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Renal, metabolic and hormonal responses to ingestion of animal and vegetable proteins

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Renal, metabolic and hormonal responses to ingestion of animal and vegetable proteins. Renal and hormonal responses were studied in a group of healthy individuals fed, in random order, for three weeks, a vegetable protein diet ($N = 10$), an animal protein diet ($N = 10$), or an animal protein diet supplemented with fiber ($N = 7$), all containing the same amount of total protein (chronic study). In seven additional subjects the acute renal, metabolic and hormonal response to ingestion of a meat or soya load of equivalent total protein content was investigated (acute study). In the chronic study GFR, RPF and fractional clearance of albumin and IgG were significantly higher on the animal than the vegetable protein diets (GFR: 121 ± 4 vs. 111 ± 4 ml/min/1.73 m^2, $P < 0.001$; RPF: 634 ± 29 vs. 559 ± 26 ml/min/1.73 m^2, $P < 0.001$; θ alb: 19.5 ± 3.1 vs. 10.2 ± 1.6 × 10^{-7}, $P < 0.01$; θ IgG: 11.6 ± 3.1 vs. 7.5 ± 1.7 × 10^{-7}, $P < 0.05$). Renal vascular resistance was lower on the animal than vegetable protein diet (82 ± 5 vs. 97 ± 5 mmHg/min/liter; $P < 0.001$). Fiber supplementation to APD did not have any effect on the renal variables measured which were indistinguishable from APD. In the acute study, GFR and RPF both rose significantly by ~16% ($P < 0.005$) and ~14% ($P < 0.05$), respectively, after the meat load, while RVR fell by ~12% ($P < 0.05$). There were no significant changes in these parameters following the soya load. Plasma glucagon showed a greater rise following the meat load (by ~65%) than after the soya load (~39%). The incremental glucagon area was significantly greater after meat than after soya (129 ± 17 vs. 70 ± 18 pg · hr · ml^{-1}, $P < 0.001$). Prostaglandin 6-keto-$PGF_{1\alpha}$ rose significantly following the meat load (baseline 3.7 ± 1.9, afterload 5.4 ± 1.7 ng/ml, $P < 0.05$) but did not change after the soya challenge (baseline 4.2 ± 1.0, afterload 3.4 ± 1.6 pg/ml, NS). No differences were found in plasma amino acid levels following the two protein loads. Thus, independently of quantity of protein, vegetable protein has significantly different renal effects from animal protein in normal humans which could be partly explained by differences in glucagon and renal vasodilatory prostaglandin secretion.

Protein intake has a profound effect on renal hemodynamics and excretory function. It has been suggested that high protein intake may have deleterious effects on the kidney, particularly in pre-existant kidney disease [1–3]. Experimental evidence indicates that restriction of dietary protein limits further damage both in animal models of renal disease and in human nephropathy [4–8]. It is becoming clear, however, that not all proteins are equal in relation to their renal effects. Lower levels of glomerular filtration rate and urinary albumin excretion have been described in vegan and lactovegetarian individuals compared to omnivorous subjects [9, 10]. In all these studies, however, the average protein intake tended to be lower in the vegan and vegetarian groups. In the subtotally nephrectomized rat model, animals fed vegetable protein had longer survival, less proteinuria and milder renal histologic damage than those which received animal protein [11]. The factors that mediate the renal hemodynamic changes after protein intake have only partially been elucidated [12–14] and no study has explored the metabolic and hormonal mediators of the renal effects of different types of protein, nor is information available on the effect of dietary protein of different sources on proteinuria. We therefore studied, in healthy subjects, the renal effects of a three week vegetable or animal protein diet and compared the acute renal response to a meat or soya protein load. We also explored some of the mediators of the putative renal changes.

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Methods

Study population

Seventeen healthy volunteers were recruited from the members of the medical research staff of the Division of Medicine at Guy's Hospital. All subjects were males aged between 22 and 40 years, within 20% of ideal body weight and with normal blood pressure (<140/90 mm Hg). No subject was taking any medication or had a past medical history of renal, endocrine, cardiovascular or other systemic disease.

Chronic diet study design

Preliminary experiments indicated that matching for major macronutrients was achievable between animal and vegetable protein diets with the exception of fiber intake. Subjects took part in three different dietary regimens for a period of three weeks each: an animal protein diet (APD), a vegetable protein diet (VPD) and an animal protein diet with fiber supplementation (APD + F). Ten subjects (mean age 35, range 30 to 40 years) participated in the APD and VPD and seven of them in the APD + F. The order of the diets was randomly determined, and there was at least one week interval between each diet to avoid carry-over effects. During each dietary period subjects

