht 1.5 - 2 keer de "safe levels" te eten, overeenkomend met de normale eiwit inname van gezonde Nederlandse kinderen. Eén kinderarts en één diëtiste controleerden en adviseerden de kinderen elke drie maanden gedurende de drie jaar dat het onderzoek duurde.
Of de kinderen het voorgeschreven dieet inderdaad volgden werd elke drie maanden gecontroleerd aan de hand van voedingslijsten, waaruit de eiwit inname werd berekend, en de serum ureum / creatinine ratio. Lengte, gewicht, bovenarm omtrek en triceps huidplooi werden elke drie maanden opgemeten om de groei van de kinderen te volgen.

De nierfunctie werd door middel van drie methoden gemeten:

1. De endogene creatinine klaring werd elke drie maanden berekend, maar is afhankelijk van een betrouwbare 24-uurs urine verzameling.
2. Voor de berekende creatinine klaring is alleen het serum creatinine gehalte en de lengte van de patiënt nodig, en er is een goede correlatie met de inuline klaring (r = 0.85).
3. Hoewel de inuline klaring wordt beschouwd als de gouden standaard voor de bepaling van de glomerulaire filtratie snelheid, is het een invasief en belastend onderzoek voor een kind. Wij hebben ons daarom geconcentreerd op de berekende creatinine klaring en de endogene creatinine klaring daar deze methoden van nierfunctie bepaling elke drie maanden herhaald konden worden. De inuline klaring is twee jaar en drie jaar na de start van het onderzoek verricht.

In *hoofdstuk drie* wordt de betrouwbaarheid van de ureum uitscheiding in 24-uurs urine onderzocht, als maat voor de eiwit inname. Gedurende dit deel van het onderzoek hadden de kinderen geen eiwitbeperking voorgeschreven gekregen. De ureum uitscheiding in 24-uurs urine had een matige correlatie met de eiwit inname berekend uit de voedingslijsten van de kinderen (r = 0.58). Als oorzaak voor deze matige correlatie werden fouten, gemaakt bij het urine verzamelen, uitgesloten door een variatie coëfficiënt binnen patiënten van 14 %, wat acceptabel is. Behoudens een grote variatie in ureum excretie tussen patiënten was er ook een grote variatie binnen de patiënten. Bij veertien patiënten met een variatie binnen de patiënt in eiwit inname van 10 % was de variatie coëfficiënt voor ureum uitscheiding 41 %. We concluderen dat ook al wordt de ureum uitscheiding in 24-uurs urine frequent gebruikt om na te gaan of een patiënt zich aan een eiwitbeperkt dieet houdt, de waarde hiervan twijfelachtig is.

In *hoofdstuk vier* wordt het effect van een eiwitbeperkt dieet op de nierfunctie en de groei van zesenvijftig kinderen met een chronische nierinsufficiëntie beschreven.
De kinderen hielden zich goed aan het voorgeschreven eiwitbeperkte dieet, gemeten aan de berekende eiwit inname van de voedingslijsten en de serum ureum / creatinine ratio.
De lengtegroei en het gewicht van de kinderen uit de eiwitbeperkte groep verschilden niet significant van de controle groep en kwamen overeen met die van gezonde Nederlandse kinderen. De bovenarm omtrek en triceps huidplooi werden ook niet beïnvloed door de langdurige eiwitbeperking. We vonden geen verschil in het voorkomen van renale osteodystrofie tussen de eiwitbeperkte groep en de controle groep.
In beide groepen waren vier kinderen die tijdens de studie aan nierfunctie vervangende

behandeling toekwamen. Er was geen significant verschil in nierfunctie tussen de eiwitbeperkte groep en de controle groep gedurende de follow-up periode van drie jaar. In beide groepen nam de gemiddelde berekende creatinine klaring langzaam af: -2.9 (± 1.0) ml/min/1.73m^2 / jaar in de eiwitbeperkte groep en -2.1 (± 0.8) ml/min/1.73m^2 / jaar in de controle groep. Er was geen significant verschil in het onstaan van microalbuminurie tussen beide groepen.
Dit onderzoek impliceert dat kinderen met chronische nierinsufficientie geen profijt hebben van een eiwitbeperkt dieet.

In *hoofdstuk vijf* worden plasma aminozuren en hun relatie met de eiwit inname van vijftig kinderen met chronische nierinsufficiëntie weergegeven.
De inname van elk van de essentiële aminozuren was ruim boven de minimaal aanbevolen dagelijkse hoeveelheid in de eiwitbeperkte groep. Er waren geen plasma aminozuren die significant lager waren en drie plasma aminozuren (serine, glycine en arginine) die significant hoger waren in de eiwitbeperkte groep vergeleken met de controle groep. Ten opzichte van gezonde kinderen hadden de kinderen met chronische nierinsufficiëntie een lagere plasma serine, asparagine en tyrosine en een hogere concentratie glycine en arginine. Deze resultaten suggereren dat het eiwitbeperkte dieet voorgeschreven in dit onderzoek veilig is. Dit dieet behoeft niet met essentiële aminozuren gesuppleerd te worden.

In *hoofdstuk zes* worden de resultaten van een onderzoek naar de aminozuur concentraties in de granulocyt en in plasma van vierentwintig kinderen met chronische nierinsufficiëntie besproken.
De resultaten werden vergeleken met aminozuur concentraties van vijftien gezonde kinderen. Bovendien werden de relaties tussen enerzijds de intracellulaire en plasma aminozuur spiegels en anderzijds eiwit inname, nierfunctie en groei onderzocht. De granulocyten werden geïsoleerd uit 10 ml bloed waarbij gebruik gemaakt werd van een dextraan - ficoll - hypaque methode.
Er was geen effect van het eiwitbeperkte dieet op de intracellulaire aminozuren. Daarentegen waren de intracellulaire concentraties van leucine, isoleucine, phenylalanine, methionine, lysine, histidine, tyrosine en arginine significant lager bij kinderen met chronische nierinsufficiëntie dan bij gezonde kinderen. Er waren geen intracellulaire en slechts twee plasma aminozuren die een significante correlatie met de glomerulaire filtratie snelheid toonden. Zeven intracellulaire aminozuren hadden een positieve correlatie, terwijl vijf plasma aminozuren een negatieve correlatie met gewichtsgroei hadden, gemeten over de laatste zes maanden voordat granulocyten isolatie plaats

