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Dietary protein, blood pressure and renal function in renal transplant recipients

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Abstract

Hypertension is highly prevalent among renal transplant recipients (RTR) and a risk factor for graft failure and cardiovascular events. Protein intake has been claimed to affect blood pressure (BP) in the general population and may affect renal function. We examined the association of dietary protein with BP and renal function in RTR. We included 625 RTR (age 53 (SD 13) years; 57 % male). Protein intake was assessed with a FFQ, differentiating between animal and plant protein. BP was measured according to a strict protocol. Creatinine clearance and albuminuria were measured as renal parameters. Protein intake was 83 (SD 12) g/d, of which 63 % derived from animal sources. BP was 136 (SD 17) mmHg systolic (SBP) and 83 (SD 11) mmHg diastolic (DBP). Creatinine clearance was 66 (SD 26) ml/min; albuminuria 41 (10–178) mg/24 h. An inverse, though statistically insignificant, association was found between the total protein intake and both SBP ($\beta = -2{\cdot}22$ mmHg per SD, $P=0{\cdot}07$) and DBP ($\beta = -0{\cdot}48$ mmHg per SD, $P=0.5$). Protein intake was not associated with creatinine clearance. Although albuminuria was slightly higher in the highest tertile of animal protein intake compared with the lowest tertile (66 *v.* 33 mg/d, respectively, $P=0{\cdot}03$), linear regression analyses did not reveal significant associations between dietary protein and albuminuria. Protein intake exceeded the current recommendations. Nevertheless, within the range of protein intake in our RTR population, we found no evidence for an association of dietary protein with BP and renal function. Intervention studies focusing on different protein types are warranted to clarify their effect on BP and renal function in RTR.

Key words: Protein intake: Renal transplantation: Blood pressure

High blood pressure (BP) is a serious health problem after renal transplantation[1–3]. It is an important risk factor for graft failure, cardiovascular events and mortality in renal transplant recipients (RTR)[3,4], and usually requires multiple antihypertensives to ensure adequate BP control. Remarkably, the mechanisms and treatment of high BP in RTR are poorly defined and management is largely derived from data in non-transplant populations. Better elucidation of the mechanisms underlying high BP in RTR is urgently needed, as emphasised recently[5]. Data in non-transplant populations consistently demonstrate an important role of diet and lifestyle in BP. Well-established dietary factors that favourably affect BP in the general population are weight reduction, reduced salt intake, moderation of alcohol intake and increased K intake[6], and in non-transplant renal patients dietary salt restriction[7,8]. In a first study on BP and dietary factors in RTR, we have recently reported a positive association between Na intake and BP[9], suggesting that modification of dietary factors can beneficially influence BP in addition to pharmacological BP regimens. Currently, interest is growing in the influence of dietary patterns and macronutrient intake, including protein, on BP. Dietary protein has also been claimed to affect BP, but the large body of literature on dietary protein and BP in the general population[10–16] is not consistent. In renal patients, dietary protein can affect renal haemodynamics as well as renal protein loss, hence modifying the course of

Abbreviations: BP, blood pressure; en%, percentage of energy; RTR, renal transplant recipients; SBP, systolic blood pressure.

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long-term renal damage[7–10]. By these mechanisms, dietary protein might also affect BP. Concern exists that high protein intake induces high intraglomerular pressure and concurrent hyperfiltration, eventually leading to kidney damage and subsequent hypertension[17,18]. Although data from intervention studies applying protein restriction in chronic kidney disease were not entirely conclusive[19,20], dietary recommendations for patients with chronic kidney disease advocate a protein intake of 0·6–0·8 g/kg per d, to decrease renal workload and help delay the progression of kidney failure[21].

Considering the vast body of studies on dietary protein in chronic kidney disease, surprisingly little data are available on the impact of dietary protein in RTR. Data on dietary habits, and on associations of dietary protein with BP and renal function in RTR are virtually lacking, and consequently, the empirical basis for the few available dietary guidelines regarding protein intake for RTR is virtually absent[22,23]. Consequently, it remains unclear, for medical practitioners as well as for RTR, what the optimal level and favourable source of dietary protein are in this population[24].

In the present study, therefore, we aimed to clarify the relationship of protein with BP and renal function in stable RTR. For this purpose, we examined dietary habits, and analysed whether the intakes of total protein and types of protein (plant and animal) were associated with BP and renal function, in a cross-sectional analysis in a Dutch patient-based cohort of 625 RTR with a functioning graft for at least 1 year.

