Renoprotective mechanisms of soy protein intake in the obese Zucker rat

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The prevalence of obesity in adults and children has risen over the past two decades and has been linked to changes in dietary habits and adaptation of a sedentary lifestyle. Traditionally, obesity has been associated with the development of renal failure and is a major established risk factor for both diabetes and cardiovascular disease. However, recent information strongly suggests that obesity itself is an independent risk factor for the development of renal injury (11). In this respect, the obesity epidemic has coincided with the increased incidence in chronic kidney disease (CKD) (11, 13, 15). Several studies have shown that obesity is associated with mesangial expansion, glomerular hypertrophy, focal segmental glomerulosclerosis, and proteinuria (15, 30). However, despite these findings, little is known about the mechanisms by which obesity induces renal damage (11).

The obese Zucker rat has been widely used to investigate the pathogenesis of nephropathy associated with obesity and type II diabetes. These animals harbor an autosomal-recessive mutation in the gene that encodes the leptin receptor, resulting in hyperleptinemia, hyperphagia, obesity, hyperlipidemia, peripheral insulin resistance, hyperinsulinemia, and impaired glucose tolerance. In addition, it has been previously demonstrated in the literature that leptin may promote renal injury through binding to the short leptin receptor, which is expressed more abundantly in the kidney than in the brain (31). Obese Zucker rats are also a good model for studying obesity-related renal disease because these animals also develop glomerular hypertension, glomerular hypertrophy, proteinuria, and focal segmental glomerulosclerosis (5, 10, 14, 30). In fact, the cause of death in these rats is often due to end-stage renal disease (14).

In comparison to casein, soy protein has been recognized to be beneficial in several models of chronic renal disease, including subtotally nephrectomized rats (47), mice with polycystic kidney disease (36), spontaneous hypercholesterolemic Imai rats (28), and rats with chronic nephritic syndrome (25). Soy protein has also been shown to be protective in obese models such as Zucker obese rats and db/db mice with type II diabetic nephropathy (20, 34, 39). Previous studies from our laboratory and others observed that soy protein diet consumption is able to reduce plasma cholesterol levels, urinary protein excretion, and the rate of progression to glomerular injury in the obese Zucker rat (20, 39). Renoprotection conferred by soy feeding has been demonstrated to be associated with restoration of urinary nitrate and nitrite excretion (UNO2/NO3V). Obese Zucker rats fed with casein exhibited a 90% reduction in UNO2/NO3V, whereas such a reduction was not present when rats were fed with a soy protein diet. Thus the renoprotective effects of a soy protein intake appear to be associated with restoration of urinary NO metabolites, suggesting that NO deficiency may contribute to progression of renal injury in this model (39).

The mechanisms by which soy protein confers renal protection, however, are unknown. Although not formally studied, it has been suggested that this renal protection could be related to high levels of phytoestrogens and L-arginine in soybean prod...
molecular events (42). The beneficial effects of isoflavones, the most common phytoestrogen in soy, have been attributed to their antioxidant and antiproliferative properties (35, 44), as well as to their actions as weak estrogens (22) and to the modulation of TGF-β1 expression at the transcriptional level (16). The present study was designed to begin to understand the mechanisms by which soy protein confers renoprotection in obese Zucker rats.

MATERIALS AND METHODS

Ten lean (Fa/Fa) and ten obese (fa/fa) Zucker rats were obtained at 5 wk of age (Harlan, Indianapolis, IN) and randomly divided into four groups: lean or obese Zucker rats fed ad libitum with a 20% casein protein diet (Fa/Fa+cas and fa/fa+cas groups, respectively) and lean or obese Zucker rats fed with a 20% soy protein diet (Fa/Fa+soy and fa/fa+soy groups, respectively). Body weight and food intake were recorded every day, and, after 160 days, the rats were euthanized by decapitation. All experiments involving animals were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Academy Press, Washington, DC, 1996) and were approved by the Institutional Animal Care and Use Committee of our Institutions.

RNA isolation and real-time PCR. Total RNA was isolated from the renal cortices of each group following the guanidine isothiocyanate-cesium chloride method (29). Integrity of isolated total RNA was examined by 1% agarose gel electrophoresis, and RNA concentration was determined by UV-light absorbance at 260 nm. Primers and probes for Kim-1, ObRa, and TGF-β1 were selected from the Applied Biosystems Assays-on-Demand ABI product (Applied Biosystems, Foster City, CA). RT-PCR-grade water.

