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RESEARCH ARTICLE

Distinctive effects of plant protein sources on renal disease progression and associated cardiac hypertrophy in experimental kidney disease

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Scope: Dietary soy protein reduces renal disease progression in a number of renal diseases, suggesting that plant compared with animal proteins may be renoprotective. The inclusion of other plant protein sources could enhance compliance of intervention diets, but the effects of other plant protein sources are not known.

Methods and results: Weanling Han:SPRD-*cy* rats with experimental polycystic kidney disease were given hemp-, pea- and soy protein-based diets compared with the standard AIN 93G diet with casein as the protein source. Kidneys from diseased rats given diets which contained soy or hemp protein compared with casein-based diets were less enlarged, had lower fluid content, smaller cyst volumes, less fibrosis, lower chemokine receptor 2 (CCR2) levels and normalized serum creatinine levels. Soy and hemp protein diets also normalized heart size, which was enlarged in diseased compared with normal rats consuming casein. Kidneys from diseased rats given pea protein compared with casein were more enlarged and had higher fluid content and cyst volumes, despite growing better and having lower serum creatinine and renal chemokine receptor 2 levels, and similar levels of renal fibrosis.

Conclusion: Not all plant proteins are equally protective in experimental kidney disease and associated cardiac hypertrophy.

Keywords:
Cardiac hypertrophy / Hemp protein / Kidney disease / Pea protein / Soy protein

1 Introduction

As the incidence of chronic kidney disease (CKD) continues to increase [1], dietary interventions as adjunct treatments to reduce uremic symptoms and other effects of CKD are becoming more attractive. Although difficult to maintain, dietary protein restriction is one effective dietary strategy to reduce the signs and symptoms of CKD [2, 3]. However, not only are low-protein diets less palatable to many patients, but the risk of protein malnutrition also is a concern in the latter stages of CKD. As an alternative or modification of dietary protein restriction interventions, therefore, there is interest in determining whether protein source differentially affects kidney disease progression and its associated effects.

There is evidence that diets with plant proteins derived from a mixture of sources attenuate the decline in renal function in renal patients and in individuals with mild renal insufficiency [4–7]. However, the studies on the effects of plant proteins on renal disease are primarily restricted to the studies of soy protein, which has been suggested as a safe alternative or partial substitute for animal protein-based diets in CKD. In nephritic patients, a vegan soy diet decreases proteinuria [8], and in diabetics a soy-based diet reduces glomerular hyperfiltration, proteinuria or albuminuria [9–14]. These findings are supported by the studies in animal models of renal disease, including *db/db* mice with

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Abbreviations: CCR2, chemokine receptor 2; **CKD,** chronic kidney disease; **MCP-1,** monocyte chemoattractant protein-1; **PCNA,** proliferating cell nuclear antigen; **PKD,** polycystic kidney disease

type 2 diabetic nephropathy [11], obese rats [15, 16], 5/6 nephrectomized Wistar rats [17, 18], Wistar rats with chronic nephrotic syndrome [19], aging Fischer 344 rats [20] and *pcy* mice with adolescent nephronophthisis [21, 22].

The effects of plant protein sources such as pea protein and hemp protein have not been studied in relation to renal health. The hypothesis of the current study, therefore, was that dietary hemp and pea protein sources would be as renoprotective as soy protein, when compared with casein, the standard protein in the AIN 93 diet for laboratory rodents and commonly used in experimental renal disease. To test this hypothesis, the Han:SPRD-*cy* rat model of renal cyst disease was used [23, 24]. Polycystic kidney disease (PKD) encompasses a number of renal cyst disorders that are primarily genetically determined, occurring in one in 500–1000 individuals [25, 26]. Feeding soy protein to Han:SPRD-*cy* rats with renal cyst disease from weaning for as short a time period as 1 wk reduces renal fibrosis [27], and longer exposure (6–8 wk) of weanling rats to soy protein reduces cyst growth, renal inflammation and serum creatinine levels [28–30]. The pathology of renal disease in the Han:SPRD-*cy*, like other forms of chronic renal disease and injury, includes increases in interstitial inflammation, fibrosis and cell proliferation [31]. The aim of the current study therefore was to test the effects of hemp, pea and soy protein compared with casein in this model of experimental kidney disease.