vond. De toename in lengte van de laatste zes maanden was significant gecorreleerd met vier plasma aminozuren, maar niet met intracellulaire aminozuur concentraties. De resultaten van dit onderzoek zouden misschien veroorzaakt kunnen worden door een defect in het transport van aminozuren over de celmembraan of een veranderd aminozuur metabolisme in de cel bij kinderen met chronische nierinsufficiëntie. Dit zou tot cellulaire ondervoeding en verminderde groei bij kinderen met preterminale nierinsufficiëntie kunnen leiden.

In *hoofdstuk zeven* bestudeerden we het effect van een eiwitbeperkt dieet en een dieet zonder restricties op de vet inname en serum lipiden van veertig kinderen met chronische nierinsufficiëntie. Hoewel de totale vet inname niet veranderde, vonden we een lagere cholesterol inname en een hogere meervoudig onverzadigde / verzadigde vetzuren ratio bij de patiënten met het eiwitbeperkte dieet. Dit wordt waarschijnlijk veroorzaakt door de restrictie van dierlijk eiwit. Dit leidt tot de vervanging van dierlijk vet door plantaardig vet in de eiwitbeperkte groep. Bovendien vonden we een toename van serum cholesterol en LDL cholesterol bij patiënten met het dieet zonder restricties, dit werd niet gevonden bij de patiënten met het eiwitbeperkte dieet. Dit suggereert een gunstig indirect effect van een eiwitbeperkt dieet op vet inname en serum lipiden.

In *hoofdstuk acht* wordt gediscussieerd over de waarde van een eiwitbeperkt dieet bij chronische nierinsufficiëntie. We concluderen dat een eiwitbeperkt dieet geen positief maar ook geen negatief effect heeft op de nierfunctie en groei van kinderen met chronische nierinsufficiëntie.

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About the author

The author of this thesis was born in Lisbon on June 2, 1957. She followed her secondary education (Atheneum B) at the Rijnlands Lyceum in Wassenaar from 1968 to 1975. She started her medical studies at Leiden University in the same year. During her studies she was a research assistant at the department of Clinical Genetics, University Hospital Leiden (1978-1979). She obtained her medical qualification in June 1982. Specialist training in Pediatrics was started in 1982 and completed in 1986 at Sophia Children's Hospital / University Hospital Rotterdam (Prof.dr. H.K.A. Visser). From 1986 to 1987 the author was a clinical fellow at the department of Pediatric Nephrology, Sophia Children's Hospital / University Hospital Rotterdam. From 1987 to 1991 she was a research fellow at the department of Pediatric Nephrology at the same hospital, on a grant provided by the Dutch Kidney Foundation. During this period the major part of this study was performed. In January 1991 she started as a (parttime) general pediatrician at the Diaconessenhuis in Voorburg. She is married and has two children Nico (1987) and Tietse (1989).

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Pediatric Nephrology

Brief report

Protein intake can not be estimated from urinary urea excretion

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Abstract. We assessed the relationship between protein intake (calculated from a 3-day prospective dietary diary) and 24-h urinary urea excretion in 37 children with chronic renal failure. Protein intake was not restricted during the investigation period. The 24-h urinary urea excretion correlated poorly with the protein intake estimated from the dietary diary ($r = 0.58$). We conclude that although it is common practice to assess compliance with a protein-restricted diet in children with chronic renal failure with a dietary diary and 24-h urinary urea excretion, the value of this assessment is questionable.

Key words: Chronic renal failure – Urinary urea excretion – Dietary diary – Protein restriction – Compliance

Introduction

As protein restriction may slow down, or even halt, the progression of chronic renal failure [1], accurate assessment of dietary compliance is important. It is well known that urinary urea excretion can be used to calculate the protein intake of adults with chronic renal failure [2–4]. Urinary urea excretion reflects approximately 70% of nitrogen loss. The remaining nitrogen loss is said to be virtually independent of nitrogen intake, suggesting that changes in nitrogen intake are reflected by changes in urinary urea excretion [2, 4]. There are, however, no comparable studies in children. The purpose of this study was to investigate whether the protein intake of children with chronic renal failure could be reliably estimated in the outpatient clinic from dietary diaries and urinary urea excretion.

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Patients and methods

Fifty-four children with chronic renal failure were asked to carry out three 24-h urine collections at home. The urine collections were performed at monthly intervals over a 3-month period. The urine was collected in acidified plastic bottles to prevent degradation of urea by urease-producing bacteria. Protein intake was not restricted during the investigation period, in order to rule out the effect of compliance with a prescribed diet. A dietician especially assigned to the study instructed parents and children how to keep a 3-day prospective dietary diary. The dietary diary consisted of 2 weekdays and 1 day at the weekend. The urine was collected in the same period. The dietician discussed the dietary diary with parents and children at the outpatient clinic to ensure that nothing was omitted. She then calculated the mean protein intake using the Dutch nutrition index [5].

Of the 54 children, only 37 successfully completed the 24-h urine collections; the remaining children were either incontinent or had great trouble completing collections. There were 26 boys and 11 girls with a mean age of 10 years 5 months (range 3 years 4 months to 16 years 11 months). Five children had glomerulopathy; 25 had either reflux nephropathy, obstructive uropathy and/or renal dysplasia; 7 children had miscellaneous diagnoses. The glomerular filtration rate (40 × length cm/creatinine μmol/l) varied from 18 to 60 ml/min per 1.73 m^2 (mean 42 ml/min per 1.73 m^2). In order to assess the accuracy of the urine collections, the same children were asked to perform five 24-h urine collections over a 15-month period. Creatinine excretion was calculated for the 30 children who where able to complete five 24-h urine collections. Urinary urea excretion was measured enzymatically as first described by Talke and Schubert [6], using an automatic chemical analyser.

