

Effect of short-term high-protein compared with normal-protein diets on renal hemodynamics and associated variables in healthy young men^{1–3}

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ABSTRACT

Background: High-protein diets are effective for weight reduction; however, little is known about the potential adverse renal effects of such diets.

Objective: The aim of our study was to compare the effect of a high-protein (HP) with a normal-protein (NP) diet on renal hemodynamics and selected clinical-chemical factors.

Design: We prospectively studied the effect of an HP diet ($2.4 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) with that of an NP diet ($1.2 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) on the glomerular filtration rate (assessed on the basis of sinistrin—an inulin analog—clearance) and renal plasma flow (*para*-aminohippuric acid clearance) by using the constant infusion technique. Filtration fraction and renal vascular resistance were calculated. Twenty-four healthy young men followed the 2 diet protocols for 7 d each in a crossover design. They were individually advised by a dietitian to achieve the planned protein intake by selecting normal foods under isocaloric conditions. Serum and urinary variables and renal hemodynamics were measured on day 7 of both diets.

Results: The glomerular filtration rate (NP: $125 \pm 5 \text{ mL/min}$; HP: $141 \pm 8 \text{ mL/min}$; $P < 0.001$) and filtration fraction (NP: $23 \pm 5\%$; HP: $28 \pm 5\%$; $P < 0.05$) increased significantly with the HP diet. Renal plasma flow was not significantly different between the HP ($496 \pm 25 \text{ mL/min}$) and NP ($507 \pm 18 \text{ mL/min}$) phases. Renal vascular resistance was not significantly different between the NP ($94 \pm 6 \text{ mm Hg} \cdot \text{mL}^{-1} \cdot \text{min}^{-1}$) and HP ($99 \pm 8 \text{ mm Hg} \cdot \text{mL}^{-1} \cdot \text{min}^{-1}$) phases. Blood urea nitrogen, serum uric acid, glucagon, natriuresis, urinary albumin, and urea excretion increased significantly with the HP diet.

Conclusions: A short-term HP diet alters renal hemodynamics and renal excretion of uric acid, sodium, and albumin. More attention should be paid to the potential adverse renal effects of HP diets. *Am J Clin Nutr* 2009;90:1509–16.

INTRODUCTION

Recent studies suggest that diets with a higher protein content ($\leq 30\%$ of total energy) induce sustained reductions in body weight and appetite (1, 2). Even in diabetic patients, a high-protein, low-carbohydrate diet was shown to lower body weight and to improve glucose and lipid metabolism within a short period (3). As a consequence, diets high in protein have become popular and are even recommended in some recent nutritional guidelines (4).

However, concerns exist as to whether a high protein intake may induce clinically relevant harmful alterations in kidney perfusion and function. Protein intake has been recognized as a modulator of renal function (5). In clinical studies, an increased protein supply has been shown to induce a transient surge in creatinine clearance determined in 24-h urine samples collected from healthy subjects (6). These data were confirmed, in part, by Wiegmann et al (7), who showed an increase in creatinine clearance after a 2-wk high-protein diet compared with a low-protein diet. However, this study failed to verify an effect of a high protein intake on glomerular filtration rate (GFR), assessed on the basis of inulin clearance (7). Studies in animals with established renal disease showed an accelerated progression of renal insufficiency with a high protein intake (8). A relation between the quantity of protein intake and the rate of renal deterioration has also been supposed in patients with chronic renal disease (9). Results from the MDRD (Modification of Diet in Renal Disease) study suggest that a restriction in dietary protein is beneficial in the prevention of prodigial renal insufficiency (10).

Unfortunately, clinical data concerning the influence of protein intake on kidney function in humans are inconsistent, and the results of previous studies have not been conclusive concerning recommendations for the optimum protein content of a healthy diet. The aim of our prospective crossover study was to compare, using an optimum study design, the effects of a high-protein diet with those of a normal-protein diet on renal function, hemodynamics, and clinical-chemical variables in healthy young men to elucidate the functional changes and the underlying mechanisms of a short-term increased oral protein load.

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Received February 8, 2009. Accepted for publication September 3, 2009.

First published online October 7, 2009; doi: 10.3945/ajcn.2009.27601.

SUBJECTS AND METHODS

This was a randomized crossover study in healthy young men. We started the study in 2006, and the first participant was screened on 27 April 2006. The first study visit of the first participant was on 10 May 2006. The last study visit of the last study participant was 23 February 2007. Twenty-four white young normotensive men between the ages of 21 and 30 y without any clinical or laboratory evidence of kidney, heart, liver, or endocrine disease during the initial screening procedure were recruited at the Life Science Campus of the Technical University of Munich, Germany. Normotension was based on 3 casual blood pressure readings with a standard sphygmomanometer at different times, with the participant sitting after 5 min at rest, and defined as average blood pressure values of $\leq 140/90$ mm Hg. The mean casual systolic and diastolic blood pressure was normotensive in all participants ($124 \pm 6/72 \pm 7$ mm Hg). Subjects with a body mass index (in kg/m^2) ≥ 25 , a serum creatinine concentration >1.2 mg/dL, micro- or macroalbuminuria (tested 3 times with test strips for the immunologic in vitro determination of urinary albumin; Mikraltest; Roche, Mannheim, Germany), antihypertensive medication use, and regular alcohol consumption and smokers were excluded. The screening procedure consisted of a physical examination, blood and urine chemistry measurements [eg, electrolytes, creatinine, blood urea nitrogen, liver enzymes, and red and white blood cell counts].