performed two 24-hour urine collections, one at the end of the second week and one at the end of the third week of study, for measurement of urea, creatinine, electrolytes, phosphate, total protein, glucose and 3-methylhistidine. The mean of the two measurements were used for calculation. At the end of each diet period subjects were admitted fasting to a Metabolic Ward for clearance studies. From 10:00 p.m. the previous night, food, tea, coffee, alcohol and smoking, but not tap water, were prohibited. On the morning of the test subjects were weighed without shoes in indoor clothing. Experiments were performed during a steady state of water diuresis as previously described [15]. Two teflon cannulae (Venflon, Viggo, AB Helsingborg, Sweden) were inserted into an antecubital vein in each arm, one for blood sampling and the other for infusion of polyfructosan (Inutest, Boheringer-Manheim, Zurich, Switzerland) and sodium paramino-hippurate (PAH; Merck Sharp and Dohme, Hoddesdon, Hertfordshire, UK) as previously reported [16]. After 60 minutes of equilibration, four exactly-timed urine collection periods of 20 minutes each were made. At the midpoint of each urine collection period, pulse rate and blood pressure (phase I/V), using a standard mercury sphygmomanometer, were taken by a single observer (PK), and blood samples were drawn for measurement of PAH, polyfructosan, glucose, urea, creatinine, total plasma protein, haematocrit, electrolytes, albumin and IgG. Urines were aliquoted into plain tubes for measurements of PAH, polyfructosan and electrolytes and into a tube containing 20 μl gelatin (10% wt/vol) and 10 μl 4 M NaOH for measurement of urinary albumin, IgG and $\beta_2$microglobulin concentration. Polyfructosan is handled by the kidney in an identical fashion to inulin, and has been shown to be strictly comparable to inulin for the determination of renal clearance [17].

Dietary prescription and assessment

The three diets were designed to be isocaloric and contained 1 g protein/kg body weight per day with 35% of the energy from fat. The VPD contained exclusively vegetable protein, although supplements of animal fats were used to maintain the P/S ratio as in APD. APD contained approximately 70% animal protein and 30% vegetable protein in order to enable an adequate carbohydrate and fiber intake. Calcium and phosphate tablets were prescribed on VPD. Fiber content was lower on APD and a supplement of fiber in the form of Lejguar (Britannia Pharmaceuticals Ltd, West Byfleet, Surrey, UK) was prescribed for APD + F, but all other nutrients remained the same as for the APD. Guar was chosen rather than a cereal fiber because the fiber on VPD was derived mainly from beans.

Dietary assessments were carried out by a nutritionist (RD) using a three-day weighed food record system. Subjects weighed and recorded all their food, using Soenhle digital scales, on two week days and one weekend day on each diet. The records and recipes were checked with the subjects then coded and analyzed using the DIET program on the University of London computer.

Protein intake was also calculated from the urinary urea nitrogen (UUN) and an estimated non-urea nitrogen (NUN) excretion of 29 mg $N \cdot kg^{-1} \cdot day^{-1}$ as follows [18]:

$$UUN + NUN = IN$$

Table 1. Amino acid composition of the meat and soya meals

	Meat meal	Soya meal	Soya/meat %
Isoleucine	4.104	3.871	94.3
Leucine	6.412	6.532	101.9
Lysine	7.310	5.000	68.4
Methionine	2.180	0.968	44.4
Cystine	1.026	1.210	117.9
Phenylalanine	3.591	4.032	112.3
Tyrosine	3.078	2.822	91.7
Threonine	3.719	3.145	84.6
Tryptophan	1.026	0.806	78.6
Valine	4.232	4.032	95.3
Arginine	5.386	6.290	116.8
Histidine	2.949	2.097	71.1
Alanine	5.130	3.387	66.0
Aspartic acid	7.694	9.435	122.6
Glutamic acid	13.850	15.402	111.2
Glycine	4.488	3.387	75.5
Proline	4.104	4.355	106.1
Serine	3.591	4.113	114.5

$$IN \times 6.25 = \text{protein intake (g/day)}$$

where IN is nitrogen intake. This formula assumes nitrogen balance over the 24-hours of urine collection.

Acute protein load study design

Seven additional healthy male subjects with mean age 31 years (range 22 to 39 yrs) took part in this study. All subjects were omnivorous and consumed their usual diet prior to the acute protein load experiments. The subject's usual food pattern was recorded using a brief dietary history and a three-day weighed food record as described above. After an overnight fast, subjects underwent an acute oral protein challenge either with 80 g of protein as ground lean cooked beef (a raw weight of approximately 390 g), or with 80 g of protein as diluted soya powder (Soya Protein Powder; Booker Health Products, West Byfleet, UK) corresponding to a weight of 96 g of pure powder. The soya powder was made up with water and a small amount of low calorie cordial as flavoring. Mineral composition and fiber contents of the two loads were similar; the meat load, however, contained more fat (17 g vs. 1 g) and less carbohydrate (0 g vs. 5 g) than the soya load. The order of the challenges was randomly allocated, four subjects receiving the meat load and three the soya load first. The amino acid composition of the two loads is given in Table 1.

The study protocol for the renal clearance experiments was similar to that described in the chronic diet study, but baseline observations were followed by the ingestion of the protein load over a 30 minute period, and by a further three hours of observation during which three exactly timed, 20-minute urine collections were made every hour. The urine collected during the 30 minute period of protein ingestion was discarded. At the midpoint of each urine collection period blood samples were drawn, pulse rate was taken, and blood pressure was measured as described above. In addition to the blood and urine measurements described in the chronic study, pre- and postprandial blood samples were collected in lithium heparin iced tubes containing trasylol 500 μl for glucagon, growth hormone and

insulin measurements, and in plain tubes for determination of amino acids. Urine was collected for prostaglandin measurements.