We investigated the relationship between the protein intake as assessed by the dietary diary and the protein intake derived from the 24-h urinary urea excretion by linear least-squares regression. In this analysis only the last measurements were used when more than one pair of results were available. Variations in urinary urea excretion (within patient) when protein intake differed by less than 10%, were assessed using analysis of variance by calculation of components of variance. Results were considered statistically significant when P was less than 0.05.

Results

Five patients completed one, 30 patients two and 2 patients three successful urine collections which coincided with the dietary diary. Protein intake as calculated from dietary records varied from 0.83 to 2.99 g/kg per day (0.13–0.48 g

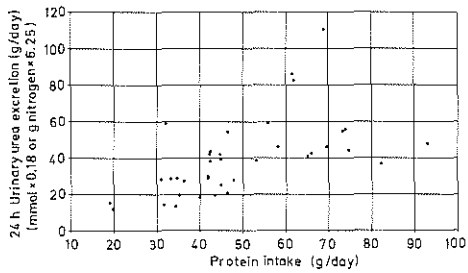

Fig. 1. Correlation between protein intake assessed from a dietary diary and 24-h urinary urea excretion; $n = 37$, $r = 0.58$, $P < 0.001$

nitrogen/kg per day), mean 1.62 g (0.26 g nitrogen)/kg per day. Urinary urea excretion varied from 2.9 to 13.3 mmol/kg per day (0.08–0.38 g nitrogen/kg per day), mean 6.9 mmol (0.20 g nitrogen)/kg per day. Two children had proteinuria of 2–2.5 g/day, the other children had no significant proteinuria (<1 g/day). The CV for creatinine excretion of 30 children calculated for each child from five 24-h urine collections, was 14%. The correlation coefficient of protein intake and urinary urea excretion was 0.58 ($P < 0.001$, Fig. 1). Besides the large between-patient variation in urinary urea excretion, there was also a large within-patient variation. In 14 patients with a within-patient variation of protein intake of less than 10%, the CV for urinary urea excretion was 41%!

Discussion

The correlation between protein intake and urinary urea excretion ($r = 0.58$) is disappointingly low in this study. Ziegler et al. [7] performed 1148 nitrogen balance studies in 123 healthy hospitalised children 1–11 years in age. They found a considerably better correlation between daily nitrogen intake and urinary nitrogen excretion ($r = 0.76$) than the correlation between protein intake and urea excretion found in our outpatient study. Coles et al. [8] reported a poor correlation ($r = 0.6$, $P < 0.001$) between protein intake and 24-h urinary nitrogen excretion in an outpatient study of 39 adults with chronic renal failure.

There are several possible reasons why the correlation between protein intake calculated from a dietary diary and protein intake derived from 24-h urinary urea excretion is poor in the outpatient setting. Large errors may be made when estimating protein intake from a dietary diary, even when as in our study diaries are written prospectively and are explained to children and parents by a dietician. A systematic flaw might be that children who eat more tend to overestimate their intake more than children who eat relatively small amounts of protein. Accurate estimation of 24-h urinary urea excretion may be affected by major errors in urine collection. However, this was not a problem in our study since the within-patient CV of 24-h creatinine excretion was 14%, which is acceptable. Non-urea urinary nitrogen excretion occurs (ammonia, creatinine, uric acid) and nitrogen is also lost in faces and sweat and due to cell loss. All these losses are relatively small with the exception of ammonia. Ammonia is formed in proportion to net acid excretion which is dependant on protein intake [9], this was not measured in our study and could be a reason for the poor correlation of protein intake assessed by dietary diary and 24-h urinary urea excretion. In adult studies non-urea nitrogen excretion varies from 23 to 34 mg/kg per day [4, 10, 11]. For a child of 25 kg body weight this amounts to 3.1 g protein (0.125 g protein/kg per day = 0.02 g nitrogen/kg per day). Changes in the urea pool, although they are not negligible, can not be held responsible for the discrepancy between protein intake estimated from a dietary diary and derived from the 24-h urinary urea excretion. For example, a change in serum urea of 2 mmol/l in a child of 25 kg, will yield a change in the urea pool of 2 mmol $\times 0.6 \times 25 = 30$ mmol urea $= 30 \times 0.18 = 5.4$ g protein (0.2 g protein/kg per day = 0.03 g nitrogen/kg per day).

Only a very small part of the daily protein intake is used for growth: 0.01 g nitrogen/kg per day = 0.06 g protein/kg per day [12]. However, the day-to-day variation might be bigger due to changes in catabolism and anabolism. Although this can not explain a systematic flaw in our data it may cause a wide range of individual observations. Since the children were on an unrestricted diet, non-compliance with a diet does not play a role in our study.

In summary, although it is common practice to use dietary diaries and 24-h urinary urea excretion for the assessment of compliance with a protein-restricted diet, this study and a study in adults [8] can not confirm its value for individual patients in the outpatient setting. The poor correlation between protein intake and 24-h urinary urea excretion may be caused by urinary ammonia excretion which is dependant on protein intake but was not measured in this study [9].

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Protein restriction in chronic renal failure

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Abstract

The aim of the study was to investigate the effect of a protein restricted diet on renal function and growth of children with chronic renal failure. In a multicentre prospective study 56 children (aged 2–18 years) with chronic renal failure were randomly assigned to the protein restricted (0·8–1·1 g/kg/day) or the control group. All children were followed up by the same paediatrician and dietitian. After a follow up period of three years there was no significant difference in glomerular filtration rate between children on a protein restricted diet and children of the control group. There was no significant difference in weight with respect to height and height SD score between the protein restricted and the control group. Compliance with the protein restricted diet, as indicated by the prospective diet diaries and the serum urea:creatinine ratio, was good. This study shows that children with chronic renal failure do not benefit from a protein restricted diet.

