Dietary Soy Protein Selectively Reduces Renal Prostanoids and Cyclooxygenases in Polycystic Kidney Disease

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Increasing evidence in human chronic kidney disease and in animal models indicates the potential utility of dietary soy protein in the treatment of this disorder. A model in which a beneficial soy protein effect has been consistently demonstrated is the Han:SPRD-*cy* rat model of polycystic kidney disease. Therefore, since dietary soy protein alters renal hemodynamics and prostanoid production, the effects of dietary soy protein on renal prostanoids and related rate-limiting enzymes were examined. Normal and diseased weanling rats were given diets containing casein or soy protein for 7 wk. At 10 wk of age, renal levels of thromboxane B_2 (TXB_2, stable metabolite of TXA_2), prostaglandin E_2 (PGE_2) and 6-keto $PGF_{1\alpha}$ (stable metabolite of PGI_2) and activities of cyclooxygenase 1 (COX1) and COX2 were elevated in diseased compared to normal kidneys. Soy protein feeding resulted in 49% lower in vitro steady-state levels of TXB_2, and 76% less 6-keto $PGF_{1\alpha}$ produced by COX1 activity in diseased kidneys, while not altering these parameters in normal kidneys. It also resulted in 47% less TXB_2 and 36% lower 6-keto $PGF_{1\alpha}$ produced by COX2 activity in diseased kidneys. The relative effect of soy protein feeding on COX2 activity was in the order of TXB_2 > 6-keto $PGF_{1\alpha}$ > PGE_2. Diseased kidneys had elevated protein and mRNA levels of cytosolic phospholipase A_2 ($cPLA_2$) and COX1 and lower levels of COX2. Dietary soy protein attenuated the protein levels of $cPLA_2$ in diseased kidneys, and reduced COX2 mRNA expression in both normal and diseased kidneys. Dietary soy protein therefore reduced the levels of specific renal prostanoids, $cPLA_2$ and COX enzymes in this model of polycystic kidney disease, a model in which soy protein has been demonstrated to reduce disease progression.

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Key words: prostaglandin; thromboxane; cyclooxygenase; soy protein; polycystic kidney disease

Introduction

Prostanoids in the kidney play an important role in maintaining normal renal function through hemodynamic effects, water and solute transport, and modulation of the renin-angiotensin system (1–3). Renal prostanoids have short half lives and normally are present at low levels, but production can rapidly increase in order to maintain normal renal homeostasis when faced with acute physiological challenges. In pathological conditions, both the types and concentrations of renal prostanoids are dramatically altered in association with inflammatory and proliferative processes in response to and as part of the renal injury (3–5). The diverse effects of prostanoids are due to specific local effects, as well as the variety of prostanoid types and receptors displaying stimulatory and inhibitory actions. For example, prostaglandin E_2 (PGE_2) has both vasodilatory and vasoconstrictory effects, depending on which of several EP (E-prostanoid) receptors are present, thus enabling it to prevent excessive variations in either direction. PGE_2 also has pro-inflammatory effects in many cells and thromboxane A_2 (TXA_2) has vasoconstrictory effects in the kidney. In addition to its hemodynamic effects, PGI_2 also can influence tubular transport processes and cell growth and death (1–3, 5). Because of these diverse effects, alterations in prostanoid production can be both detrimental and protective.

Prostanoid production is initiated and regulated in part by the rate of arachidonic acid release from cellular lipid membranes by phospholipase A_2 (PLA_2). Further control of the rate of prostanoid production is mediated by conversion of arachidonic acid to prostaglandin H_2 (PGH_2) by cyclooxygenase (COX)-1 or COX2. Subsequent conversion to prostanoids such as TXA_2 (which is rapidly broken down to

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TXB_2), PGI_2 (which is rapidly converted to 6-keto $PGF_{1\alpha}$), and PGE_2 is catalyzed by synthases specific to each prostanoid (5, 6).

The use of dietary soy protein in the treatment of kidney disease is gaining interest, with studies in humans and rodents demonstrating improvements in the progression of various renal diseases with this dietary intervention (7–15). Vegetable protein compared to meat protein has a moderating effect on renal hyperfiltration (16–18), and in a study of early nephropathy associated with obesity, dietary soy protein reduced the production of 6-keto $PGF_{1\alpha}$ (10). Inhibition of prostanoids by COX2 inhibitors appears to be beneficial in many forms of renal disease (19–26), but this is not always the case, as inhibition of prostanoids by NSAIDS has long been associated with nephrotoxicity (6, 27, 28). Furthermore, some selective COX2 inhibitors may increase cardiovascular risk (29, 30). Therefore, using a model of kidney disease which is responsive to dietary soy protein intervention and which has documented alterations in prostanoid production as well as COX and $cPLA_2$ enzymes (31–34), the objective of the current study was to determine whether dietary soy protein alters renal prostanoid levels and these enzymes in normal and diseased kidneys.

