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Very low protein diet supplemented with ketoanalogs improves blood pressure control in chronic kidney disease

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Blood pressure (BP) is hardly controlled in chronic kidney disease (CKD). We compared the effect of very low protein diet (VLPD) supplemented with ketoanalogs of essential amino acids (0.35 g/kg/day), low protein diet (LPD, 0.60 g/kg/day), and free diet (FD) on BP in patients with CKD stages 4 and 5. Vegetable proteins were higher in VLPD (66%) than in LPD (48%). LPD was prescribed to 110 consecutive patients; after run-in, they were invited to start VLPD. Thirty subjects accepted; 57 decided to continue LPD; 23 refused either diet (FD group). At baseline, protein intake (g/kg/day) was 0.79±0.09 in VLPD, 0.78±0.11 in LPD, and 1.11±0.18 in FD ($P<0.0001$). After 6 months, protein intake was lower in VLPD than LPD and FD (0.54±0.11, 0.78±0.10, and 1.04±0.21 g/kg/day, respectively; $P<0.0001$). BP diminished only in VLPD, from 143±19/84±10 to 128±16/78±7 mm Hg ($P<0.0001$), despite reduction of antihypertensive drugs (from 2.6±1.1 to 1.8±1.2; $P<0.001$). Urinary urea excretion directly correlated with urinary sodium excretion, which diminished in VLPD (from 181±32 to 131±36 mEq/day; $P<0.001$). At multiple regression analysis ($R^2=0.270$, $P<0.0001$), BP results independently related to urinary sodium excretion ($P=0.023$) and VLPD prescription ($P=0.003$), but not to the level of protein intake. Thus, in moderate to advanced CKD, VLPD has an antihypertensive effect likely due to reduction of salt intake, type of proteins, and ketoanalogs supplementation, independent of actual protein intake.

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Elevated blood pressure (BP) is the most frequent complication of chronic kidney disease (CKD).[1] Its correction is a prominent intervention because uncontrolled hypertension is a recognized determinant of progression of renal damage;[2–5] it also represents a major cause for the elevated cardiovascular morbidity and mortality detected in these patients.[6–9] Accordingly, current clinical practice guidelines strongly suggest reducing BP to less than 130/80 mm Hg.[10]

The achievement of BP goal in CKD in the 'real world' of clinical practice, however, remains dramatically low even in the presence of multidrug antihypertensive therapy including inhibitors of renin-angiotensin system.[1,11] This likely occurs because in CKD patients hypertension is mainly related to the impaired ability of the kidney to appropriately excrete dietary salt.[1,5] The consequent sodium retention, which is enhanced by pharmacological treatment with vasodilator agents, definitely precludes an optimal control of BP.[5] On the other hand, short-term studies have shown that the sole dietary restriction of salt, that is, in the absence of changes of protein intake, markedly diminishes BP in patients with moderate to advanced CKD.[12,13] This non-pharmacological intervention, moreover, enhances the antihypertensive and antiproteinuric effect of renin-angiotensin system inhibitors.[14,15]

Unfortunately, the implementation of low sodium diet in nephrology clinics is very low.[1] This critical problem can be explained by the observation that moderate protein restriction, that is, the most frequent non-pharmacological intervention in these patients, is not coupled with a significant reduction of salt intake.[1,16] However, the effect on dietary sodium intake of a marked dietary restriction of protein, such as that obtained by means of a supplemented very low protein diet (VLPD), remains undefined. Indeed, in well-motivated CKD patients, VLPD seems to be more efficacious than standard low protein diet (LPD) in reducing signs and symptoms of uremia, and postponing the need of dialysis independent of the rate of glomerular filtration rate (GFR) decline.[17–19] Therefore, it is possible to hypothesize that VLPD, besides reducing levels of urea, phosphate,

and other uremic toxins, may also limit the intake of salt,[19] and, consequently, the degree of extracellular volume expansion.

This hypothesis, even though extremely intriguing and of great potential clinical impact, has never been verified in CKD patients. To this aim, we assessed in hypertensive patients with moderate to advanced CKD, the antihypertensive effect of a VLPD supplemented with essential amino acids and ketoanalogs, as compared with standard LPD and unrestricted diet.

RESULTS

One hundred and fourteen patients were selected on the basis of inclusion criteria. We excluded four patients because of neoplastic disease ($n=3$) or infectious disease ($n=1$). At the end of the run-in period of standard LPD, 27% of patients chose the VLPD, whereas 52% of them preferred to remain at LPD and 21% refused either diet (free diet (FD) group). The main demographic and clinical characteristics of patients in VLPD, LPD, and unrestricted FD groups were similar (Table 1). Throughout the 6 months of follow-up, no patient dropped off the study and none progressed toward end-stage renal disease.

At baseline, the achievement of recommended target (BP < 130/80 mm Hg) was similarly poor in the three groups (Table 2). The mean number of prescribed antihypertensive drugs was generally greater than two. The most common agents prescribed were angiotensin-converting enzyme inhibitors and angiotensin II blockers; the distribution of the main antihypertensive drugs (antagonists of renin-angiotensin system and diuretics) was similar in the three groups. During follow-up, VLPD patients showed a significant reduction of both systolic and diastolic BP values after 3 and 6 months and a consequent increase in the number of patients reaching the BP target. Better BP control occurred in the presence of a significant reduction of the number of antihypertensive drugs. In contrast, BP control remained unmodified in LPD and FD patients.