Methods

Design and study population

We conducted an observational study to perform cross-sectional analyses in a large, single-centre RTR cohort. We invited all RTR (≥18 years) with a functioning graft for at least 1 year who visited our outpatient clinic between November 2008 and March 2011. RTR were all transplanted in our centre, had sufficient knowledge of the Dutch language and had no history of drug or alcohol addiction, as reported in their patient files. RTR were on standard antihypertensive and immunosuppressive therapy. Of 817 initially invited RTR, 707 (87 %) signed written informed consent to participate in the present study. We excluded all patients with missing dietary data, leaving 625 RTR eligible for analysis. The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the Institutional Review Board (METc 2008/186). The routine regimen included no specific dietary counselling, except for discouraging excess Na intake and encouraging losing weight in overweight individuals. Patients with diabetes were counselled as appropriate to adapt their dietary habits to achieve normoglycaemia.

Assessment of protein intake

Dietary intake was assessed using a semi-quantitative FFQ that inquired about the intake of 177 food items during the last month. For each item, the frequency was recorded in times per d, week or month. The number of servings per frequency was expressed in natural units (for example, slice of bread or apple) or household measures (for example, cup or spoon). The questionnaire was self-administered and filled out at home. At the day of the visit to the outpatient clinic, all FFQ were checked for completeness by a trained researcher and inconsistent answers were verified with the patients. Total energy and nutrient intake per d was calculated using a computerised Dutch food composition table taking seasonal variation into account[25]. Because RTR have only sparsely been subject to nutritional studies thus far, we checked consistency of our data on protein intake in our population by comparing the estimated protein intake with 24 h urinary urea excretion. Therefore, all participants were carefully instructed to collect a 24 h urine sample according to a strict protocol. Urinary urea excretion was considered as a marker reflecting dietary total protein intake and was used to calculate protein intake according to the method of Maroni and colleagues taking also proteinuria into account (protein intake (g/d) = (0·18 × urinary urea excretion in mmol per 24 h) + 15 + urinary protein excretion in g per 24 h)[26,27]. In addition, excretion of several urinary components was measured to infer dietary intake of additional dietary nutrients such as Na and K.

Outcome measurements

All measurements were performed during a morning visit to the outpatient clinic. Fasting BP (mmHg) was measured according to a strict protocol. Participants were left alone in a room in half-sitting position while systolic BP (SBP), diastolic BP (DBP) and mean arterial pressure were measured with a semi-automatic device (Dinamap® 1846; Critikon). Measurements were performed every minute for 15 min and values of the last three measurements were averaged.

Blood was drawn after an 8–12 h overnight fasting period in the morning after completion of 24 h urine collection. Renal function was assessed by 24 h urinary creatinine clearance (ml/min), calculated as time-factored urinary creatinine concentration (mg/min) divided by plasma creatinine concentration (mg/ml). Serum creatinine levels were determined using a modified version of the Jaffé method (MEGA AU 510; Merck Diagnostica). Plasma and urinary concentrations of electrolytes and urea were measured using routine clinical laboratory methods, as were serum cholesterol and HbA1c. Urinary albumin concentration was determined by nephelometry (Dade Behring Diagnostic). Total urinary protein concentration was analysed using the Biuret reaction (MEGA AU 510; Merck Diagnostica). Proteinuria was defined as urinary protein excretion ≥0·5 g/24 h.

Collection of risk factor data

Information on patients' health status, medical history and medication use was obtained from patient records. Questionnaires were used to obtain information on smoking behaviour and alcohol intake. Participants were classified as current smokers, former smokers or never smokers. Alcohol intake was assessed based on a self-reported number of beverages

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consumed weekly, converted into g ethanol/d and divided into quartiles (no alcohol, 0–10 g/d, 10–30 g/d and >30 g/d). Body weight and height were measured with participants wearing indoor clothing without shoes. BMI was calculated as weight divided by height squared (kg/m^2).

Statistical analyses

Data analysis was performed using SPSS version 18.0 software (SPSS, Inc.). Normality was tested with the Kolmogorov–Smirnov test and skewed data were normalised by logarithmic transformation (i.e. albuminuria and proteinuria). Protein intake (total, plant and animal) was adjusted for total energy intake according to the residual method which is based on an isoenergetic principle[28]. Characteristics of the study population and data on dietary intake were calculated in tertiles of energy-adjusted total protein intake. Data are presented as means and standard deviations, unless stated otherwise.

We used multivariable linear regression models to obtain the regression coefficients for BP and renal parameters per SD of energy-adjusted protein intake (total, plant and animal) in RTR. Our basic model (model 1) included age (continuous) and sex. In the second model, we further adjusted for BMI (continuous), SBP (continuous; only applied in analyses for the association between protein intake and renal function), smoking behaviour (never/ever/current), alcohol consumption (no alcohol, 0–10 g/d, 10–30 g/d and >30 g/d), use of antihypertensive medication (number of drugs; continuous) and time since transplantation (years; continuous). In the final model, we additionally adjusted for total energy intake (continuous; kJ/d), urinary Na, K (all continuous; mmol/24 h), intake of Ca, Mg (continuous; mg/d), carbohydrates, SFA and PUFA (all continuous; g/d).