Each subject participated in 2 strictly controlled 7-d diet phases with either a high-protein (HP) or a normal-protein (NP) diet in random order. The NP diet contained $1.2 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$, and the high protein diet contained $2.4 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$. After a washout phase of 7–10 d, during which subjects consumed their usual diet, the participants were allocated to the other diet. Measurements of renal hemodynamics, 24-h blood pressure (Spacelabs Medical, Redmond, WA), and biochemical and neurohumoral variables were performed on day 7 of both the NP and the HP diets in all participants. Albuminuria was quantitatively determined with an immunochemical complex reaction by using a specific antibody test (Dade Behring, Marburg, Germany). We recorded 24-h systolic and diastolic blood pressures at the end of each protein diet period for all participants. Twenty-four-hour urine collections with analysis of 24-h creatinine clearance were performed in each subject on day 7 of the HP and NP diet periods. The protocol was approved by the Ethical Committee of the Technical University Munich, Germany. Each subject gave his written informed consent.

Diet protocol

During the recruitment phase, all participants were asked to record their dietary food intake for 7 d. Only those who had a normal protein intake between 1.0 and $1.5 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ were included. During the active intervention periods, the participants were individually advised and monitored by a dietitian to achieve the planned protein intake by selecting normal foods without specific restrictions in carbohydrate and fat intakes. To achieve the intervention goals, the individual food preferences of the participants were considered. In particular, the increase in protein intake was mainly achieved by increasing the consumption of foods from animal sources including milk and milk products. Participants were also asked to completely avoid alcoholic drinks during the study period. Salt restriction was not

practiced. Food intake was recorded daily by the participants during both intervention periods, and dietary adherence was monitored via phone calls by an experienced dietitian. The macronutrient intake was analyzed by using a commercially available software program (version 5.2, PRODI, Freiburg, Germany) based on the largest database of German foods. Adherence to the dietary protein instructions was also assessed by measuring urea nitrogen appearance in the 24-h urine samples on day 7 during both the NP and HP phases.

Measurements of systemic and renal hemodynamics

To determine renal hemodynamics, we used the constant infusion technique without urine collection (11). Renal plasma flow was assessed on the basis of *para*-aminohippuric acid clearance (PAH; aminohippurate sodium; Merck & Co, Whitehouse Station, NJ), and the GFR was assessed on the basis of sinistrin clearance (Inutest, Linz, Austria), which is a polyfructosan and an analog of inulin (12). For the infusion of both indicators, a venous infusion line was inserted via the left vena basilica. After administration of an intravenous loading dose of both tracer substances, a continuous infusion of the tracer substances was administered. The bolus dose of both indicators (18 mg PAH/kg ; $45 \text{ mg sinistrin/kg}$) was calculated according to the distribution volumes of both indicators (12) and infused over 15 min. Subsequently, a constant infusion dose of both substances was calculated (0.75 g PAH/h ; $1.5 \text{ g sinistrin/h}$), and a continuous infusion of both tracers was started. The principle of this method is based on the observation that, under steady state conditions between infusion and renal excretion, the excreted amounts of inulin and PAH are equal to the infused doses of each indicator. On the basis of this assumption, the clearances of the tracer substances can be calculated by using the following equation:

$$\text{Clearance (mL/min)} = \text{rate of infusion (mg/min)} / P(\text{mg/mL}) \quad (1)$$

where P is the plasma concentration of each indicator. This technique has been shown to be a valid and reliable method for determining renal hemodynamics (11, 13–15). This concept of the constant infusion technique requires an equilibration after infusion and the maintenance of a constant volume of distribution during the phase of tracer infusion. The priming dose as well as a sustained infusion have been shown to reach a steady state 1.5 h after the loading dose (14). Under steady state conditions (1.75 h after the loading dose), blood samples from an intravenous line on the opposite arm were drawn for the measurement of *para*-aminohippurate and sinistrin. Concentrations of *para*-aminohippurate were measured by the method of Pratt and Marshall as modified by Smith et al (16). Sinistrin was measured indirectly by enzymatic degradation of sinistrin into fructose by using inulinase and subsequently measuring fructose with an enzymatic method (10139106035; r-biopharm; Roche, Darmstadt, Germany). The laboratory technique used to measure sinistrin was reported previously (17). It has been shown in many studies that sinistrin is exhaustively degraded to fructose, and its concentration is a valid assessment of GFR via the constant infusion technique (18, 19). The filtration fraction was calculated as the quotient of clearance of sinistrin/clearance of

