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Comunicación breve

High-protein diets and renal status in rats

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Abstract

Introduction: **High-protein (HP) diets might affect renal status. We aimed to examine the effects of a HP diet on plasma, urinary and morphological renal parameters in rats.**

Material and methods: **Twenty Wistar rats were randomly distributed in 2 experimental groups with HP or normal-protein (NP) diets over 12 weeks.**

Results and discussion: **Final body weight was a 10% lower in the HP group ($p < 0.05$) whereas we have not observed differences on food intake, carcass weight and muscle ashes content. No significant clear differences were observed on plasma parameters, whereas urinary citrate was an 88% lower in the HP group ($p = 0.001$) and urinary pH a 15% more acidic ($p < 0.001$). Kidney wet mass was ~22 heavier in the HP group ($p < 0.001$). Renal mesangium area was a 32% higher in the HP group ($p < 0.01$). Glomerular 1 and 2 were also ~30 higher in the HP diet ($p < 0.01$ and $p < 0.05$, respectively) and glomerular area a 13% higher ($p < 0.01$).**

Conclusion: **High-protein diet promoted a worse renal profile, especially on urinary and morphological markers, which could increase the risk for developing renal diseases in the long time.**

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Key words: *High-protein diet. Plasma. Urine. Kidney. Renal morphology. Rats.*

DIETAS HIPERPROTEICAS Y ESTADO RENAL EN RATAS

Resumen

Introducción: **Las dietas hiperproteicas (HP) pueden afectar la función renal. El objetivo del presente estudio fue examinar los efectos de una dieta HP sobre parámetros renales plasmáticos, urinarios y morfológicos en ratas.**

Material y métodos: **Veinte ratas Wistar fueron distribuidas aleatoriamente en 2 grupos experimentales con dieta HP o normoproteicas durante 12 semanas.**

Resultados y discusión: **El peso corporal final fue un 10% inferior en el grupo de dieta HP ($p < 0,05$) mientras que no se han observado diferencias en la ingesta de comida, peso de la carcasa del animal y el contenido muscular de cenizas. No se observaron claras diferencias en los parámetros plasmáticos, mientras que el citrato urinario fue de un 88% inferior en el grupo de dieta HP ($p = 0,001$) y el pH urinario un 15% más ácido ($p < 0,001$). El peso del riñón en sustancia fresca fue un 22% más pesado en el grupo de dieta HP ($p < 0,001$). El Área mesangial fue un 32% mayor en el grupo HP ($p < 0,01$). El floculo glomerular 1 y 2 fueron también ~ 30 mayores en la dieta HP ($p < 0,01$ y $p < 0,05$, respectivamente) y el área glomerular un 13% mayor ($p < 0,01$).**

Conclusión: **Una dieta hiperproteica promueve un peor perfil renal, especialmente en los marcadores urinarios y morfológico, que podrían aumentar el riesgo de desarrollar enfermedades renales a largo plazo.**

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Palabras clave: *Dieta hiperproteica. Plasma. Orina. Riñón. Morfología renal. Ratas.*

Abbreviations

ANOVA: Analysis of variance.
CKD: Chronic kidney disease.
ER: Endoplasmic reticulum.
GFR: Glomerular filtration rate.
HRT: Hypertrophy resistance training.
LDH: Lactate dehydrogenase.
MCP-1: Monocyte chemoattractant protein-1.
N: Nitrogen.
SEM: Standard error of the mean.

Introduction

In the last few years, the use of high-protein (HP) diets (i.e. «The Dr. Dukan diet») is gaining in popularity among the general population. Indeed, HP diets are increasingly being recommended as one of the management strategies for weight control in overweight and obese individuals[1-3]. High-protein diets

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appear to reduce appetite, energy intake, body weight, and fat deposition at the same time that improve plasma lipid profile[4-5]. In view of the high prevalence of obesity, type 2 diabetes, and metabolic syndrome[6], it is important to understand the effects of high levels of protein intake on health. This is particularly important for the kidney, because the above mentioned patients are characterized by renal hyperfiltration and increased risk of kidney disease[7-9].

Despite the antiobesity effects of HP diets, the impact of such diets on renal status remains unclear[10-12]. The potentially harmful effects of dietary proteins on renal function are believed to be due to the 'overwork' induced by such diets on the kidneys. Indeed, HP diets cause elevation of glomerular filtration rate (GFR) and hyperfiltration[11]. However, some authors affirm that the link between protein-induced renal hypertrophy or hyperfiltration and the initiation of renal disease in healthy individuals has not been clearly demonstrated[13]. This hyperfiltration could have deleterious consequences in diseased kidneys[14], however, in healthy individuals, the impact of consuming HP on renal health is unknown[10, 13]. Nevertheless, a few studies have observed that the exposure of rodents[15-16], cats[17] or pigs[18] to long-term HP diets results in glomerular hyperfiltration with renal morphologic injuries such as glomerular hypertrophy, and a greater prevalence of renal pathological changes.