Variation of dietary intake of protein and salt and of fractional urinary excretion of sodium (FENa), associated to

Table 1 | Basal characteristics of patients at VLPD, LPD, and FD

	VLPD	LPD	FD
N	30	57	23
Age, years	58.0 ± 16.1	56.3 ± 15.7	56.3 ± 15.6
Male gender, *n* (%)	18 (60)	29 (51)	14 (61)
BMI, kg/m^2	23.9 ± 2.5	25.5 ± 4.3	24.2 ± 2.4
CrCl, ml/min/1.73 m^2	17.1 ± 5.5	18.2 ± 6.0	17.6 ± 5.3
Renal Disease, %			
Glomerulonephritis	33.3	33.3	21.7
Hypertensive nephropathy	16.7	14.0	17.4
Diabetic nephropathy	3.3	10.5	21.7
PKD	23.3	19.3	8.7
Other or unknown	23.3	22.8	30.4

BMI, body mass index; CrCl, 24 h-measured creatinine clearance; FD, free diet; LPD, low protein diet; PKD, polycystic kidney disease; VLPD, very low protein diet.

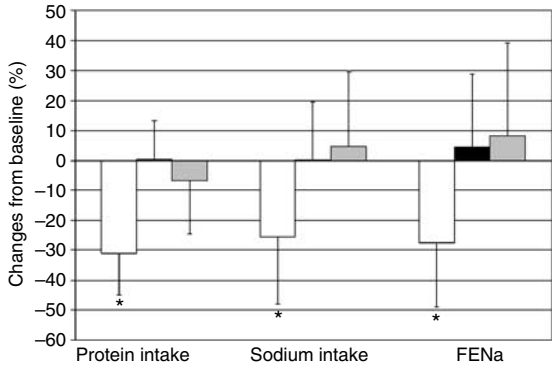

Figure 1 | Percent changes of protein intake, salt intake, FENa from baseline to 6 months, in patients at VLPD (white bars), LPD (black bars), and FD (gray bars). *$P<0.001$ vs LPD and FD.

Table 2 | Dietary protein and salt intakes and management of hypertension at baseline and during follow-up in patients at VLPD, LPD, and FD

	VLPD			LPD			FD		
	Baseline	3 months	6 months	Baseline	3 months	6 months	Baseline	3 months	6 months
PI, g/kg/day	0.79 ± 0.09	0.57 ± 0.19[a,b]	0.54 ± 0.11[b,c]	0.78 ± 0.11	0.77 ± 0.12	0.78 ± 0.10	1.11 ± 0.18	1.06 ± 0.18	1.04 ± 0.21
SI, mEq/day	181 ± 32	143 ± 38	131 ± 36[b,c]	170 ± 50	161 ± 57	166 ± 44	170 ± 60	175 ± 64	171 ± 51
SBP, mm Hg	143 ± 19	130 ± 17[b]	128 ± 16[b,c]	140 ± 21	138 ± 16	136 ± 15	141 ± 18	141 ± 19	139 ± 15
DBP, mm Hg	84 ± 10	80 ± 6	78 ± 7[b,c]	87 ± 10	86 ± 7	86 ± 7	85 ± 7	84 ± 5	83 ± 8
MBP, mm Hg	103 ± 11	96 ± 8[a,b]	95 ± 7[a,b]	105 ± 12	103 ± 8	102 ± 8	104 ± 9	103 ± 7	102 ± 8
BP < 130/80, *n* (%)	2 (7)	4 (13)	9 (30)[d]	4 (7)	2 (3)	2 (3)	2 (9)	2 (9)	1 (4)
Drugs, *n*	2.6 ± 1.1	1.7 ± 1.1[b]	1.8 ± 1.2[b]	2.0 ± 1.1	1.8 ± 1.2	2.1 ± 1.3	2.1 ± 1.2	1.7 ± 1.2	2.0 ± 1.1
Anti AII, *n* (%)	19 (63)	17 (57)	17 (57)	23 (40)	32 (56)	27 (47)	9 (39)	13 (56)	12 (52)
Diuretics, *n* (%)	15 (50)	16 (53)	18 (60)	24 (42)	26 (46)	29 (51)	10 (43)	9 (39)	11 (48)
FENa, (%)	8.6 ± 4.0	7.1 ± 5.1	6.2 ± 3.4[b]	7.5 ± 3.4	6.6 ± 5.4	7.6 ± 3.7	7.5 ± 3.8	7.8 ± 2.3	7.7 ± 3.5

Anti AII, ACE-inhibitors and angiotensin II blockers; BP, blood pressure; DBP, diastolic blood pressure; Drugs, antihypertensive drugs; FD, free diet; FENa, fractional excretion of sodium; LPD, low protein diet; MBP, mean blood pressure; PI, protein intake; SBP, systolic blood pressure; SI, sodium intake; VLPD, very low protein diet.
[a]$P<0.001$ vs baseline.
[b]$P<0.0001$ vs LPD and FD.
[c]$P<0.01$ vs LPD and FD.
[d]$P<0.001$ vs baseline and 3 months.

BP changes, are depicted in Table 2 and Figure 1. At baseline, the urinary urea excretion was similar in VLPD (6.5 ± 1.5 g/day) and LPD (6.2 ± 1.3 g/day), whereas it was higher in FD (9.4 ± 1.5 g/day, $P < 0.001$ vs other groups). During the study, protein intake decreased in VLPD patients. This was coupled with a significant reduction of both salt intake and FENa. In contrast, no significant modification of these parameters was observed in the other two groups. Indeed, the values of urinary urea and sodium excretion at 6 months directly correlated in VLPD patients ($n = 30$; $R = 0.453$, $P = 0.012$) (Figure 2), whereas no correlation was found in LPD and FD.