PAH, and renal vascular resistance (RVR) was calculated by using the following equation (20):

$$\begin{aligned} \text{RVR (mm Hg} \cdot \text{mL}^{-1} \cdot \text{min}^{-1}) \\ = [\text{mean arterial blood pressure} \\ - 12] \times 723 / \text{effective renal plasma flow} \\ \times (1 - \text{hematocrit}) \end{aligned} \quad (2)$$

For a proper interpretation of the results, it has to be recognized that the clearances of sinistrin and as inulin and the true GFR are not identical, but are approximate values (21). It has been determined that the PAH clearances are $\approx 20\%$ higher than the true renal plasma flow (11). PAH is metabolized and conjugated to a certain extent in humans. This extrarenal loss of PAH has to be considered for the determination of renal plasma flow via the constant infusion technique. This bias is constant, because the extraction of PAH is $\approx 90\%$. The accuracy of the method is less in patients with markedly reduced renal function (11). In our study, we selected patients with normal renal function, which has been documented by the measurement of 24-h urinary creatinine clearance before entry into the study.

During the laboratory phase (constant infusion technique), systolic and diastolic blood pressures were measured every 10 min with an oscillometric device (Dinamap Pro 100V2; Criticon, Norderstedt, Germany).

Sample collection

Fasting blood samples were collected in the morning on the last day of the NP and HP diets. We measured red and white blood cell counts and serum creatinine, blood urea nitrogen, uric acid, electrolyte, glucose, triglyceride, insulin, glucagon, plasma renin, angiotensin II, and total, LDL-, and HDL-cholesterol concentrations. Specific urinary gravity was analyzed with a spot urine probe. Twenty-four-hour urine samples were collected for the measurement of pH, sodium, nitrogen, albumin, and daily urea nitrogen excretion by using established methods. Fractional sodium excretion (Fe-Na) was calculated as follows:

$$\text{Fe} - \text{Na} (\%) = U_{\text{Na}} \times V / P_{\text{Na}} \times \text{GFR} \quad (3)$$

where U_{Na} is urinary sodium concentration (mmol/L), V is the urinary volume excretion rate ($\mu\text{L} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$), and P_{Na} is the plasma sodium concentration (mmol/L).

Analysis of plasma amino acids

In a subgroup of 9 participants, the circulating amino acid patterns in 40 μL of the fasting plasma samples collected on day 7 of both protein diets were stored at -80°C until analyzed. A targeted quantitative metabolomics approach was used to identify the amino acid profile in plasma. A liquid chromatography-tandem mass spectrometric platform (3200 QTrap, Applied Biosystems, Darmstadt, Germany) was used for the analysis of amino acids and derivatives, including some dipeptides, with the iTRAQ-labeling method by using commercially available test kits (AA45/32 Physiologic Reagent Kit; Applied Biosystems).

Statistical analysis

The values are presented as means \pm SDs. All data were analyzed by using SPSS/PC version 16.0 (22). The variables were normally distributed. Student's t test for paired and unpaired data (HP compared with NP diet) was used. To examine the effects of the diets on quantitative outcomes of interest, sequential analyses for crossover studies, as proposed by Hills and Armitage (23), were conducted. All tests were performed in an explorative manner, and the significance level was set at $\alpha = 0.05$.

RESULTS

Clinical characteristics

Of the 32 young men initially screened, 25 fulfilled all of the inclusion criteria and 24 completed the study; one participant was excluded because of an allergic skin reaction to sinistrin exposure. The clinical data of the study subjects are presented in **Table 1**. During the NP and the HP diet phases, body mass index remained stable in all participants: 22.3 ± 2.0 and 22.5 ± 2.0 , respectively (NS).

Protein and macronutrient intakes during the 2 experimental periods are shown in **Table 2**. During the NP diet, a mean protein intake of $1.18 \pm 0.04 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ was recorded, whereas a significantly elevated protein intake of $2.46 \pm 0.09 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ was documented under the HP diet. There was no difference in total energy intake during the 2 study periods (Table 2). Adherence to the diet was also assessed by measuring urea nitrogen excretion in the 24-h urine sample, which was significantly higher in the HP diet phase than in the NP diet phase (urea nitrogen excretion on day 7 of HP: 13915 ± 2275 compared with $9094 \pm 1599 \text{ mg NP/d}$; $P < 0.01$) (**Table 3**). Twenty-four-hour urine collection was completed in 22 of the 24 participants. The excretion of creatinine in the 24-h urine samples was not different during the 2 diet phases (NP: $1796 \pm 292 \text{ mg/d}$; HP: $1989 \pm 381 \text{ mg/d}$; $P = 0.12$) (Table 3).