Methods

Animals and Diets. The tissues for analyses in this study were obtained from rats in a previously published study that demonstrated the efficacy of dietary soy protein in delaying renal disease progression in this rat model of polycystic kidney disease (34). Han:SPRD-*cy* rats [also known as PKD/Mhm(*cy*/+)] were obtained from our breeding colony that is derived from animals that were provided by B.D. Cowley (University of Kansas Medical Center, Kansas City, KS, USA). This model develops renal cyst disease and is an excellent model for exploring potential preventative and therapeutic strategies to slow renal disease progression and modify renal damage (35, 36). The experimental protocol was in accordance with Canadian Council of Animal Care guidelines and was approved by the Institutional Animal Care and Use Committee. Normal (+/+) and diseased (Cy/+) male rats from dams that had been given the AIN 93G diet were weaned at three weeks of age, randomly divided into 2 groups, and assigned to either a soy protein diet (20 g soy protein isolate/100 g diet) or a casein diet (20 g casein/100 g diet) for seven weeks. Diets were based on the AIN 93G laboratory rodent diet modified with corn oil as the lipid source and were identical in composition with the exception that casein was replaced by an equivalent amount of heat-treated soy protein isolate. At the end of the feeding period, animals were anesthetized with carbon dioxide and decapitated. The kidneys were removed, weighed, and immediately frozen in liquid nitrogen and stored at –80°C until analyses.

Renal Prostanoid Levels and COX Activity. Lyophilized right kidney from each rat was homogenized in Tyrodes buffer and Triton X-100 as described (32, 33). Aliquots of 180 μL were dispensed into chilled microcentrifuge tubes containing 20 μL Tyrodes buffer with vehicle (1% ethanol) or 0.1 μM SC560, a COX1 selective inhibitor (Cayman Chemical Company, Ann Arbor, MI), and incubated for another 30 min on ice. The following four conditions were employed: i) time 0 with no inhibitor, to determine endogenous kidney levels of prostanoids; ii) 60 min incubation at 37°C with no inhibitor, to determine in vitro steady-state levels of prostanoids; iii) 10 min at 37°C with no inhibitor, to determine total COX activity; and iv) 10 min incubation at 37°C with 0.1 μM SC560, to determine COX2 activity. COX1 activity was calculated by the difference between total COX (condition 3) and COX2 (condition 4) activities. The incubation times and concentrations of SC560 were determined from published time course studies which demonstrated that prostanoid production was linear for the first 10 min, steady-state levels of prostanoids were achieved by 30–40 min of incubation and that 0.1 μM SC560 inhibits >90% of COX1 activity under these conditions, while not inhibiting COX2 (33). After stopping the reaction with acetylsalicylic acid (ASA) (Sigma, St. Louis, Missouri), samples were analyzed for PGE_2 and the stable metabolites of TXA_2 (TXB_2) and PGI_2 (6-keto $PGF_{1\alpha}$) as described (32, 33).

Immunoblotting and Real-Time RT-PCR. Steady-state levels of $cPLA_2$, COX1 and COX2 proteins were determined by immunoblotting and quantification of immunoreactive bands by densitometry as described (31, 33). Levels of mRNA were determined by real-time RT-PCR using primers as described for COX1 and COX2 (33). For $cPLA_2$, the sense primer sequence was 5′-GACTTTTCTG-CAAGGCCAAG-3′ and the anti-sense primer was 5′-CTTCAATCCTTCCCGATCAA-3′.

Statistical Analyses. Results were analyzed by 2 × 2 ANOVA (genotype × diet) using SAS software (SAS Institute, Cary, North Carolina). Main effects and simple differences were considered significant at $P < 0.05$ and interactions at $P < 0.10$. If interactions were present, simple effects were determined by the Least Significant Differences test. Normality of the data was tested using the Shapiro-Wilk statistic ($W > 0.01$ for normality) and log transformed if it did not follow a normal distribution. If normality was not achieved after log transformation, differences were determined using non-parametric analysis (Wilcoxin rank sum test, $P < 0.10$). All data are expressed as means ± the standard error of the mean. Protein and mRNA levels of enzymes determined by immunoblotting and real-time PCR are expressed relative to the level observed in the kidneys from normal rats given casein based diets.