No significant difference was detected in dietary compliance between VLPD and LPD patients. Indeed, when considering acceptable a difference of less than 0.2 g/kg/day between prescribed and achieved protein intakes, 77 and 63% of VLPD and LPD patients, respectively, adhered to the diet at 6 months; similar results were obtained at 3 months (data not shown). The analysis of dietary diaries revealed that the sources of proteins exceeding prescription were mostly represented by proteins of vegetable origin, such as pasta and bread in both VLPD and LPD groups. In either LPD or VLPD group, no patient had a protein intake lower than that prescribed.

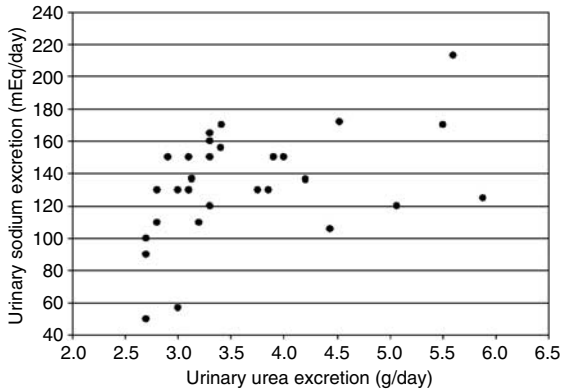

Figure 2 | Relationship between values of 24-h urinary urea and sodium excretion at 6 months in 30 patients at VLPD diet ($R = 0.453$, $P = 0.012$).

Changes of urinary sodium excretion did not differ between diuretic-treated and diuretic-untreated patients (-20 ± 29 and -24 ± 25%, respectively, $P = 0.744$). Similarly, the presence of polycystic kidney disease did not account for the observed changes in urinary sodium excretion; in fact, no significant difference in the changes of urinary sodium excretion became apparent between patients with and without polycystic kidney disease in either VLPD and LPD groups ($P = 0.208$ and $P = 0.534$, respectively). Overall, no association was found between renal disease and changes in sodium excretion.

Modifications of renal function parameters and cardio-renal risk factors during the study are reported in Table 3. Creatinine clearance did not vary throughout follow-up in either group. Only in VLPD patients, blood urea levels markedly decreased in parallel with the reduction of urinary urea excretion. Similarly, the urinary excretion of phosphate decreased only in VLPD patients (from 610 ± 226 to 341 ± 182 mg/day at 6 months, $P < 0.0001$), but not in LPD and FD patients (data not shown). The mean values of triglycerides and cholesterol decreased only in VLPD group; calcium–phosphate product diminished exclusively in VLPD patients, mainly because of the decrease of plasma levels of phosphate (from 4.5 ± 1.0 to 3.5 ± 0.7 mg/dl at 6 months, $P < 0.0001$); similarly, parathyroid hormone (PTH) decreased in VLPD, but not in LPD or FD group; also proteinuria significantly diminished only in VLPD group. In contrast, urinary excretion of potassium did not differ in the three groups either at baseline or during the follow-up.

The different dietary regimens did not affect the nutritional status; as reported in Table 3, in fact, body weight and serum levels of albumin did not change; also transferrin values and daily urinary creatinine excretion did not change during the follow-up in any group of patients (data not

Table 3 | Renal function and cardio-renal risk parameters at baseline and after 6 months in patients at VLPD, LPD, and FD

	VLPD		LPD		FD	
	Baseline	6 months	Baseline	6 months	Baseline	6 months
Body weight, kg	67.5 ± 10.2	67.1 ± 11.0	67.8 ± 13.6	68.0 ± 13.9	65.1 ± 7.3	65.6 ± 7.3
GFR, ml/min/1.73 m^2	17.1 ± 5.5	17.8 ± 6.6	18.2 ± 6.0	17.7 ± 7.0	17.6 ± 5.3	16.1 ± 5.8
Urea, mg/dl	146 ± 39	48 ± 19[a,b]	146 ± 48	145 ± 44	160 ± 37	165 ± 34
Albumin, g/dl	3.9 ± 0.4	3.9 ± 0.4	4.0 ± 0.3	4.0 ± 0.4	3.9 ± 0.4	4.0 ± 0.3
Hemoglobin, g/dl	11.6 ± 0.8	11.5 ± 0.8	11.6 ± 1.2	11.6 ± 0.9	11.5 ± 1.2	11.3 ± 1.0
TC, mg/dl	223 ± 36	169 ± 26[a,b]	216 ± 38	206 ± 36	214 ± 39	217 ± 36
TG, mg/dl	170 ± 40	140 ± 28[a,b]	176 ± 63	167 ± 37	170 ± 38	217 ± 36
CaxP, mg^2/dl^2	41 ± 10	31 ± 8[b,c]	38 ± 6	40 ± 5	38 ± 5	39 ± 5
PTH, pg/ml	175 ± 115	109 ± 73[a,d]	168 ± 114	170 ± 108	190 ± 72	189 ± 82
UK, mEq/day	52 ± 17	51 ± 17	48 ± 13	48 ± 14	48 ± 14	49 ± 15
Proteinuria, g/day	1.34 ± 1.2	0.87 ± 0.8[a]	1.43 ± 1.55	1.29 ± 1.4	0.79 ± 0.9	0.86 ± 0.7

CaxP, calcium–phosphorus product; FD, free diet; GFR, 24-h measured creatinine clearance; LPD, low protein diet; PTH, parathyroid hormone; TC, total cholesterol; TG, triglycerides; UK, urinary potassium excretion; VLPD, very low protein diet.
[a]$P < 0.0001$ vs baseline.
[b]$P < 0.001$ vs LPD and FD.
[c]$P < 0.001$ vs baseline.
[d]$P < 0.01$ vs LPD and FD.

