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Journal of Nutritional Biochemistry 19 (2008) 255–262
Journal of Nutritional Biochemistry

Dietary soy protein reduces early renal disease progression and alters prostanoid production in obese *fa/fa* Zucker rats

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Received 23 December 2006; received in revised form 8 March 2007; accepted 27 March 2007

Abstract

With the rising incidence of obesity and the metabolic syndrome, obesity-associated nephropathy also has increased. One of the earliest pathologies in the development of this nephropathy is glomerular hyperfiltration and hypertrophy. Dietary soy protein (SP) ameliorates disease progression in several models of renal disease, and vegetable sources of protein, as compared to animal sources of protein, alter renal hemodynamics. Therefore, the effect of dietary SP on early renal disease and prostanoid production was examined in the obese *fa/fa* Zucker rat. Rats, 6 weeks of age, were given diets containing 17% protein from either SP or egg white (EW) for 8 weeks. Feed consumption and body and kidney weights were significantly greater in *fa/fa* rats as compared to lean rats. The *fa/fa* rats also had 139% more proteinuria and kidneys with 43% larger glomeruli. SP feeding did not alter body weights or proteinuria but did result in 6% lower kidney weights (g/100 g body weight) and 16% smaller glomeruli in *fa/fa* rats. Cyclooxygenase activity as determined by 6-keto prostaglandin $F_{1\alpha}$ (6-keto $PGF_{1\alpha}$) synthesis was lower in *fa/fa* rats given SP-based diets as compared to those given EW-based diets. Ratios of renal thromboxane (TX) B_2/6-keto $PGF_{1\alpha}$ and PGE_2/6-keto $PGF_{1\alpha}$ were higher, while TXB_2/PGE_2 levels were not different in rats given SP diets as compared to those given EW diets, also indicating that dietary SP reduced renal 6-keto $PGF_{1\alpha}$ levels. These findings suggest that attenuation of early glomerular hypertrophy in young obese *fa/fa* rats by dietary SP may be mediated by the lower levels of 6-keto $PGF_{1\alpha}$ since this would be expected to reduce glomerular hyperfiltration.

Keywords: Dietary soy protein; Obesity-associated nephropathy; Glomerular hypertrophy; Prostanoids; 6-keto prostaglandin $F_{1\alpha}$; Thromboxane B_2

1. Introduction

Obesity has become an international epidemic associated with increased risk of a number of disorders including metabolic and cardiovascular diseases such as diabetes and hypertension. Not only do both of these conditions increase the risk for renal disease, but obesity also independently increases the risk of obesity-associated nephropathy (OAN) [1]. Obesity increases glomerular filtration, apparently by causing dilatation of the afferent arteriole while not affecting the efferent arteriole [2]. This leads to increased glomerular capillary pressure causing hyperfiltration, glomerular enlargement, thickening of the glomerular basement membrane, mesangial expansion and proteinuria. In the long term, these changes result in fibrosis in both the glomerulus and in the tubulointerstitial tissue [1–3].

Prostanoids are known to be important regulators of renal hemodynamics and may be involved in early kidney changes that ultimately result in OAN. In addition to regulating renal blood flow, renin secretion and GFR, prostaglandin (PG) I_2 also is involved in the regulation of tubular transport processes and cell growth and death in the kidney. PGE_2 generally has been considered to have vasodilatory effects that increase GFR and to regulate tubular transport, but it can also have vasoconstrictory effects. This ability to modulate renal vascular tone and tubular transport by binding to

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doi:10.1016/j.jnutbio.2007.03.001

different receptors (designated E-prostanoid) is thought to allow PGE_2 to prevent extreme physiologic alterations in either direction. Thromboxane A_2 (TXA_2) has a vasoconstrictory effect that decreases GFR [4–6].

The incidence of obesity in children is rising, with 17% of U.S. children and 10% of children worldwide now being considered obese [7,8]. Individuals who have a body mass index ≥25 kg/m^2 at age 20 have a threefold increased incidence of kidney failure [9], illustrating the impact of obesity in children on later renal health. Therefore, early detection and treatment at the very beginning stages of renal disease associated with obesity are important.

Dietary protein level affects the progression of renal diseases, in part via alterations in prostanoid production [10–13]. The source of dietary protein also can affect disease progression. This has been observed with dietary soy protein (SP), which has been shown to reduce disease progression in a number of models of renal disease [14–18]. Renal hemodynamics is altered by soy or vegetable protein compared to animal protein sources, possibly by altering prostanoid production [19–25]. Therefore, the effect of dietary SP on early renal disease progression and prostanoid production in OAN was examined in the obese *fa/fa* Zucker rat.