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Hyperfiltration, the adaptive response to the loss of functioning kidney mass, is thought to be detrimental to long term kidney function because of the development of glomerulosclerosis.[1] It is postulated that protein restriction can decrease hyperfiltration and slow down progression of chronic renal failure.[2] Studies have shown a beneficial effect of a protein restricted diet on renal function in laboratory rats with chronic renal failure.[3-5] However the growth of some rats on a very low protein diet was impaired.[6]

It is still unknown whether protein restriction can slow down progression of chronic renal failure in man. For children with chronic renal failure one prospective, but not controlled, study has demonstrated a favourable effect of a protein restricted diet on renal function.[7] A prospective randomised European multicentre study in children is now in progress.[8] In addition, the potential benefit of protein restriction for children has to be balanced against the risk of retarded growth. We present the results of a three year follow up of a prospective randomised study of the effect of a protein restricted diet on renal function and growth in children with chronic renal failure.

Patients and methods

GENERAL OUTLINE

In order to minimise interobserver variations one single paediatrician and one dietitian visited the patients in the different centres throughout the course of the study. We regard this as an essential aspect of the study.

PATIENTS

To be eligible for the study the children (age 2–18 years) had to be under treatment for chronic renal failure (glomerular filtration rate (GFR) 15–60 ml/min/1·73m²) for at least six months. Excluded from the study were children with cystinosis, oxalosis, lupus erythematosus, severe hypertension (blood pressure exceeding 110% of the 97th centile according to Andre and Deschamps[9]), and other severe diseases and children on corticosteroid, growth hormone, or erythropoietin treatment. Altogether 56 children were studied. Progressive renal failure was defined as a significant decrease in GFR with respect to the last six to 12 months before the study.

RANDOMISATION

After an observation period of three months the children were randomly assigned to a protein restricted group (n=27) or a control group (n=29) after stratification for treatment centre. Both groups were well balanced with respect to various baseline characteristics (see the table).

DIET

The children in the protein restricted group were advised to reduce their protein intake to the safe levels of the World Health Organisation (WHO), which vary according to age and gender between 0·8 and 1·1 g/kg/day.[10] The children in the control group were advised to eat at least 1·5 to 2·0 times the safe levels of protein intake, according to age, which equals the normal protein intake for healthy Dutch children.[11] For all children the target energy intake was at least 100% of the energy requirement advised by the WHO.[10] A paediatrician and dietitian assigned to the study visited the children at the different outpatient clinics every three months.

DIETARY ASSESSMENT

The children and their parents were asked to provide a prospective dietary diary once every

Patient characteristics. Data given are numbers of patients or means (SD)

	Protein restricted group (n=27)	*Control group (n=29)*
Age (years)	10·2 (4·7)	9·3 (4·5)
Sex (M/F)	18/9	23/6
GFR (ml/min/1·73 m²)	42 (14)	38 (14)
Diagnosis:		
Glomerulopathy	5	5
Uropathy	17	18
Miscellaneous	5	6
Progressive renal failure	11	10
Weight for length (%)	100 (17)	97 (11)
Height SD score	−1·16 (1·70)	−1·57 (1·17)

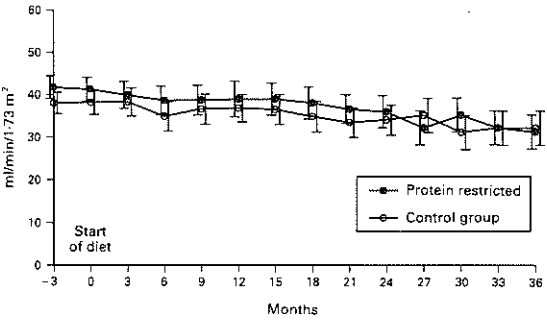

Figure 1 Calculated creatinine clearance: 40×length (cm)/creatinine (μmol/l). Bars show SEM.

three months that covered two days during the week and one day in the weekend. The dietitian then discussed the dietary diary with parents and children to be sure that nothing was omitted. Protein and energy intakes were calculated using the Dutch Nutrition Index[12] and were adjusted if they did not correspond to the prescribed amount.

The serum urea:creatinine ratio was measured every three months. Twenty four hour urinary urea excretion is not a reliable method for assessment of nitrogen intake and was therefore not used to assess dietary compliance.[13]

RENAL FUNCTION

Once every three months renal function was assessed by means of the endogenous creatinine clearance and the calculated creatinine clearance (40 × length in cm/plasma creatinine in μmol/l).[14] Endogenous creatinine clearance was computed from 24 hour urine collections and plasma creatinine. For the children who were put on renal replacement treatment or died during the study, creatinine clearance and the calculated creatinine clearance were counted as 0 ml/min/1·73 m² for the remainder of the study. Endogenous creatinine clearance and calculated creatinine clearance are relatively easy non-invasive procedures for assessment of renal function in children and could therefore be determined at regular three month intervals. Because it is a more invasive procedure, determination of the inulin clearance was only performed after two and three years. This provided validation of the endogenous creatinine clearance and calculated creatinine clearance. Inulin clearance was measured by administration of a continuous inulin infusion and timed collection of urine and plasma samples during a standardised period of the day.

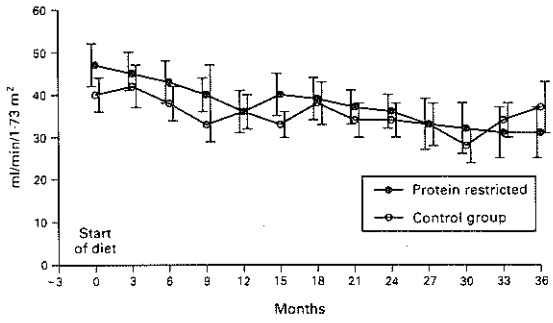

Figure 2 Endogenous creatinine clearance. Bars show SEM.