To allow for non-linear associations, general linear model analyses were used to investigate the associations of tertiles of energy-adjusted protein intake (total, plant and animal) with BP and renal function in RTR. Per tertile of energy-adjusted protein intake, the estimated mean values of BP, creatinine clearance and albuminuria were calculated as well as the *P*-trend across tertiles. Multivariable analyses were repeated with aforementioned adjustments. Within all statistical analyses, a two-sided *P* value less than 0·05 was considered to indicate statistical significance.

Results

Population characteristics

The mean age of the present study population was 53 (SD 13) years and 57 % were male. Mean BMI was 26·7 (SD 4·8) kg/m^2, with 59 % of the patients being overweight. Mean SBP was 136 (SD 17) mmHg, mean DBP was 83 (SD 11) mmHg and 91 % of the cohort had hypertension (i.e. BP ≥ 140/90 mmHg or use of antihypertensive medication). Of the 625 RTR, seventy-two (11 %) were not using any antihypertensive drugs, while 198 (30 %) used one antihypertensive drug, 231 (35 %) used two and 159 (24 %) used three or more different antihypertensive drugs. Calcineurin inhibitors were used in 57 % of RTR, 2 % of RTR used mammalian target of rapamycin inhibitors and 83 % of RTR were on proliferation inhibiting therapy. The median prednisolone dose was 10 (7·5–10·0) mg/d.

The diet contained 83 (SD 12) g/d of energy-adjusted protein (corresponding to 15·5 % of an individual's total energy intake (en%) or 1·1 (SD 0·3) g/kg per d), of which 52 (SD 13) g/d (9·9 en%) derived from animal origin and 31 (SD 6) g/d (5·7 en%) from plant origin (mean animal:plant ratio approximately 2:1). The distribution of plant protein intake and animal protein intake per group of total protein intake (g/d) is shown in Fig. 1. The percentage of protein intake declined from 57 % in the lowest group to 28 % in the highest group. The animal:plant ratio in the lowest group of total protein intake was 0·75 *v.* 2·5 in the highest group of total protein intake.

Based on the Maroni formula, total protein intake was 85 (SD 21) g/d (approximately 1·1 (SD 0·3) g/kg per d), which did not significantly differ from the protein estimate derived from the FFQ (P=0·3). The mean intake of energy, Ca, Mg and P were 9100 (SD 2665) kJ/d 2175 (SD 637) kcal/d, 1049 (SD 378) mg/d, 331 (SD 90) mg/d and 1521 (SD 331) mg/d respectively. The mean urinary excretion of Na and K was 157 (SD 62) and 73 (SD 24) mmol/24 h, respectively. Of the energy intake, 36 en% derived from fat (saturated fat 13 en%; monounsaturated fat 12 en%; polyunsaturated fat 8 en%) and 46 en% came from carbohydrates. The mean intake of fibre was 22 (SD 7) g/d.

The patient characteristics by tertiles of total energy-adjusted protein intake are shown in Table 1. RTR in the highest tertile of energy-adjusted protein intake were likely to be older, to have a higher BMI and higher urinary urea excretion levels, whereas the prevalence of males and smokers was lower with higher energy-adjusted protein intake. With higher protein intake, RTR tended to increase the intake of animal protein rather than that of plant protein, both in absolute (g/d) and relative (en%) amounts.

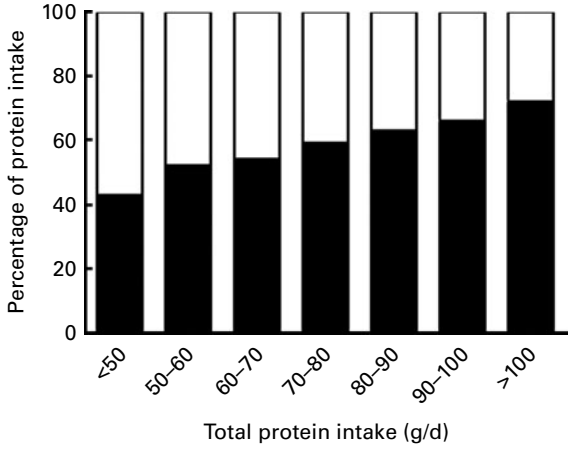

Fig. 1. Distribution of plant protein intake (□) and animal protein intake (■) per group of total protein intake (g/d). The percentage of plant protein intake declined from 57 % in the lowest group to 28 % in the highest group. The animal:plant ratio in the lowest group of total protein intake was 0·75 *v.* 2·5 in the highest group of total protein intake.