Effect on systemic and renal hemodynamics

The 24-h mean systolic and diastolic blood pressure was normotensive, and there was no significant difference on day 7 between the HP and the NP diets (systolic blood pressure: 118 ± 7 and $117 \pm 7 \text{ mm Hg}$, respectively; diastolic blood pressure: 69 ± 7 vs $69 \pm 4 \text{ mm Hg}$, respectively). Creatinine clearance measured in 24-h urine samples was significantly elevated

TABLE 1

Clinical characteristics of the 24 healthy male study participants at study entry

Clinical characteristic	Mean \pm SD
Age (y)	24.1 ± 2
Weight (kg)	75.6 ± 5
Height (m)	1.84 ± 6
BMI (kg/m^2)	22.3 ± 2.0
Blood pressure (mm Hg)	
Systolic	124 ± 6
Diastolic	72 ± 7
Waist circumference (cm)	75 ± 4

TABLE 2

Total energy and macronutrient intakes during a 7-d normal-protein (NP) and high-protein (HP) diet in 24 healthy normal-weight men¹

Intake	NP diet	HP diet	<i>P</i> value ²
Total energy (kcal/d)	2736 ± 386	2743 ± 369	NS
Protein (g · kg ⁻¹ · d ⁻¹)	1.18 ± 0.04	2.46 ± 0.09	<0.001
Protein (g/d)	88 ± 8	181 ± 16	<0.001
Animal sources	51 ± 11	149 ± 15	<0.001
Plant sources	33 ± 8	32 ± 8	NS
Energy (% of total)	13.3 ± 1.8	26.6 ± 2.9	<0.001
Carbohydrate (g/d)	328 ± 63	294 ± 58	<0.01
Total fat (g/d)	115 ± 20	89 ± 23	<0.01
Sodium (mg/d)	2482 ± 913	2737 ± 945	<0.05

¹ All values are means ± SDs. For all participants, 7-d diet protocols were analyzed.

² Derived by using a Student's *t* test for multiple comparisons in an explorative manner; significance was set at $\alpha = 0.05$.

during the HP compared with the NP diet (126 ± 27 mL/min compared with 115 ± 25 mL/min; $P < 0.05$). Renal plasma flow assessed on the basis of PAH clearance was not different between the NP and HP phases (507 ± 18 mL/min compared with 496 ± 25 mL/min; NS) (Table 4, Figure 1). GFR, assessed on the basis of sinistrin clearance, however, was significantly elevated in the HP diet phase compared with the NP diet phase (141 ± 8 mL/min compared with 125 ± 5 mL/min; $P < 0.001$) (Table 4, Figure 1). Analysis of the study groups separately according to the sequence of HP and NP diet phases (group 0: first the NP diet and then the HP diet; group 1: first the HP diet and then the NP diet), it was confirmed that there was no period effect. Renal vascular resistance of the participants was not different between the NP and HP diet phases (99 ± 8 mm Hg · mL⁻¹ · min⁻¹ compared with 94 ± 6 mm Hg · mL⁻¹ · min⁻¹; NS) (Table 4, Figure 1). The filtration fraction was significantly higher with the HP diet (HP: $28 \pm 5\%$; NP: $23 \pm 5\%$; $P < 0.05$) (Figure 2).

During the entire laboratory experimental phase, mean systolic and diastolic blood pressure measured with an oscillometric device was normotensive, and there was no difference in systolic and diastolic blood pressure between the HP and NP periods (HP: $126 \pm 5/75 \pm 6$ mm Hg; NP: $127 \pm 5/77 \pm 8$ mm Hg; NS).

TABLE 3

Urinary chemical variables in healthy men who consumed a normal-protein (NP) or a high-protein (HP) diet¹

	NP diet	HP diet	<i>P</i> value ²
Urinary albumin (mg/24 h)	8.7 ± 7	18.3 ± 7	<0.05
Urine pH	6.45 ± 0.39	5.79 ± 0.47	<0.05
Specific gravity	1020 ± 10	1020 ± 5	NS
Sodium excretion (mmol/d)	173.4 ± 64	215.6 ± 53	<0.05
Urea nitrogen excretion (mg/d)	9094 ± 1599	13915 ± 2275	<0.01
Creatinine excretion (mg/d)	1796 ± 292	1989 ± 381	0.12

¹ All values are means ± SDs. Excretion of albumin, sodium, urea nitrogen, and creatinine was measured in 22 participants who completed 24-h urine collections. Urinary pH and specific weight were measured by using a spot urine probe in 24 participants.

² Derived by using a Student's *t* test for multiple comparisons in an explorative manner; significance was set at $\alpha = 0.05$.

Effect of protein intake on selected clinical-chemical and neurohumoral variables

The HP diet did not influence serum electrolytes, lipids, or creatinine. However, blood urea nitrogen and uric acid concentrations were significantly greater with the HP diet than with the NP diet (Table 5). Furthermore, excretion of urinary sodium and urea nitrogen was significantly elevated during the HP diet. Urinary pH was significantly lower in the HP than in the LP diet phase. The 24-h urinary albumin excretion rate was significantly higher during the HP diet than during the NP diet (Table 3).

There was no significant effect of the HP diet on plasma renin, angiotensin II, aldosterone, and insulin concentrations, whereas serum glucagon concentrations were significantly higher after the HP than after the NP diet phase (79 ± 16 compared with 72 ± 11 ng/L; $P < 0.05$; Table 6). In a subgroup of 9 participants, an analysis of the amino acid pattern in plasma samples on day 7 of each diet was performed. As shown in Table 7, a significant decrease in circulating glycine and L-alanine concentrations was observed under the HP compared with the NP diet, whereas circulating sarcosine concentrations increased moderately with the HP diet.