Reverse transcription (RT) was carried out using 2.5 μg of total RNA from renal cortices. Before the RT reaction, total RNA was heated at 65°C for 10 min. RT was performed at 37°C for 60 min in a total volume of 20 μl using 200 U of the Moloney murine leukemia virus reverse transcriptase (GIBCO, Carlsbad, CA), 100 pmol of random hexamers (GIBCO), 0.5 mM of each dNTP (Sigma Chemical, St. Louis, MO), and 1× RT buffer (75 mM KCl; 50 mM Tris-HCl; 3 mM MgCl2; 10 mM DTT, pH 8.3). Samples were heated at 95°C for 5 min to inactivate the reverse transcriptase and diluted to 40 μl with PCR-grade water.

The mRNA levels of kidney injury molecule 1 (Kim-1), short leptin receptor (ObRa), and TGF-β1 were quantified by real-time PCR on the ABI Prism 7300 Sequence Detection System (TaqMan; Applied Biosystems, Foster City, CA). RT buffer (75 mM KCl; 50 mM Tris-HCl; 3 mM MgCl2; 10 mM DTT, pH 8.3). Samples were heated at 95°C for 5 min to inactivate the reverse transcriptase and diluted to 40 μl with PCR-grade water.

The mRNA levels of kidney injury molecule 1 (Kim-1), short leptin receptor (ObRa), and TGF-β1 were quantified by real-time PCR on the ABI Prism 7300 Sequence Detection System (TaqMan; Applied Biosystems, Foster City, CA). FAM or VIC dye-labeled probes were selected from the Applied Biosystems Assays-on-Demand ABI product line and specifically used to detect and quantify cDNA sequences selected from the Applied Biosystems Assays-on-Demand ABI product line and specifically used to detect and quantify cDNA sequences.

Urinary Kim-1. Urinary Kim-1 protein was measured using the Microsphere-based Luminter xMAP technology (40). This technique is an adaptation of the recently developed and validated sandwich ELISA assay (41). For quantitation of urinary Kim-1 ectodomain, 30 μl of 24-h urine samples were analyzed in duplicate.

Western blot analysis. Renal proteins were isolated by homogenization and used for immunoblot analysis with rabbit endothelial nitric oxide synthase (eNOS) antibody, phospho-eNOS T495 antibody, or phospho-eNOS S1177 antibody, all used at a dilution of 1:2,500 (Alpha Diagnostics, San Antonio, TX). To control protein loading and transfer, all membranes were simultaneously probed with an actin antibody (1:2,500) and secondary antibody (donkey anti-goat IgG- horseshad peroxidase, Santa Cruz Biotechnology, Santa Cruz, CA). Proteins were detected using an enhanced chemiluminescence kit (Amer sham, Buckinghamshire, UK) and autoradiography, following the manufacturer’s recommendations. All Western blot analyses were performed within the linear range of protein loading and antibody use. The bands were scanned for densitometric analysis.

Urinary glutathione peroxidase determination. Urinary glutathione peroxidase activity was assayed in the form of nitrotrietazolium blue reacting substance (TBARS) as previously reported (26). Briefly, after homogenization of the tissue, the reaction was performed in a 0.8% aqueous solution of nitrotrietazolium blue in 15% TCA and heated at 95°C for 45 min. The mixtures were then centrifuged at 3,000 g for 15 min. The absorbance of the supernatant was read at 532 nm. TBARS was quantified using an extinction coefficient of 1.56 × 105 M−1 cm−1 and expressed as nmol of TBARS per milligram of protein. Protein concentration was estimated using the Bradford method.

Antioxidant enzyme activity. Renal tissue was homogenized in 50 mM potassium phosphate, 0.1% Triton, pH 7.0. Crude homogenates were centrifuged at 15,300 revolution/min for 30 min at 4°C and used to assess antioxidant enzyme activities. Total protein concentration was measured by Lowry’s method (19).

Catalase activity assay. Catalase activity was determined according to the Aebi method (1). This method is based on measuring the decomposition of H2O2 by catalase at an optical density of 240 nm at times 0 and 15 s (first-order kinetics) given the equation k = (1/ΔA) (2.3 × log A1/A2), where A is the first-order reaction rate constant and t is the time interval of decrease of H2O2. An enzyme activity unit was defined as the degradation of 1 μmol H2O2 per second per mg tissue protein, and the enzyme activity was expressed as k/mg protein.