Results

Prostanoid levels were higher in the diseased compared to normal kidneys. Endogenous and in vitro steady-state levels of prostanoids were ~5 times and ~10 times higher for TXB_2, ~2 times higher for PGE_2, and ~4 times and ~5

Table 1. Effects of Dietary Soy Protein on Endogenous and In Vitro Steady-State Levels of Prostanoids in Normal and Diseased Kidneys of Han:SPRD-*cy* Rats

	Normal		Disease		
Genotype Diet	Casein	Soy protein	Casein	Soy protein	Effects
Endogenous level (ng/mg protein)					
TXB_2	0.26 ± 0.02[1]	0.26 ± 0.02	1.26 ± 0.07	1.12 ± 0.08	Genotype ($P < 0.0001$)
PGE_2	1.34 ± 0.34	1.17 ± 0.12	2.72 ± 0.54	2.00 ± 0.21	Genotype ($P = 0.0003$)
6-keto $PGF_{1\alpha}$	1.87 ± 0.25	1.88 ± 0.07	7.37 ± 0.68	6.71 ± 0.55	Genotype ($P < 0.0001$)
Total	3.72 ± 0.72	3.36 ± 0.22	11.40 ± 1.19	9.82 ± 0.77	Genotype ($P < 0.0001$)
Steady-state level (ng/mg protein)					
TXB_2	$0.46^{a,2}$ ± 0.06	0.47^{a} ± 0.02	6.12^{c} ± 0.92	3.11^{b} ± 0.49	Genotype × diet
PGE_2	2.66 ± 0.34	2.53 ± 0.21	6.75 ± 1.31	5.14 ± 0.51	Genotype ($P < 0.0001$)
6-keto $PGF_{1\alpha}$	8.21 ± 0.98	6.91 ± 0.37	41.89 ± 4.49	31.80 ± 4.13	Genotype ($P < 0.0001$)
Total	10.43 ± 1.07	9.91 ± 0.38	56.29 ± 5.98	40.05 ± 4.64	Genotype ($P < 0.0001$)

[1] Data is presented as mean ± SEM ($n = 8$–9).
[2] Where interactions are present, values in rows that contain different superscripts are significantly different.

times higher for 6-keto $PGF_{1\alpha}$ (Table 1) in diseased compared to normal kidneys. Feeding dietary soy protein did not alter baseline levels of prostanoids in normal kidneys. However, in diseased kidneys the effect of dietary soy protein on these parameters was specific for in vitro steady-state levels of TXB_2, which was 49% lower in diseased kidneys from rats fed soy protein compared to casein.

COX activity in normal kidneys was predominantly (>90%) due to the COX2 isoform (Table 2). Compared to normal rat kidneys, total COX activity was higher, depending on the prostanoid used to determine activity: for TXB_2, PGE_2 and 6-keto $PGF_{1\alpha}$, the elevations were 9 times, 2 times and 6 times, respectively. Higher COX1 activity in diseased kidneys resulted in elevated production of TXB_2 and 6-keto $PGF_{1\alpha}$ while higher COX2 activity resulted in elevated production of all three prostanoids when compared to normal kidneys.

Dietary soy protein intervention selectively reduced the elevated COX activities in diseased kidneys, while not altering these enzyme activities in normal kidneys (Table 2). COX1 activity was significantly lower (by 76%) in diseased kidneys from soy protein compared to casein fed rats when determined by production of the major prostanoid, namely 6-keto $PGF_{1\alpha}$. On the other hand, soy protein feeding did not alter TXB_2 and PGE_2 production by COX1. COX2, as well as total COX activity was 40–50% lower in kidneys from soy protein fed compared to casein fed rats when measured by TXB_2, 6-keto $PGF_{1\alpha}$ or total prostanoid production, but not when measured by PGE_2 production.