Table 4 | Multiple regression analysis with mean BP at the end of the study as dependent variable in stages 4 and 5 CKD patients

	β Coefficient	P-value
Constant	94.817	0.0001
Age	0.075	0.119
Gender (female as reference)	−0.302	0.845
eGFR	−0.134	0.175
Number of antihypertensive drugs	1.256	0.101
Diuretic use	−0.191	0.900
Protein intake	−3.882	0.397
Supplemented VLPD	−6.692	0.003
Sodium intake	0.696	0.023

BP, blood pressure; CKD, chronic kidney disease; eGFR, GFR estimated by Cockroft-Gault equation; VLPD, very low protein diet.
Model summary: R^2=0.270, P=0.0001.

shown). Body weight was similar in diuretic-treated and untreated patients in VLPD, LPD, and FD groups during the study.

On the basis of these results, we carried out a multiple regression analysis to determine the independent role of the main clinical characteristics on achieved mean BP at 6 months (Table 4). The model, which explained 27% of variance of mean BP, identified sodium intake ($P = 0.023$) and prescription of supplemented VLPD ($P = 0.003$) as the sole independent predictors of BP level at 6 months. Indeed, the effects of age, gender, GFR, number of antihypertensive drugs, use of diuretic, and protein intake were not significant.

DISCUSSION

This prospective study provides first-time evidence that in patients with CKD stages 4 and 5, the VLPD supplemented with ketoanalogs induces a marked and sustained decrease of BP in the presence of reduction of prescribed antihypertensive drugs. This effect was specifically related to the prescription of VLPD, as standard LPD, similarly to the unrestricted diet, was coupled with the maintenance of higher BP levels. Such a peculiar benefit of VLPD was observed in patients who started with inadequate BP control at baseline in spite of multidrug antihypertensive therapy.

Specifically, after the 6-month period of follow-up, VLPD helped in achieving BP target (<130/80 mm Hg) from 7 to 30% of patients and led to a sustained decrement in BP, mainly owing to a large reduction, by 14 mm Hg on average, in systolic BP. The maintenance of a similar reduction in systolic BP is particularly relevant to prevent cardiovascular events in high-risk patients, such as in the case of our patients.[20] In addition, adequate BP control in the predialysis phase of CKD effectively prevents cardiovascular mortality during the subsequent dialysis period.[21]

A possible explanation to this beneficial effect of VLPD derives from the contemporaneous examination of salt intake and FENa (Figure 1). We observed a significant decrement of these two parameters exclusively in VLPD patients. The reduction of FENa, in the presence of stable GFR and diminished sodium intake, indicates increased tubular sodium reabsorption secondary to extracellular volume contraction.[5,12,13] It is, therefore, reasonable to hypothesize that VLPD led to a reduction of volume expansion secondary to the diminished salt intake. Indeed, in agreement with the hypothesis of Guyton and Coleman on the salt sensitivity of BP in essential hypertension,[22] a positive salt balance raises osmotic pressure, increases water intake, and thus rapidly causes hypervolemia. The consequent increase of peripheral vascular resistances, in turn, leads to the steady hypertensive state. In CKD patients, the extracellular volume expansion secondary to impaired sodium excretion occurs since the early phase of the disease.[23] Consequently, effective correction of arterial hypertension is difficult to obtain in these patients, even by multidrug therapy, if salt intake is not restricted.[5] Indeed, also efficacious decrement of sodium intake is hard to achieve in CKD patients, as in non-CKD hypertensive subjects.[1,5] The present study evidences that standard moderate protein restriction is not associated with any beneficial effect on BP control with respect to unrestricted protein diet. Although a parallel variation of protein and salt intake during administration of restricted protein diets has been previously hypothesized,[19] no investigator has ever formally addressed this issue. This study shows for the first time that in CKD patients a direct relationship between protein and sodium intake occurs during VLPD, but not during LPD (Figure 2). Taken together, these data indicate that extracellular volume expansion, which is a key determinant of uremia-related hypertension,[10,24–26] can be substantially corrected by means of severe protein restriction because of the concomitant significant reduction of sodium intake. Specifically, the estimation of NaCl content of low protein products showed that VLPD provides almost 2 g/day of NaCl less than LPD. Such an amount accounts for most of the difference detected in urinary Na excretion during follow-up between VLPD and LPD patients (Table 2).

The observed high extent of antihypertensive response to moderate sodium restriction may be an unexpected finding. As compared with CKD patients, in fact, in hypertensive patients with normal GFR, a greater reduction of sodium intake is associated with much lower decrements in systolic and diastolic BP values.[27,28] On the other hand, the antihypertensive response to salt reduction, the so-called salt sensitivity of BP, greatly increases in CKD in parallel with worsening of renal function, because of the dependent increment of the extracellular volume expansion.[5,12,13,29] Therefore, in this study, a sustained decrease of sodium intake of about 3 g NaCl/day secondary to the shift from LPD to VLPD, was associated with a decrement of mean BP of about 8–9 mm Hg; similarly, Koomans *et al.*[13] found that in advanced CKD a greater acute decrease of sodium intake of about 6 g/day, in the absence of changes in protein intake, led to a greater decrement of mean BP of about 12 mm Hg. On the other hand, a previous study by our group indicates that the isolated restriction of protein intake, that is, in the presence of constantly normal salt intake, does not induce any change of mean BP values in CKD patients.[30] Overall,

these data identify the moderate reduction of salt intake, with the dependent partial correction of extracellular volume expansion, as a relevant determinant of BP decrease during VLPD. In addition, other authors have evidenced that moderate dietary sodium restriction rapidly normalizes BP in patients with systolic hypertension also by improving large elastic artery compliance.[31,32]