GPx activity. GPx activity was indirectly detected by a method previously described by Lawrence and Burk (17). GPx requires reduced glutathione (GSSG), which is regenerated by glutathione reductase using oxidized glutathione (GSH), a process that consumes NADPH. The activity of glutathione peroxidase was defined as micromoles of NADPH oxidized per minute, taking into account that the millimolar absorption coefficient for NADPH at 340 nm is 6.221·mM−1·cm−1. The results are expressed as U/mg protein.

SOD activity. SOD activity was measured through the inhibition of nitrotetrozolium blue (NBT) reduction by O2− generated through the xanthine-xanthine oxidase system as previously reported (17). One SOD activity unit was defined as the amount of enzyme promoting 50% inhibition of NBT reduction in 1 ml of reaction solution per milligram tissue protein. Results are expressed as U/mg protein.

Serum estradiol determination. Serum estradiol concentrations were determined by radioimmunoassay using a commercial rat kit from DPC Coat-a-count (TKE22). Intra- and interassay coefficients of variation were 3% and 2%, respectively. Sensitivity of the radioimmunoassay was 0.37 pg/ml. Each serum sample was assayed in duplicate.

Histological studies. After appropriate dehydration, kidney slices were embedded in paraffin, sectioned at 3 μm, and stained with routine methods: hematoxylin/eosin, periodic acid-Schiff, and Masson trichromatic. In Masson-stained slides, the degree of tubulointerstitial fibrosis was evaluated by morphometry as previously reported by our laboratory (8). For this purpose, 10 cortex fields (magnification ×200) were randomly selected in sections of kidney from the different experimental groups. The images were recorded using a digital camera incorporated in a Nikon microscope, and the affected areas were delimited and quantified by using eclipse net processing and analysis software (Nikon Instruments, Melville, NY). Tubulointerstitial fibrosis consisted of extracellular matrix expansion and collagen deposition together with distortion and collapse of the tubules; fibrosis was evidenced by blue coloration.
in Masson stain. The proportion of fibrosis was calculated by dividing the area of interstitial fibrosis by the total area per field, excluding the glomerular and luminal tubular areas. In addition, the glomerular size was evaluated by measuring the diameter as previously described (26). For this purpose, five to six images of different renal cortex fields were recorded, and the glomerular diameter was measured in the digitalized microphotographs (magnification ×100). The histological analysis was performed without knowledge of the experimental group in which each kidney belonged. The degree of tubulointerstitial fibrosis from each rat was correlated with their corresponding renal TGF-β mRNA levels.

Statistical analysis. Comparison among the groups for continuous data was made by using ANOVA. When ANOVA showed a statistically significant difference, a group-by-group comparison was performed using a t-test with Bonferroni’s correction for multiple comparisons. The differences in the ranks of glomerular diameter among the groups were evaluated by a contingency analysis, and the differences were tested using Fisher’s exact test. Statistical significance was defined as a P value <0.05.

RESULTS

Effect of soy and casein protein feeding on glomerular injury. We have observed that obese Zucker rats fed a casein protein diet developed progressive renal disease that was significantly abrogated when the rats were fed a soy protein diet (39). Similar to our previous observations, at the end of the study, the creatinine clearance was 0.42 ± 0.1 ml/min in obese Zucker rats fed with a casein protein diet and 1.0 ± 0.1 ml/min in rats fed with a soy protein diet (P < 0.05), whereas in lean Zucker rats fed with casein or soy diets the values were 1.4 ± 0.1 and 1.3 ± 0.1 ml/min, respectively.