2. Materials and methods

2.1. Animals and diets

Twenty lean and 20 male obese *fa/fa* Zucker rats were purchased from Harlan (Indianapolis, IN) at 5 weeks of age, acclimated for 1 week and then randomly divided into four groups in a 2×2 design. Ten lean and 10 *fa/fa* rats were given diets containing equal amounts of protein in the form of egg white (EW) or SP as the animal or vegetable protein diets, respectively (Table 1). Body weights were recorded weekly, and feed intake was recorded throughout the study. Urine was collected in metabolic cages 1 week prior to the end of the feeding period. After 8 weeks, the rats were fasted overnight (12 h) and killed the following morning by CO_2 anesthesia followed by decapitation. All procedures were approved by the University of Manitoba Animal Care Committee and adhered to the guidelines of the Canadian Council on Animal Care. Trunk blood was collected for serum analysis. Serum and urine creatinine were measured colorimetrically using commercial kits (Sigma-Aldrich, Oakville, Canada) and, along with urine volume, were used to calculate creatinine clearance. Urine protein was determined using the Bradford protein assay method with bovine serum albumin as a standard [26].

Table 1
Composition of experimental diets

Ingredients[a]	Diet (g/kg)	
	EW	SP
Cornstarch	363.0	383.8
Maltodextrin	132.0	132.0
Sucrose	100.0	100.0
EW[b]	212.5	–
SP[c]	–	197.7
Cellulose	50.0	50.0
Mineral mix (AIN 93G)	35.0	35.0
Vitamin mix (AIN 93VX)	10.0	10.0
Choline	2.5	2.5
Biotin mix[d]	10.0	10.0
tert-Butylhydroquinone	0.014	0.014
Soy oil	85.0	79.0

[a] Ingredients were supplied by Harlan Teklad (Madison, WI), except for *tert*-butylhydroquinone (Aldrich Chemical Company, Milwaukee, WI) and cornstarch (Best Foods, Etobicoke, ON, Canada).
[b] EW contains 80% protein.
[c] SP contains 86% protein and 3 g oil/100 g isolate. Therefore, less soy oil was added to the SP diet.
[d] Biotin mix=200 mg biotin/kg cornstarch.

2.2. Glomerular size

The right kidney was sliced longitudinally, and half of the kidney was placed in 10% phosphate-buffered formalin prior to embedding in paraffin and sectioning at 5 μm. Kidney sections were placed in xylene to remove the paraffin and stained with hematoxylin and eosin. Using a camera (Spot Diagnostic Instruments, Inc., Sterling Heights, MI) mounted on an Olympus BX60 microscope (Olympus Optical Company, Hamburg, Germany), slides were analyzed using the ×20 objective. Using standard stereological techniques developed by Weibel [27], we determined mean glomerular volume by measuring the maximum glomerular diameter of 30 randomly chosen glomeruli per kidney. The radius was then used to estimate glomerular volume using the following formula: mean glomerular volume=$\beta/K(\pi r^2)^{3/2}$. The value of the coefficients β and K is based on assumptions made for the maximum diameter of spheres (β=1.38) and the distribution bias of section location (K=1.10) [28]. The observer was blinded to treatments for all analyses.

2.3. Prostanoid production and cyclooxygenase activity

Production of prostanoids and determination of cyclooxygenase (COX) isoform activities were analyzed as described [29]. Briefly, lyophilized left kidneys from each rat were homogenized in fresh Tyrode's buffer and incubated under the following conditions: (a) 0 min with no inhibitor for determination of endogenous levels of prostanoid production, (b) 60 min incubation at 37°C with no inhibitor for determination of steady state in vitro prostanoid production, (c) 10 min incubation at 37°C with no inhibitor for determination of total COX activity, (d) 10 min incubation at 37°C with 0.1 μM SC560 (Cayman, Ann Arbor, MI) for determination of COX-2 activity. COX-1 activity was determined by the difference between total COX (Condition c) and COX-2 (Condition d) activities.