DISCUSSION

We compared, under strict and controlled nutritional conditions, the effects of short-term HP and NP diets in a crossover design and measured the renal hemodynamic response using the validated constant infusion technique in healthy young men. We found significantly different effects on renal hemodynamics, with an increase in GFR and the filtration fraction even after a short-term high protein intake, whereas renal plasma flow and renal vascular resistance remained unchanged. Other major findings of this experimental study were that the HP diet resulted in an elevation of blood urea nitrogen, uric acid, and plasma glucagon concentrations. Furthermore, the extent of albumin excretion in the 24-h urine sample increased with the HP diet. Thus, this study showed that a short-term HP diet affects the renal hemodynamic response in healthy young men. Elucidation of the renal effect of a high nutritional protein intake is of interest in connection with the surfeit of protein in developed countries. In previous studies, it was shown that protein intake modulates renal function (5). Lew et al (24) found that the extent of protein intake has a direct and quantitative effect on endogenous creatinine clearance in young healthy subjects with various habitual protein intakes. There was a significant relation between urea nitrogen excretion and creatinine clearance, which suggests that creatinine clearance is not a fixed function (24). The effect of a chronic increased dietary protein intake was studied with a similar design by using endogenous creatinine clearance as a measure of GFR in healthy volunteers (25). It was found that creatinine clearance increased the more that the daily protein intake increased, reflecting the important role of protein intake as a control variable for creatinine clearance (25). Analysis of these studies indicated that an assessment of the variables of the different methods used for the measurement of creatinine clearance and GFR is essential. Wiegmann et al (7) investigated the effect of changes in a chronic HP diet (1.6 g/kg body weight) compared with an NP diet (0.5 g/kg) on renal function, assessed on the basis of creatinine clearance and inulin clearance. This study confirmed the effect of an HP intake to increase creatinine clearance, but did not succeed

TABLE 4

 Glomerular filtration rate (GFR), renal plasma flow (RPF), and calculated renal vascular resistance (RVR) in 24 men according to the sequence of the 2 diet phases¹

	Group 0: NP→HP ²		Group 1: HP→NP ³	
	<i>n</i>	Mean ± SD	<i>n</i>	Mean ± SD
GFR under NP (mL/min)	13	125.7 ± 4.70	11	124.1 ± 5.08
GFR under HP (mL/min)	13	141.3 ± 7.82	11	139.6 ± 8.20
RPF under NP (mL/min)	13	506.5 ± 15.54	11	507.5 ± 19.36
RPF under HP (mL/min)	13	501.2 ± 25.45	11	490.5 ± 25.43
RVR under NP (mm Hg · mL ⁻¹ · min ⁻¹)	13	99.0 ± 7.97	11	97.5 ± 9.02
RVR under HP (mm Hg · mL ⁻¹ · min ⁻¹)	13	93.9 ± 5.51	11	94.3 ± 7.34

¹ All values are means ± SDs. GFR was assessed on the basis of sinistrin clearance (mL/min), RPF was assessed on the basis of *para*-aminohippuric acid (mL/min), and RVR was calculated. NP, normal protein; HP, high protein.

² Group 0: first consumed the NP diet and then the HP diet.

³ Group 1: first consumed the HP diet and then the NP diet.

in demonstrating an effect of changes in protein intake on GFR (7). In our study, the GFR rose significantly with the HP diet, whereas renal plasma flow and renal vascular resistance were not affected. Discerning these hemodynamic results, one can hypothesize that a vasodilator response with hyperemia induced by the protein load may have caused the rise in GFR. However, a vasodilator response may cause an increase in glomerular hydrostatic pressure and an increase in renal blood flow. In our study, renal plasma flow and renal vascular resistance were not affected. Therefore, in the presence of an unchanged renal plasma flow, a change in the permeability with an increase of the ultrafiltration coefficient has to be considered as the presumable mechanism for the functional renal effects with a transient elevation in GFR under high protein intakes. This augmentation of the basal GFR may reflect the renal hemodynamic reserve modulated in healthy subjects by protein intake (6). These insights agree with the results of micropuncture studies, which showed a significantly higher glomerular capillary ultrafiltration coefficient in Munich-Wistar rats fed isocaloric diets with a high protein content as opposed to a low protein content (26). It is possible that functional alterations of the mesangium resulting in

a transitory increase of GFR could be induced by protein intake (6). In this context, the L-arginine nitrous oxide pathway plays a major role in increasing the glomerular ultrafiltration coefficient. Nitrous oxide produced by the macula densa controls glomerular hemodynamics indirectly through the tubuloglomerular feedback and is involved in the regulation of glomerular capillary blood pressure (27, 28). An enhanced protein load may increase Na⁺/K⁺ ATPase activity in the medullary thick ascending limb, which enhances the urinary concentrating ability and alters the tubuloglomerular feedback mechanism (29). Moreover, hormonal and neurohumoral effects caused by a protein-rich diet have to be taken into account in the increase of glomerular filtration. Plasma glucagon concentrations were significantly higher with the HP diet. High glucagon concentrations have been shown to increase the GFR under certain pathophysiologic conditions (30). However, it is not clear what threshold of glucagon concentration is required to produce renal hemodynamic effects in humans. In contrast, there was no difference in plasma renin and angiotensin II concentrations during the HP and NP diets, which indicates no systemic stimulation of the renin-angiotensin system with an HP diet.