According to the results of multivariate analysis (Table 4), we can reasonably exclude that the variation of total protein intake *per se*, as estimated by the daily urinary urea excretion, can have an independent role in the reduction of BP. Protein intake, as it was used for this analysis, is only a quantitative parameter that does not distinguish the quality of proteins; indeed, the ratio of vegetable to animal proteins was higher in VLPD than in LPD. This observation is of interest as a significant inverse relationship between intake of vegetable proteins and BP levels has been documented.[33] Thus, the major portion of vegetable proteins in the VLPD may have affected the BP. In addition, because of the significant effect of VLPD prescription, we can hypothesize that the supplement of ketoanalogs/amino acid in VLPD may also play a direct role on BP control, possibly by inducing a vasodilator effect through the increase of plasma concentrations of the respective branched-chain essential amino acids.[34,35]

Other diet-related and -unrelated lifestyle modifications, such as weight loss, increased potassium intake, reduction in the plasma levels of cholesterol, phosphate, and PTH, might lower BP.[33,36,37] There are also some suggestions that intensive cholesterol reduction by statin administration may lower BP by improving artery stiffness and that pharmacological correction of calcium–phosphate metabolism and secondary hyperparathyroidism may contribute to decreasing BP in CKD.[36,37] In our study, no change in body weight and urinary potassium excretion was detected during follow-up. Unlike this, we observed a significant reduction of plasma cholesterol likely related to the lower content of cholesterol in VLPD vs other diets; similarly, the lower intake of phosphate in VLPD, as indicated by the lower urinary excretion, likely accounted for the reduction of phosphatemia and the consequent improvement of secondary hyperparathyroidism.

The therapeutic advantage of VLPD was not limited to the better control of BP. During VLPD, in fact, we observed a significant reduction in proteinuria, which is an independent predictor of renal and cardiovascular outcome.[38] The antiproteinuric response to VLPD was likely related to the amelioration of glomerular hypertension owing to the lowering of systemic BP, and also to the reduction of protein and salt intake *per se*, as suggested by different authors.[38–41] Of note, according to Klosa *et al.*,[42] a reduction of proteinuria of at least 35% associated with a reduction in BP, as observed in the present study, predicts a better renal and cardiovascular prognosis over the long run.

A limitation of the study is represented by the non-randomized assignment of different protein regimens; therefore, minor selection biases cannot be excluded. However, the number of enrolled patients was sufficient to avoid any significant difference in the main demographic and clinical basal characteristics among the three groups. On the other hand, the spontaneous clustering of patients in the three groups allowed us to evaluate the rate of acceptance for either diet; to our knowledge, this is the first study evaluating the implementation of dietary protein restriction on single patient-level basis. We found that the choice of LPD almost doubled that of VLPD (52 vs 27%). Nonetheless, compared to LPD, in VLPD patients the adherence to the diet was not reduced; indeed, almost two-third of patients were compliant to the diet in either group. These findings overall indicate that intensive protein restriction represents a major change in lifestyle and is accepted by only a minority of CKD patients, even when they are greatly motivated because renal function is close to the pre-dialysis value; however, once accepted by the patient, compliance to this diet is quite high.

In conclusion, this prospective study represents the first evidence that in moderate to advanced CKD, the VLPD diet supplemented with ketoanalogs, as compared to either standard LPD or unrestricted diet, allows a marked and sustained improvement of BP control which is associated with a significant decrement in the extent of proteinuria. This beneficial effect, rather than being dependent on the effective amount of ingested proteins, appears to be directly dependent to the specific characteristics of the VLPD (type of proteins, cholesterol, and phosphate content), and also to ketoanalog supplementation, as well as to the concomitant reduction of salt intake with consequent partial correction of extracellular volume expansion.

These findings support the daily efforts of nephrologists in challenging the reluctance of CKD patients to change their lifestyle, especially when considering the elevated cardiovascular risk associated with renal disease.

MATERIALS AND METHODS

Subjects

The study was performed in three outpatient nephrology clinics. Consecutive incident CKD patients in the period 1 January to 31 December 2004 were enrolled according to the following inclusion criteria: age $\geqslant$18 years, measured creatinine clearance less than 30 ml/min/1.73 m^2, and arterial hypertension, defined as either use of antihypertensive drugs or BP greater than 130/80 mm Hg in the absence of antihypertensive drugs. Exclusion criteria were pregnancy, history of dialysis/renal transplantation, malignant disease, infectious disease, use of immunosuppressive drugs, and acute changes of renal function.