Measurements and calculations

Plasma and urinary inulin were measured after perchloric acid hydrolysis using a centrifugal analyzer (Cobas Mira, Roche Diagnostica, Welwyn Garden City, UK) as previously described [19]. Plasma and urine PAH were measured using the method of Bratton and Marshall [20] adapted for use on a Cobas Bio centrifugal analyzer (Roche Diagnostica). Sodium, potassium, phosphate, urea and creatinine were measured in urine and plasma using a multichannel autoanalyzer (Hitachi, BCL, Lewes, UK). Glucose was measured by a glucose oxidase method (Yellow Springs Analyzer, YSI Inc., Ohio, USA), hematocrit using routine Coulter counter and total protein by refractometry. Plasma albumin and IgG were measured on the Cobas Bio analyzer, urinary albumin and IgG by ELISA technique modified from the method of Voller, Bidwell and Bartlett [21], and urinary β_2-microglobulin by RIA (Phadebas, β_2Microtest, Pharmacia Diagnostics AB, Uppsala, Sweden). Plasma glucagon, growth hormone and insulin as previously described [22]. Amino acid concentrations were measured in serum using a high pressure liquid chromatography technique (HPLC; Perkin Elmer, Padova, Italy), and 3-methylhistidine was determined on unhydrolyzed urine by an amino acid analyzer. For prostaglandin measurement urine samples were extracted and submitted to silicic acid chromatography before radioimmunoassay. The PGE_2 fraction was analyzed by radioimmunoassay with a highly specific rabbit antibody (Institute Pasteur, Paris, France) and [^{3}H] PGE_2 as tracer. The 6-keto-$PGF_{1\alpha}$ fraction, the stable hydrolysis product of PGI_2, was analyzed using a commercial kit (Neck-0.25; New England Nuclear, Boston, Massachusetts, USA) with 6-keto-$^{125}PGF_{1\alpha}$ as tracer. The inter- and intra-assay coefficient of variation for PGE_2 were 10% and 15%, and for 6-keto-$PGF_{1\alpha}$ 6% and 11%, respectively. TxB_2, the stable degradation product of TxA_2, was determined by radioimmunoassay [23, 24].

GFR and RPF were calculated as the clearance of polyfructosan and PAH, respectively, using the standard formula UV/P and corrected to 1.73 m^2 body surface area. Albumin and IgG excretion rates were calculated as urinary concentration times urine flow rate, and their fractional clearance was obtained by dividing their clearance by the GFR. Renal vascular resistance (RVR) was calculated as MBP × (1 − Hct)/RPF where, MBP is mean blood pressure (diastolic blood pressure + ⅓ pulse pressure) and Hct is hematocrit. Results in the chronic study represent the mean of the four measurements taken during the four 20-minute experimental periods. In the acute study the mean of measurements were made within each one hour period, four at baseline, and three for each of the three hourly periods following the protein load. "After meal" indicates the mean of all values following the protein challenge. The area under the curve was calculated using the trapezoid rule.

Statistical analysis

Data were analyzed by analysis of variance for cross-over designs with repeated measurements, using the Genstast statistical package (Rothamsted Experimental Station, UK).

Differences between dietary periods at baseline and subsequent time periods were tested for difference using the *t*-statistic, correcting the significance level for multiple comparisons (Bonferroni's adjustment).

Values for albumin and IgG clearance and excretion rate were logarithmically transformed before calculation because of their positively skewed distribution. Data are presented as mean ± SEM unless otherwise stated. A significant difference was taken as $P < 0.05$.

Table 2. Mean ± SEM dietary intake during vegetable protein diet (VPD), animal protein (APD), and APD with fiber supplement (APD + F)

	APD	APD + F	VPD
Energy *kcals/day*	1893 ± 107	1774 ± 144	2022 ± 156
Total protein *g/day*	80 ± 5	81 ± 10	76 ± 5
Animal protein *g/day*	56 ± 6	58 ± 11	0 ± 0[b]
Vegetable protein *g/day*	24 ± 7	23 ± 2	76 ± 5[b]
Fat *g/day*	79 ± 17	74 ± 11	76 ± 18
P/S ratio	0.47 ± 0.04	0.70 ± 0.15	0.78 ± 0.05[a]
Carbohydrate *g/day*	194 ± 26	176 ± 20	251 ± 17[c]
Fiber *g/day*	19 ± 3[d]	37 ± 2	31 ± 3

Values are represented for the 7 subjects who performed all 3 experiments. (Values for the 10 subjects who took part in APD and VPD were closely comparable and not shown).
[a] $P < 0.04$ VPD vs. APD
[b] $P < 0.04$ VPD vs. APD or APD + F
[c] $P < 0.04$ VPD vs. APD + F
[d] $P < 0.04$ APD vs. APD + F

Results

Dietary assessment

The protein intake assessed by the three-day weighed food records (APD 80 ± 5, APD + F 81 ± 10, VPD 76 ± 5 g/day) was similar to that calculated from urinary urea excretion (80 ± 8, 86 ± 7, 72 ± 6 g/day) and did not differ significantly between the three diets. Energy and fat intake were comparable during the three diet periods, but carbohydrate intake tended to be higher on the VPD. Fiber intake was similar on VPD and APD + F but lower during APD. P/S ratio was significantly lower on APD (Table 2).