2 Materials and methods

2.1 Animals and diets

Han:SPRD-*cy* rats (also known as PKD/Mhm [*cy*/+] rats) were derived from our breeding colony, which originated from the colony of Dr. Benjamin Cowley, University of Kansas Medical Center, Kansas City [23]. Prior to weaning, rats were genotyped and 12–14 diseased (cy/+) and four to five controls (+/+) were randomly assigned to diets based on either casein, hemp, pea or soy protein. Diet ingredients except pea and hemp protein were purchased from Dyets (Bethlehem, PA) or Harlan Teklad (Madison, WI). Pea protein derived from Canadian Yellow Peas was generously provided by Nutri-Pea (Portage la Prairie, Manitoba, Canada) and hemp protein from hemp seed by Sepallo Food Ingredients (Barrhead, Alberta, Canada). The latter contains less than 0.3% Δ^9-tetrahydrocannabinol and is sold as a safe supplement for human consumption. Pea and soy proteins underwent heat treatment to inhibit trypsin activity present in legumes. The casein-based diet was the standard AIN-93G laboratory rodent diet which has 17% casein protein by weight [32]. The amounts of protein, on an as is basis, in each source were tested [33], and found to be 79, 70, 78 and 79%, respectively, for casein, hemp, pea and soy protein. To make the other diets, equivalent amounts of protein from hemp, pea or soy protein were substituted for the casein. To correct for the differences in protein concentration, the amount of cornstarch was adjusted in order to produce isonitrogenous (17% protein) and isocaloric (3.9 kcal/g) diets. The protein sources were all delipidated and contained only trace amounts of lipid from these sources. To balance the amino acid concentrations of the other diets with the casein diet, the following amounts of crystalline amino acids were added to the diets: 0.18% cystine, 0.4% lysine and 0.09% methionine to the hemp protein diet; 0.22% cystine, 0.27% methionine, 0.04% threonine and 0.06% tryptophan to the pea protein diet; 0.15% cystine, 0.24% methionine and 0.02% threonine to the soy protein diet. For the details of amino acid compositions and levels of P, K and Na in the diets, see Supporting Information Table 1. All animal procedures were approved by the University of Manitoba Committee on Animal Care and were in accordance with the guidelines of the Canadian Council on Animal Care.

All rats were offered diet ad libitum and weighed weekly. One week prior to termination, a subset of rats ($n = 5$) were lightly anesthetized with isofluorane gas and blood pressure was determined by tail-cuff plethysmography. At the end of the feeding period, rats were anesthetized with a ketamine/zylazine mix and heart blood was collected and bladder urine and tissues were removed. The left kidney was sectioned into halves longitudinally across the hilum, and one half was fixed in 10% buffered formalin for morphological and histological analyses. The remaining tissues were frozen at −80°C until analysis.

2.2 Biochemical analysis

Serum creatinine was measured using a method developed by Heinegard and Tiderstrom and adapted for a micro-assay procedure using 96-well plates [34]. Urinary protein was measured using the Bradford method for total protein [35].

Right kidneys were lyophilized to determine the fluid and dry matter content. Free monocyte chemoattractant protein-1 (MCP-1) levels in the kidney and serum were determined by ELISA in duplicate according to the manufacturers' instructions (Biosource, Camarillo, CA). The frozen half of the left kidney was lyophilized and a representative sample was homogenized in 100 volumes of ice-cold homogenization buffer with protease inhibitors, centrifuged at 100 000 × *g* for 30 min at 4°C and the supernatant was taken for MCP-1 analysis. The buffer was identical to the buffer described below for immunoblotting. The amount of MCP-1 present in kidney tissue was expressed per milligram of renal protein, determined by the Bradford method [35].

2.3 Histology and image analysis

The formalin-fixed left kidney was embedded in paraffin, sectioned at 5 μm and was processed using our previously

described methods for histological and immunohistochemical analyses [29, 36, 37]. Transverse tissue sections (including cortex, medulla and papilla) were stained with Masson's trichrome stain which permits image analysis measurement using a standard incandescent microscope light source.

After being captured using a SPOT junior CCD camera by random stage movement through the sections, images were analyzed using the Image Pro software (Media Cybernetics, Silverspring, MD) as described previously [29, 36, 37]. Briefly, the portion of tissue section occupied by open area (cyst) or blue stain (fibrosis) was measured through a series of images (at 10 × objective) starting from a random field of tissue section until the entire kidney was measured. An average of 25 measurements from whole kidney cross-sections was collected. All measurements were carried out in a blinded fashion. Proportional cyst and fibrosis areas were multiplied by kidney weight to estimate volume as described previously [29, 36, 37]. Measurements of fibrosis were corrected to solid tissue areas of sections to avoid underestimation of these variables due to empty cystic areas in these sections.