Table 1. Patient characteristics across tertiles of energy-adjusted total protein intake
(Mean values and standard deviations; number of patients and percentages)

	Tertiles of energy-adjusted protein intake (g/d)						
	I (*n* 208)		II (*n* 209)		III (*n* 208)		
	Mean	SD	Mean	SD	Mean	SD	*P*
Protein intake							
Absolute amount (g/d)	71	7	83	3	96	7	
Relative amount (en%)	13·1		15·6		17·8		
Demographics							
Sex (% male)	61		58		51		0·06
Age (years)	50	14	54	12	56	12	<0·001
Weight (kg)	78	16	79	15	83	17	0·01
Length (cm)	174	10	173	10	173	9	0·4
BMI (kg/m^2)	25·7	4·6	26·3	4·4	27·7	5·0	<0·001
Current smokers (%)	15		14		10		0·27
Time since transplantation (years)							0·22
Median	6·5		5·1		5·1		
IQR	3·1–12·4		1·6–12·5		1·3–11·9		
Dietary intake							
Energy intake							0·024
kcal/d	2255	774	2085	564	2185	548	
kJ/d	9441	3240	8729	2361	9148	2294	
Animal protein							<0·001
Absolute amount (g/d)	40	9	52	6	65	10	
Relative amount (en%)	7·6		9·9		12·1		
Plant protein							0·19
Absolute amount (g/d)	31	7	31	5	31	6	
Relative amount (en%)	5·6		5·7		5·7		
Fat (g/d)	92	44	84	27	88	29	0·052
Carbohydrate (g/d)	269	90	237	71	242	68	<0·001
Ca intake (mg/d)	865	334	1001	293	1279	366	<0·001
Mg intake (mg/d)	316	101	323	84	358	78	<0·001
Fibre intake (g/d)	22·2	7·9	22·0	6·6	23·1	5·7	0·21
Alcohol intake (g/d)*							0·06
Median	2·0		3·5		2·0		
IQR	0·02–11·6		0·05–13·7		0·05–13·7		
Medication use							
Antihypertensives (%)	86		85		93		0·015
Number of antihypertensives*							0·97
Median	2		2		2		
IQR	1–2		1–2		1–3		
CNI							0·77
n	119		116		122		
%	57		56		59		
mTOR inhibitors							0·24
n	6		1		3		
%	3		0·5		1		
Haemodynamic parameters							
SBP (mmHg)	136	16	137	18	135	18	0·53
DBP (mmHg)	83	11	84	11	82	11	0·053
Mean arterial pressure (mmHg)	100	11	102	13	100	12	0·14
Renal function parameters							
Serum creatinine (μmol/l)							0·21
Median	128		121		126		
IQR	103–171		99–155		99–156		
Creatinine clearance (ml/min)	63	27	68	25	66	25	0·12
eGFR (ml/min per 1·73 m^2)	52	21	54	21	56	20	0·26
Urinary albumin excretion (mg/24 h)							0·57
Median	36·1		38·4		50·0		
IQR	9·7–176		8·5–149		11·9–202		
Proteinuria (≥0·5 g/24 h)							0·98
n	45		48		45		
%	22		23		22		
Serum parameters							
Urea (mmol/l)							0·09
Median	9·1		9·0		10·1		
IQR	6·9–13·5		7·0–12·7		8·1–13·9		
Cholesterol (mmol/l)							0·47
Median	4·9		5·1		5·1		

Table 1. *Continued*

	Tertiles of energy-adjusted protein intake (g/d)						
	I (*n* 208)		II (*n* 209)		III (*n* 208)		
	Mean	SD	Mean	SD	Mean	SD	*P*
IQR	4·3–5·7		4·4–5·8		4·4–5·8		
HbA1c (%)	5·8	0·7	6·0	0·8	6·2	0·9	<0·001
Uric acid (mmol/l)	0·44	0·12	0·43	0·11	0·44	0·12	0·84
Urinary excretions (mmol/24 h)							
Phosphate	23·4	8·6	25·6	8·7	26·2	8·9	<0·001
Urea	344	97	397	111	430	119	<0·001
Na	142	54	167	66	161	61	<0·001
K	67·4	23·4	74·3	24·7	77·8	24·4	<0·001
Creatinine	11·4	3·6	11·7	3·2	11·7	3·3	0·29
Net acid excretion	41·3	20·0	46·7	20·2	48·1	22·3	0·003

en%, percentage of energy; IQR, interquartile range; CNI, calcineurin inhibitors; mTOR, mammalian target of rapamycin; SBP, systolic blood pressure; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HbA1c, glycated Hb.
* Among users.