In order to determine whether the relative proportions of renal prostanoids were altered by disease, prostanoid

Table 2. Effects of Dietary Soy Protein on Prostanoids Produced by COX Activities in Normal and Diseased Kidneys of Han:SPRD-*cy* Rats

	Normal		Disease		
Genotype Diet	Casein	Soy protein	Casein	Soy protein	Effects
Total COX activity (ng/min/mg protein)					
TXB_2	0.04^{a} ± 0.01	0.03^{a} ± 0.00	0.38^{c} ± 0.05	0.20^{b} ± 0.04	Genotype × diet
PGE_2	0.13 ± 0.02	0.11 ± 0.01	0.28 ± 0.05	0.22 ± 0.02	Genotype ($P < 0.0001$)
6-keto $PGF_{1\alpha}$	$0.29^{a,2}$ ± 0.04	0.28^{a} ± 0.03	2.11^{c} ± 0.24	1.18^{b} ± 0.17	Genotype × diet
Total	$0.44^{a,2}$ ± 0.06	0.43^{a} ± 0.04	2.68^{c} ± 0.33	1.61^{b} ± 0.21	Genotype × diet
COX1 activity (ng/min/mg protein)					
TXB_2	0.00 ± 0.00	0.00 ± 0.00	0.04 ± 0.01	0.03 ± 0.01	Genotype ($P = 0.0004$)
PGE_2	0.01 ± 0.01	0.01 ± 0.00	0.03 ± 0.00	0.02 ± 0.02	—
6-keto $PGF_{1\alpha}$	$0.03^{a,2}$ ± 0.01	0.00^{a} ± 0.02	0.25^{b} ± 0.05	0.06^{a} ± 0.06	Genotype × diet
Total	0.04 ± 0.01	0.01 ± 0.01	0.34 ± 0.06	0.25 ± 0.13	Genotype ($P = 0.0010$)
COX2 activity (ng/min/mg protein)					
TXB_2	0.03^{a} ± 0.01	0.03^{a} ± 0.00	0.32^{c} ± 0.04	0.17^{b} ± 0.02	Genotype × diet
PGE_2	0.11 ± 0.02	0.10 ± 0.01	0.23 ± 0.03	0.21 ± 0.03	Genotype ($P < 0.0001$)
6-keto $PGF_{1\alpha}$	$0.26^{a,2}$ ± 0.03	0.28^{a} ± 0.02	1.61^{c} ± 0.19	1.04^{b} ± 0.13	Genotype × diet
Total	$0.39^{a,2}$ ± 0.05	0.42^{a} ± 0.03	2.21^{c} ± 0.27	1.42^{b} ± 0.16	Genotype × diet

[1] Data is presented as mean ± SEM ($n = 8$–9).
[2] Where interactions are present, values in rows that contain different superscripts are significantly different.

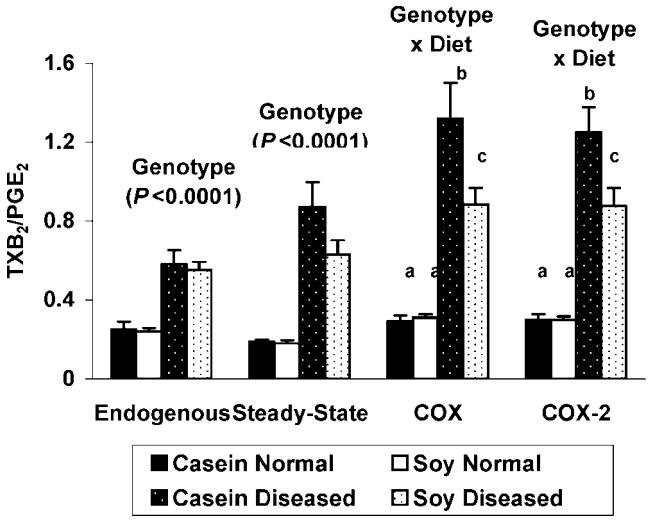

Figure 1. Renal TXB_2 to PGE_2 ratios in normal and diseased rats fed casein or soy protein. Ratios were determined under the following conditions: endogenous levels; 60 min incubations (in vitro steady-state levels); and produced by total COX and COX2 activities. Where interactions are present, values with different letters within each assay condition are significantly different ($P < 0.05$).