Design of the study

The study was a prospective, controlled study. A 3-month period of run-in preceded the study; during this period, all new CKD stages 4 and 5 patients who met the inclusion criteria were prescribed a standard LPD (0.60 g protein/kg body weight/day). In each patient, we verified the stability of creatinine clearance measured in three separate visits (coefficient of variation $<$5%), and the nutritional and metabolic status. After run-in was completed, we proposed to all patients the supplemented VLPD (0.30 g of protein/kg body weight/day). Patients refusing VLPD were invited to remain on their standard LPD. If also this latter choice was not accepted, the patient

was included in the FD group. All patients were prescribed at least 30 kcal/kg/day of energy; a lower intake of calories was considered in those with body mass index $>30\,kg/m^2$. VLPD diet was supplemented with a mixture of ketoanalogs and essential amino acids (Alfa Kappa; Shire Italia, Firenze, Italy) administered at the dose of one pill per 5 kg body weight, in order to maintain the neutral nitrogen balance even at the lower protein intakes, thus to increase the efficiency of nitrogen utilization and to maintain a good nutritional status.[43] Each pill contained calcium keto-isoleucine 67 mg, calcium keto-leucine 101 mg, calcium keto-alanine 68 mg, calcium keto-valine 86 mg, calcium hydroxyl-methionine 59 mg, L-lysine monoacetate 105 mg, L-threonine 53 mg, L-histidine 38 mg, and L-tyrosine 30 mg. Inclusion of the amino acids by the oral supplements as an additional source of nitrogen, resulted in a mean total protein prescription (from food and supplements) of 0.35 g/kg/day in the VLPD group. The content of cholesterol in the VLPD and LPD diets was 60–80 and 90–130 mg/day, respectively. The two diets had the same content of NaCl (less than 1 g/day) when considering fresh food shared by the two diets, that is, milk, meat, fish, fresh cheese, and vegetables/fruit; whereas NaCl content differed when examining bakery products (bread and analogs) and pasta. Specifically, owing to both the difference in sodium content between protein and no-protein pasta, and bakery products and the difference in the amount of such food between the two diets, the daily salt amount was almost 2 g higher in LPD vs VLPD (i.e. in the diet containing 1900 kcal, which was the most frequently prescribed, the whole sodium content was 1260 mg/day (53 mEq = 3.1 g NaCl) and 540 mg/day (22 mEq = 1.3 g NaCl), respectively, in LPD and VLPD diets). All patients at the first visit received the common indications to minimize added salt in order to keep daily sodium intake <100 mmol. As a further difference, the two diets contained a different percentage of vegetable proteins that was equal to 48% in LPD and 66% in VLPD, respectively.

Antihypertensive therapy was prescribed to all patients during the run-in period to obtain the BP target of levels $<130/80$ mm Hg; it included angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, calcium channel blockers, diuretics (oral furosemide at the daily dose of 50 mg in patients with GFR ranging from 30 to 15 ml/min/1.73 m^2, and 75 mg in patients with GFR lower than 15 ml/min/1.73 m^2), β-blockers, or α-blockers. During the study, the number of antihypertensive drugs was reduced if systolic BP reached values <115 mm Hg or in the presence of symptomatic hypotension. Changes in the number of antihypertensive drugs were made after examining the clinical and laboratory data.

During the run-in, patients were trained to correctly collect the 24-h urine. Patients underwent a complete study including blood withdrawal and urine collection, at baseline and after 3 and 6 months. They underwent a clinical evaluation every month, including the measurement of body weight and BP, with eventual adjustment of therapy, and the dietetic counseling by a skilled dietician including evaluation of the adherence to the prescribed diet. There was at least 1 month period between change of therapy and laboratory determinations to allow the achievement of steady state for sodium balance and to avoid changes of sodium excretion eventually related to change in diuretic prescription. Patients ended the observation if creatinine clearance decreased to less than 7 ml/min/1.73 m^2 or in the case of development of uremic complications requiring dialysis treatment.[44]

Measurements and calculations

During the physician's visit (0800–1000 h), clinical BP was measured, according to the recommendations of the European Society of Hypertension,[45] in a quiet environment with a mercury sphygmomanometer with the patient in a sitting position after 5 min of rest. Systolic and diastolic BP values (Korotkoff phases I and V, respectively) represented in each visit the mean of three different readings measured at 5-min intervals. In each patient, measurements were obtained by the same physician during the whole study. Measurements of clinical BP were performed on the morning just before the administration of drug(s). Mean BP was calculated by using the formula $(2 \times \text{diastolic pressure}) + \text{systolic pressure}/3$. Body mass index was calculated as body weight (kg)/square value of the height (m).

At each time point of the study, we measured in blood samples urea, creatinine, sodium, potassium, phosphate, calcium, intact PTH, total proteins, albumin, total cholesterol, triglycerides, transferrin, and hemoglobin. In the 24-h urinary collection, we measured the excretion of urea, creatinine, sodium, potassium, phosphate, and proteins; the daily urine collection was considered inaccurate and discarded if the value of measured creatinine excretion was outside the 60–140% range of the value calculated according to Dwyer and Kenler.[46] The FENa was calculated as 24-h urinary excretion of sodium (urinary concentration of sodium (mEq/l) $\times$ urinary output (ml/24 h)) $\times$ 100/(GFR (ml/24 h) $\times$ plasma sodium concentration (mEq/l)).

Serum albumin, serum and urinary levels of creatinine, urea, sodium, phosphate and potassium were measured by autoanalyzer (Olympus AU 400; Olympus Italia, Segrate, Italy). Measurement of proteinuria was performed by pyrogallol red-molibdate method. Creatinine in plasma and urine was measured by means of modified kinetic Jaffè reaction. Hemoglobin was measured by Coulter counter (Coulter Electric, Hialeah, FL, USA). PTH level was assessed by standard radioimmunoassay method using two affinity-purified goat antibodies specific for two different regions of the PTH molecule (PTH 1–34 and PTH 39–84) (Sorin, Saluggia, Italy).