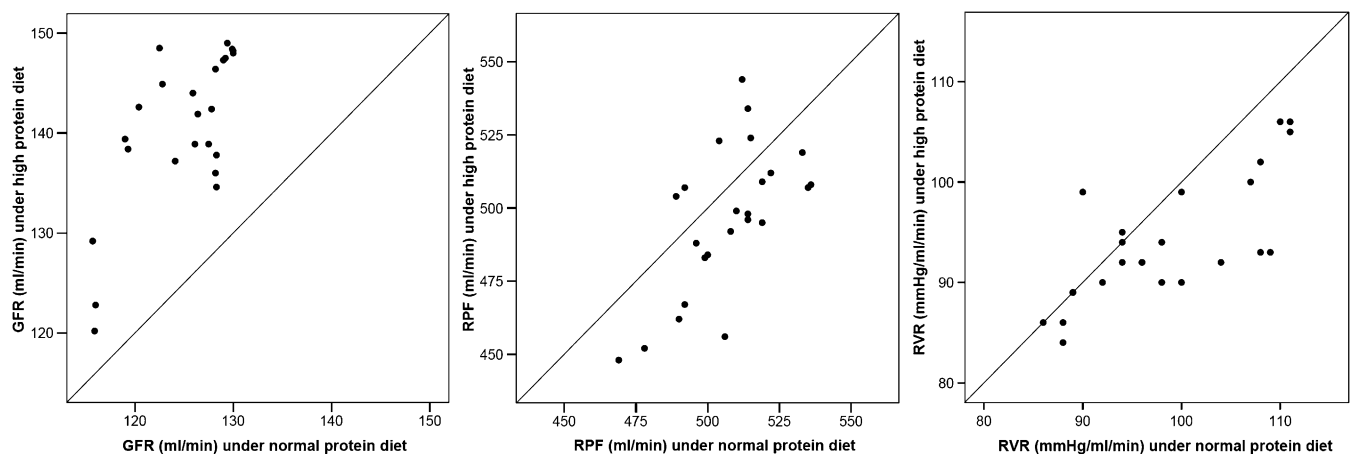


FIGURE 1. Glomerular filtration rate (GFR) assessed on the basis of sinistrin clearance, renal plasma flow (RPF) assessed on the basis of *para*-aminohippuric acid, and calculated renal vascular resistance (RVR) in 24 men during normal-protein compared with high-protein diets. The GFR was significantly higher with the high-protein than with the normal-protein diet ($P < 0.001$; 95% CI: $-19.3, -11.7$), whereas RPF and RVR were not significantly different between the high-protein and normal-protein diet phases (RPF: $P = 0.096$, 95% CI: $-1.9, 23.2$; RVR: $P = 0.084$, 95% CI: $-1.1, 16.4$). The data were tested with sequential analyses for crossover studies as proposed by Hills and Armitage (23) and are presented as scatterplots.

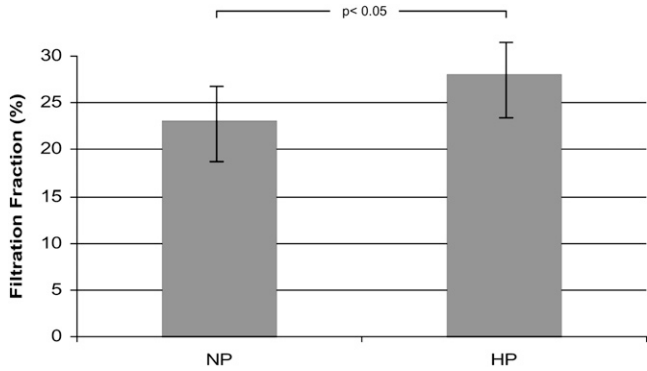


FIGURE 2. Mean (\pm SD) filtration fraction in 24 healthy men during the normal-protein (NP) compared with the high-protein (HP) diet. Student's *t* test for multiple comparisons was used in an explorative manner; significance was set at $\alpha = 0.05$.

In previous animal studies, rats fed a high-protein diet had an increase in overall kidney size with both glomerular and tubular enlargement (31). Thus far, the concept that hemodynamic changes in response to dietary protein induce structural abnormalities in the kidneys is largely based on animal studies (5). The results of clinical studies concerning the effect of dietary protein intake on functional and structural renal changes are not consistent and often suffer from methodologic limitations. Therefore, we applied a refined method, the constant infusion technique, to measure inulin clearance, which is accepted as a highly reliable method for the determination of the GFR (32). In addition, we selected a rather homogenous group of healthy young and normotensive men with a normal body mass index to minimize potential confounding by interfering factors such as age (33), body mass index (34), and sex. Furthermore, adherence to each diet was ascertained by close telephone monitoring and measurement of urinary urea nitrogen excretion, which was significantly higher with the HP than with the NP diet. Under these carefully selected conditions, our study likely had a high validity to assess the effect of dietary protein on renal function and related factors.