GROWTH AND NUTRITIONAL ASSESSMENT

Every three months weight, height, upper arm circumference, and triceps skinfold thickness were measured by the same observer. Weight is expressed as weight for height percentage and height as height SD score for healthy Dutch children.[15] Delta height SD score was computed as height SD score−height SD score at the time of randomisation. For the children who were put on renal replacement treatment or died during the study, the last weight, height, upper arm circumference, and triceps skinfold thickness were carried forward. Serum albumin and transferrin concentrations were measured every three months as parameters of protein malnutrition.[16]

MISCELLANEOUS PARAMETERS

Systolic and diastolic blood pressure and haemoglobin, serum uric acid, and bicarbonate concentrations were measured every three months.

STATISTICS

Data given are mean (SEM). Mean (SD) is given in case it was felt necessary to show the distribution of outcomes. Differences in the means of various parameters between the two groups were assessed for statistical significance using the Mann-Whitney test. In addition, for repeated measurements analysis of variance (BMDP, module 5V) was used to compare changes in the course of time in endogenous creatinine clearance, calculated creatinine clearance, weight with respect to height, height SD score, upper arm circumference, and triceps skinfold thickness. The p values given are two sided; 5% was considered the limit of significance.

Results

One child from the control group with mesangiocapillary glomerulonephritis received a course of high dose corticosteroids because of a rapidly progressive phase of his disease three months after randomisation. This child was considered non-evaluable and was excluded from further analysis. None of the other 55 children dropped out of the study during the first two years. In the last year of the study one child from the control group and seven children from the protein restricted group dropped out of the study. Four of these children received growth hormone treatment; this was an exclusion criterion and four children could not comply with the protein restricted diet any longer.

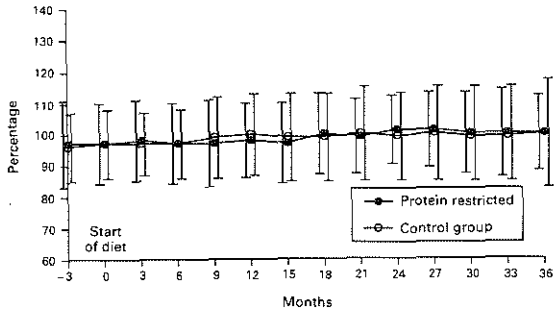

Figure 3 Weight with respect to height. Bars show SD.

RENAL FUNCTION

In the protein restricted group as well as in the control group four children reached end stage renal disease during the course of the study. Creatinine clearances could be calculated for 42 (75%) of the 55 patients; for the remaining children urine could not be collected because of incontinence. There was no significant difference between the experimental and the control group in either calculated creatinine clearance or endogenous creatinine clearance during the three year follow up period (figs 1 and 2). In both groups the mean glomerular filtration rate slowly declined: −2·9 (1·0) ml/min/1·73 m²/year for the protein restricted group and −2·1 (0·8) ml/min/1·73 m²/year for the control group.

Inulin clearance was measured after two years in 45 patients (six refused, four had reached end stage renal disease) and after three years in 33 patients. The mean inulin clearances after two and three years were 38 (16) and 36 (15) ml/min/1·73 m² respectively for the protein restricted group, which did not significantly differ from 36 (15) and 32 (14) ml/min/1·73 m² for the control group. Calculated creatinine clearance and endogenous creatinine clearance correlated significantly with inulin clearance: r=0·85 and r=0·78, respectively.

GROWTH AND NUTRITIONAL ASSESSMENT

There was no significant difference between the control group and the protein restricted group in weight with respect to height (fig 3), delta height SD score (fig 4), upper arm circumference, triceps skinfold thickness, and serum albumin and transferrin concentrations during the course of the study.

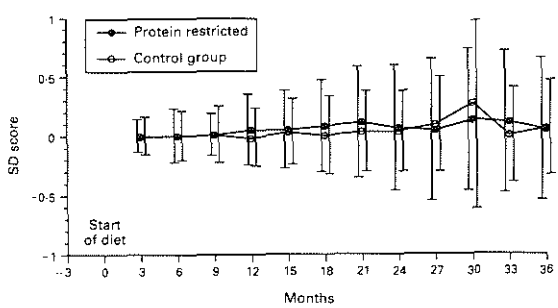

Figure 4 Delta height SD score. Bars show SD.

DIETARY ASSESSMENT

During the three year follow up period, the mean protein intake of the children in the protein restricted group, calculated from the prospective diet diaries and expressed as a percentage of the prescribed diet, was approximately 100% (fig 5). The mean protein intake of the children in the control group always exceeded 100% of the prescribed 1·5–2·0 times the safe levels for protein intake that approximates the normal protein intake of Dutch children.[11] Energy intake of all children, except one in the control group, was adequate (>80% of the advised amount) and there was no significant difference between the children in the protein restricted group and those of the control group. The serum urea:creatine ratio was significantly lower during the entire study period for the protein restricted group with respect to the control group (fig 6).

MISCELLANEOUS PARAMETERS

There was no significant difference between the protein restricted and the control group in systolic or diastolic blood pressure or in the haemoglobin and bicarbonate concentrations. Uric acid before randomisation was significantly higher in the protein restricted group: 0·41 (0·10) mmol/l compared with 0·37 (0·07) mmol/l for the control group. This difference did not change during the three year follow up period.