Daily salt intake in grams per day was calculated dividing the 24-h urinary sodium excretion by 17.1.[10,12,27] Dietary protein intake was estimated from the 24-h urea nitrogen excretion according to Maroni *et al.*[47]

Statistical analysis

Continuous variables were expressed as mean $\pm$ s.d. and compared with analysis of variance except for variables with non-normal distribution (identified by Shapiro–Wilk test) that was analyzed with Kruskall–Wallis test. The changes of continuous variables during the follow-up (intra-group comparisons) were assessed by means analysis of variance for repeated measurements or by Friedman's test for those with non-normal distribution. Categorical variables were expressed as percentage and compared by using χ^2 test. Linear correlation analysis and multiple regression analysis were also used; the latter was applied to evaluate the relationships between achieved mean BP and main clinical variables, considering age, antihypertensive drugs, protein intake, sodium intake, and estimated GFR as continuous variables, and gender, supplemented VLPD, and diuretic treatment as categorical variables. A two-tail P-value <0.05 was considered statistically significant.

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REFERENCES

1. De Nicola L, Minutolo R, Chiodini P *et al.* for the TABLE in CKD Study Group. Global approach to cardiovascular risk in chronic kidney disease: reality and opportunities for intervention. *Kidney Int* 2006; **69**: 538–545.
2. Bakris GL, Weir MR, Shanifar S *et al.* Effects of blood pressure level on progression of diabetic nephropathy. Results from the RENAAL Study. *Arch Int Med* 2003; **163**: 1555–1565.
3. Jafar TH, Stark PC, Schmid CH *et al.* for the AIPRD Study Group. Progression of chronic kidney disease: the role of blood pressure control, proteinuria, and angiotensin-converting enzyme inhibition. *Ann Inter Med* 2003; **139**: 244–253.
4. Bakris GL, Williams M, Dworkin L *et al.* Preserving renal function in adults with hypertension and diabetes: a consensus approach: National Kidney Foundation Hypertension and Diabetes Executive Committees Working Group. *Am J Kidney Dis* 2000; **36**: 646–661.
5. De Nicola L, Minutolo R, Bellizzi V *et al.* for the TABLE in CKD Study Group. Achievement of target blood pressure levels in chronic kidney disease: a salty question? *Am J Kidney Dis* 2004; **43**: 782–795.
6. Sarnak MJ, Levey AS, Schoolwert AC *et al.* Kidney disease as a risk factor for development of cardiovascular disease. A statement from the American Heart Association Councils on kidney in cardiovascular disease, high blood pressure research, clinical cardiology, and epidemiology and prevention. *Circulation* 2003; **108**: 2154–2169.
7. Keith DS, Nichols GA, Gullion CM *et al.* Longitudinal follow-up and outcomes among a population with chronic kidney disease in a large managed care organization. *Arch Inter Med* 2004; **164**: 659–663.
8. Go SA, Chertow GM, Fan D *et al.* Chronic kidney disease and the risks of death, cardiovascular events and hospitalization. *N Engl J Med* 2004; **351**: 1296–1305.
9. Sarnak MJ, Greene T, Wang X *et al.* The effect of a lower target blood pressure on the progression of kidney disease: long-term follow-up of the modification of diet in renal disease study. *Ann Int Med* 2005; **142**: 342–351.
10. K/DOQI clinical practice guidelines on hypertension and antihypertensive agents in chronic kidney disease. *Am J Kidney Dis* 2004; **43**(Suppl 1): 11–13.
11. Marin R, Fernandez-Vega F, Gorrostidi M *et al.* for COPARENAL Study Investigators. Blood pressure control in patients with chronic renal insufficiency in Spain: a cross-sectional study. *J Hypertension* 2006; **24**: 395–402.
12. Cianciaruso B, Bellizzi V, Minutolo R *et al.* Renal adaptation to dietary sodium restriction in moderate renal failure resulting from chronic glomerular disease. *J Am Soc Neprol* 1996; **7**: 306–313.
13. Koomans HA, Roos JC, Dorhout Mees EJ *et al.* Sodium balance in renal failure: a comparison with normal subjects under extremes of sodium intake. *Hypertension* 1985; **7**: 714–721.
14. Navis G, De Jong PE, Donker AJM *et al.* Moderate sodium restriction in hypertensive subjects: renal effects of ACE-inhibition. *Kidney Int* 1985; **31**: 815–819.
15. Houlihan C, Allen TJ, Baxter AL *et al.* A low-sodium diet potentiates the effects of losartan in type 2 diabetes. *Diabetes Care* 2002; **25**: 663–671.
16. Cianciaruso B, Capuano A, D'Amaro E *et al.* Dietary compliance to a low protein and phosphate diet in patients with chronic renal failure. *Kidney Int* 1989; **36**(S27): S173–S176.
17. Walser M, Hill S. Can renal replacement be deferred by a supplemented very low protein diet? *J Am Soc Nephrol* 1999; **10**: 110–116.
18. Aparicio M, Chaveau P, De Precigout V *et al.* Nutrition and outcome on renal replacement therapy of patients with chronic renal failure treated by a supplemented very low protein diet. *J Am Soc Nephrol* 2000; **11**: 708–716.