The incubation conditions were determined from previous time-course studies that demonstrated that the

production of prostanoids is linear for the first 10 min of incubation, that steady-state levels of prostanoids in vitro are achieved by 30–40 min of incubation and that a concentration of 0.1 μM SC560 inhibits more than 90% of COX-1 activity but does not inhibit COX-2 at all [29]. Reactions were stopped by adding cold acetylsalicylic acid to the sample incubation, vortexing and centrifuging at 12,000×*g* at 4°C for 5 min. The supernatant was removed for determination of PGE_2, 6-keto PGF_{1a} (stable metabolite of PGI_2) and TXB_2 (stable metabolite of TXA_2), using commercial enzyme immunoassay kits (Cayman).

2.4. Immunoblotting

Steady-state protein levels of cytosolic phospholipase A_2 ($cPLA_2$), COX-1 and COX-2 were determined as described [30]. Half of the left kidney, frozen and stored at −80°C at termination, was lyophilized, and 20 mg was homogenized in 100 volumes of ice-cold homogenization buffer (50 mM Tris–HCl, pH 7.2; 250 mM sucrose; 2 mM EDTA; 1 mM EGTA; 50 μM NaF; 100 μM Na orthovanadate; 1 μg/ml soybean trypsin inhibitor; 144 μM 4-benzene-sulfonyl fluoride; 25 μg/ml aprotinin; 25 μg/ml leupeptin; 25 μg/ml pepstatin; and 10 mM β-mercaptoethanol). Homogenates were centrifuged at 100,000×*g* for 30 min at 4°C, and the supernatant (cytosolic fraction) was removed. The remaining pellet was resuspended in 20 volumes of homogenization buffer containing 1% Triton X-100 (Sigma, St. Louis, MO), incubated on ice for 10 min and then centrifuged at 100,000×*g* for 30 min at 4°C. The resulting supernatant (particulate fraction) was collected. Cytosolic and particulate fractions were subjected to SDS-PAGE as described [30]. After SDS-PAGE, proteins were transferred to PVDF, blocked and incubated with primary antibodies to $cPLA_2$ (Santa Cruz Biotechnology Inc., Santa Cruz, CA), COX-1 and COX-2 (Cayman). Following this, blots were incubated for 1 h at room temperature with a peroxidase-conjugated secondary antibody. Immunoblots were incubated with ChemiGlow (Alpha Innotech Corporation, San Leandro, CA), and image analysis and quantification of immunoreactive bands were performed using the Fluorchem FC digital imaging system (Alpha Innotech Corporation).

2.5. Quantitative RT-PCR

Total RNA for real-time RT-PCR was extracted from 20 mg of lyophilized kidney. Primers for RT-PCR were chosen using Primer 3 software [31]. Oligonucleotide sequences were as follows: for $cPLA_2$: sense, 5′-GACTTTTCTGCAAGGCCAAG-3′; antisense, 5′-CTTCAATCCTTCCCGATCAA-3′; for COX-1: sense, 5′-GCCTCGACCACTACCAATGT-3′; antisense, 5′-AGGTGGCATTCACAAACTCC-3′; for COX-2: sense, 5′-TACCCGGACTGGATTCTACG-3′; antisense, 5′-TTCGAAGGAAGGGAATGTTG-3′. Real-time RT-PCR reactions were performed with SYBR green on a Cepheid Smart Cycler II (Cepheid, Sunnyvale, CA) sequence detection system. Products were verified by melting curve analysis. Relative amounts of mRNA were determined by comparing cycle threshold (CT) values for equal amounts of amplified RNA and calculated using the formula $2^{\Delta CT}$ as described [29].

2.6. Statistical analysis

Data were assessed for normality using the Shapiro–Wilk statistic and for homogeneity of variance using Levene's Test for Homogeneity of Variance. Data that were not normally distributed were log transformed. A two-way analysis of variance was used to analyze main effects and interactions. If interactions were present ($P<.05$) or if the main effects were marginal ($.05<P<.10$), least significant difference (LSD) tests were performed to test for differences between groups. Data were analyzed using SAS (SAS Institute, version 9.1, Cary, NC).

3. Results

3.1. SP-based diets as compared to EW-based diets result in less renal and glomerular hypertrophy in fa/fa rats

At the end of the feeding period, *fa/fa* rats had consumed approximately 50% more diet and were significantly larger than lean rats (Table 2). Rats grew equally well on both diets, despite the slightly lower feed intake (7%) in *fa/fa* rats given the SP-based diet as compared to those given the EW-based diet. The *fa/fa* rats had larger kidneys than lean rats at the end of the study on a weight basis, but relative to body weights, the kidney weights in *fa/fa* rats were smaller than in lean rats.