Protein intake and blood pressure

Intake of energy-adjusted protein (total, animal and plant) tended to be inversely associated with BP, although the level of significance was not reached (Table 2). After adjustment for potential confounders, the regression coefficients for SBP were −2·22 (P=0·07), −1·07 (P=0·37) and −1·41 (P=0·19) per SD increase of total, animal and plant protein, respectively. On secondary regression analyses with tertiles of energy-adjusted protein intake (total, animal and plant) instead of with continuous values of protein intake, in that way forcing more contrast in protein intake, the findings remained essentially similar. The median total protein intake in the lowest tertile was 73·0 g/d compared with 94·0 g/d in the highest tertile. Although SBP was 3·9 mmHg lower in the highest tertile of protein intake (133·8 *v.* 137·7 mmHg in the lowest tertile), this difference did not reach statistical significance (P=0·2). Similar trends were found for the associations of animal and plant protein with BP (data not shown).

Protein intake and renal function

Table 3 shows the regression coefficients for the association between energy-adjusted protein intake (total, animal and plant) and renal function, reflected by creatinine clearance, albuminuria and proteinuria. The total protein intake was significantly associated with creatinine clearance, independent of age and sex (β = 2·17 per SD increase of protein intake; P=0·05). However, the fully adjusted models for the association between protein intake and creatinine clearance yielded insignificant regression coefficients of 0·19 ml/min (P=0·9), 0·17 ml/min (P=0·9) and 0·03 ml/min (P=0·9) per SD increase of total, animal and plant protein, respectively. Also, protein intake was not associated with albuminuria or proteinuria in this RTR cohort, regardless of both protein type and the adjustments that were made (Table 3). In order to consider the effect of calcineurin inhibitors, we also performed an adjustment for calcineurin inhibitors use in linear regression analyses, which did not essentially change the findings in the complete cohort.

Table 2. Regression coefficients for the association between energy-adjusted total protein intake and blood pressure in renal transplant recipients

(β-Coefficients per standard deviation of the exposure variable)

			SBP (mmHg)		DBP (mmHg)		MAP (mmHg)	
Exposure variable	SD	Model*	*β*	*P*	*β*	*P*	*β*	*P*
Total protein (g/d)	12·0 g/d	1	−0·39	0·59	−0·21	0·63	−0·27	0·59
		2	−0·84	0·25	−0·40	0·39	−0·54	0·28
		3	−2·22	0·07	−0·48	0·54	−1·06	0·22
Animal protein (g/d)	13·1 g/d	1	0·23	0·74	−0·13	0·77	−0·01	0·98
		2	−0·22	0·76	−0·34	0·46	−0·29	0·55
		3	−1·07	0·37	−0·47	0·53	−0·67	0·41
Vegetable protein (g/d)	5·8 g/d	1	−1·22	0·08	−0·12	0·78	−0·49	0·31
		2	−1·14	0·10	−0·05	0·92	−0·41	0·40
		3	−1·41	0·19	0·14	0·83	−0·37	0·62

SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure.
* Model 1: adjusted for age and sex; model 2: additionally adjusted for BMI, smoking behaviour, alcohol intake, antihypertensive drugs and time since transplantation; model 3: additionally adjusted for total energy intake, urinary Na and K excretion, intake of Ca, Mg, carbohydrates, SFA and PUFA.

Table 3. Regression coefficients for the association between energy-adjusted total protein intake and renal function parameters in renal transplant recipients

(β-Coefficients per standard deviation of the exposure variable)

			Creatinine clearance (ml/min)		Albuminuria (log mg/24 h)		Proteinuria (log g/24 h)	
Exposure variable	SD	Model*	β	P	β	P	β	P
Total protein (g/d)	12·0 g/d	1	2·17	0·05	0·07	0·37	0·01	0·97
		2	2·00	0·07	0·09	0·27	0·02	0·77
		3	0·19	0·90	0·12	0·30	0·01	0·91
Animal protein (g/d)	13·1 g/d	1	1·59	0·14	0·06	0·50	0·01	0·86
		2	1·30	0·23	0·05	0·56	0·01	0·95
		3	0·17	0·91	0·08	0·47	0·01	0·99
Vegetable protein (g/d)	5·8 g/d	1	0·73	0·48	0·02	0·77	0·04	0·61
		2	1·05	0·32	0·07	0·35	0·02	0·87
		3	0·03	0·99	0·05	0·67	0·02	0·84

*Model 1: adjusted for age and sex; model 2: additionally adjusted for BMI, SBP, smoking behaviour, alcohol intake, antihypertensive drugs and time since transplantation; model 3: additionally adjusted for total energy intake, urinary Na and K excretion, intake of Ca, Mg, carbohydrates, SFA and PUFA.