The present study aimed to examine the plasma, urinary and morphological renal effects of HP diets in rats.

Materials and methods

Animals and experimental design

A total of 20 young albino male Wistar rats were allocated into two groups (n=10), with HP or normal-protein (NP) diet. The animals, with an initial body weight of 148±6 g, were housed in individual stainless steel metabolism cages designed for the separate collection of urine. The cages were located in a well-ventilated thermostatically controlled room (21±2ºC), with relative humidity ranging from 40 to 60%. A 12:12 light-dark (08.00-20.00 h) cycle was implemented. Throughout the experimental period all rats had free access to distilled water and the animals consumed the diet *ad libitum*. One week prior to the experimental period, the rats were allowed to adapt to the experimental conditions.

Body weight was measured weekly for all animals at the same time and the amount of food consumed by each rat was registered daily.

On week 11, a 12-hour urine sample from each animal was collected for biochemical analysis. Urine volumes were recorded and samples were transferred into graduated centrifuge tubes for the posterior pH, Ca, and citrate analysis.

At the end of the experimental period, the animals were anaesthetized with ketamine-xylacine and sacrificed by cannulation of the abdominal aorta. Blood was collected (with heparin as anticoagulant) and centrifuged at 3000 rpm for 15 minutes to separate plasma that was frozen in liquid N and stored at -80ºC. Carcass weight was recorded. Carcass is the weight of the slaughtered animal's cold body after being skinned, bled and eviscerated, and after removal the head, the tail and the feet. Kidneys were extracted, weighed, and immediately the left one was introduced in formalin for the posterior histological analysis.

All experiments were undertaken according to Directional Guides Related to Animal Housing and Care (European Community Council, 1986) [19], and all procedures were approved by the Animal Experimentation Ethics Committee of the University of Granada.

Experimental diet

Formulation of the experimental diet is presented in table I. The diet was formulated to meet the nutrient requirements of adult rats following the recommendations of the American Institute of Nutrition (AIN-93M)[20], with slight modifications. We have selected a 45% of protein level for the HP diet group, following previous studies in which HP diet was compared with NP diets in rats[4-5,21], whereas a 10% of protein content was chosen for the NP diet group. Commercial soy protein isolate was used as the only source of protein since this protein source is widely available and used by sportsmen. Inclusion of 45% protein level in the diet was done at the expense of complex carbohydrates (wheat starch). Prior to diet preparation, total protein concentration of the commercial isolate was measured. Total N content was 12.4±0.7 g/100g of dry matter, which corresponds to a 77.5% of richness. Total protein concentration of the experimental diet was also assayed, with values of 44.1±2.2% and 9.8±0.4% respectively, for the HP and NP diet.

Chemical analyses

Total N of the soy protein supplement was determined according to Kjeldahl's method. Crude protein

Table I
Nutritional composition of the experimental diets

Nutritional Composition (g/100 g DM)	*Normal-protein*	*High-protein*
Soy protein supplement	13.1	57.4
Mineral mix (AIN-93M-MX)	3.5	3.5
Vitamin mix (AIN-93-VX)	1	1
Fat (olive oil)	4	4
Choline chloride	0.25	0.25
Cellulose	5	5
Starch	62.4	28.6
Methionine	0.5	–
Sucrose	10	–

DM, dry matter

was calculated as N × 6.25. Urine Ca content was determined by atomic absorption spectrophotometry using a PerkinElmer Analyst 300 spectrophotometer (PerkinElmer, Wellesley, MA, USA). Analytical results were validated by standard reference materials CRM-189, CRM-383, and CRM-709.

Urinary pH was analyzed with a bench pH-meter (Crison, Barcelona, Spain) and urinary citrate with a commercial kit (Spinreact, S.A. Gerona, España). Plasma total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, urea, total proteins, albumin and lactate dehydrogenase (LDH), were measured with a Hitachi-Roche p800 autoanalyzer.

Histological analysis

Left-kidney samples were fixed in buffered 4% formalin and embedded in paraffin. Afterwards, four-micrometer-thick sections were obtained and stained with 1% Picro-sirius red F3BA (Gurr, BDH Chemicales Ltd, Poole, United Kingdom)[22]. This technique allows the visualization of connective fibers deep red stained on a pale yellow background[22]. The sections were assessed by optical microscopy. Forty images per sample were captured: twenty of the glomerulus to determine the morphometry and the intraglomerular connective tissue and twenty of the tubulointersticial area to measure the interstitial connective tissue. All images were acquired with 20× objective and analyzed with the Fibrosis HR® software[23]. This image analysis application allowed us to automatically quantify morphometric parameters by using various image-processing algorithms[23].