Discussion

Although it is common practice to advise a protein restricted diet for children with chronic renal failure, this study cannot support the hypothesis that protein restriction can preserve renal function at least during an observation period of three years. Although eight children had to stop the study during the third year, the results in the last year of the study resemble those found in the preceding two years. The results of our study are in accordance with those reported by Rosman *et al*[17] and Locatelli *et al*,[18] who found no beneficial effect of a protein restricted diet in larger randomised studies of adults (248 patients[18] and 456 patients[18] with a degree of chronic renal failure similar to that in our study. Only Ihle *et al* found a favourable effect of a protein restricted diet (0·4 g/kg/day) in a randomised study of 65 adult patients with severe progressive chronic renal failure (serum creatinine 350–1000 μmol/l).[16] But in his study the patients with a protein restricted diet lost significantly more weight and had significantly lower concentrations of serum transferrin and a significantly lower total lymphocyte count, each parameter indicating malnutrition, compared with the control group. Possibly the protein restriction prescribed in our study was not severe enough to protect against haemodynamically mediated glomerular injury when there is a

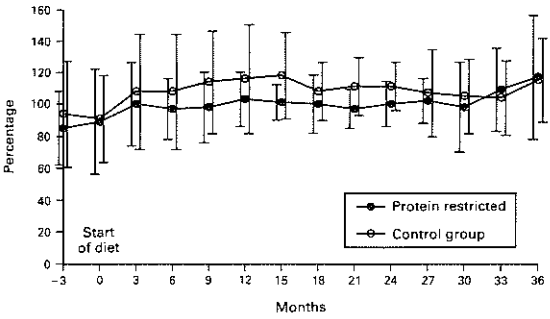

Figure 5 Protein intake. Bars show SD.

significant reduction in functioning nephrons. As children with chronic renal failure are already at risk for growth retardation, however,[20-22] caution is essential when a protein restricted diet is recommended. The protein restriction followed in our study was combined with an adequate caloric intake and did not cause growth impairment. It does not appear advisable to reduce protein intake below the sale levels recommended by the WHO for protein intake for children in the process of growth, but prospective controlled studies are not available.[23 24] Although the children and their parents were extensively coached and supported during the entire study, diet compliance may not always have been optimal but was in our opinion the best to be achieved. The protein restricted group as a whole had significantly lower serum urea:creatinine ratios compared with the control group.

Those who advocate a low protein diet for the management of patients with renal failure argue that there is an improvement in the patient's wellbeing after a reduction in blood urea concentration. Although this was not extensively studied, our impression was that a few patients in our study did feel better after reducing their protein intake, but in most children this could not be found.

We conclude that the protein restricted diet used in this study has no obvious effect on renal function in children with chronic renal failure. But it would be too early to state that there is no effect after a more extended observation period. It would also be of interest to analyse the effect of a low protein diet after stratification for underlying primary renal diseases. The relatively small number of our patients did not allow to do this but the analysis of the 200 patients of the European multicentre study did not show such difference, at least for the first study year.[8] One further important conclusion can be drawn from the study. Long term protein restriction according to the safe levels of the WHO does not negatively influence body growth of these children if adequate amounts of energy are supplied.

When the GFR has fallen below 60 ml/min/1·73 m^2 the single nephron GFR has already reached its maximum. It is unlikely that any intervention can prevent or even halt the inexorable focal and segmental glomerulosclerosis that has been abundantly documented in rats and in a few human studies. The next step might be investigating the effect of a protein restricted diet in patients with a near normal glomerular filtration rate. However the disadvantages of keeping to such an unpleasant diet must be balanced against the risk for such a patient to progress to end stage renal disease.

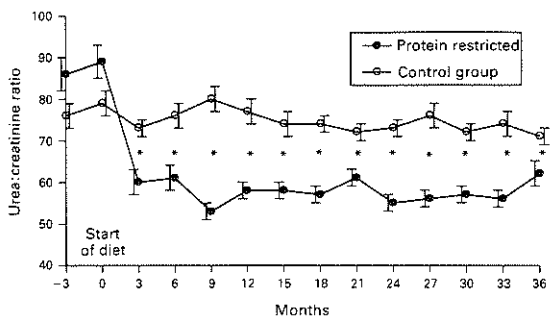

*Figure 6 Serum urea:creatinine ratio assessed three months before to 36 months after randomisation. *p<0·05 = statistical difference between the protein restricted group and control group.*

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Parts of this study were presented to the 8th Congress of the International Pediatric Nephrology Association, September 1989, Toronto, Canada; to the 24th Congress of the European Society of Paediatric Nephrology, October 1990, Rome, Italy; to the 6th International Congress of Nutrition and Metabolism in Renal Disease, August 1991, Harrowgate, UK, and to the 9th Congress of the International Pediatric Nephrology Association, September 1992, Jerusalem, Israel.

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Protein Restriction Affects Fat Intake and Serum Lipids in Children with Chronic Renal Failure

Abstract

Cardiovascular complications in patients with chronic renal failure have been associated with the hyperlipidemia present in many of these patients. Since a protein-restricted diet is often prescribed in an attempt to preserve renal function, we performed a randomized controlled study in children with chronic renal failure on the effect of a protein-restricted diet on fat intake and serum lipid profiles. Although total fat intake did not change, we found a lower cholesterol intake and a higher polyunsaturated/saturated fat ratio in the patients with the protein-restricted diet. This is probably caused by the restriction of animal protein which results in the replacement of animal fat by vegetable fat in the protein-restricted group. Moreover, we observed an increase of plasma cholesterol and low-density lipoprotein cholesterol in patients with a normal protein intake which was absent in the protein-restricted group. This suggests a favourable effect of the institution of a protein-restricted diet on lipid intake and plasma profile.

Key Words
Children
Cholesterol
Chronic renal failure
Fat
Protein-restricted diet

Introduction

Hyperlipidemia is frequently observed in adult [1, 2] and pediatric patients [3, 4] with end-stage renal disease and has been proposed as one of the main risk factors associated with the high prevalence of cardiovascular complications in adult patients on maintenance dialysis [1]. Conditions associated with high levels of low-density lipoprotein (LDL) cholesterol are associated with increased atherosclerosis [5]. In addition, there is an inverse relationship between circulating high-density lipoprotein (HDL) cholesterol and coronary heart disease, suggesting that the type of transporting lipoproteins, rather than the concentrations of the circulating lipids determines the rate of development of atherosclerosis [6]. Finally, diseases characterized by increased plasma triglyceride levels also seem to have a higher incidence of atherosclerosis [5].

Patients with chronic renal failure are often prescribed a protein-restricted diet in order to preserve renal function [7]. We studied the baseline fat intake of children with chronic renal failure and the effect of the institution of a protein-restricted diet on the fat intake and the resulting plasma lipid levels.