2.4 Western immunoblotting

Western immunoblotting of proliferating cell nuclear antigen (PCNA) and chemokine receptor 2 (CCR2) was performed as described previously [38, 39], with minor changes. Briefly, 30 mg of lyophilized kidney was homogenized in 100 volumes of ice-cold homogenization buffer containing protease inhibitors, centrifuged at 100 000 × *g* for 30 min at 4°C and the supernatant was removed. Antibodies to PCNA (1:200) and CCR2 (1:10 000) were purchased from Santa Cruz Biotechnology, Santa Cruz, CA. Image analysis and quantification of immunoreactive bands were performed using the Fluorchem Q digital imaging system (Alpha Innotech, San Leandro, CA). A reference kidney homogenate was loaded on each gel in duplicate so that the results could be compared across gels. Dose–response curves were used to determine the linear range of response (14 μg of protein was used).

2.5 Statistical analyses

Data were analyzed using SAS version 9.2 (SAS, Cary, NC) by two-way (diet, genotype) ANOVA for all data except for data from histological analysis of cyst area and fibrosis which included only the analysis of diseased kidneys, in which case one-way (diet) ANOVA was used. ANOVA was followed by protected LS-means tests only if the main or interaction effects were significant in the two-way ANOVA, or if the overall one-way ANOVA *F*-test was significant. All reported *p*-values are two-sided and significance was set at $p < 0.05$. Normality of the data was tested using a plot of actual versus predicted residuals and the Shapiro–Wilk *W*-statistic on the residuals. Data were normalized by logarithmic transformation if necessary. All means are presented as means ± SEM. Pearson's correlation was used to test the correlations between MCP-1 and CCR2.

3 Results

3.1 Effects on growth and kidney pathology

At the end of the study control, normal rats given the four different types of dietary proteins had similar body weights, indicating that all had sufficient dietary protein to maintain normal growth (Table 1). In the diseased rats, those given the plant proteins had similar body weights as the controls, but the diseased rats given casein were ~30 g lighter than the controls. Kidney weights were three to four times greater in diseased rats compared with controls. The extent of kidney enlargement in diseased rats was greater in the rats given casein or pea protein, as compared with those given the hemp or soy protein diets. Compared with diseased kidneys from rats given the casein diet, kidney weights (relative to body weights) from rats given the hemp and soy protein diets were 17 and 21% lower, respectively, and kidneys from rats given the pea protein diet were 27% higher.

The lower mass of the diseased kidneys from rats given the hemp and soy protein compared with the casein diet was due to 14 and 18% less fluid since the dry matter content did not differ between these diets (Table 1). On the other hand, there was not only 31% more renal fluid in diseased kidneys from rats given pea protein compared with casein, but the dry matter content was also 20% higher. Renal fluid and dry matter content were not different in normal rats given the different diets. The results of cyst morphometry analysis were consistent with the fluid levels, as cyst volume was 33% higher in kidneys from pea protein compared with casein-fed rats, and 29 and 39% lower in kidneys from rats given the hemp and soy protein diets, respectively (Fig. 1, Supporting Information Fig. 1). The level of renal fibrosis also indicated that the hemp and soy protein diets were protective, with 51 and 35% less fibrosis, respectively, in diseased kidneys from these rats compared with the casein-fed rats. Renal fibrosis in the pea protein-fed rats was not different from the casein-fed rats (Fig. 2, Supporting Information Fig. 2).

3.2 Effects on kidney function and inflammation

At the end of the feeding period, serum creatinine levels were 84 and 78% higher in the diseased compared with control rats given the casein and pea protein diets, respectively (Table 2). On the other hand, serum creatinine levels in hemp and soy protein fed rats were not different in diseased compared with controls. Proteinuria was also 42%

Table 1. Effects of dietary protein source on body weights, tissue weights and blood pressure

