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FULL COMMUNICATION

Whey Versus Soy Protein Diets and Renal Status in Rats

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Virginia A. Aparicio,[1] Elena Nebot,[1] Mohammed Tassi,[2] Daniel Camiletti-Moirón,[1] Cristina Sanchez-Gonzalez,[1] Jesús M. Porres,[1] Pilar Aranda[1]

[1]Department of Physiology, School of Pharmacy and Institute of Nutrition and Food Technology, University of Granada, Granada, Spain.
[2]Department of Pathologic Anatomy and Institute of Regenerative Biomedicine, School of Medicine, University of Granada, Granada, Spain.

ABSTRACT Different dietary protein sources can promote different renal statuses. We examined the effects of whey protein (WP) and soy protein (SP) intake on plasma, urinary, and morphological renal parameters in rats. One hundred and twenty Wistar rats were randomly distributed into 2 experimental groups fed with either WP or SP diets over 12 weeks. These diets were based on commercial WP or SP isolates. The urinary calcium content was higher in the WP diet compared to the SP diet group ($P < .001$) whereas the urinary citrate level was lower ($P < .001$). The urinary pH was more acidic in the WP diet group compared to the SP diet group ($P < .001$); however, no differences were observed between the groups for any of the renal morphological parameters analyzed (all, $P > .05$) or other plasma renal markers such as albumin or urea concentrations. The increase of acid and urinary calcium and the lower urinary citrate level observed in the WP diet group could increase the incidence of nephrolithiasis compared to the SP diet group. Despite the WP showed poorer acid-base profile, no significant morphological renal changes were observed. These results suggest that the use of SP instead of WP appears to promote a more alkaline plasma and urinary profile, with their consequent renal advantages.

KEY WORDS: acidosis kidney protein rats renal morphology soy protein urine whey

INTRODUCTION

The use of protein supplements with over 80% protein concentrates or over 90% protein isolates have become popular among the population.[1] Whey protein (WP) is the liquid that remains after milk has been curdled and strained to remove the caseins, and it contains proteins, lactose, vitamins, minerals, and traces of fat. WP represents 20% of the total protein content of milk and has been reported to have positive effects on bone, muscle, blood, brain, pancreas, cancer, metabolism, and the immune system wound healing, learning, and aging.[2,3] Soy protein (SP) is a vegetable-based high-quality protein, with a protein digestibility corrected amino acidic score of 1. Furthermore, SP also has a high arginine/lysine ratio, which is associated with lower insulin secretion compared to animal proteins.[4,5] Soy protein contains isoflavones, which act as weak estrogens that inhibit tyrosine kinase-dependent signal transduction processes and function as cellular antioxidants.[4,5]

Soy protein is low in sulphuric amino acids, therefore some nutritional advantages could be obtained by replacing animal-based foods for soy foods.[5] A relative excess of animal protein ingestion (acidic load from sulphur-containing amino acids) can induce intracellular acidosis that stimulates hypocitraturia, which is often accompanied by hypercalciuria.[6–8] Hypocitraturia and hypercalciuria both contribute to the formation of calcium-containing kidney stones.[6,9]

The renal effects of SP have been widely studied, but the results are controversial and inconclusive. Moreover, while some studies reported a protective role of SP on renal health,[10–14] other studies failed to demonstrate any significant improvements.[15,16] In the case of WP, no studies have analyzed their effects to date, at least in a normal-protein concentration. Therefore, the present study aimed to further examine the effects of WP versus SP intake on plasma, urinary, and morphological renal parameters in rats. This study thoroughly analyzed potential changes in renal morphology in response to different protein sources (SP or WP diet), which had not previously been comprehensively studied.

MATERIALS AND METHODS

Animals and experimental design

A total of 120 young male Wistar rats were allocated into two experimental groups that were fed with either whey

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Address correspondence to: Virginia A. Aparicio, PhD, Department of Physiology, School of Pharmacy and Institute of Nutrition and Food Technology, Campus Universitario de Cartuja s/n, University of Granada, Granada 18071, Spain, E-mail: virginiaparicio@ugr.es

(n = 60) or soy (n = 60) protein for 12 weeks. Animals, with an initial body weight of 165 □8 g were housed from day 0 of the experiment in individual stainless steel metabolism cages designed for separate collection of feces and urine. The cages were located in a well-ventilated, thermostatically controlled room (21 □2□C), with relative humidity ranging from 40% to 60%. A 12:12 light-dark cycle (08.00□ 20.00 h) was implemented. Throughout the experimental period, all rats had free access to double-distilled water and the animals consumed the diets (WP or SP) ad libitum.