Table 2
Effects of dietary SP on feed intake, body and kidney weights, proteinuria and creatinine clearance in obese *fa/fa* Zucker rats

Parameters	Lean EW	Lean SP	*fa/fa* EW	*fa/fa* SP	Effects
Total feed intake (g)	1022±24[c]	1101±15[c]	1666±51[a]	1551±36[b]	Interaction
Body weight (g)	328±5	349±9	561±13	568±8	Genotype
Kidney weight (g)	2.33±0.08	2.34±0.05	3.03±0.09	2.83±0.05	Genotype
Kidney weight (g/100 g body weight)	0.71±0.02	0.67±0.01	0.54±0.01	0.50±0.01	Diet, genotype
Urinary protein (mg)/Creatinine (mg)	1.37±0.24	1.77±0.21	3.01±0.40	4.48±0.69	Genotype
Serum creatinine (μmol/L)	38.9±2.7	43.3±1.8	34.5±3.5	38.0±1.8	No effect
Creatinine clearance(ml/min)	1.47±0.16	1.63±0.14	1.41±0.22	1.17±0.10	No effect

Values are expressed as mean±S.E.M. (n=9–10/group).
Values in a row with different superscripts are significantly different ($P<.05$).

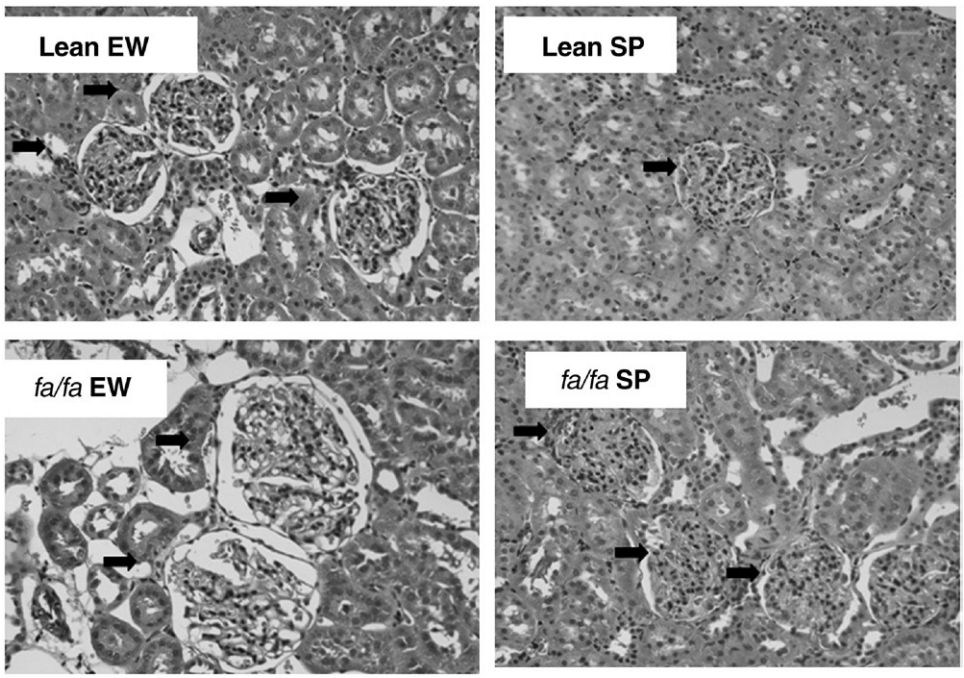

Fig. 1. Kidney cross sections stained with hematoxylin and eosin from lean and *fa/fa* rats given either EW or SP diets. Arrows point to glomeruli.

There was a significant effect of diet with rats given SP diets as compared to those given EW diets; that is, the former group had 6% lower kidney weights relative to body weights.

One of the earliest structural changes in OAN as a result of increased glomerular pressure and filtration is an increase in glomerular size. Glomerular size was elevated in *fa/fa* rats as indicated by the 43% larger mean glomerular volumes in these rats compared to lean rats (Figs. 1 and 2). This increased size was mitigated by SP feeding, which resulted in 16% lower mean glomerular volumes compared to the rats given EW-based diets. These early kidney changes in glomerular size were reflected in the higher proteinuria in the *fa/fa* rats, indicating the initial stages of compromised kidney function. However, serum creatinine and creatinine clearance were not yet altered in this early stage of OAN. Furthermore, dietary protein source also did not influence any of these markers of renal function.