	Normal (+/+) rats				Diseased (*cy*/+) rats			
	Casein	Hemp	Pea	Soy	Casein	Hemp	Pea	Soy
n	4	5	5	4	14	13	13	12
Initial body weight (g)	56 ± 3	54 ± 4	58 ± 1	58 ± 1	56 ± 2	57 ± 2	56 ± 1	56 ± 1
Final body weight (g)	392 ± 8^{bc}	393 ± 13^{bc}	397 ± 7^{bc}	381 ± 7^{b}	360 ± 5^{a}	405 ± 4^{c}	390 ± 4^{b}	395 ± 5^{bc}
Kidney weight (g)	3.09 ± 0.11^{a}	2.98 ± 0.09^{a}	3.06 ± 0.04^{a}	2.91 ± 0.06^{a}	9.51 ± 0.37^{c}	8.88 ± 0.45^{bc}	13.12 ± 0.43^{d}	8.25 ± 0.35^{b}
Kidney weight (g/100 g BW)	0.79 ± 0.01^{a}	0.76 ± 0.01^{a}	0.77 ± 0.01^{a}	0.76 ± 0.01^{a}	2.64 ± 0.09^{c}	2.19 ± 0.11^{b}	3.36 ± 0.11^{d}	2.09 ± 0.08^{b}
Kidney fluid content (g/100 g BW)[a)]	0.32 ± 0.01^{a}	0.30 ± 0.00^{a}	0.31 ± 0.01^{a}	0.31 ± 0.01^{a}	1.12 ± 0.05^{c}	0.96 ± 0.05^{b}	1.47 ± 0.04^{d}	0.92 ± 0.04^{b}
Kidney dry weight (g/kg BW)[a)]	0.88 ± 0.01^{a}	0.79 ± 0.01^{a}	0.84 ± 0.02^{a}	0.86 ± 0.00^{a}	1.40 ± 0.04^{b}	1.39 ± 0.03^{b}	1.68 ± 0.04^{c}	1.43 ± 0.04^{b}
Liver weight (g/100 g BW)	3.93 ± 0.12	3.86 ± 0.07	3.89 ± 0.07	3.73 ± 0.03	3.80 ± 0.05	3.72 ± 0.05	3.72 ± 0.05	3.70 ± 0.04
MAP (mmHg)[b)]	129 ± 6^{a}	115 ± 8^{a}	114 ± 7^{a}	120 ± 9^{a}	134 ± 6^{ab}	127 ± 6^{a}	154 ± 11^{b}	135 ± 4^{ab}

All parameters have a significant diet × genotype interaction except initial body weight (BW), liver weight and mean arterial pressure (MAP). There were no differences in initial body weight. There was only a significant genotype effect for liver weight ($p = 0.0155$) and MAP ($p = 0.0021$). Means with different superscript letters are significantly different ($p < 0.05$).

a) Data from right kidney.

b) $n = 5$ for each group for MAP measurement only.

Figure 1. Effects of dietary protein source on renal cyst volume in diseased rats. Han:SPRD-*cy* rats were given diets containing isonitrogenous levels of protein from casein, hemp, pea or soy protein for 8 wk. Kidney sections were stained with hematoxylin and eosin for cyst area measurement. Values with differing subscripts are significantly different ($p < 0.05$).

Figure 2. Effects of dietary protein source on renal fibrosis volume in diseased rats. Han:SPRD-*cy* rats were given diets containing isonitrogenous levels of protein from casein, hemp, pea or soy protein for 8 wk. Kidney sections were stained with Masson's trichrome stain for fibrosis measurement. Values with differing subscripts are significantly different ($p < 0.05$).

higher in diseased compared with control rats, but differences due to diet were not significant.

To assess renal cell proliferation and inflammation, PCNA and MCP-1/CCR2 were determined. Quantitative immunoblotting revealed a genotype effect of PCNA levels, with PCNA being twice as high in diseased compared with control kidneys. However, the differences due to dietary treatments were not significant (Table 2, Supporting Information Fig. 3). Diseased compared with control kidneys had 78% higher levels of CCR2 overall and in diseased kidneys CCR2 levels were higher in the casein-fed rats compared with rats given any of the other diets (Table 2, Supporting Information Fig. 4). Reflective of binding to CCR2, free MCP-1 was lower in diseased kidneys, with the kidneys from diseased rats given casein and pea protein diets having approximately one-third the level of free MCP-1 compared with controls on the same diets. Free MCP-1 levels were approximately twice as high in the diseased kidneys of rats given the hemp and soy protein diet compared with the casein or pea protein diet, but were still 39 and 33% lower than their respective controls on the same diets. The levels of serum MCP-1 were not affected by diet or genotype. Interestingly, the inverse relationship between unbound