The rats' body weights were measured weekly and at the same time of day, and the amount of food consumed by each rat was registered daily.

On week 11, a urine sample from each animal was collected for biochemical analysis. The urine volumes were recorded and samples were transferred into graduated centrifuge tubes for pH, calcium, and citrate analysis. At the end of the experimental period, the animals were anaesthetized with ketamine-xylazine and sacri□ced by cannulation of the abdominal aorta. Blood was collected (with heparin as anticoagulant) and centrifuged at 3,000 rpm for 15 min to separate the plasma that was subsequently removed, frozen in liquid Nitrogen, and stored at - 80□C. The carcass weights were recorded, and the left kidneys were extracted, weighed, and immediately stored in formalin for subsequent histological analyses.

All experiments were performed according to the Directional Guides Related to Animal Housing and Care (European Community Council, 1986),[17] and followed the Canadian Council on Animal Care (CCAC) guidelines. All procedures were approved by the Animal Experimentation Ethics Committee of the University of Granada.

Experimental diets

Experimental diets were formulated to meet the nutrient requirements of rats (NRC, 1995)[18] based on the AIN-93M formulation described by Reeves et al., but included modi□cations in the protein source and content and the oil source (Table 1).[19] A 10% of protein content was chosen according to the American Institute of Nutrition (AIN-93M).[19] Commercial WP or SP isolates were used as the sole sources of protein since these proteins are widely available and used by sportsmen and people interesting on losing weight or improve health.

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Total N content of the commercial WP isolates was 11.8 □0.6 g/100 g of dry matter and corresponded to 73.8% protein content. Total N content of the commercial SP isolate was 12.4 □0.7 g/100 g of dry matter, which corresponded to 77.5% protein content.

The total protein content of the experimental diets was 10.4 □0.6% for the WP diet and 9.8 □0.4% for the SP diet. These values are adequate for our experimental design.

Chemical analyses

The total N of the WP and SP supplements was determined according to Kjeldahl's method. Crude protein amounts were calculated as N □6.25. Bone, diets, and feces ashes were prepared by calcination at 500□C to a constant weight.

Table 1. Composition of the Experimental Diets

Nutritional composition (g/100 g DM)	Whey protein diet	Soy protein diet
Whey protein supplement	13.8	-
Soy protein supplement	-	13.1
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	61.7	62.4
Methionine	0.5	0.5
Sucrose	10	10
Mineral composition of the experimental diets (mg/g DM)		
Ca (mg/g DM)	5.39 (0.23)	6.08 (0.76)
P (mg/g DM)	2.50 (0.10)	3.63 (0.13)
Mg (mg/g DM)	0.52 (0.02)	0.49 (0.02)
Zn (1g/g DM)	27.3 (0.70)	23.0 (0.15)
Sulphur aminoacids of the protein supplement (g/100 g)		
L-methionine	2.2	0.78
L-cysteine	2.2	0.78

DM, dry matter; Ca, Calcium; P, Potassium; Mg, Magnesium; Zn, Zinc.

Calcium, magnesium and zinc content in urine, diets, and feces were determined by atomic absorption spectrophotometry using a Perkin Elmer Analyst 300 spectrophotometer (Perkin Elmer, Wellesley, Massachusetts, USA). Analytical results were validated using standard references certi□ed materials CRM-189 (wholemeal starch; Community Bureau of Reference, Geel, Belgium), CRM-383 (haricot beans; Community Bureau of Reference), and CRM-709 (pig feed; Community Bureau of Reference). Phosphorus content in diets and feces was determined using the methodology described by Chen et al.[20]

The urinary pH was analyzed using a bench pH-meter (Crison, Barcelona, Spain) and the urinary citrate level was analyzed using a commercial kit (Spinreact, S.A. Gerona, Spain). The plasma urea, total protein, calcium, phosphorous, magnesium, albumin, and creatinine concentrations were measured using a Hitachi-Roche p800 autoanalyzer (Roche Diagnostics Corp., Indianapolis, Indiana, USA).