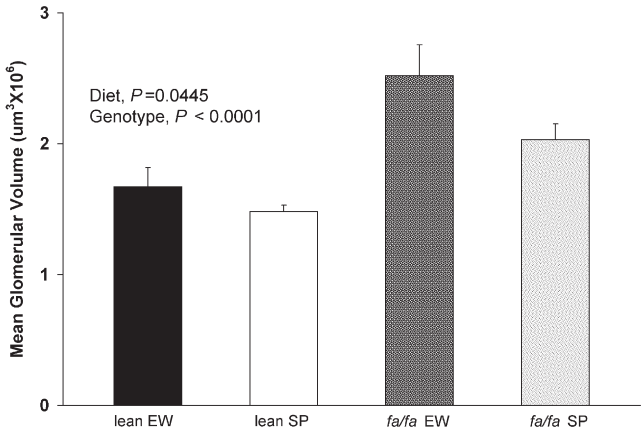

Fig. 2. Glomerular size in lean and *fa/fa* rats given either EW or SP diets. Values are mean±S.E.M. (n=8–10/group).

3.2. SP feeding results in less 6-keto $PGF_{1\alpha}$ generated by COX in fa/fa rats

To determine the potential role of prostanoids in the attenuation of early renal disease by SP, we determined prostanoid levels and COX activities. The prostanoid present at the highest level in kidneys from both lean and *fa/fa* rats was 6-keto $PGF_{1\alpha}$, which contributed to more than half of the total endogenous prostanoids or those produced in kidney homogenates in vitro (Table 3). TXB_2 levels were the lowest, contributing to only 1% of endogenous prostanoids

Table 3
Effects of dietary SP on endogenous and steady-state in vitro prostanoid levels in kidneys of *fa/fa* Zucker rats

	Lean EW	Lean SP	*fa/fa* EW	*fa/fa* SP	Effects
Endogenous (ng/mg protein)					
TXB_2	0.15±0.02	0.16±0.02	0.14±0.02	0.14±0.02	No effect
PGE_2	1.07±0.20	1.07±0.19	0.58±0.08	0.71±0.10	Genotype
6-keto $PGF_{1\alpha}$	1.86±0.26	1.77±0.28	1.77±0.28	1.18±0.14	No effect
Steady state (ng/mg protein)					
TXB_2	0.16±0.02	0.18±0.03	0.24±0.04	0.21±0.03	Genotype
PGE_2	2.97±0.34	3.31±0.54	2.72±0.45	2.94±0.40	No effect
6-keto $PGF_{1\alpha}$	13.97±1.65	15.37±2.96	20.86±3.54	13.98±1.70	No effect

Values are expressed as mean±S.E.M. (n=9–10/group).

and ~6% of those synthesized in vitro, while PGE_2 levels were intermediate. Diet had no effect on these prostanoid levels; however, *fa/fa* rats had reduced endogenous PGE_2 and elevated steady-state levels of TXB_2, as compared to lean rats.

Renal COX activity, in both lean and *fa/fa* rat kidneys, was due primarily to the COX-2 isoform as can be seen in the similar levels of total COX and COX-2 activities (Table 4). Renal prostanoid levels due to COX activities were generally not altered by diet or genotype, with the exception of renal 6-keto $PGF_{1\alpha}$ levels produced by COX activity, which were elevated in *fa/fa* rats given EW-based diets. The SP-based diet normalized this alteration, as renal 6-keto $PGF_{1\alpha}$ levels in rats on SP-based diets were similar to those in the lean rats.

3.3. Diet and genotype alter renal prostanoid ratios

In order to probe further the possible effects of dietary SP on prostanoids, we calculated and compared ratios to determine whether there were changes in the amounts of individual prostanoids relative to others. Changes in the vasodilatory prostaglandins relative to the vasoconstrictory thromboxanes could affect renovascular tone and consequently influence glomerular filtration. Renal TXB_2/6-keto $PGF_{1\alpha}$ ratios were significantly higher in kidneys from rats given SP diets as compared to those given EW diets, indicating that dietary SP reduced either 6-keto $PGF_{1\alpha}$ and/or increased TXB_2 levels (Fig. 3). Similarly, PGE_2/6-keto $PGF_{1\alpha}$ ratios were generally higher in kidneys from rats given SP-based diets (particularly in *fa/fa* rats) as compared to those given the EW-based diets, also indicating that dietary SP reduced renal 6-keto $PGF_{1\alpha}$ and/or increased PGE_2 levels (Fig. 4). Renal TXB_2/PGE_2 ratios, on the other hand, were altered by genotype but not by diet (Fig. 5). It is not likely that the levels of the latter (TXB_2, PGE_2) prostanoids are altered by diet, as the individual prostanoid measurements indicate no differences