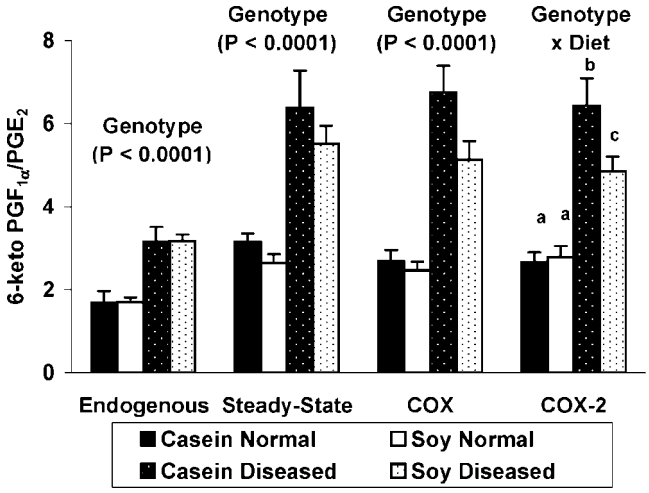

Figure 3. Renal 6-keto $PGF_{1\alpha}$ to PGE_2 ratios in normal and diseased rats fed casein or soy protein. Ratios were determined under the following conditions: endogenous levels; 60 min incubations (in vitro steady-state levels); and produced by total COX and COX2 activities. Where interactions are present, values with different letters within each assay condition are significantly different ($P < 0.05$).

ratios were calculated. The ratio of vasoconstrictory to vasoconstrictory prostanoids will reflect the overall effect of interventions and diseases on renal function and physiology. The higher ratios of TXB_2/PGE_2 (Fig. 1) and TXB_2/6-keto $PGF_{1\alpha}$ (Fig. 2) demonstrate that the change in TXB_2 is greater than either PGE_2 or 6-keto $PGF_{1\alpha}$ in diseased compared to normal kidneys. The higher ratios of 6-keto $PGF_{1\alpha}$ / PGE_2 (Fig. 3) demonstrate that the change in 6-keto $PGF_{1\alpha}$ is greater than PGE_2 in diseased compared to normal kidneys. Hence, the relative differences in prostanoid levels due to the presence of disease was in the order of $TXB_2 >$ 6-keto $PGF_{1\alpha} > PGE_2$.

Soy protein feeding did not alter the relative proportions of endogenous and in vitro steady-state levels of prostanoids. The ratios of COX2 activities determined by production of prostanoids, however, were altered by dietary soy protein intervention. The COX2 activity ratios for TXB_2/PGE_2, TXB_2/6-keto $PGF_{1\alpha}$ and 6-keto $PGF_{1\alpha}/PGE_2$ were ~20–30% lower in kidneys from soy fed compared to casein fed rats. Hence, the effect of soy protein on COX2 activity was in the order of $TXB_2 >$ 6-keto $PGF_{1\alpha} > PGE_2$ produced.

With respect to the key enzymes in prostanoid biosynthesis, mRNA levels of $cPLA_2$ and COX1 were higher and COX2 levels were lower in diseased kidneys (Figs. 4–6). These alterations were generally reflected in the protein levels, although only significantly for $cPLA_2$ in the particulate fraction and for COX2. Dietary soy protein significantly attenuated the higher steady-state protein levels

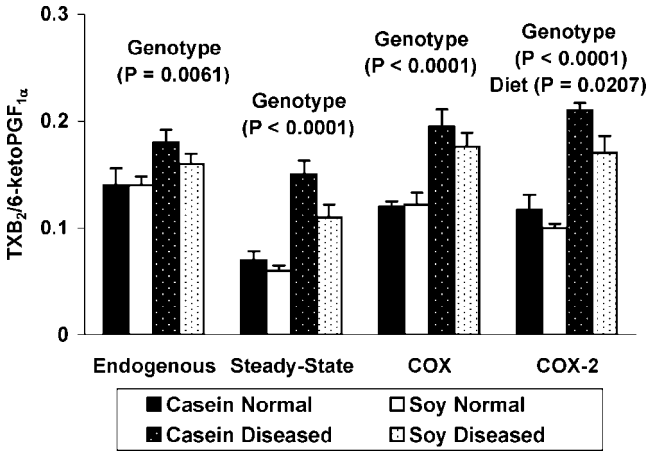

Figure 2. Renal TXB_2 to 6-keto $PGF_{1\alpha}$ ratios in normal and diseased rats fed casein or soy protein. Ratios were determined under the following conditions: endogenous levels; 60 min incubations (in vitro steady-state levels); and produced by total COX and COX2 activities.