Statistical analysis

Results are presented as mean and standard error of the mean. Differences between HP and NP diet groups were analyzed by ANOVA; with final body weight, food intake and muscle, urinary, plasma and renal morphology parameters as dependent variables. All analyses were conducted with the Statistical Package for Social Sciences (SPSS, version 19.0 for Windows; SPSS Inc., Chicago, IL), and the level of significance was set at 0.05.

Results

The effects of the HP diet on final body weight, food intake, muscle, plasma and urinary parameters are shown in table II.

Final body weight, food intake and muscle ashes content

Final body weight was a 10% lower in the HP group ($p<0.05$). No differences were observed in food intake, carcass weight, and muscle ashes content (all, $p>0.05$).

Plasma and urinary parameters

No significant differences were observed on plasma lipid profile as well as in the rest of renal plasma markers measured (all, p>0.05).

Urinary citrate was an 88% lower in the HP group ($p=0.001$) and urinary pH a 15% more acidic (p<0.001).

The effects of HP diet on kidney weight and morphology are shown in table III.

Kidney weight and morphology

Kidney wet mass, as expressed in absolute value as well as expressed referred to final body weight or carcass weight, was ~22 higher in the HP group (all, $p<0.001$). No differences between groups were observed on kidney interstitial connective tissue.

Renal mesangium area was a 32% higher in the HP group (p<0.01). Glomerular tuft 1 and 2 were also ~30 higher in the HP diet ($p<0.01$ and p<0.05, respectively) and glomerular area a 13% higher in the HP diet ($p<0.01$).

Discussion

The findings of the present study show: i. HP diet significantly reduced body weight but without clearly improving plasma lipid profile. ii. Urinary citrate and

Table II
Effects of high-protein diet on plasma and urinary parameters

	High-protein diet	*Normal-protein diet*	*P*
Final body weight (g)	317.6 (10.6)	350.4 (8.41)	0.025
Carcass weight (g)	178.9 (4.93)	170.9 (5.49)	0.299
Food intake (g/day)	15.56 (0.21)	14.10 (0.71)	0.067
Longissimus dorsi ashes (g/100 g DM)	4.31 (0.11)	4.46 (0.05)	0.226
Plasma			
Total cholesterol (mg/dl)	53.1 (2.1)	54.0 (5.3)	0.744
LDL-cholesterol (mg/dl)	5.34 (1.1)	5.29 (1.1)	0.974
HDL-cholesterol (mg/dl)	13.65 (0.88)	15.70 (4.8)	0.714
Triglycerides (mg/dl)	43.9 (7.5)	66.8 (8.6)	0.063
Urea (mg/dl)	28.90 (1.30)	25.04 (1.55)	0.080
Total Proteins (g/dl)	5.30 (0.10)	5.61 (0.07)	0.878
Albumin (mg/dl)	2.65 (0.23)	2.73 (0.25)	0.822
Lactate Dehydrogenase (u/L)	575 (73)	677 (76)	0.373
Urine			
Urinary Calcium (mg/day)	0.80(0.17)	0.51 (0.08)	0.130
Urinary Citrate (g/L)	0.35 (0.08)	2.88 (0.55)	0.001
Urinary pH	6.29 (0.11)	7.25 (0.17)	<0.001
Urinary volume (ml)	3.92 (0.52)	2.75 (0.36)	0.083

Values expressed as mean (standard error of the mean). DM, dry matter.

Table III
Effects of high-protein diet on kidney morphology

	High-protein diet	*Normal-protein diet*	*P*
Kidney (g) (mean right and left)	1.18 (0.04)	0.92 (0.03)	<0.001
Kidney (g/100 g body weight)	0.375 (0.015)	0.264 (0.011)	<0.001
Kidney (g/100 g carcass)	0.66 (0.016)	0.54 (0.011)	<0.001
Kidney interstitial connective tissue (%)	3.33 (0.23)	2.71 (0.40)	0.197
Kidney interstitial connective tissue area (μm²)	4245 (287)	3657 (548)	0.355
Mesangium area (μm²)	6178 (442)	4172 (475)	0.006
Glomerular tuft I area (μm²)	10154 (705)	6616 (838)	0.005
Glomerular tuft II area (μm²)	20891 (1258)	14573 (1857)	0.011
Glomerular area (μm²)	46590 (1404)	40405 (1061)	0.002

Values expressed as mean (standard error of the mean).

pH were drastically lower in the HP group, which could constitute a favorable environment for kidney stones formation in high-risk patients. iii. The increase of kidney weight observed in the HP groups was accompanied by higher renal mesangiums, glomerular tufts and areas. Therefore, HP diet promoted a worse morphological renal profile.