To further characterize the protective effects of soy protein, we evaluated the glomerular diameter in lean and obese Zucker rats fed long-term casein or soy protein diets. To this end, glomerular sizes were separated into six different groups covering the range from 80 to 180 μm. The data in Fig. 1A and B demonstrate that the glomerular diameter size distribution was similar in lean Zucker rats fed with casein or soy. The majority of glomeruli diameters are in the range of 101 to 120 μm (41 and 38.9%, respectively), and a lesser proportion are in the range of 81 to 100 μm (18.8 and 18.8 7%, respectively), 121 to 140 μm (26.6 and 26.7%, respectively), or 141 to 160 μm (13.7 and 5.4%, respectively). Given these data, the histograms in the lean experimental groups exhibit a typical bell-shaped Gaussian distribution, as previously observed in Wistar rats (26). In contrast, obese Zucker rats fed with casein demonstrated a diameter distribution shifted to the right, reflecting glomerular hypertrophy (Fig. 1C). In this group, glomerular diameters measured as follows: 35.2% in the range of 121 to 140 μm, 24.8% in 141 to 160 μm, 6.4% above 160 μm, and only 27.2% between 100 and 120 μm. The majority of these differences were statistically significant compared with lean rats fed with the casein diet. In contrast, obese Zucker rats fed with a soy protein diet exhibited a lesser glomerular hypertrophy as evidenced by a left-shift in glomerular size distribution (Fig. 1D). This group exhibited a greater proportion of glomerular diameter in the range of 80 to 100 μm (11%) and a lesser proportion in the range of 140 to 160 μm (18.5%) and above 160 μm (1.1%). This beneficial effect of soy protein compared with casein was not associated with changes in serum estradiol concentration levels. Thus serum estradiol levels in obese Zucker rats fed with either casein or soy protein were 11.5 ± 4.2 and 6.6 ± 3.2 pg/ml (P = NS), respectively. Similar values were observed in lean animals 15.5 ± 6.0 and 15.0 ± 6.8 pg/ml, respectively.

The renoprotective effect of soy protein feeding was also evidenced by evaluation of Kim-1 expression, which we have previously demonstrated to be a sensitive marker of renal injury (26, 41). Figure 2, A and B, depicts renal mRNA and urinary protein Kim-1 levels, respectively. A significant increase in Kim-1 mRNA and protein levels (13- and 4.8-fold, respectively) were seen in obese Zucker rats fed a casein protein diet. Kim-1 upregulation was partially prevented by soy protein feeding in the obese group.

Phosphorylation of eNOS at T495 is reduced by soy protein diet. To begin to understand how soy protein feeding is associated with lesser injury in obese Zucker rats, we assessed eNOS protein levels and phosphorylation status. Figure 3A

![Fig. 1. Glomerular diameter distributions in lean control groups fed with casein or soy diets are shown in A and B, respectively. Obese Zucker rats fed with casein or soy diets are represented in C and D, respectively. The statistical significance was tested by contingency analysis and square chi. *P < 0.05 vs. the same range in lean rats feeding with casein, and **P < 0.05 vs. the same range in obese Zucker rats fed with casein.](http://ajprenal.physiology.org/Downloaded from http://ajprenal.physiology.org/)
shows an immunoblot of renal eNOS as well as the densitometric analysis normalized to actin in all experimental groups (lean or obese rats fed with casein or soy protein over 160 days). The eNOS protein levels were similar in both lean and obese Zucker rats, and the type of diet provided did not influence expression of the enzyme. It has been shown that eNOS activity may be both positively and negatively regulated at the posttranscriptional level via phosphorylation. eNOS phosphorylation at Ser1177 increases the activity of the enzyme, whereas phosphorylation at Thr495 inactivates the enzyme (23, 24, 33). As shown in Fig. 3B, the phospho-eNOS Ser1177/actin ratio was similar among the four experimental groups. In contrast, consumption of a soy protein diet was associated with a reduction in the inactivating eNOS phosphorylation by ~50%, as illustrated in Fig. 3C. Thus, in obese Zucker rats fed with casein, the phospho-eNOS Thr495/actin ratio was 1.7 ± 0.4 compared with 0.8 ± 0.2 in rats fed with soy protein (P = 0.001). This difference in eNOS Thr495 phosphorylation was not observed in lean Zucker rats fed with casein or soy diets, in which comparable values were observed (1.8 ± 0.5 and 1.7 ± 0.4, respectively; P = NS). These data revealed that decreased NO generation in obese rats fed with casein is not associated with reduced eNOS expression levels or with eNOS phosphorylation status. Thus decreased eNOS levels or activity are unlikely to explain the observed marked reduction of \( \text{UNO}_2/\text{NO}_3 \).