Renal function

Chronic diet study. Glomerular filtration rate and renal plasma flow were significantly lower, by ~8% and ~12%, respectively, on VPD than APD (GFR 111 ± 4 vs. 121 ± 4 ml/min/1.73 m^2; $P < 0.001$; RPF 559 ± 26 vs. 634 ± 29 ml/min/1.73 m^2; $P < 0.001$). Renal vascular resistance was higher by ~15% on VPD (VPD vs. APD; 97 ± 5 vs. 82 ± 5 mm Hg/min/liter; $P < 0.001$), while filtration fraction was similar on the two diets (VPD vs. APD; 0.20 ± 0.01 vs. 0.19 ± 0.02; Fig. 1). Mean blood pressure was similar during the two diet periods (VPD vs. APD; 91 ± 1 vs. 89 ± 2 mm Hg). Twenty-four hour urinary albumin excretion rate [median (range)] was ~58% lower during VPD [VPD vs. APD 6.5 (2.6 to 12.2) vs. 14.1 (7.8 to 35.8) mg/24 hrs; $P = 0.003$] and the fractional clearance of albumin was reduced by ~48% on VPD ($P = 0.008$; Fig. 2). Similarly, fractional clearance of IgG fell on average 35% from 11.6 ± 3.1 to 7.5 ± 1.7 × 10^{-7}, $P < 0.05$, but excretion of β_2-microglobulin, an indicator of proximal tubular reabsorption

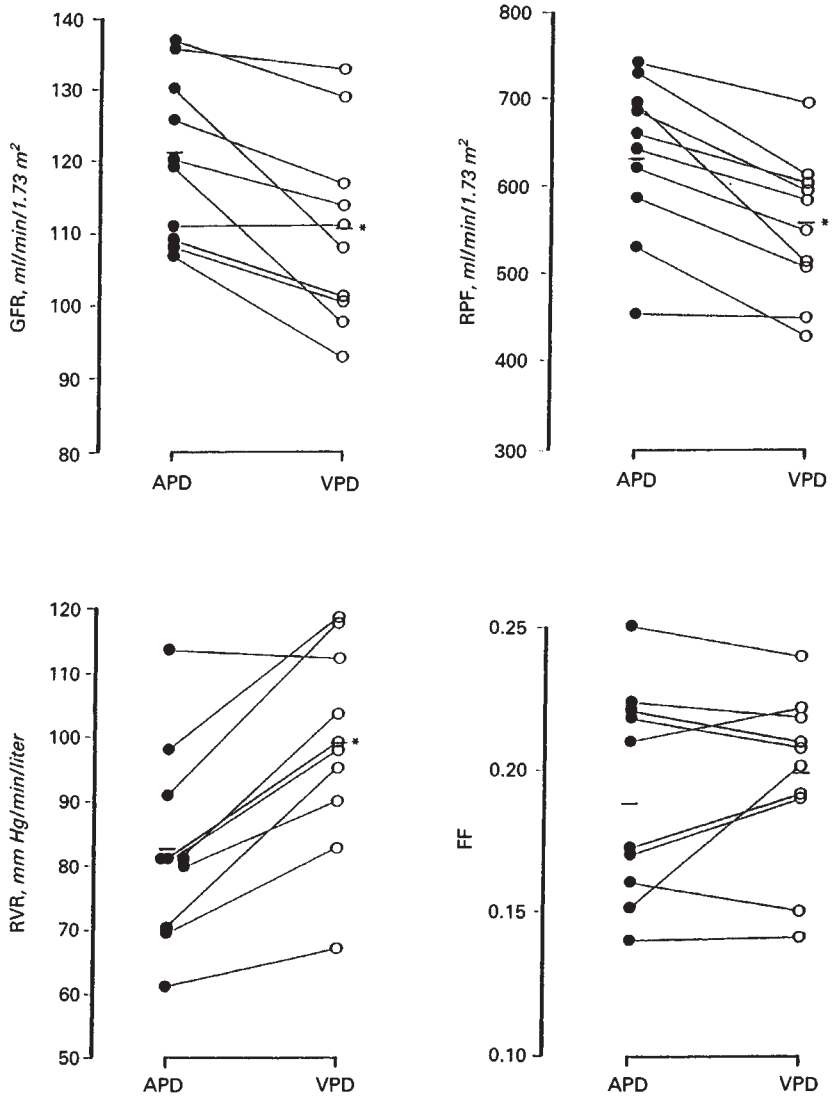

Fig. 1. *Glomerular filtration rate (GFR), renal plasma flow (RPF), renal vascular resistance (RVR) and filtration fraction (FF) after 3 weeks of animal (APD ●) or vegetable (VPD ○) protein diet in 10 healthy subjects.* $^*P < 0.001$ APD vs. VPD.

capacity, was not different (APD 89 ± 13 vs. VPD 90 ± 18 ng/min).

In seven subjects, addition of fiber to the APD to the same amount as in the VPD did not result in significant alteration of GFR (APD vs. APD + F 123.8 + 4.9 vs. 124.3 ± 4.7 ml/min/1.73 m^2), RPF (632 ± 39 vs. 635 ± 30 ml/min/1.73 m^2) or albumin excretion rate [median (range)] [30.5 (7.9 to 35.8) vs. 12.7 (8.1 to 30.1) mg/24 hrs]. All these variables persisted significantly higher than on VPD, excluding the possibility that the effect of VPD on renal function was due to the extra fiber content of the diet or to a higher P/S ratio. P/S ratio was in fact similar between APD + F and VPD.

In all ten subjects no differences were found between APD and VPD in plasma concentration of urea (5.4 ± 0.4 vs. 5.1 ± 0.4 mmol/liter), creatinine (84 ± 4 vs. 81 ± 4 μmol/liter), total protein (72 ± 2 vs. 70 ± 1 g/liter) and glucose (4.7 ± 0.2 vs. 4.6 ± 0.1 mmol/liter), and in urinary excretion of urea (333 ± 34 vs. 330 ± 22 mmol/24 hr), creatinine (15 ± 2 vs. 16 ± 2 mmol/24 hr) and phosphate (25 ± 2 vs. 21 ± 4 mmol/24 hr). Addition of fiber to APD did not significantly modify these parameters. Urinary excretion of 3-methylhistidine, an indicator of meat intake [25], was significantly higher during APD than VPD (303 ± 28 vs. 176 ± 26 mmol/24 hr; $P < 0.001$), confirming compliance with the diets. To explore the mechanisms and possible mediators of the strikingly different renal changes on VPD, we performed a series of acute studies.