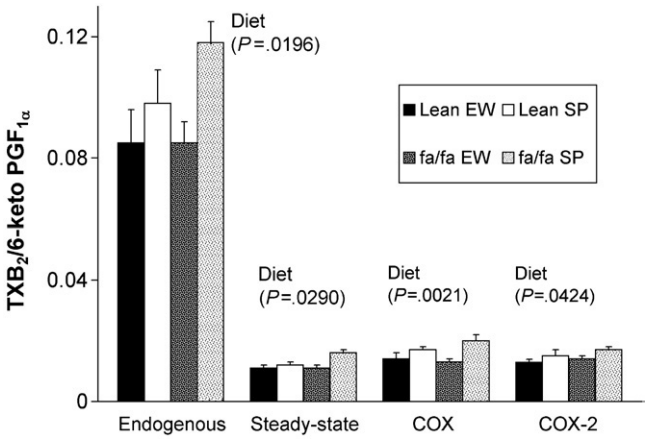

Fig. 3. TXB_2/6-keto $PGF_{1\alpha}$ ratios in kidneys of lean and *fa/fa* rats given either EW or SP diets (n=8–10/group).

in levels between dietary treatments. Taken together, these results indicate that renal 6-keto $PGF_{1\alpha}$ levels are reduced in rats given SP diets while PGE_2 and TXB_2 levels are not altered.

3.4. COX-2 protein and mRNA levels are higher in fa/fa rat kidneys as compared to lean rat kidneys

With respect to the rate-limiting enzymes responsible for the production of prostanoids, the COX isoforms were present only in the particulate fraction, while $cPLA_2$ was present in both the cytosolic and particulate fractions. Neither $cPLA_2$ nor COX-1 levels were altered by genotype or diet (Figs. 6 and 7). COX-2 protein and mRNA levels, however, were elevated in kidneys from *fa/fa* rats as compared to lean rats, as determined by Western immunoblotting (Fig. 6) and real-time RT-PCR (Fig. 7), respectively. Dietary SP did not alter the amount of COX-2 protein, but

Table 4
Effects of dietary SP on total COX and COX-2 activities in kidneys of *fa/fa* Zucker rats

	Lean EW	Lean SP	*fa/fa* EW	*fa/fa* SP	Effects
Total COX (ng/min/mg protein)					
TXB_2	0.01±0.00	0.01±0.00	0.02±0.00	0.02±0.00	No effect
PGE_2	0.20±0.02	0.25±0.05	0.22±0.04	0.24±0.04	No effect
6-keto $PGF_{1\alpha}$	0.86 ± 0.14^{ac}	0.83 ± 0.17^{bc}	1.24 ± 0.21^{a}	0.77 ± 0.10^{bc}	Diet *
COX-2 (ng/min/mg protein)					
TXB_2	0.01±0.00	0.01±0.00	0.02±0.00	0.02±0.00	No effect
PGE_2	0.19±0.02	0.24±0.04	0.22±0.04	0.21±0.03	No effect
6-keto $PGF_{1\alpha}$	0.86±0.13	0.88±0.15	1.17±0.16	0.88±0.11	No effect

Values are expressed as mean±S.E.M. (n=9–10/group).
Values in a row with different superscripts are significantly different (P<05).
* Marginal diet effect (P=.0628). Therefore, LSD tests were performed to test for differences between groups.

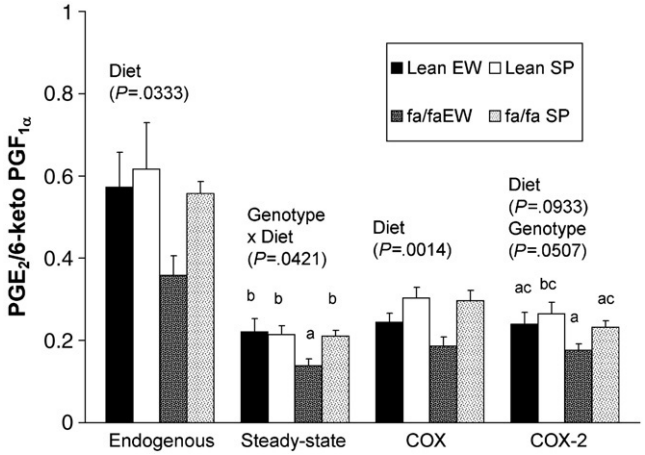

Fig. 4. PGE_2/6-keto $PGF_{1\alpha}$ ratios in kidneys of lean and *fa/fa* rats given either EW or SP diets. Means within each experimental condition with differing letters are significantly different (n=8–10/group).