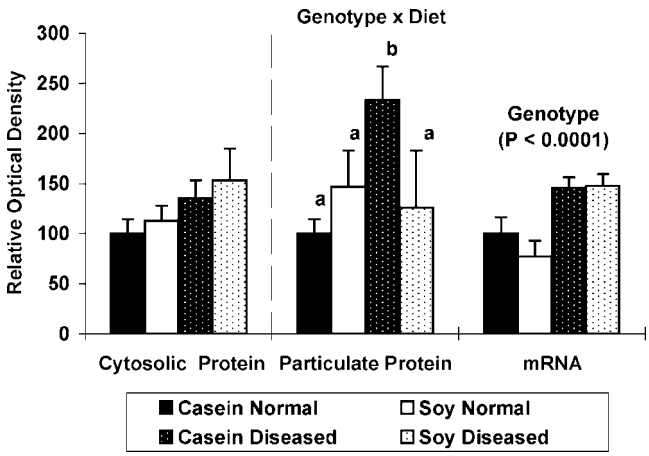

Figure 4. Renal $cPLA_2$ protein and mRNA expression in normal and diseased rats fed casein or soy protein. Where interactions are present, values with different letters within each assay condition are significantly different ($P < 0.05$).

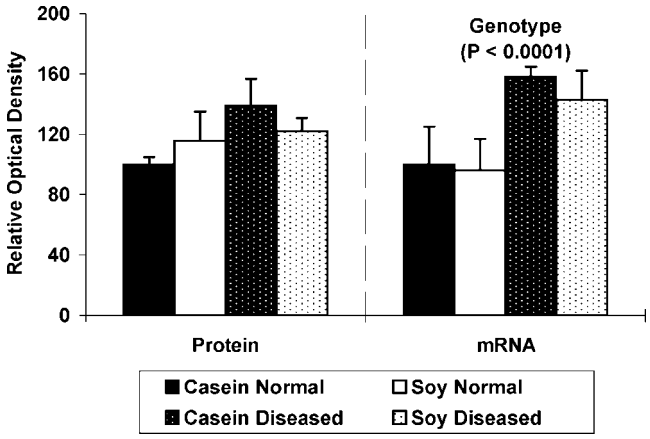

Figure 5. Renal COX1 protein and mRNA expression in normal and diseased rats fed casein or soy protein.

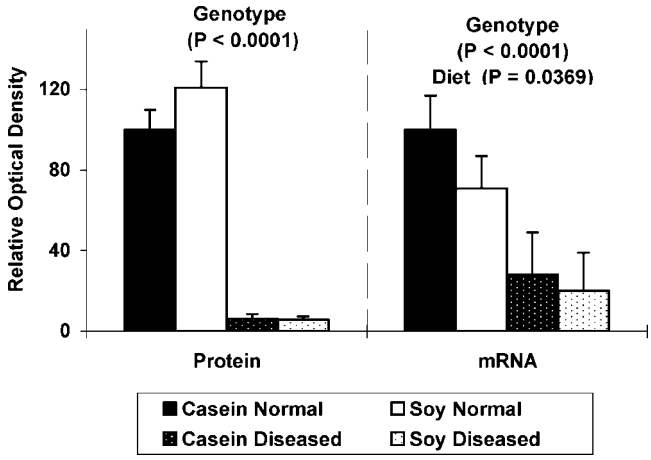

Figure 6. Renal COX2 protein and mRNA expression in normal and diseased rats fed casein or soy protein.

of $cPLA_2$ in the particulate fraction in the diseased kidneys (Fig. 4). With respect to dietary soy protein effects on gene expression, COX2 mRNA levels were affected by the dietary treatment, with soy protein feeding resulting in a further reduction in COX2 gene expression. This was an overall main effect, indicating that soy protein feeding reduced COX2 gene expression in both normal and diseased rats (Fig. 6).

Discussion

The results of this study demonstrate that both disease and dietary soy protein intervention have specific effects on renal prostanoid production in this model of kidney disease. The moderating effects of dietary soy protein are primarily exhibited in the diseased kidney and are specific for prostanoid type. Prostanoid production and activities and levels of related rate-limiting enzymes are altered in many forms of renal disease, with some prostanoids having protective effects, while others contribute to the pathology. Therefore, pharmacologic inhibitors of the specific prostanoids are actively being pursued as potential drugs to treat renal diseases. The results herein demonstrate that dietary soy protein, which slows disease progression in this model (34), has specific effects on prostanoids and related enzymes in the kidney.