Table 2. Effects of dietary protein source on serum, urine and kidney biochemistry

	Normal (+/+) rats				Diseased (*cy*/+) rats			
	Casein	Hemp	Pea	Soy	Casein	Hemp	Pea	Soy
n	4	5	5	4	14	13	13	12
Serum								
Creatinine (mg/dL)	0.50 ± 0.05^{ab}	0.40 ± 0.05^{a}	0.40 ± 0.04^{a}	0.62 ± 0.07^{bc}	0.92 ± 0.07^{d}	0.51 ± 0.06^{ab}	0.71 ± 0.05^{c}	0.52 ± 0.03^{ab}
MCP-1 (pg/mL)	140 ± 8	128 ± 9	105 ± 3	150 ± 13	152 ± 11	134 ± 15	137 ± 11	141 ± 9
Urine								
Protein (mg/g creatinine)	0.38 ± 0.06	0.24 ± 0.12	0.34 ± 0.13	0.29 ± 0.10	0.54 ± 0.07	0.43 ± 0.08	0.50 ± 0.06	0.33 ± 0.07
Kidney								
MCP-1 (pg/mg protein)	159 ± 18^{c}	174 ± 9^{c}	162 ± 18^{c}	144 ± 7^{c}	52 ± 5^{a}	85 ± 9^{b}	57 ± 6^{a}	97 ± 7^{b}
CCR2 (arbitrary units)	0.62 ± 0.13^{ab}	0.33 ± 0.05^{a}	0.68 ± 0.18^{ab}	0.44 ± 0.08^{ab}	1.22 ± 0.10^{c}	0.73 ± 0.16^{b}	0.85 ± 0.11^{b}	0.79 ± 0.16^{b}
PCNA (arbitrary units)	0.55 ± 0.07	0.52 ± 0.08	0.60 ± 0.03	0.53 ± 0.05	1.15 ± 0.06	1.07 ± 0.07	1.17 ± 0.04	1.06 ± 0.05

ANOVA effects are as follows: diet × genotype interactions for serum creatinine and kidney MCP-1; no effect of diet or genotype for serum MCP-1; a genotype effect for proteinuria ($p = 0.0165$) and PCNA ($p < 0.0001$); a diet ($p = 0.0378$) and genotype ($p = 0.0004$) effect for CCR2. Means with different superscript letters are significantly different ($p < 0.05$).

Figure 3. Correlation of renal CCR2 with MCP-1 in diseased rats. Han:SPRD-*cy* rats were given diets containing isonitrogenous levels of protein from casein, hemp, pea or soy protein for 8 wk.

MCP-1 and CCR2 levels was true for diseased kidneys (Fig. 3), but not in controls.

3.3 Effects on the liver and heart

Liver weights were lower in the diseased compared with control rats, but there were no diet effects on this tissue (Table 1). On the other hand, heart weights were higher in diseased rats given the casein and pea protein diets. As shown in Fig. 4, the hearts in diseased rats compared with controls were 11 and 15% higher in rats on the casein and pea protein diets, respectively. In comparison, no heart enlargement was observed in the diseased rats given either the hemp or the soy protein diets when compared with normal rats on the same diets. Blood pressure demonstrated a genotype effect, with diseased rats having 15% higher blood pressure ($p = 0.0021$) overall compared with controls. In the diseased rats, blood pressure was higher in rats given pea compared with hemp protein, with blood pressure being intermediate in rats given the casein and soy protein diets (Table 1).

4 Discussion

This study demonstrates that plant sources of protein do not have equal effects on the progression of kidney disease in experimental PKD. The previous studies have demonstrated that soy protein compared with animal protein (usually casein in experimental models) retards kidney disease progression. Hemp protein can now be included as a potential beneficial plant protein for kidney health, whereas pea protein should be regarded with caution in this regard until tested further. Further studies in other animal models, including nonhuman primates, are needed prior to extending these findings to the human population. In the Han:SPRD-*cy* rat, those given diets which contained either soy protein or hemp protein exhibited slower disease progression when compared with diseased

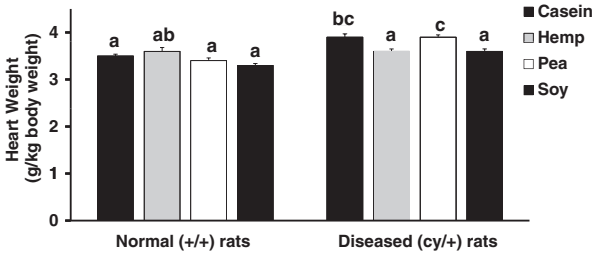

Figure 4. Effects of dietary protein source on heart weights. Han:SPRD-*cy* rats were given diets containing isonitrogenous levels of protein from casein, hemp, pea or soy protein for 8 wk. Values with differing subscripts are significantly different ($p < 0.05$).

rats given either the casein- or the pea protein-based diets. In contrast to these two plant protein sources, disease progression in the rats given the pea protein was similar, worse or not different when compared with rats given the casein diet, depending on the measure of disease progression.