With tertiles of energy-adjusted protein intake (total, animal and plant) rather than continuous variables, the results were essentially similar; creatinine clearance was 62·4 ml/min in the lowest tertile of energy-adjusted protein intake (median intake 73·0 g/d) *v.* 66·3 ml/min in the highest tertile of energy-adjusted protein intake (median intake 94·0 g/d; *P*-trend=0·2). Also, differentiation between animal and plant protein did not alter previous findings (data not shown). With albuminuria, we did not observe significant differences across increasing tertiles of total protein intake (*P*=0·15). However, with respect to the intake of energy-adjusted animal protein, a significant trend was found. The highest tertile, with a median protein intake of 63 g/d, had 66 mg/24 h albuminuria, compared with 33 mg/24 h albuminuria in the lowest tertile with a median animal protein intake of 41 g/d (*P*-trend=0·03). This was independent of age, sex, BMI, SBP, smoking behaviour, alcohol intake, antihypertensive drugs, time since transplantation and dietary factors. These differences in albuminuria were not seen across tertiles of plant protein intake.

Discussion

To date, no evidence is available regarding the optimal level of protein intake and its favourable source (i.e. animal or plant protein) in stable RTR. Therefore, we examined dietary habits in RTR, with the main purpose to study whether dietary protein was associated with BP and renal function parameters in a large, single-centre RTR cohort. In the present analyses among 625 RTR with a functioning graft for at least 1 year, the average protein intake was 83 (SD 12) g/d (approximately 1·1 (SD 0·3) g/kg per d), thus exceeding the recommended values for RTR. The intake of other relevant dietary factors, i.e. Na, P, fibre and intake and composition of fat, was not in compliance with dietary recommendations either. Dietary protein (total, plant and animal) was not associated with BP or creatinine clearance. Although an adverse renal effect of animal protein intake was suggested by a higher albuminuria in the highest tertile of animal protein intake compared with the lowest tertile, no continuous relationship was found in linear regression analysis.

This is the first study providing detailed information on dietary habits in RTR. Several methodological aspects of the nutritional assessment deserve to be addressed. First, estimation of animal and plant protein intake was assessed by FFQ, based on self-report, which may have led to misclassification due to inadequacies in dietary recall. However, estimations of total protein intake based on FFQ were similar to estimations based on urinary urea excretion. We therefore do not expect much bias from misclassification regarding animal and plant protein either. Additionally, during the study, a dietary diary was kept for three consecutive days in a subgroup of sixty RTR and dietary data of both FFQ and diaries were compared. Pearson's correlations between FFQ and diaries were 0·72 for energy intake, 0·64 for protein intake, 0·50 for fat intake and 0·69 for carbohydrate intake. These correlation coefficients were comparable with those observed in previous studies analysing the validity of FFQ in population-based cohort studies[29]. Second, the present analyses are based on cross-sectional data with protein intake and BP being measured at the same moment. This makes it difficult to assess the temporal relationships in a potential association. For instance, patients with renal function decline might restrict their protein intake, which might have manipulated potential associations. This is, however, unlikely, because no active intervention on protein intake is advocated in RTR when renal function decreases, until dialysis is restarted, to prevent induction of protein malnutrition in the face of continued immunosuppression. Third, it might be hypothesised that, as a result of heterogeneity of the RTR population (e.g. pharmacological regimens, diversity in allograft vintage), significant associations of dietary protein with BP might go unnoticed. However, despite possible blurring of potential associations due to these factors, classical factors associated with BP in the general population such as age, sex, BMI and Na intake were significantly positively associated with BP in our RTR

population[9], thus supporting the power of the present study to identify determinants of BP in the current clinical context. The strengths of the present study include the fact that, to our knowledge, this is the first study examining the association of dietary protein with BP and renal function in a large, stable RTR population, with the obvious limitation, however, of its single-centre nature that limits its generalisability. Extensive data collection made it possible to adjust for many potential confounders, including Na intake reflected by urinary Na excretion. Previous studies, in line with the present study, have shown a firm and inextricable association between protein intake and Na intake cross-sectionally[8,30], which therefore makes it difficult to distinguish between the effects of the separate dietary components on BP and renal function.

Our dietary inventory allows a detailed assessment of the dietary habits of the RTR population, albeit in a single-centre set-up. The dietary habits of our RTR generally are not quite optimal, as shown from their intake of macronutrients as well as Na and P, which deviate from the available recommendations. Accordingly, dietary habits can be considered logical targets for intervention in RTR, but this renders it all the more important to reinforce the empirical basis in this population.

Protein intake and blood pressure

No significant associations of dietary protein with BP in RTR were seen, which suggests that dietary protein, within the range of intake in our population, is well tolerated in stable RTR. However, it might be hypothesised that the absence of a significant association is explained by the relatively small range of protein intake in our population. The SD of unadjusted mean total protein intake in RTR was 20 g/d, which is smaller than the SD of 27 g/d in a big sample of the Dutch general population[31]. RTR usually have a history of long-term exposure to strict dietary restrictions, especially during the dialysis period, and this may have modulated the eating habits of this specific population to a fairly homogeneous pattern, which could mask a potential association of protein intake with BP. Nevertheless, repeating our multivariate analyses in tertiles instead of per SD, forcing more contrast in exposure, did not reveal significant associations either. Future studies could include RTR from different populations and countries to acquire a larger variation in protein intake or intervene on protein intake, by isoenergetic exchange for other macronutrients.