The described alterations appear to be moderate and to be part of the adaptive response of the kidney to dietary changes; nev-

ertheless, there is concern that chronic exposure to a high-protein diet may have undesired adverse effects on kidney function. An increased GFR with a high-protein diet may accelerate chronic kidney disease progression, particularly in those with abnormal kidney function (35). This process is usually associated with an increase in albuminuria and blood pressure, which may increase the risk of atherosclerotic complications. Albuminuria has been recognized as a risk factor not only for a poor renal prognosis but also for an impaired cardiovascular disease prognosis (36). Urinary albumin was elevated in the present study during the HP diet. This finding may be associated with a higher GFR under HP conditions. Thus, it is presumable that the increase in albumin excretion may have been due to a reduction in tubular reabsorption under the HP conditions. An altered amino acid profile may affect the proximal tubules, where plasma albumin normally filtered through the glomerulus is almost completely reabsorbed, which leads to the observed change in the reabsorption of albumin. Third, it cannot be ruled out that the degree of albuminuria was influenced, at least in part, by the higher sodium intake during the HP phase, because it has been recognized that a high sodium intake can increase microalbuminuria independently of blood pressure (37).

HP diets are also known to increase the risk of nephrolithiasis and to cause various metabolic alterations. Hyperuricemia and a reduction in urinary pH, also observed in our study, are recognized risk factors for nephrolithiasis (9). Experimental data provide evidence that uric acid may be an important mediator of renal disease and progression (38). Elevated uric acid may cause, in part, endothelial dysfunction, linking hyperuricemia with hypertension, the metabolic syndrome, and kidney disease (39, 40). Physiologic studies have shown a blunted renal vascular responsiveness to angiotensin II by serum uric acid as an independent predictor, consistent with results from experimental hyperuricemia showing an activated intrarenal renin-angiotensin system (41).

The results of our study primarily reflect the physiologic and largely adaptive changes in renal function to an HP load and their clinical significance is far from clear. A limitation to the interpretation of the results of our study was the fact that salt restriction was not a requirement during the HP or NP diet phases.

TABLE 5
Blood clinical-chemical variables at study entry and on day 7 of the high-protein (HP) and normal-protein (NP) diet in 24 healthy male study participants¹

Serum	Screening	NP diet	HP diet	P value ²
Sodium (mmol/dL)	140 \pm 1.3	138.8 \pm 1.6	139.6 \pm 2.1	NS
Potassium (mmol/dL)	4.2 \pm 0.2	3.9 \pm 0.4	3.9 \pm 0.4	NS
Phosphate (mg/dL)	3.6 \pm 0.5	3.6 \pm 0.4	3.6 \pm 0.5	NS
Calcium (mmol/dL)	2.4 \pm 0.1	2.4 \pm 0.1	2.4 \pm 0.1	NS
Blood urea nitrogen (mg/dL)	14 \pm 2	14 \pm 2	19 \pm 4	<0.05
Creatinine (mg/dL)	1.1 \pm 0.1	1.1 \pm 0.1	1.1 \pm 0.1	NS
Uric acid (mg/dL)	5.3 \pm 1.0	5.4 \pm 1.0	5.7 \pm 1.0	<0.05
Triglycerides (mg/dL)	—	89 \pm 39	85 \pm 44	NS
Total cholesterol (mg/dL)	—	142 \pm 15	150 \pm 18	NS
HDL cholesterol (mg/dL)	—	48 \pm 15	46 \pm 11	NS
LDL cholesterol (mg/dL)	—	94 \pm 22	96 \pm 20	NS
Hematocrit (%)	45.5 \pm 2.2	45.4 \pm 2.1	45.5 \pm 2.1	NS
Hemoglobin (g/dL)	14.9 \pm 0.7	14.7 \pm 0.7	14.8 \pm 0.7	NS

¹ All values are means \pm SDs.
² Derived by using a Student's *t* test for multiple comparisons in an explorative manner; significance was set at $\alpha = 0.05$.

TABLE 6

Plasma hormonal and neurohumoral variables in 24 men who consumed a normal-protein (NP) or a high-protein (HP) diet¹

Plasma	NP diet	HP diet	<i>P</i> value ²
Renin ($\mu\text{g/L}$)	15.8 \pm 9	12.2 \pm 10	NS
Angiotensin II (ng/L)	10.2 \pm 9	9.1 \pm 11	NS
Aldosterone ($\mu\text{g/L}$)	156.0 \pm 81	136.8 \pm 54	NS
Insulin (mIU/L)	5.8 \pm 4	5.5 \pm 4	NS
Glucagon (ng/L)	72.0 \pm 11	79.2 \pm 16	<0.05

¹ All values are means \pm SDs.

² Derived by using a Student's *t* test for multiple comparisons in an explorative manner; significance was set at $\alpha = 0.05$.