In diseased compared to normal kidneys the relative difference in TXB_2 was the greatest among the three prostanoids examined. Levels of the vasoconstrictory TXA_2 are increased in conditions in which glomerular filtration rate (GFR) is reduced such as kidney transplant rejection, hepatorenal syndrome and systemic lupus erythematosus (6, 37). Selective inhibition of TXA_2 synthesis is associated with increased GFR, improved renal function and prevention of renal histologic damage in several models of renal disease (37–39). In the Han:SPRD-*cy* rat, COX-2 inhibition results in inhibition of prostaglandin production, including TXA_2, and also improves renal histology (32). In the current study, soy protein feeding was associated specifically with lower in vitro steady-state levels of TXB_2 in diseased animals. Further, dietary soy protein lowered COX2 activity, but not COX1 activity producing TXB_2, perhaps reflecting the fact that the major COX isoform activity producing TXB_2 in the diseased kidneys is COX2.

In contrast to thromboxanes, prostaglandins generally are vasodilators, with PGI_2 being the major prostaglandin synthesized in the kidney. Prostaglandins are predominantly protective mediators in the kidney, particularly when faced with physiological and pathological challenges that result in vasoconstriction. In polycystic kidneys, enlarged renal cysts compromise renal function, alter intrarenal circulation in renal ischemia areas and activate the renin-angiotensin system. Hence, the increased renal prostaglandin synthesis in diseased rats observed herein may counteract the vasoconstriction caused by angiotensin and TXA_2 and elevate the decreased GFR, and thus contribute to renal homeostasis. Dietary soy protein intervention maintained the increased endogenous and steady-state levels of prostaglandins in diseased kidneys, while renal steady-state TXB_2 levels were lower in soy protein fed diseased rats. Although soy protein feeding resulted in reduced renal prostaglandin production by COX2 activity, the lower renal COX2 activity associated with the TXB_2/PGE_2 and TXB_2/6-keto $PGF_{1\alpha}$ ratios in rats given soy protein compared to casein also indicates a shift in balance towards the vasodilatory prostanoids. Whether these effects were due directly to dietary soy protein or resulted indirectly from improvements in renal disease progression due to dietary soy protein remains to be determined. On the one hand, the lack of effect of soy protein on prostanoids in normal kidneys may suggest that the effect is indirect, while on the other hand the significant effect of soy protein on COX2 gene expression suggests otherwise. It appears that dietary effects may be manifested only when renal homeostasis is challenged and prostanoid production is altered. It should be noted that the effects are not due to differences in protein intake, as the protein intake was similar in both groups (34).

The soy protein induced changes in prostanoid production observed herein also may be related to the reduced renal epithelial cell proliferation and apoptosis, cyst growth and renal fibrosis observed when soy protein is given to these rats (11, 34). In cultured mesangial cells, PGE_2 and PGI_2 inhibit cell proliferation and extracellular matrix deposition, while TXA_2 stimulates cell proliferation and mesangial cell matrix production (40, 41).

Dietary soy protein attenuated the higher protein levels of particulate $cPLA_2$ in the diseased kidneys, suggesting that less active $cPLA_2$ is available for releasing available substrate for COX activity. Changes in the level of kidney COX1 protein and mRNA due to the disease state or in response to dietary soy protein were consistent with changes in the activity of this isoform. On the other hand, protein and mRNA levels of COX2 are lower in diseased rat kidneys, but its activity is elevated. This discordance between COX2 expression and activity in the kidney is consistent with our previous findings in this model (32, 33) but not in a model of obesity-associated nephropathy in which we used the same methodology (10). This discordance also has been reported in some, but not other kidney disorders (22, 42–44), and may reflect a feedback mechanism by prostanoids on COX2 activity in some conditions. In contrast to the disease effect of lowering COX2 expression while increasing its activity, soy protein feeding reduced both COX2 gene expression and activity. Thus, in the same kidneys, the mode of COX2 regulation by disease appears to be different than its regulation by dietary intervention; this interesting finding warrants further investigation.

Recent studies have found that COX2 inhibitors are associated with an increased risk of cardiovascular events and several of these drugs have been removed from the market (29, 30). In contrast, dietary soy protein may be a safe adjunct for treatment of polycystic kidney disease since it not only delays disease progression and preserves renal function, but it also decreases COX activities and selectively reduces renal prostanoids. The potential application of therapeutic interventions involving dietary soy protein in patients with polycystic kidney disease, without the untoward effects of pharmacologic inhibitors, remains to be determined.

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