Histological analysis

The left-kidney samples were □xed in 4% buffered formalin and embedded in paraf□n. Subsequently, four-micrometer-thick sections were obtained and stained with 1% Picro-sirius red F3BA (Gurr, BDH Chemicales Ltd., Poole, UK).[21] This technique facilitates the visualization of connective □bers as deep red stains on a pale yellow background.[21] The sections were assessed by optical microscopy. Forty images per sample were captured: 20 images of the glomerulus to determine the morphometry and the intraglomerular connective tissue, and 20 images of the

tubulointerstitial area to measure the interstitial connective tissue. All images were acquired using the 20 objective and analyzed with the Fibrosis HR software.[22] This image analysis application allowed us to automatically quantify morphometric parameters by using various image-processing algorithms.[22]

We estimated the following eight morphological variables that we describe for the better understanding of the present results: 1. Percentage of interstitial connective tissue in reference to the image area, excluding the glomerular area (the connective tissue that is in the gap over the Bowman's capsule); 2. The area of interstitial connective tissue (including Bowman's capsule). The Fibrosis HR software divides glomerular tufts into two categories: ``glomerular tuft I'' and ``glomerular tuft II.'' The variable ``glomerular tuft I'' corresponds to the renal corpuscle excluding the Bowman's capsule. The variable ``glomerular tuft II'' corresponds to the renal corpuscle excluding the Bowman's capsule and considering the area of the capillary lumens and urinary spaces in the glomerulus; 3. Glomerular tuft I area; 4. Glomerular tuft II area; 5. Glomerular tuft I percentage (percentage of glomerular tuft I related to the glomerular area); 6. Glomerular tuft II percentage (percentage of glomerular tuft II related to the glomerular area). 7. Mesangial area; 8. Glomerular area.

Statistical methods

The results are presented as mean and standard error of the mean. Differences between WP and SP diet groups were analyzed using the Student's t-test where the nal body weight, urine, plasma, and renal parameters were the dependent variables. All analyses were conducted using the Statistical Package for Social Sciences (SPSS, version 19.0 for Windows; SPSS Inc., Chicago, Illinois, USA), and the level of statistical signi cance was set at P .05.

RESULTS

The effects of whey and SP diets on the nal body and muscle weight, food intake, plasma and urinary parameters are shown in Table 2.

Food intake, nal body weight, carcass weight, and muscle weight

No differences in the nal body weight, carcass weight, and food intake were observed between the WP and the SP diet groups. Gastrocnemius was heavier in the WP diet group (P = .029).

Plasma and urinary parameters

The total plasma proteins concentration was higher for the WP diet compared to the SP diet (P = .001). The plasma albumin and urea concentrations were similar for the WP and the SP diet groups (2.78 0.14 vs. 2.69 0.19, P = .734 and 31.7 1.1 vs. 30.3 1.1, P = .383).

Urinary calcium, as expressed in mg per liter as well as in mg per day, was higher in the WP group when compared to the SP diet group (both, P < .001), whereas the urinary citrate was lower (P < .001). The urine pH was more acidic in rats that consumed the WP diet compared to the group that consumed the SP diet (P < .001). Urinary volume was also higher in rats that consumed the WP diet compared to the group that consumed the SP diet (P = .020).

The effects of the WP and SP diets on kidney weight and morphology are shown in Table 3.

Table 2. Effects of Whey and Soy Protein on Plasma and Urinary Parameters

	Source of protein		
	Whey	Soy	P
Final body weight (g)	328.5 (4.0)	324.0 (3.7)	0.408
Food intake (g/day)	16.4 (0.24)	15.8 (0.27)	0.125
Carcass weight (g)	168.2 (2.1)	173.2 (2.0)	0.090
Gastrocnemius (g N/100g DM)	14.0 (0.18)	13.2 (0.25)	0.029
Plasma Urea (mg/dL)	31.7 (1.1)	30.3 (1.1)	0.383
Plasma total proteins (g/dL)	5.60 (0.06)	5.30 (0.06)	0.001
Plasma Albumin (mg/dL)	2.78 (0.14)	2.69 (0.10)	0.734
Plasma Phosphorous (mg/dL)	6.35 (0.22)	6.87 (0.32)	0.184
Plasma Magnesium (mg/dL)	2.25 (0.27)	2.62 (0.17)	0.232
Urinary Calcium (mg/L)	3.35 (0.24)	2.16 (0.10)	0.001
Urinary Calcium (mg/day)	0.74 (0.07)	0.46 (0.05)	<.001
Urinary Citrate (g/L)	0.83 (0.14)	1.80 (0.17)	<.001
Urinary pH	6.34 (0.04)	6.72 (0.04)	<.001
Urinary volume (mL)	4.03 (0.32)	3.05 (0.24)	0.020