Protein and renal function

The potentially deleterious effect of dietary protein on renal function, suggested by several previous studies[17,18,32–34], is ascribed to the induction of high intraglomerular pressure and concurrent hyperfiltration. This adverse phenomenon of dietary protein was not so pronounced in the present study in stable RTR, as appears from the non-significant regression coefficients resulting from the present statistical analyses. We did not see higher creatinine clearance or higher albuminuria or proteinuria in RTR with a higher total protein intake. However, a slightly, but significantly, higher albuminuria was seen in the highest tertile of animal protein, independent of age, sex, BMI, BP and dietary factors such as energy and Na intake. This significant association was not seen in tertiles of plant protein, suggesting that it is not protein *per se* that could influence albuminuria, but that differences exist between types of protein. One other study addressing the association between protein intake and renal function in RTR was performed by Bernardi *et al.* They studied the role of long-term dietary protein restriction on renal graft function in forty-two post-transplant patients with signs of chronic rejection[33]. Patients with moderate protein intake (0·73 (SD 0·11) g/kg) maintained unchanged renal graft function, whereas patients with a high-protein diet (1·4 (SD 0·23) g/kg) ended up with a significantly lower graft function. However, during enrolment, all patients received similar dietary recommendations and compliance with protein restriction was not pre-specified but based on urinary urea excretion. At the end of the study, patients were compared in two groups stratified by compliance status. Moreover, the low-protein diet was provided in combination with a low-Na and low-lipid diet which makes it complicated to isolate the effects of moderate protein intake on renal function.

In conclusion, we found no clear-cut association of dietary protein with BP or creatinine clearance within the ranges of protein intake in our population consisting of 625 stable RTR. Although RTR in the highest tertile of animal protein intake had a higher urinary albumin excretion compared with RTR in the lowest tertile, no continuous association was found between animal protein intake and albuminuria. In general, dietary habits in our RTR deviated from the available guidelines, with intake of protein, saturated fat, Na and P being higher, and intake of polyunsaturated fat, carbohydrates and fibre being lower than recommended. These data prompt for further studies addressing the role of dietary factors in the cardiovascular and renal risk in RTR, including the effects of intervention studies.