Acute protein load study. After the meat challenge GFR increased significantly by ~16% and RPF by ~14% ($P < 0.005$ and $P < 0.005$, respectively). By contrast, the soya load produced no significant change in these variables (Fig. 3). Filtration fraction did not change with either load (meat: baseline 0.19 ± 0.01 vs. afterload 0.19 ± 0.01; soya: 0.19 ± 0.01 vs. 0.20 ± 0.01). Renal vascular resistance fell significantly after the meat load but remained unmodified after soya. (meat: baseline 90 ± 3 vs. afterload 79 ± 4; $P < 0.05$, soya: 90 ± 4 vs. 93 ± 6 mm Hg/min/liter; NS). Mean blood pressure fell after the meat challenge though not significantly (87 ± 6 to 85 ± 7 mm Hg), but was unchanged following soya ingestion (87 ± 5 and 87 ± 6 mm Hg). Fractional clearance of albumin and IgG were similar at baseline before the acute protein challenge. After

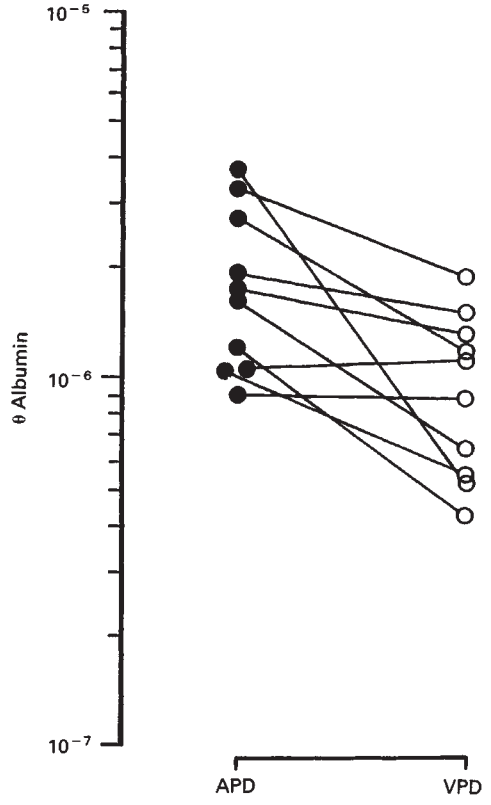

Fig. 2. *Fractional clearance of albumin (θ Alb: log scale) after 3 weeks of animal (APD ●) or vegetable (VPD ○) protein diet in 10 healthy subjects.*

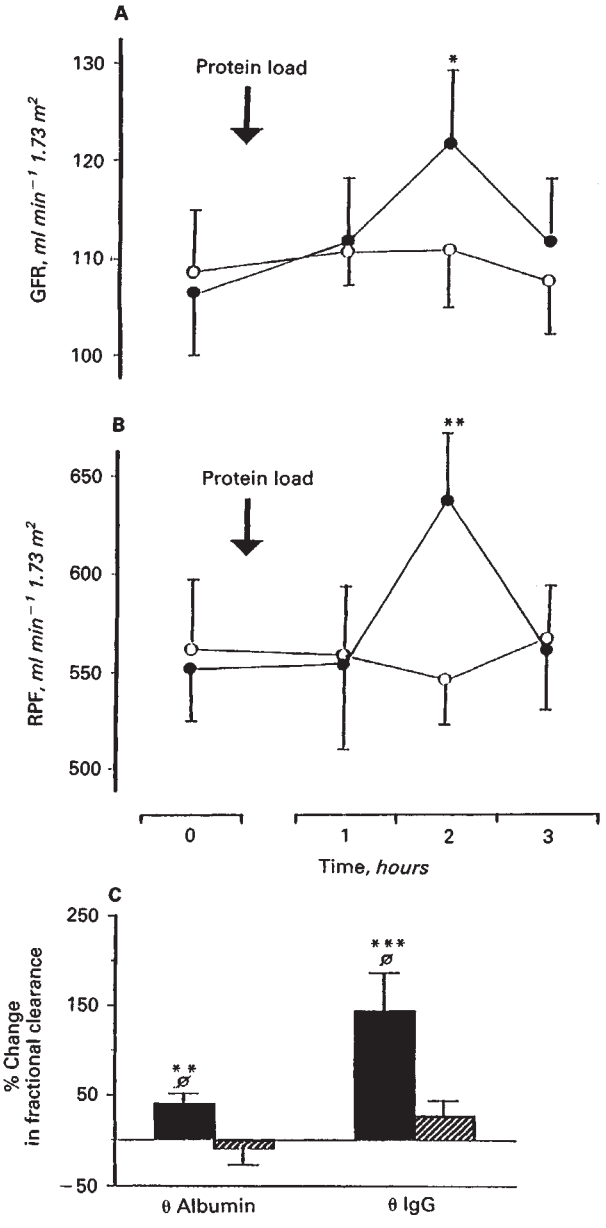

Fig. 3. *Mean ± SE glomerular filtration rate (GFR) (A), renal plasma flow (RPF) (B) and percent changes in fractional clearance of albumin and IgG (C) after meat (●, ■) or soya (○, ▨) challenge in 7 healthy subjects.* $^{*}P < 0.005$ vs. baseline; $^{**}P < 0.05$ vs. baseline; $^{***}P < 0.01$ vs. baseline; ø $P < 0.05$ vs. soya.