19. Mitch WE. Beneficial responses to modified diets in treating patients with chronic kidney disease. *Kidney Int* 2005; **67**(S94): S133–S135.
20. Ogden LG, He J, Lydick E, Whelton PK. Long-term absolute benefit of lowering blood pressure in hypertensive patients according to the JNC VI risk stratification. *Hypertension* 2000; **35**: 539–545.
21. Lucas MF, Quereda C, Teruell JL *et al.* Effect of hypertension before beginning dialysis on survival of hemodialysis patients. *Am J Kidney Dis* 2003; **41**: 814–821.
22. Guyton AC, Coleman TG. Quantitative analysis of the pathophysiology of hypertension. *J Am Soc Nephrol* 1999; **10**: 2248–2258.
23. Bellizzi V, Scalfi L, Terracciano V *et al.* Early changes in bioelectrical estimates of body composition in chronic kidney disease. *J Am Soc Nephrol* 2006; **17**: 1481–1487.
24. Vasawada N, Agarwal R. Role of excess volume in the pathophysiology of hypertension in chronic kidney disease. *Kidney Int* 2003; **64**: 1772–1779.
25. Brater DC. Diuretic therapy. *N Engl J Med* 1998; **339**: 387–395.
26. Ritz E, Dikow R, Morath C, Schwenger V. Salt – a potential 'uremic toxin'? *Blood Purif* 2006; **24**: 63–66.
27. Sacks FM, Svetkey LP, Vollmer WM *et al.* Effects on blood pressure of reduced dietary sodium and Dietary Approaches to Stop Hypertension (DASH) diet: DASH –Sodium Collaborative Research Group. *N Engl J Med* 2001; **344**: 3–10.
28. He FJ, Mac Gregor GA. Effect of modest salt reduction on blood pressure: a meta-analysis of randomized trials: implications for public health. *J Hum Hypertens* 2002; **16**: 761–770.
29. Koomans HA, Roos JC, Boer P *et al.* Salt sensitivity of blood pressure in chronic renal failure: evidence for renal control of body fluid distribution in man. *Hypertension* 1982; **4**: 190–197.
30. Cianciaruso B, Bellizzi V, Capuano A *et al.* Short-term effects of low protein-normal sodium diet on renal function in chronic renal failure. *Kidney Int* 1994; **45**: 852–860.
31. Gates PE, Tanaka H, Hiatt WR, Seals DR. Dietary sodium restriction rapidly improves large elastic artery compliance in older adults with systolic hypertension. *Hypertension* 2004; **44**: 35–41.
32. Seals DR, Tanaka H, Clevenger CM *et al.* Blood pressure reductions with exercise and sodium restriction in postmenopausal women with elevated systolic pressure: role of arterial stiffness. *J Am Coll Cardiol* 2001; **38**: 506–513.
33. Appel LJ, Brands MW, Daniels SR *et al.* Dietary approaches to prevent and treat hypertension. A scientific statement from the American Heart Association. *Hypertension* 2006; **47**: 296–308.
34. Mitch WE, Abras E, Walser M. Long-term effects of a new ketoacid-amino acid supplement in patients with chronic renal failure. *Kidney Int* 1982; **22**: 48–53.
35. Magri P, Rao MA, Cangianiello S *et al.* Early impairment of renal hemodynamic reserve in patients with asymptomatic heart failure is restored by angiotensin II antagonism. *Circulation* 1998; **98**: 2849–2854.
36. Rostand SG, Drueke TB. Parathyroid hormone, vitamin D and cardiovascular disease in chronic renal failure. *Kidney Int* 1999; **56**: 383–392.
37. Ferrier KE, Muhlmann MH, Baguet JP *et al.* Intensive cholesterol reduction lowers blood pressure and large artery stiffness in isolated systolic hypertension. *J Am Coll Cardiol* 2002; **39**: 1020–1025.
38. Remuzzi G, Benigni A, Remuzzi A. Mechanisms of progression and regression of renal lesions of chronic nephropathies and diabetes. *J Clin Invest* 2006; **116**: 288–296.
39. Mitch WE, Remuzzi G. Diets for patients with chronic kidney disease, still worth prescribing. *J Am Soc Nephrol* 2004; **15**: 234–237.
40. Weir MR, Fink JC. Salt intake and progression of chronic kidney disease: an overlooked modifiable exposure? A commentary. *Am J Kidney Dis* 2005; **45**: 176–188.
41. Gansevoort RT, De Zeeuw D, De Jong PE. Additive antiproteinuric effect of ACE inhibition and a low-protein diet in human renal disease. *Nephrol Dial Transpl* 1995; **10**: 497–504.
42. Klosa N, Bakris G. Lessons learned from recent hypertension trials about kidney disease. *Clin J Am Soc Nephrol* 2006; **1**: 229–235.
43. Walser M, Coulter AW, Dighe S, Crantz FR. The effect of ketoanalogues of essential amino acids in severe chronic uremia. *J Clin Invest* 1973; **52**: 2865–2877.
44. Di Iorio BR, Minutolo R, De Nicola L *et al.* Supplemented very low protein diet ameliorates responsiveness to erythropoietin in chronic renal failure. *Kidney Int* 2003; **64**: 1822–1828.
45. O'Brien E, Asmar R, Beilin L *et al.* on behalf of the European Society of Hypertension Working Group on Blood Pressure Monitoring. European Society of Hypertension recommendations for conventional, ambulatory and home blood pressure measurement. *J Hypertens* 2003; **21**: 821–848.
46. Dwyer J, Kenler SR. Assessment of nutritional status in renal disease. In: Mitch WE, Klahr S (eds). *Nutrition and the Kidney*, 2nd edn. Little, Brown and Company: Boston, MA, 1993 pp 61–95.
47. Maroni BJ, Steinman TI, Mitch VE. A method for estimating nitrogen intake of patients with chronic renal failure. *Kidney Int* 1985; **27**: 58–65.