The sodium intake was significantly higher parallel to the HP intake in the HP phase. It is known that that dietary sodium intake can influence not only the tubular reabsorption of sodium but also—via the tubuloglomerular feedback mechanism—the GFR, especially in salt-sensitive individuals (42). Therefore, we cannot rule out that the higher sodium intake during the HP phase could possibly have affected renal hemodynamics. The higher sodium consumption associated with the HP diet has led correctively to increased natriuresis. This corrective natriuresis after increased sodium intake can be elicited without any change in arterial blood pressure (43). The fact that the measurement of renal plasma flow may be influenced by a modified PAH secretion induced by the HP diet needs to be considered when interpreting the results concerning the significant changes in filtration rate and the lack of change in plasma. In our setting, it cannot be excluded that an HP

intake alters tubular function with an impaired secretion of PAH. The specific gravity of urine was not different during the 2 diet phases. Nevertheless, the results have to be interpreted cautiously, and a possible effect of the HP diet on tubular function must be considered. Over the 2 study periods, the volume state of the participants was balanced. This was supported by stable clinical signs in the physical examination as well as unchanged hematocrit and hemoglobin values in both the NP and HP study phases.

In view of the frequent use of HP diets for the management of obesity and the increased vascular vulnerability of these patients due to associated conditions such as hypertension and type 2 diabetes, the issue of what constitutes an optimum diet and macronutrient composition has important public health implications. In a 6-mo prospective dietary intervention study in obese subjects, an HP diet resulted in an increase in GFR from baseline of 5 mL/min, whereas albuminuria remained unchanged (44). In contrast, our data in young healthy men may support the notion that an HP diet increases the GFR and albuminuria. Therefore, it is worthwhile to more closely evaluate renal function in obese subjects consuming HP diets and, at least, to exclude those with early signs of impaired kidney function, eg, based on the urinary albumin excretion rate, because an impairment in renal function with an HP diet may be particular pronounced in individuals with preexisting chronic kidney disease (35).

In conclusion, our study provides evidence that an HP diet in healthy young men induces significant changes in the GFR, the filtration fraction, albuminuria, serum uric acid, and urinary pH values, whereas other indicators of renal function remained unchanged. Although the clinical significance of these findings is unclear, it is recommended that more attention be paid to the monitoring of renal function in humans consuming HP diets, because long-term effects cannot be excluded at present. Nevertheless, more detailed and long-term studies on the consequences of HP diets to kidney function are urgently needed.

The extremely valuable contributions of Christine Hinsky (Else Kröner-Fresenius Center for Nutritional Medicine) and Bernhard Haller (Institute for Medical Statistics and Epidemiology, Technical University Munich) are gratefully acknowledged.

The authors' responsibilities were as follows—HF and HH: designed the study and conducted the investigations; HH and RB: assessed renal and systemic hemodynamics and laboratory variables; RB and JG: coordinated the study; HH, JG, and UA-G: outlined the diet protocol, performed the dietary advisory, and calculated the nutritional facts; HD: analyzed the amino acid pattern and analyzed the results; and HF, HH, and UH: analyzed the data. All investigators were involved in the writing of the manuscript. All of the authors stated that they had no conflict of interest.

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TABLE 7

Amino acid concentrations (in $\mu\text{mol/L}$) in plasma on day 7 of the normal-protein (NP) and high-protein (HP) diets in 9 participants¹

Amino acid	NP diet	HP diet	<i>P</i> value ²
L-Alanine	448 \pm 113	400 \pm 19	0.05
Glycine	285 \pm 56	243 \pm 42	0.009
L-Valine	280 \pm 46	301 \pm 53	0.11
L-Leucine	152 \pm 24	157 \pm 24	0.44
L-Isoleucine	83 \pm 14	87 \pm 17	0.27
L-Serine	132 \pm 20	127 \pm 22	0.40
Threonine	142 \pm 20	139 \pm 28	0.59
L-Methionine	19 \pm 8	19 \pm 9	0.86
L-Homocysteine	2.9 \pm 0.1	2.9 \pm 0.1	0.56
L-Aspartic acid	19 \pm 4	19 \pm 4	0.85
L-Asparagine	67 \pm 10	63 \pm 9	0.09
L-Glutamic acid	112 \pm 23	110 \pm 24	0.76
L-Glutamine	632 \pm 70	615 \pm 67	0.45
L-Arginine	100 \pm 20	96 \pm 19	0.40
L-Lysine	188 \pm 24	196 \pm 34	0.36
L-Histidine	93 \pm 10	93 \pm 12	0.95
L-Phenylalanine	70 \pm 10	70 \pm 11	0.93
L-Tyrosine	72 \pm 11	72 \pm 13	0.84
L-Tryptophan	63 \pm 8	63 \pm 10	0.90
L-Proline	239 \pm 75	236 \pm 66	0.77
Hydroxyproline	15 \pm 7	13 \pm 4	0.23
Sarcosine	1.8 \pm 0.5	2.1 \pm 0.5	0.02
L-Citrulline	34 \pm 8	36 \pm 9	0.35
Ornithine	79 \pm 15	77 \pm 15	0.62
Taurine	123 \pm 27	121 \pm 19	0.78

¹ All values are means \pm SDs.

² A 2-sided *t* test for paired data was used for statistical comparison.

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