meat ingestion fractional albumin clearance rose by ~40% ($P < 0.05$) and IgG by ~144% ($P < 0.001$), changes not seen after the soya load (Fig. 3). Fractional clearance of albumin tended in fact to fall after soya (θ alb meat vs. soya: $P < 0.05$). Plasma protein concentrations (meat: baseline 69 ± 4 vs. afterload 68 ± 4; soya: 70 ± 4 vs. 68 ± 6 g/liter), hematocrit (meat: baseline 44 ± 3 vs. afterload 43 ± 2; soya: 43 ± 2 vs. 42 ± 3%) and urine flow (meat: baseline 16 ± 2 vs. afterload 16 ± 4; soya 15 ± 3 vs. 16 ± 4 ml/min) were unchanged by ingestion of either protein. Plasma concentrations of sodium and potassium and urinary excretion rates of potassium showed similar responses to meat or soya ingestion (P_{Na^+}, meat: baseline 135 ± 1, aftermeal 137 ± 1; soya: 135 ± 1 vs. 136 ± 1 mmol/liter; P_{K^+}, meat: baseline 3.8 ± 0.1, aftermeal 4.1 ± 0.1, soya: 3.8 ± 0.1 vs. 4.1 ± 0.2 mmol/liter; U_{K^+}, meat: baseline 53 ± 12 vs. aftermeal 65 ± 13, soya 39 ± 7 vs. 47 ± 13 μmol/min). Urinary excretion of sodium tended to increase after meat ingestion, but this did not reach statistical significance (U_{Na^+}, meat: baseline 181 ± 36 vs. aftermeal 305 ± 93; $P = 0.09$; soya: 131 ± 21 vs. 158 ± 28 μmol/min). After the meat meal urinary creatinine rose significantly by the second hour (baseline 8.1 ± 1.1 vs. 2nd hour 10.6 ± 0.4 μmol/min, $P < 0.05$), but the increase in plasma creatinine just failed to reach significance (baseline 97 ± 2 vs. 2nd hour 102 ± 3 μmol/liter, $P = 0.07$). No changes were seen after the soya load in these variables.

Plasma and urinary urea both rose by the third hour following each meal, (P_{urea}, meat: baseline 4.9 ± 0.4 vs. 5.3 ± 0.3; $P = 0.07$; soya: 4.2 ± 0.5 vs. 5.3 ± 0.4 mmol/liter; $P = 0.002$; U_{urea}: meat: 272 ± 34 vs. 384 ± 43; $P = 0.006$; soya: 246 ± 58 vs. 366 ± 67 μmol/min, $P = 0.006$). Plasma levels of the 10 amino acids measured were similar at baseline and rose comparably following the two protein loads (Fig. 4). This set of data indicates that no incomplete or delayed absorption of protein occurred with the soya load.

Plasma glucagon concentration was similar at baseline (meat vs. soya: 73 ± 38 vs. 80 ± 41 pg/ml), but a greater rise by ~65% occurred at two hours after the meat meal, to 136 ± 37 pg/ml, than after the soya load when the increase was ~39%, to 112 ±