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Table 1. Characteristics at the time of first blood sampling of the protein-restricted and control groups expressed as mean values (SD)

Patient characteristics	Protein restricted	Control group
Number of patients	23	25
Age, years	10.9 (5.0)	10.4 (4.4)
Gender (boys, girls)	17, 6	19, 6
Diagnosis (1, 2, 3)[1]	3, 16, 4	3, 16, 6
Weight for length, %	101 (16)	98 (13)
Height standard deviation	−1.28 (1.70)	−1.31 (1.00)
Glomerular filtration rate, ml/min/1.73 m²	40 (17)	37 (16)

[1] Diagnosis; 1 = glomerulopathy; 2 = reflux nephropathy; obstructive uropathy and/or dysplasia; 3 = miscellaneous.

Patients and Methods

Forty-eight children with chronic renal failure who participate in a prospective randomized study on the effect of a protein-restricted diet on renal function and growth were studied. Age at the first blood sampling for the present study varied from 2 years and 6 months to 18 years and 8 months (mean age, 10 years and 8 months). There were 36 boys and 12 girls. Mean (SD) glomerular filtration rate (40 × length (cm)/creatinine (μmol/l)) was 39 (16) ml/min/1.73 m², range 9–69 ml/min/1.73 m² [8]. The diagnoses were: glomerulonephritis 6; obstructive uropathy, reflux nephropathy and or renal dysplasia 31; miscellaneous 11.

The children were first stratified according to renal disease, progression of renal disease and centre and then were randomly assigned a protein restricted diet or a diet without restrictions (table 1). The children in the protein-restricted group (n = 23) were advised to eat the safe levels for protein intake advised by the World Health Organization 1985, which varies from 0.8 to 1.1 g/kg/day, according to the age and gender of the child [9].

The children in the control group (n = 25) were advised to eat the usual protein intake for healthy Dutch children, which corresponds to 1.5–2.0 times the safe levels for protein intake according to their age and weight [10]. For all children we aimed at a caloric intake of at least 100% of the energy requirement as recommended by the World Health Organization [9]. Supplementation of calories was preferably advised as polyunsaturated and sometimes as saturated fat (for example, whole fat products instead of 'light' products, more vegetable margarine on a sandwich, toasted bread can take more margarine, baked potatoes with mayonnaise, add unwhipped cream or butter/margarine to food).

The children and their parents were instructed and asked to keep a prospective diet diary every 3 months consisting of 2 days during the week and 1 day at the weekend. A dietician especially assigned to the study talked the diary over with parents and children at the outpatient clinic to ensure no omissions. Protein, calorie, carbohydrate, fat and cholesterol intake and the polyunsaturated fat/saturated fat ratio (p/s ratio) were calculated using the Dutch Nutrition Index [11]. If protein or caloric intake calculated from the diet diary of a child did not correspond to the prescribed quantity, the parents were instructed how to adjust the intake. Blood was drawn in the fasting state for serum lipids on two occasions. The first time was at a mean of 11.4 months (range 3–15 months) after randomization, and the second time was 12 months later. Serum total cholesterol was measured with an enzymatic reagent kit (Boehringer Mannheim) calibrated with a human calibrator serum to ensure accurate results [12]. The HDL fraction was

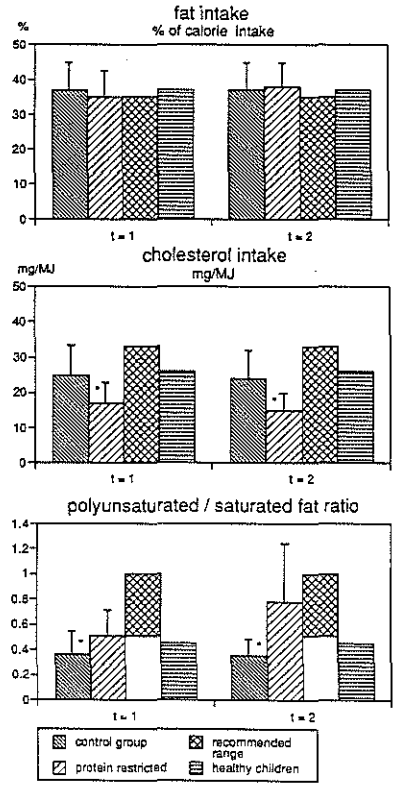

Fig. 1. Mean fat intake expressed as a percentage of caloric intake (upper panel), cholesterol intake (middle panel) and p/s ratio (lower panel) of the control group, the protein-restricted group, recommended for healthy Dutch children and eaten by healthy Dutch children. t = 1 = First measurement; t = 2 = second measurement. Bars denote SD. *p < 0.05 between groups.

Table 2. Means (SD) of food intake and of serum lipids

	Sample	Control group	Protein-restricted group	Recommended[1]	Significance of difference between groups
Diet					
Calorie, % of	1	106 (27)	97 (24)	100	n.s.
recommended by WHO	2	105 (18)	95 (21)		n.s.
Carbohydrate, % of caloric	1	52 (8)	59 (10)	55	n.s.
intake	2	53 (8)	53 (7)		n.s.
Fat, % of caloric intake	1	37 (7)	35 (7)	30–35	n.s.
	2	37 (7)	38 (6)		n.s.
Cholesterol, mg/MJ	1	25 (8)	17 (6)	< 33	$p < 0.001$
	2	24 (8)	15 (5)		$p < 0.001$
p/s ratio	1	0.36 (0.19)	0.51 (0.20)	0.5–1.0	$p = 0.02$
	2	0.35 (0.12)	0.78* (0.46)		$p < 0.01$
Serum					
Cholesterol, mmol/l	1	4.52 (0.60)	4.48 (0.95)	3.6–6.3	n.s.
	2	4.77* (0.81)	4.51 (0.70)		n.s.
Triglycerides, mmol/l	1	1.38 (0.88)	1.62 (0.76)	0.3–1.6	n.s.
	2	1.37 (0.73)	1.71 (1.12)		n.s.
HDL cholesterol, mmol/l	1	1.25 (0.39)	1.20 (0.55)	0.7–2.0	n.s.
	2	1.20 (0.35)	1.09 (0.36)		n.s.
LDL cholesterol, mmol/l	1	2.63 (0.54)	2.66 (0.79)	< 3.5	n.s.
	2	2.93* (0.60)	2.61 (0.70)		n.s.
VLDL cholesterol, mmol/l	1	0.62 (0.39)	0.73 (0.34)	0.13–0.48	n.s.
	2	0.62 (0.33)	0.77 (0.50)		

*$p < 0.05$, second value is higher than the first. Sample 1 = First measurement; sample 2 = second measurement.