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

Kidney weight and morphology

The kidney wet mass, as expressed in an absolute value, was lower in the WP group compared to the SP group (P = .015), but there was no difference when the kidney wet

Table 3. Effects of Whey and Soy Protein on Kidney Morphology

	Source of protein		
	Whey	Soy	P
Kidney (g) (mean right and left)	1.00 (0.02)	1.06 (0.01)	0.015
Kidney (g/100 g body weight)	0.320 (0.006)	0.333 (0.004)	0.130
Kidney (g/100 g carcass)	0.602 (0.009)	0.613 (0.006)	0.377
Kidney interstitial connective tissue (%)	3.41 (0.24)	3.28 (0.16)	0.683
Kidney interstitial connective tissue area (μm^2)	4456 (304)	4246 (205)	0.594
Mesangium (%)	64.9 (1.11)	63.9 (0.75)	0.457
Mesangium area (μm^2)	5951 (376)	5425 (254)	0.281
Glomerular tuft I (%)	21.6 (1.36)	19.5 (0.92)	0.226
Glomerular tuft I area (μm^2)	9445 (626)	8704 (423)	0.361
Glomerular tuft II (%)	45.8 (2.79)	43.3 (1.88)	0.492
Glomerular tuft II area (μm^2)	19791 (1235)	19078 (834)	0.656
Glomerular area (μm^2)	43779 (778)	44953 (525)	0.244

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

mass was expressed relative to the final body weight or carcass weight.

No differences between groups were observed in any of the renal morphological parameters analyzed (all, $P > .05$).

DISCUSSION

The results of the present study demonstrate that rats fed with the WP diet displayed a poorer urinary acid-base homeostasis profile when compared to rats fed with the SP diet, and this may promote a higher risk of nephrolithiasis. Despite these differences, there were no observed renal morphological changes after the intervention period.

Renal pathologies result in clinically relevant disturbances of protein metabolism. Limitation of ingested protein, particularly from animal sources, is crucial in order to slow the progression of chronic kidney disease and impaired renal function.[23] Moreover, of particular importance for kidney health is the maintenance of acid/base homeostasis.[23] The catabolism of dietary protein generates ammonium ion and sulphates from sulphur-containing amino acids.[24] Urine pH is an indicator of dietary acid-base balance. Welch et al. investigated the relationship between urine pH and dietary acid-base load (potential renal acid load) in 22,034 men and women aged 39–78 years.[25] A more alkaline diet (lower potential renal acid load) based on high fruit and vegetable intake and lower consumption of meat was significantly associated with a more alkaline urine pH.[25] In the present study, the SP groups showed a more alkaline pH than the WP diet group, probably due to the lower content of sulfur amino acids of the SP supplement, and therefore, a lower potential renal acid load. In addition, decreased urinary pH, hypocitraturia and hypercalciuria, are recognized risk factors for kidney stone formation, specifically by increased urinary saturation of calcium salts.[6,9] Dietary calcium content of the present study design was at the recommended levels and it was similar in both diets. Urinary calcium excretion is strongly related to net renal acid excretion.[24] In our study, the WP diet increased urinary calcium excretion and decreased the urine pH and citrate levels, which could be also explained by the higher content in sulfur amino acids in the WP supplement. Therefore, animals on a WP diet could be at an increased risk of nephrolithiasis than those that consume the SP diet.

Notably, the effects of ingested proteins also depend on the presence of other nutrients in the diet. High intakes of fruits and vegetables are associated with a reduced risk of kidney stone formation in high-risk patients.[26] This beneficial effect of fruits and vegetables is probably due to their high content in potassium and magnesium. Potassium has been identified as a major stimulator of urinary citrate excretion, which is an inhibitor of calcium stone formation.[27] Therefore, the alkaline content and potassium richness of fruits and vegetables are also positively linked to reduced calcium excretion and hence, reduced kidney stone formation in high-risk patients.[26,28]