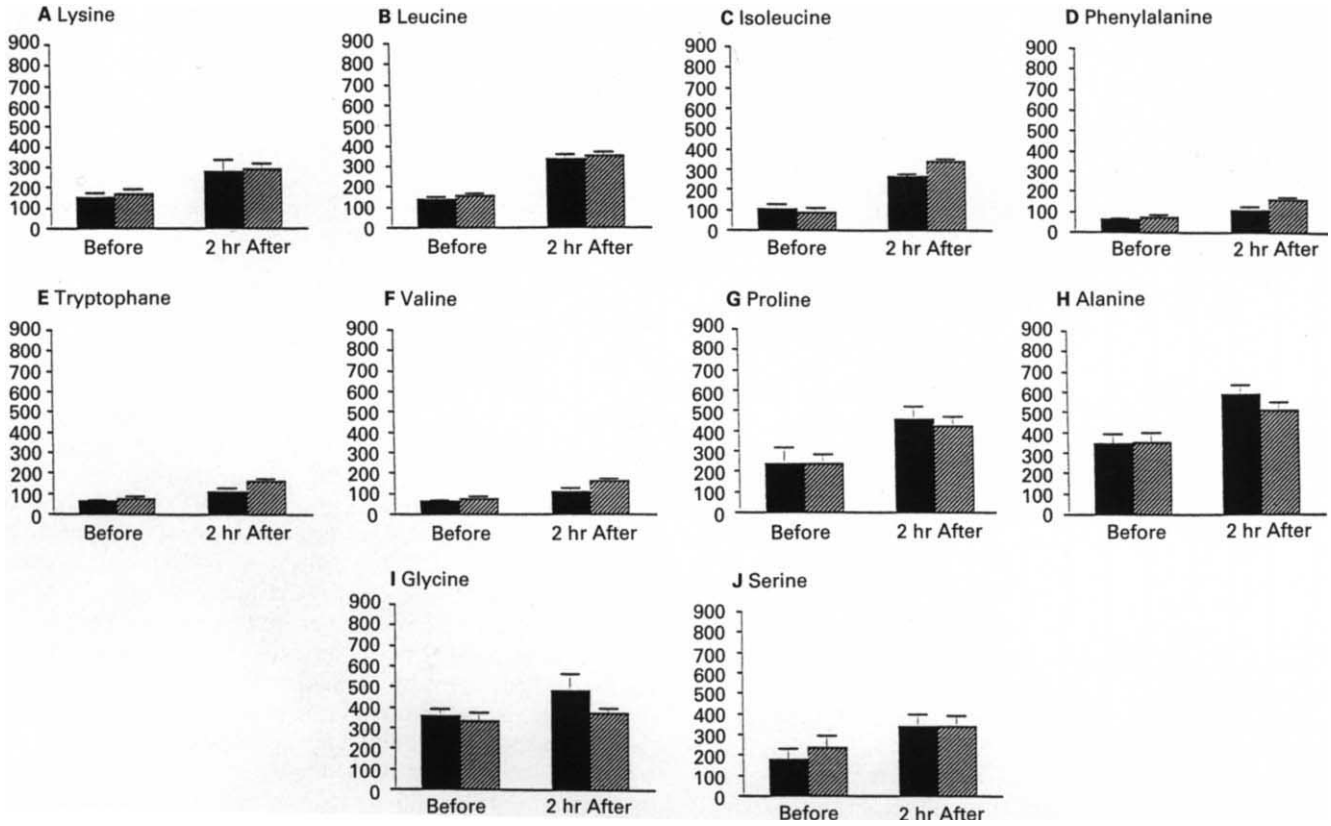

Fig. 4. *Mean ± SE plasma concentration (mmol/liter) of 10 amino acids before and after meat or soya ingestion in 7 healthy subjects.*

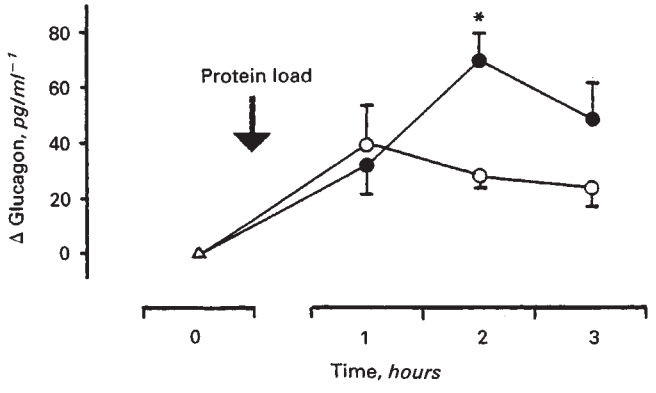

Fig. 5. *Mean ± SE plasma glucagon increment (pg · ml^{-1}) above baseline after meat (●) or soya (○) challenge in 7 healthy subjects. *P* < 0.05 meat vs. soya.

28 pg/ml ($P < 0.05$ meat vs. soya; Fig. 5). The incremental area after the meal was significantly greater for the meat than the soya meal (129 ± 17 vs. 70 ± 18 pg · hr · ml^{-1}, $P < 0.001$).

No significant differences were found between the two loads in plasma insulin or growth hormone concentrations at baseline (meat vs. soya insulin 3.5 ± 0.2 vs. 3.2 ± 0.1 mU/liter; growth hormone 0.40 ± 0.08 vs. 0.47 ± 0.06 ng/ml) or after the meal (insulin 8.1 ± 2.2 vs. 7.2 ± 1.6 mU/liter; growth hormone 1.61 ± 0.74 vs. 1.01 ± 0.38 ng/ml). The area under the curve following the two loads was similar. Urinary excretion of prostaglandins PGE_2 and 6-keto-$PGF_{1\alpha}$ were similar at baseline, with maximal increase of ~35% ($P < 0.08$) and ~68% ($P < 0.03$), respectively, following the meat meal and only of ~13% (NS) and ~10% (NS) after soya. There were no significant changes in urinary TxB_2 excretion following either meal (Table 3), suggesting that vasodilatory rather than vasoconstrictive prostaglandins were differently affected by the two protein loads.

Discussion

The treatment of chronic renal failure by conventional low protein diets is marred by problems of compliance [26]. Therefore it becomes important to explore alternatives of dietary protein modification which, while maintaining a normal protein intake, afford the same renal sparing effect as animal protein-restricted regimens. Recent studies in healthy humans [27] and in a rat model of renal disease [11] have strongly suggested that the type of protein may be important in regulating renal function. Vegan subjects, who eat no protein of animal origin, have lower glomerular filtration rate and urinary albumin excretion than omnivorous individuals; however, they also eat a smaller quantity of protein, and there are important variations of other macronutrients in their diet [9, 10]. Subtotally nephrectomized rats fed a vegetable protein diet develop less proteinuria and renal histological damage than animals receiving casein [11].