Soy protein feeding reduces oxidative stress. To explore whether the NO deficiency in casein-treated rats was related to an increase in free radical generation, renal lipoperoxidation was measured in all groups. Figure 4 demonstrates that there was a statistically significant increase in renal lipoperoxidation levels in obese Zucker rats fed with casein by more than fivefold, suggesting that greater oxidative stress may contribute to the renal injury observed in this model. Interestingly, soy protein feeding completely prevented this increase in liperoxidation levels in obese Zucker rats. Accordingly, the mean levels of lipoperoxidation in obese Zucker rats fed with casein and soy were 1.7 ± 0.3 and 0.5 ± 0.1, respectively. In

![Fig. 2. mRNA levels of kidney injury molecule (Kim-1, A) and urinary Kim-1 shedding (B) in lean and obese Zucker rats fed with casein (cas) (open bars) or soy (solid bars) during the 160-day trial using real-time RT-PCR and the sandwich ELISA assay, respectively. mRNA values were normalized to housekeeping gene expression (18s). Each bar represents mean value ± SE. *P < 0.05 vs. all groups.](http://ajprenal.physiology.org/)

![Fig. 3. Endothelial nitric oxide synthase (eNOS) expression and phosphorylation were assessed by Western blot analysis using specific eNOS and phospho-eNOS (p-eNOS) antibodies against lysates isolated from the renal cortices of lean and obese Zucker rats at the end of the study (160 days). A: total eNOS. B: p-eNOS Ser1177. C: p-eNOS Thr495. Top: representative images of eNOS, eNOS Ser1177, p-eNOS Thr495, and actin immunoblots, respectively. Bottom: optical density (OD) ratio between eNOS and actin bands for casein-fed rats (open bars) and soy-fed rats (solid bars). The results are expressed as means ± SE. Each group was formed by 5 rats. *P < 0.05 vs. all groups.](http://ajprenal.physiology.org/)
addition, soy feeding did not have any effect on renal lipoperoxidation in lean rats. These results suggest that soy protein feeding prevented oxidative stress in obese Zucker rats.

To investigate whether the reduction in renal lipoperoxidation correlated with an improvement in the antioxidant system, the activity of catalase, GPx, and SOD were evaluated and are depicted in Fig. 5, A, B, and C, respectively. Catalase activity (Fig. 5A) was significantly reduced in obese Zucker rats fed with casein compared with lean rats fed a similar diet. Mean catalase activity values were 0.09 ± 0.01 vs. 0.14 ± 0.01 k/mg protein, respectively (P < 0.05). Interestingly, soy feeding did not just normalize catalase activity, but instead appeared to enhance it compared with the other two casein groups (0.22 ± 0.003 k/mg protein). In contrast to these findings, GPx (Fig. 5B) and SOD activities (Fig. 5C) remained unchanged between the obese and lean groups and were not affected by diet. Taken together, the results from Figs. 4 and 5 demonstrate that soy protein feeding prevents oxidative stress.

Effect of soy and casein protein intake on ObRa and TGF-β mRNA levels. We observed that obese Zucker rats fed with casein and soy exhibit a significant increase in leptin plasma levels (1,251 ± 132.2 and 1,407 ± 104.1 pmol/l, respectively) compared with leptin values in lean rats (256.3 ± 25). Since leptin levels were similarly increased in obese Zucker rats fed with casein or soy protein diets, we hypothesized that the difference between these groups could be at the level of short leptin receptor (ObRa) expression in the kidney. As such, we assessed mRNA levels for this receptor using real time RT-PCR with primers directed against a unique fragment exclusive to the short receptor and not a part of other leptin receptors (for review, see Ref. 49). As shown in Fig. 6A, we observed that obese Zucker rats fed a casein protein diet exhibited higher ObRa mRNA levels compared with the other groups. The mean values (normalized to 18S, as a control gene) were 2.3 ± 0.30 and 1.0 ± 0.02, respectively (P < 0.05). Intriguingly, leptin short receptor was not overexpressed in obese animals fed with a soy protein diet (1.0 ± 0.30). With regards to this finding, it has been demonstrated that activation of the short leptin receptor initiates a cascade of events that result in an increase of TGF-β transcription in glomerular endothelial and mesangial cells (12, 48). Accordingly, we observed that obese Zucker rats showed significantly higher TGF-β mRNA levels than lean Zucker rats as is represented in Fig. 6B. However, obese Zucker rats fed a casein protein diet had an approximately threefold increase in TGF-β mRNA levels compared with lean rats (4.0 ± 0.5 vs. 1.0 ± 0.1). Obese Zucker rats fed a soy protein diet exhibited lesser mRNA levels of this cytokine compared with casein 2.3 ± 0.5 vs. 4.0 ± 0.5 respectively, P < 0.05. Similarly, tubulointerstitial fibrosis was significantly less in obese Zucker rats fed with soy compared with casein protein, 11 ± 1.1 vs. 5 ± 0.4%. Since TGF-β expression has been associated with the development of tubulointerstitial fibrosis, we performed a correlation analysis between the expression of this cytokine and the area affected by fibrosis. As shown in Fig. 6C, there was a statistically significant correlation between TGF-β mRNA levels and tubulointerstitial renal fibrosis, with an r value of 0.82, and a P value of <0.0001. These results suggest that increased short leptin receptor expression in obese Zucker rats fed with casein is associated with an increase in TGF-β. In addition, tubulointerstitial injury is highly correlated with the extent of the elevation of the cytokine TGF-β.