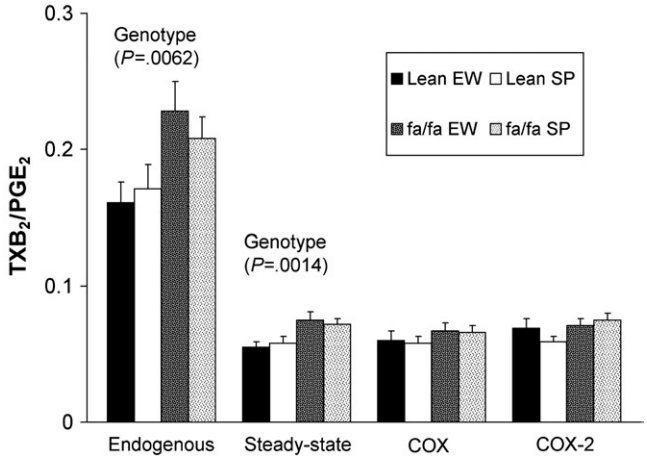

Fig. 5. TXB_2/PGE_2 ratios in kidneys of lean and *fa/fa* rats given either EW or SP diets (n=8–10/group).

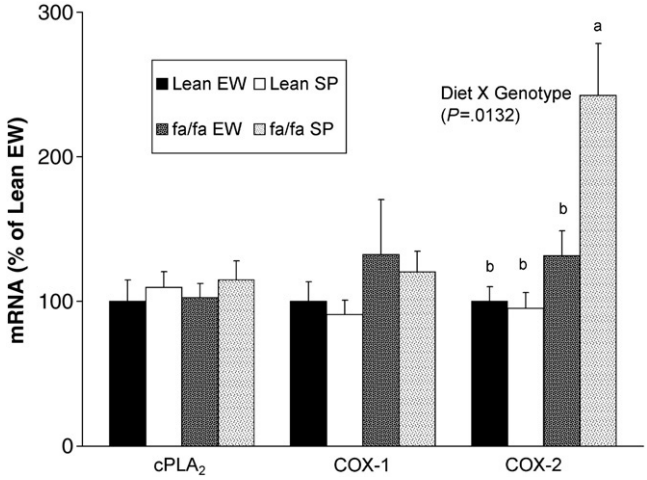

Fig. 7. $cPLA_2$, COX-1 and COX-2 mRNA levels in lean and *fa/fa* rats given EW and SP diets. Data (n=8–10/group) are expressed relative to the mean value obtained in kidneys obtained from the lean EW group. Means for COX-2 with differing letters are significantly different.

gene levels were altered by diet in *fa/fa* rats as compared to lean rats.

4. Discussion

This study demonstrates the beneficial effect of dietary SP intervention on early renal disease progression associated with obesity in the obese *fa/fa* Zucker rat. The SP diet provides protection against the initiation of glomerular injury, as demonstrated by reduced glomerular size in *fa/fa* rats given SP diets as compared to those given EW diets. Glomerular hypertrophy is one of the earliest signs of kidney disease and is an independent risk factor for the progression of renal disease. The beneficial effect of SP on glomerular size observed herein in 14-week-old rats is consistent with a previous observation in 24-week-old obese *fa/fa* Zucker rats [18]. Because of the age difference in rats between the previous study and the present study, the kidney disease had progressed further in the previous study, as evidenced by the significantly elevated proteinuria in the older rats. The authors of the previous study and others also noted that the SP diet resulted in reduced proteinuria as the disease progressed, with the difference becoming greater as *fa/fa* rats become older [18,32]. Hence, the lack of significant effects on renal function in the current study is likely due to the fact that this study examined dietary effects in the very early stages of renal disease in these rats. However, the current study does demonstrate that dietary SP has beneficial renal effects in the earliest development of glomerular hypertrophy in OAN, as this study was initiated shortly after the obese phenotype becomes apparent.