[1] Recommended food intake for Dutch children [10], serum values of healthy Dutch children and adults [16].

isolated by precipitating apo-B-containing lipoproteins with a 4% tungstate solution with Mg^2 ions present, followed by centrifugation. HDL cholesterol was then quantitated in the supernatant liquid [13]. The laboratory is standardized in the CDC/NHBLI lipid standardization program. Triglycerides were measured with an enzymatic reagent kit (first measurement: Boehringer Mannheim; second measurement: Technicon). Both assays were calibrated with the same human calibrator serum [14]. VLDL cholesterol and LDL cholesterol were calculated with the Friedewald equation [15]. The values obtained in our patients were compared to values of healthy Dutch children for serum cholesterol (3.6–6.3 mmol/l) and HDL cholesterol (0.7–2.0 mmol/l) [16]. Serum triglycerides, VLDL and LDL cholesterol concentrations were compared to values for healthy adults in the absence of reference values for children determined with our own laboratory methods in 100 blood donors: 0.3–1.6, 0.13–0.48 and < 3.5 mmol/l, respectively. This comparison was valid because of the standardized laboratory methodology [17].

Differences in mean levels between groups were assessed for statistical significance using the Mann-Whitney test. Wilcoxon's test was used to calculate if differences between the first and second measurement within the groups were significant. Five percent was considered the limit of significance.

Results

According to the diet diaries, mean (SD) protein intake in the protein-restricted group was 0.94 (0.13) g/kg/day corresponding to 98% of the advised amount of protein. Mean (SD) protein intake in the control group was 1.98 (0.54) g/kg/day which is 113% of the advised protein intake. The caloric intake of the protein-restricted group was 97% (24%) of energy requirement as advised by the World Health Organization and 106% (27%) for the control group, and did not differ significantly. The intake of carbohydrates expressed as percentage of caloric intake did not differ significantly between the protein-restricted and the control groups: 59% (9.8%) for the protein-restricted group and 52% (8.3%) for the control group. Fat intake expressed as percentage of total caloric intake did not differ significantly between the protein-restricted and the control groups, and resembles the fat intake as advised for Dutch children (maximal 30–35%) [11] (fig. 1, table 2). Although the choles-

terol intake (mg/MJ) was significantly lower in the protein-restricted group opposed to the control group, it was within advised limits (fig. 1, table 2). The p/s ratio was significantly higher in the protein-restricted group compared to the control group on both occasions (fig. 1, table 2). The recommended p/s ratio of 0.5–1 is only attained by the protein-restricted group. The children in the protein-restricted group have a significantly higher p/s ratio at the second measurement compared to the first assessment, 1 year earlier.

Mean serum cholesterol, HDL cholesterol and LDL cholesterol levels of the children with the protein-restricted diet as well as the children with the unrestricted diet were not significantly different from the mean values of healthy Dutch children. One patient from the control group and 3 patients from the protein-restricted group had values below the lower reference limit for serum cholesterol. None had higher values. All patients had normal values for HDL cholesterol except one in the control group (too low) and one in the protein-restricted group (too high). One patient from the protein-restricted and one from the control group had values above normal for LDL cholesterol. Significantly more children than the expected 5% – from both the protein-restricted and the control groups – had serum triglycerides and VLDL cholesterol above reference limits for healthy adults.

Although serum triglycerides were higher in the protein-restricted compared to the control group, this difference did not reach significance.

During the observation period of 12 months there was a significant rise of serum cholesterol and LDL cholesterol values in the control group, this could not be found in the protein-restricted group (table 2).

Discussion

As fat has a relatively high caloric content in a small volume, its prescription can be advantageous for children with a low caloric intake and a poor appetite, commonly seen in children with chronic renal failure. Futhermore, in attempting to preserve renal function with a protein-restricted diet, caloric intake is often boosted by the use of fat, but the consequence should not be an increased development of atherosclerosis and more cardiovascular complications. Data from this study show that cholesterol intake is significantly lower and the p/s ratio is significantly higher in the protein-restricted group as compared to the control group. This may result from the children in the protein-restricted group eating less meat and milk, which are of animal origin and contain more cholesterol and saturated fat. Moreover, supplementation of the protein-restricted diet mostly with polyunsaturated fat also had a favourable effect on the p/s ratio. On the other hand, the mean values for cholesterol intake of the children from the control group are still within the advised limit for healthy Dutch children [11]. Serum cholesterol and LDL cholesterol concentrations do not differ significantly between both groups, but serum cholesterol as well as LDL cholesterol levels did rise significantly in the control group during the 1-year follow-up period. Protein restriction had no effect on carbohydrate intake which may explain that no significant differences in fasting serum triglycerides were found. Serum triglycerides were high in both groups, which may be caused by the decreased activity of lipoprotein lipase and a decreased triglyceride clearance in chronic renal failure [6].

Although from these results we cannot conclude that a diet without restrictions is a risk factor for cardiovascular disease in children with chronic renal failure, the rise in 1-year of serum cholesterol and LDL cholesterol does suggest an unfavourable effect on fat metabolism compared to a protein-restricted diet. The results of our study suggest that cholesterol intake and polyunsaturated/saturated fat ratio recommendations for healthy children may not apply for children with chronic renal failure. We postulate that it is advisable for children with chronic renal failure who are on a free diet to restrict cholesterol intake (possibly < 20 mg/MJ) in view of the possible development of atherosclerotic complications at a later stage in their lives.

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