In this study we demonstrate that a period of vegetable protein diet produces significant changes in renal function independently of the daily amount of protein in a group of healthy individuals. Glomerular filtration rate, renal plasma

Table 3. Prostaglandin urinary excretion rate (pg/min) in 7 normal subjects at baseline and after a protein load with meat and soya

		Time *hr*			
		Baseline	1	2	3
PGE_2	Meat	223 ± 38	302 ± 44	301 ± 57	241 ± 55
	Soya	214 ± 54	205 ± 47	243 ± 55	191 ± 61
6-keto-$PGF_{1\alpha}$	Meat	3691 ± 1979	6198 ± 2183[a]	5895 ± 1968[a]	4155 ± 1013[b]
	Soya	4233 ± 1021	4636 ± 1947	3440 ± 1757	2197 ± 992
TxB_2	Meat	776 ± 236	699 ± 116	815 ± 188	634 ± 84
	Soya	618 ± 105	709 ± 185	542 ± 125	445 ± 95

[a] $P < 0.03$ vs. baseline
[b] $P < 0.01$ meat vs. soya

flow and the fractional clearance of albumin were markedly reduced during vegetable protein intake. The renal vasodilatory effect of chronic meat ingestion was abolished by vegetable protein feeding as indicated by a consistent rise in renal vascular resistance. Several lines of evidence suggest that the type of protein was responsible for this effect. The amount of total protein and phosphate intake was comparable between the diets; the addition of fiber to the APD did not alter renal hemodynamics or protein excretion, and fat and energy intakes were also similar on APD and VPD. The P/S ratio was lower on APD than VPD, but this is unlikely to be responsible for the renal changes because the renal differences persisted between VPD and APD + F in which the P/S ratio was similar. There was relatively more carbohydrate in VPD, but carbohydrates do not appear to have any direct effect on renal function [28]. It is unlikely that differences in food absorption were implicated as the 24-hour urinary urea excretion was similar on the two diets, and plasma protein concentration and body weight did not change. The reduction in fractional clearance of plasma protein during VPD is of particular interest as it indicates that the type of ingested protein affects the renal handling of protein in addition to glomerular hemodynamics. The fall in protein clearance could be the result of a reduced filtration or an increased tubular reabsorption. That these changes in protein clearance are likely to be glomerular in origin is supported by the unchanged urinary excretion of β_2-microglobulin in this study and in previous reports in which modification of protein intake had been applied [13, 15, 16]. The change in the type of protein could have affected membrane size and charge selectivity as well as the pressure gradient across the glomerular barrier, as recently suggested by studies with low protein diet [4, 13].

As the results of the chronic study were insufficient to explain the mechanisms of the renal effect of a vegetable protein diet, a set of experiments involving protein loads with meat or vegetable protein were designed to gain a better insight into the possible mediators of these effects. The renal response to an equivalent amount of protein ingested in the form of meat or soya was strikingly different. A meat meal produced the expected vasodilatory response, with a fall in the RVR and a rise in GFR and RPF as previously described [16]. In contrast, soya loading had virtually no effect on these parameters, and produced no significant vasodilation. Similar results on the GFR, as measured by creatinine clearance, were obtained in a preliminary study by Dhaene et al, who gave Fortimel as an alternative protein preparation to meat to a group of normal individuals [27]. Of relevance is the finding that soya loading did not result in an increase in the clearance of albumin and IgG as seen after a meat meal. The proteinuric effect of meat ingestion has been described previously in normal and diabetic subjects, and appears to be mediated by perturbations in glomerular permselectivity and hemodynamics [29, 30]. That by changing the type of protein in the meal this proteinuric effect could be abolished was unknown. The equivalent rise in the urinary urea excretion and in serum amino acid concentration after both protein loads excludes the possibility of incomplete or delayed absorption of protein from the soya meal. Changes in total plasma protein, hematocrit and arterial pressure were also similar after the two protein loads.

Our data suggest that differences in plasma amino acid levels are unlikely to have contributed to the different renal responses to meat or soya ingestion. However, a role of amino acids cannot be entirely excluded. There is evidence that the dispersive release and the absorption kinetics of amino acids from soya and animal protein differ [31] and the differences, though small, in amino acid composition between the soya and meat loads may partly be responsible for the different renal effects. In vivo renal uptake of amino acids involves glutamine, proline, glycine, alanine and tryptophan [32], the last three of which were over-represented in the meat meal. The modality by which these amino acid differences play a role in the increase in GFR is unlikely to be by direct effect, in that infusion of a complete mixture of amino acids has no effect per se on renal hemodynamics [33, 34].

The explanation of the different renal responses to meat or soya derived protein are more likely to reside in the different hormonal responses elicited by the two challenges. The glucagon and renal vasodilatory prostaglandin response seen after meat ingestion was significantly blunted after the soya meal. Both these hormones have been implicated in the meat-induced renal hemodynamic changes, and prostaglandins have been suggested as possible mediators of the proteinuria of high protein feeding [14, 35–37]. That vasodilatory prostaglandins are likely to be the final effector of the renal hemodynamic and proteinuric response to meat ingestion in normal subjects has been recently demonstrated in studies in which the renal response to a meat meal was abolished by the administration of indomethacin [24]. The pattern of glucagon secretion and urinary prostaglandin production in response to soya ingestion could therefore provide an explanation for the different renal effects of vegetable protein ingestion. The hormonal mediation of these effects is further supported by studies in which infusion of somatostatin abolished the renal response to amino acid infusion [33]. The reason for the different hormonal response to

soya and meat must, at present, remain speculative and requires further investigation. It is possible that other substances contained in soya proteins, such as saponins, isoflavonoids, genestein, trypsin inhibitors and diadzein, may exert hormonal inhibitory effects and be responsible for the renal effects observed [38].

The type of protein ingested is therefore crucial to the pattern and magnitude of the renal response elicited. Vegetable proteins seem to induce renal changes comparable to those obtained by reducing the total amount of protein in the diet and prevent the vasodilatory and proteinuric effects of meat. These effects appear to be mediated by hormonal changes involving glucagon secretion and renal prostaglandin production. Protein modified, rather than protein restricted, diets may prove advantageous in the long-term treatment of chronic renal failure.

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