Most of the latest studies suggest that dietary SP may reduce renal disease progression in a number of renal diseases.[12–14] In the study by Aukema and Gauthier,[14] kidneys from rats with polycystic kidney disease given diets which contained SP compared with casein diets were less enlarged, had lower fluid content, smaller cyst volumes, less fibrosis, lower chemokine receptor 2 levels, and normalized serum creatinine levels. The authors concluded that SP compared with animal proteins might be renoprotective. Similarly, an also in a rodent model, Hwang et al.[12] investigated the effect of SP and egg white-based diets on early renal disease in the obese fa/fa Zucker rat. Soy protein feeding did not alter proteinuria but did result in 6% lower kidney weights and 16% smaller glomeruli. Finally, in a human experimental model, Azadbakht and Esmaillzadeh investigated the effects of SP on renal-related markers among 14 type 2 diabetic patients with nephropathy.[13] One diet contained 0.8 g/kg protein (70% animal and 30% vegetable proteins), and a similar diet contained the same amount of protein with 35% animal protein, 35% SP, and 30% other vegetable proteins for 7 weeks. The inclusion of SP reduced urinary urea nitrogen, proteinuria, blood sodium, and serum phosphorus compared with animal protein. However, and in agreement with our results urea levels were not significantly changed in SP versus animal protein consumption.[13] Similarly, other studies concluded that SP prevents inflammation and early nephropathic changes in rats with metabolic syndrome secondary to the attenuation of NF-kappaB activation.[11] Soy protein supplements also improved insulin sensitivity and markedly attenuated renal basement membrane changes in fructose diet-fed rats, and the authors concluded that these findings provide evidence to support the use of dietary SP in patients with diabetic kidney disease.[10,11]

No study before this has examined the renal effects of W[illegible] at least in a normal-protein concentration. However, our group previously found higher kidney weight, urinary volume, calciuria, and acidity with a parallel reduction in the urinary excretion of citrate following a high-whey protein diet.[7] These more acute effects could be explained by the higher protein amount [illegible]ad of by the source of protein, and we did not compare WP with SP diets.

In the study by Phisitkul et al.,[29] animal proteins promoted a progressive decline in the glomerular filtration rate (GFR) of the remnant kidney associated with metabolic acidosis and an endothelin-mediated increase in renal acidification. As has been described above, we observed lower urinary acidosis in the SP compared to the WP diet. These authors showed that rats on a casein diet had baseline metabolic acidosis and developed a progressive decline in the GFR after renal mass reduction. Dietary SP did not induce baseline metabolic acidosis and rats with remnant kidney on a SP diet had no decrease in their GFR.[29] On the other hand, some researchers did not observe beneficial effects when using SP instead of animal protein when attempting to attenuate proteinuria.[15,16] In agreement with these reports, we have not observed morphological advantages when using SP instead of WP. To note is that we have used WP, which is a protein with functional properties. In this regard, Haraguchi et al. compared the biological quality of a commercial WP (similar to the used in the present study) with casein protein.[2] Despite observing how W[illegible]

improved all the biological parameters studied, (as well as those of albumin, total protein, total cholesterol, and glucose concentrations), the authors did not observe hepatic or renal dysfunctions, as in agreement with our findings.

Limitations and strengths

The present study has several limitations: First, the present physiological results obtained in rodents must be confirmed in human subjects. Specifically, the effects upon rodents observed during 3 months cannot be directly extrapolated to the potential effects over decades in human subjects. Second, we used a single source of purified protein (whey or soy), which do not exactly reflect the human diet. Third, the measurement of GFR or some renal molecular markers, such as cystatin C, neutrophil gelatinase-associated lipocalin (NGAL), IL-18, kidney injury molecule-1 (KIM-1), or osteopontin would have been of interest for the interpretation of the present study results. However, the current study involved the use of a large number of rats and the thorough analysis of renal morphology, which had not been previously fully studied.

Overall, the increase in urinary acidity and calcium and the decrease of urinary citrate due to the WP diet consumption can increase the risk of kidney stone formation in the long term. However, no significant effects on kidney morphology were observed. Consequently, the inclusion of SP instead of WP or other animal protein sources in diets and supplements can be useful especially for subjects at higher risk of nephrolithiasis.

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AUTHOR DISCLOSURE STATEMENT

The authors declare they have no competing financial interest.

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AU1: ``Sánchez C, PhD'' removed from Author list. Not documented in metadata. Please confirm inclusion.
AU2: Changed lettered items to numbered items to maintain consistency with ``. the following eight morphological variables.''
AU3: Confusing wording. Changed to, ``No study before this has examined the renal effects of WP.''
AU4: Changed ``accused'' to ``acute.'' Please confirm that your meaning is still conveyed.
AU5: Sentence edited to improve clarity. Please read and confirm that your meaning is still conveyed.