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References

1. First MR, Neylan JF, Rocher LL, *et al.* (1994) Hypertension after renal transplantation. *J Am Soc Nephrol* **4**, 8 Suppl., S30–S36.
2. Kasiske BL, Anjum S, Shah R, *et al.* (2004) Hypertension after kidney transplantation. *Am J Kidney Dis* **43**, 1071–1081.
3. Fernandez-Fresnedo G, Escallada R, Rodrigo E, *et al.* (2002) Proteinuria is an independent risk factor of cardiovascular disease in renal transplant patient. *Transplant Proc* **34**, 367.
4. Aakhus S, Dahl K & Wideroe TE (2004) Cardiovascular disease in stable renal transplant patients in Norway: morbidity and mortality during a 5-yr follow-up. *Clin Transplant* **18**, 596–604.
5. Marasa M, Remuzzi G & Cravedi P (2011) Hypertension after kidney transplantation: an important, but still neglected issue. *J Hypertens* **29**, 2310–2311.
6. Appel LJ, Brands MW, Daniels SR, *et al.* (2006) Dietary approaches to prevent and treat hypertension: a scientific statement from the American Heart Association. *Hypertension* **47**, 296–308.
7. Vogt L, Waanders F, Boomsma F, *et al.* (2008) Effects of dietary sodium and hydrochlorothiazide on the antiproteinuric efficacy of losartan. *J Am Soc Nephrol* **19**, 999–1007.
8. Slagman MC, Waanders F, Hemmelder MH, *et al.* (2011) Moderate dietary sodium restriction added to angiotensin converting enzyme inhibition compared with dual blockade in lowering proteinuria and blood pressure: randomised controlled trial. *BMJ* **343**, d4366.
9. van den Berg E, Geleijnse JM, Brink EJ, *et al.* (2012) Sodium intake and blood pressure in renal transplant recipients. *Nephrol Dial Transplant* **27**, 3352–3359.
10. Liu L, Ikeda K, Sullivan DH, *et al.* (2002) Epidemiological evidence of the association between dietary protein intake and blood pressure: a meta-analysis of published data. *Hypertens Res* **25**, 689–695.
11. Appel LJ, Sacks FM, Carey VJ, *et al.* (2005) Effects of protein, monounsaturated fat, and carbohydrate intake on blood pressure and serum lipids: results of the OmniHeart randomized trial. *JAMA* **294**, 2455–2464.
12. Elliott P, Stamler J, Dyer AR, *et al.* (2006) Association between protein intake and blood pressure: the INTERMAP Study. *Arch Intern Med* **166**, 79–87.
13. Altorf-van der Kuil W, Engberink MF, Brink EJ, *et al.* (2010) Dietary protein and blood pressure: a systematic review. *PLoS One* **5**, e12102.
14. King AJ & Levey AS (1993) Dietary protein and renal function. *J Am Soc Nephrol* **3**, 1723–1737.
15. Hostetter TH (1986) Human renal response to meat meal. *Am J Physiol* **250**, F613–F618.
16. Giordano M, Castellino P, McConnell EL, *et al.* (1994) Effect of amino acid infusion on renal hemodynamics in humans: a dose–response study. *Am J Physiol* **267**, F703–F708.
17. Metges CC & Barth CA (2000) Metabolic consequences of a high dietary-protein intake in adulthood: assessment of the available evidence. *J Nutr* **130**, 886–889.
18. Brenner BM, Meyer TW & Hostetter TH (1982) Dietary protein intake and the progressive nature of kidney disease: the role of hemodynamically mediated glomerular injury in the pathogenesis of progressive glomerular sclerosis in aging, renal ablation, and intrinsic renal disease. *N Engl J Med* **307**, 652–659.
19. Levey AS, Greene T, Sarnak MJ, *et al.* (2006) Effect of dietary protein restriction on the progression of kidney disease: long-term follow-up of the Modification of Diet in Renal Disease (MDRD) Study. *Am J Kidney Dis* **48**, 879–888.
20. Klahr S, Levey AS, Beck GJ, *et al.* (1994) The effects of dietary protein restriction and blood-pressure control on the progression of chronic renal disease. Modification of Diet in Renal Disease Study Group. *N Engl J Med* **330**, 877–884.
21. Beto JA & Bansal VK (2004) Medical nutrition therapy in chronic kidney failure: integrating clinical practice guidelines. *J Am Diet Assoc* **104**, 404–409.
22. Martins C, Pecoits-Filho R & Riella MC (2004) Nutrition for the post-renal transplant recipients. *Transplant Proc* **36**, 1650–1654.
23. Fry K, Patwardhan A, Ryan C, *et al.* (2009) Development of evidence-based guidelines for the nutritional management of adult kidney transplant recipients. *J Ren Nutr* **19**, 101–104.
24. Chadban S, Chan M, Fry K, *et al.* (2010) The CARI guidelines. Protein requirement in adult kidney transplant recipients. *Nephrology (Carlton)* **15**, Suppl. 1, S68–S71.
25. Stichting NEVO (2006) *Nederlands voedingsstoffen bestand: NEVO tabel 2006 (Dutch Nutrient Databank: NEVO Table 2006)*. The Hague: Voorlichtingsbureau voor de Voeding.
26. Masud T, Manatunga A, Cotsonis G, *et al.* (2002) The precision of estimating protein intake of patients with chronic renal failure. *Kidney Int* **62**, 1750–1756.
27. Maroni BJ, Steinman TI & Mitch WE (1985) A method for estimating nitrogen intake of patients with chronic renal failure. *Kidney Int* **27**, 58–65.
28. Willett WC, Howe GR & Kushi LH (1997) Adjustment for total energy intake in epidemiologic studies. *Am J Clin Nutr* **65**, Suppl. 4, 1220S–1228S, discussion 1229S–1231S.
29. Klipstein-Grobusch K, den Breeijen JH, Goldbohm RA, *et al.* (1998) Dietary assessment in the elderly: validation of a semiquantitative food frequency questionnaire. *Eur J Clin Nutr* **52**, 588–596.
30. Vegter S, Perna A, Postma MJ, *et al.* (2012) Sodium intake, ACE inhibition, and progression to ESRD. *J Am Soc Nephrol* **23**, 165–173.
31. Zo eet Nederland (1998) *Resultaten van de Voedselconsumptiepeiling 1997–1998 (Results of the Food Consumption Survey 1997–1998)*. The Hague: Dutch Nutrition Center.
32. Fouque D, Laville M & Boissel JP (2006) Low protein diets for chronic kidney disease in non diabetic adults. *Cochrane Database of Systematic Reviews,* issue 2, CD001892.
33. Bernardi A, Biasia F, Pati T, *et al.* (2003) Long-term protein intake control in kidney transplant recipients: effect in kidney graft function and in nutritional status. *Am J Kidney Dis* **41**, 3 Suppl. 1, S146–S152.
34. Rosenberg ME, Salahudeen AK & Hostetter TH (1995) Dietary protein and the renin–angiotensin system in chronic renal allograft rejection. *Kidney Int Suppl* **52**, S102–S106.