Under our experimental conditions, the HP diet slightly reduced body weight, which is in agreement with other studies[4-5]. However, a recent systematic review has observed that the long-term effect of HP diets on weight loss is neither consistent nor conclusive[3]. Moreover, in contrast to what has been reported by other authors[4-5], we have not observed a reduction on food intake and neither a significant better lipid profile. To note is that plasma triglycerides were a 34% lower in the HP diet fed group, which is clinically relevant, but without statistical signification (p=0.063).

Plasma urea concentrations can increase when HP diets are consumed[14, 24-25]. In the present study, we have observed a close to the signification (p=0.08) 15% higher plasma urea concentrations in the HP diet group. When more urea is excreted, more urea needs to be filtered, and maybe this can be on the basis of the 13% higher glomerular area observed in our groups fed with the HP diet.

Accordingly to the evidence reported previously by our group[24,26] and by other studies[18, 27-29] we have observed a 22% increase in kidney fresh mass weight of rats after 12 weeks of HP diet consumption. Hammond and James[29] found a 26-32% increase in kidney fresh mass weight of rats after 2 weeks of HP diet consumption, which they attributed to the strong effects on blood urea N and totally daily N filtration rate exerted by HP consumption. Some studies performed in rodents[27-28] or pigs[18] have observed histological damage with HP diets in the long term. In the study by Jia et al.[18], whole plant and animal proteins in proportions that mimicked human diets were given to pigs. Adult female pigs received either NP or HP (15 or 35% of energy from protein, respectively) isocaloric diets for either 4 or 8 months. The HP compared with the NP diet resulted in enlarged kidneys at both 4 and 8 months. Renal and glomerular volumes were 60–70% higher by the end of the study. These enlarged kidneys had greater evidence of histological damage, with 55% more fibrosis and 30% more glomerulosclerosis. Similarly, we have observed morphological impairment in our HP diet fed group, with a 32% higher renal mesangium area, a ~30% higher floccules areas and a 13% higher glomerular area in the HP diet groups, but without significant more renal interstitial connective tissue. To note is that we have not observed renal fibrosis.

In the above mentioned study by Jia et al.[18], plasma concentrations of homocysteine and renal monocyte chemoattractant protein-1 (MCP-1) were extremely higher in the HP-fed groups[18], which could explain the larger kidneys observed. Renal inflammation is induced via release of proinflammatory chemokines, such as MCP-1, which plays an important role in the recruitment of inflammatory cells into the kidney[30]. Infiltrating inflammatory cells interact with renal cells, causing them to synthesize excessive extracellular matrix, ultimately resulting in the development of kidney interstitial connective tissue[30-33].

In contrast, other authors have observed that in long interventional studies performed in humans, including overweight or obese healthy subjects, without preexisting renal dysfunction, the HP diet did not adversely affect renal function, whether it increased GFR and kidney size[34] or whether it did not[35].

Kidney plays a central role in protein metabolism. Thus, disease states of the kidney invariably result in clinically relevant disturbances of protein metabolism. Conversely, processes regulated by the kidneys are directly affected by dietary protein intake[36]. The amount and composition of ingested proteins have a direct impact on renal function, especially in a state of diseased kidneys. Consequently, limitation of ingested protein, particularly from animal sources, is crucial in order to slow the progression of chronic kidney disease and impaired renal function[36]. Relative excess of animal protein ingestion (acid load from sulphur-containing amino acids) might produce intracellular acidosis[37]. Intracellular acidosis stimulates urinary hypocitraturia, that is often accompanied by urinary hypercalciuria[37], which is strongly related to net renal acid excretion[38]. A decrease in urinary pH, hypocitraturia and hypercalciuria, are recognized risk factors for kidney stone formation, principally by increasing urinary saturation of calcium salts[21,37]. In our study, the HP diet increased plasma urea and urinary excretion of Ca (close to the statistical signification), at the same time that strongly decreased urinary pH and citrate. Therefore, those animals could be at a higher risk for nephrolithiasis. Furthermore, urine acidification is also a characteristic of visceral obesity and the

metabolic syndrome, and thus, HP diets may be associated with various metabolic abnormalities in visceral obesity[39].

Something to consider is that the effect of proteins also depends on the presence of other nutrients in the diet. High intakes of fruits and vegetables are associated with a reduced risk for stone formation in high-risk patients[40]. This beneficial effect of fruits and vegetables is probably due to their high content in potassium and magnesium. Potassium stimulates urinary excretion of citrate, which is an inhibitor of calcium stones formation[40-41].

Conclusion

The HP diet consumption promoted, in general, a worse urinary and morphological renal profile, whereas plasma parameters were less clearly affected (showed lower sensitivity to the diet). HP diet significantly reduced body weight but without a parallel improvement on plasma lipid profile. Urinary citrate and pH were drastically reduced by the HP diet, which could constitute a favorable environment for nephrolithiasis in high-risk patients. Finally, the increase of kidney weight, renal mesangiums, glomerular tufts and areas by the HP diet could compromise renal health in the long time.

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