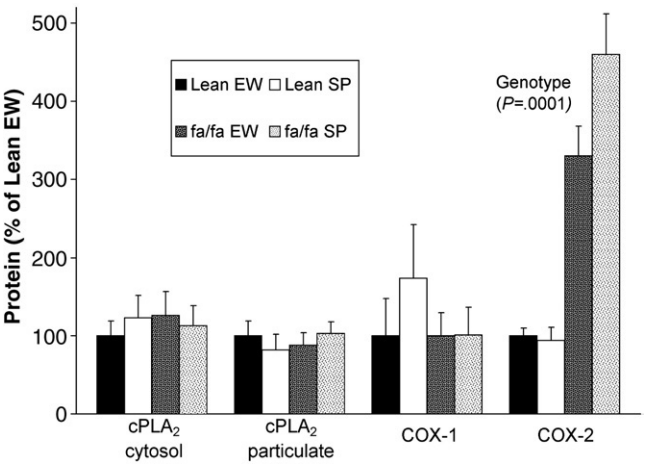

Fig. 6. Protein levels of $cPLA_2$ in the cytosolic and particulate renal cell fractions and COX-1 and COX-2 (present only in the particulate fraction) in lean and *fa/fa* rats given EW and SP diets. Data (n=8–10/group) are expressed relative to the mean value obtained in kidneys obtained from the lean EW group.

The current study also used EW as the source of animal protein, whereas previous studies [18,32] employed a casein-based diet as the animal protein comparison diet. Hence, the advantage of the SP diet in the current study or in the previously reported studies was not simply due to an inherent deficiency in the comparison diets. In fact, dietary SP has now been demonstrated to have beneficial effects on glomerular hypertrophy in the *fa/fa* Zucker rat when compared to two sources of high-quality animal protein.

OAN is characterized by glomerular hyperfiltration, increased glomerular pressure and size and subsequent development of fibrosis [2]. The current findings suggest that the effect of dietary SP on renal 6-keto $PGF_{1\alpha}$ may be a mechanism by which SP mediates its beneficial effect on glomerular size. The effect of SP on prostanoids may be analogous to the effects of low-protein diets on prostanoids and filtration in rat kidneys. Low-protein diets, as compared to high-protein diets, ameliorate the increases in prostanoid production and hyperfiltration in renal diseases [10–13]. With respect to the SP effect, plant proteins, as compared to animal proteins, alter renal hemodynamics and result in lower filtration rates [21,23–25,33]. We have observed that

dietary SP can alter disease progression and prostanoid production in rats in the very early stages of renal cyst disease [17,22]. The reduction in 6-keto $PGF_{1\alpha}$ production in the *fa/fa* rats given the SP diet, in particular the reduction in this vasodilatory prostanoid when compared to the vasoconstrictory TXB_2, as reported herein, may result in a reduction in the afferent arteriole dilatation and subsequently increased glomerular capillary pressure, which causes glomerular hyperfiltration in obesity.

In the present study, there were no significant diet or genotype effects for $cPLA_2$ or COX-1 protein and mRNA levels. In contrast, COX-2 protein levels were elevated in *fa/fa* rats, consistent with previous findings [34–36]. Interestingly, while there was no significant dietary effect on protein content, COX-2 mRNA was elevated in kidneys from *fa/fa* rats given SP as compared to those given EW. Thus, the protein production and gene expression do not necessarily reflect COX-2 activity levels. These counter-intuitive findings have been observed with renal COX-2 in some (but not in other) studies on models of renal disease [29,35,37–40]. This may reflect a regulatory feedback mechanism in some situations, which result in increased COX-2 expression when activity is reduced, and warrants further investigation.

In conclusion, the present study demonstrates that dietary SP attenuates early renal development of glomerular hypertrophy in the obese *fa/fa* Zucker rat. This effect is associated with lower 6-keto $PGF_{1\alpha}$ levels, suggesting that one of the mechanisms by which SP may mediate its beneficial effects may be via reduction of the afferent arteriole dilation and the subsequently increased glomerular pressure in obesity. Further exploration of dietary SP as an option for treating the early development of OAN is therefore warranted, particularly so with the rising incidence of childhood obesity.

Acknowledgments

This study was supported by the Natural Sciences and Engineering Research Council.

The authors thank Dielle Herchak, Rob Diakiw and Jennifer Zahradka for providing assistance with the animal care and Danielle Stringer and Natasha Ryz for animal care and clinical laboratory analyses. Equipment support from the Manitoba Institute of Child Health is also gratefully acknowledged.

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