The identification of hemp protein as potentially protective in CKD provides another plant protein besides soy protein which can be used to provide more optimum diets for patients with CKD. Restriction of dietary protein has long been used in the conservative treatment of CKD and its associated metabolic abnormalities [2, 3] although its effects in PKD are still controversial. In the Han:SPRD-*cy* rat model and in another model of renal cyst disease (the *pcy* mouse), low-protein diets reduce disease progression [21, 40, 41], whereas in humans the data are less clear [42, 43]. To reduce protein intake, plant proteins often are used in place of animal sources of protein, but an impediment to compliance is the restrictive nature of these diets. Having a greater variety of plant protein sources that are beneficial for the renal diet will therefore increase the ability of patients to comply with this dietary regimen.

The reasons for the varying effects of different plant protein sources are not clear. Amino acid composition differences between the protein sources in the current studies do not appear to be the main reason, as the hemp and soy proteins do not have any amino acid profiles in common that differentiate them from the casein and pea protein sources. For example, vasodilatory effects of arginine could play a role in renal hyperfiltration effects and arginine is reported to be renoprotective in some renal conditions but not others [44, 45]. Hemp protein has higher arginine than casein and pea protein, but the level of arginine in soy protein is not different from the latter two sources. Soy isoflavones and other phytochemicals have also been suggested as the renoprotective agents in plant foods [46, 47], but we have recently demonstrated that soy protein depleted of isoflavones by ethanol extraction also slows disease progression in this model of kidney disease [48].

The higher level of phosphorous has also been suggested as a mediator of the damaging effects of high-protein diets derived from animal foods since animal proteins are typically higher in this mineral [49]. However, the diet with the highest phosphorus levels (hemp diet) was one of the most protective diets, whereas the soy and casein diets had similar phosphorus levels but very different effects on disease progression. Similarly, sodium levels varied by as much as three-fold, yet the two diets with similarly high-sodium levels (pea and soy protein) had much different effects on disease progression and blood pressure. Hence, although the amino acid profile, phytochemical content or mineral levels may contribute to the individual effects of plant protein sources, not any of these appears to be a common factor in beneficial effects of hemp and soy protein compared with casein and pea protein in this study.

Infiltration and activation of white blood cells is an important modulator of inflammation and fibrosis in many diseases, including renal disease [50, 51]. The MCP-1/CCR2 axis is one of several components that are important in renal disease and inhibition of the CCR2 receptor reduces pathology in several types of renal injuries [52–56]. In the current study, it is not clear whether the lower level of CCR2 combined with the higher level of free MCP-1 in kidneys from rats given the hemp and soy protein compared with the casein-based diets is a direct or an indirect effect of disease amelioration in rats given these two diets. However, the fact that free MCP-1 levels in the blood are not altered by diet or genotype suggests that the effect is kidney specific.

Cardiovascular complications are often associated with CKD and are the most common cause of death in CKD patients [57–59]. In the current study, diseased rats given the casein and pea protein diets had enlarged hearts compared with controls, suggesting that cardiac hypertrophy had taken place. On the contrary, heart size was not different in diseased compared with controls given the hemp and soy protein diets. In addition to these data, the blood pressure data also suggest that the latter diets provide protection to cardiovascular health in the context of renal disease. Interestingly, the pea protein diet had higher levels of sodium than the casein diet, which may have contributed to the higher blood pressure in these rats. However, other factors are also likely to be involved, as the soy diet had also similar sodium content as the pea protein diet but not the elevated blood pressure.

In conclusion, individual plant protein sources have different effects on renal disease progression in the Han:SPRD-*cy* rat model of kidney disease. This may have implications for patients who substitute plant proteins for animal proteins in the conservative management of CKD.

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The authors have declared no conflict of interest.

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