DISCUSSION

In this study, we demonstrated that long-term soy protein consumption was more effective than a diet with casein as a
SOY RENOPROTECTIVE MECHANISMS IN THE OBESE ZUCKER RAT

There is considerable evidence in experimental and in clinical studies that NO deficiency contributes to the progression of CKD (for review, see Ref. 2). Accordingly, we observed that renal injury in obese Zucker rats fed with casein was associated with a reduction in NO metabolite excretion, and this reduction was prevented by long-term soy protein intake (39). To investigate the mechanism by which the soy protein diet reestablished NO metabolites in obese Zucker rats, eNOS expression and phosphorylation were evaluated. No difference in the level of expression of eNOS at the protein level was observed among the groups. However, it has been shown that eNOS activity can be regulated by its phosphorylation state, even in the absence of any change in the amount of eNOS protein expressed. In total, six eNOS putative phosphorylation sites have been reported in the literature. The most physiologically relevant eNOS phosphorylation sites are Ser1177 (stimulatory) and Thr495 (inhibitory). Our data demonstrated that a soy diet was able to significantly reduce the inactivating Thr495-phosphorylation, suggesting that a soy diet may increase eNOS activity in obese Zucker rats. However, it is unlikely that this change by itself could explain the large reduction in NO production and increased renal protection induced by soy.

It is widely appreciated that NO availability is reduced by an increased formation of reactive oxygen species (ROS). Therefore, a change in equilibrium between NO and ROS might contribute to enhanced progression of renal disease (for review, see Refs. 2 and 50). Hence, the effects of casein and soy on renal lipoperoxidation were evaluated. As previously reported, in obese Zucker rats, renal injury is associated with a significant increase in oxidative stress (6). Interestingly, in this study, we observed that a soy diet abrogated an increase in renal lipoperoxidation levels in obese Zucker rats. This outcome could contribute to increased NO bioavailability and reduced renal progression injury. To determine whether the observed protection was mediated by an increase in the renal antioxidant system, the effect of soy on antioxidant enzymes in the kidney was evaluated. The cellular antioxidant system is composed of enzymes such as catalase, glutathione peroxidase, and superoxide dismutase, which transform free radicals into less stable molecules. However, in this study, we observed that the cellular antioxidant system was not significantly affected by soy protein intake (39).

In this study, we observed that obese Zucker rats fed with a casein diet exhibited glomerular hypertrophy and that glomerular size was partially normalized in rats fed a soy diet, suggesting that the kind of protein may play an important role in the development of glomerular hypertrophy. A reduction in renal injury was also confirmed by assessing Kim-1 expression levels as a marker of renal injury (26, 27, 40). We observed a significant reduction in Kim-1 mRNA and protein levels in both the kidney and urine of rats fed with soy protein; these findings were in accord with our histological observations at tubular interstitial level (Fig. 6C), supporting the idea that soy protein reduces renal damage in the obese Zucker rat.

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reactive molecules. In fact, catalase activity was observed to be reduced in obese rats fed with casein, an effect that was not only reversed by soy but also significantly enhanced compared with lean Zucker rats (Fig. 5). Our results suggest that soy preserves renal cellular structure and helps maintain the antioxidant system, thus reducing oxidative stress commonly seen in this model of renal injury.

In addition, it has been previously demonstrated in the literature that leptin may promote renal injury through binding to the short leptin receptor, which is expressed more abundantly in the kidney than in the brain (31). In endothelial cells, it has been shown that leptin induces oxidative stress in human endothelial cells (4). Therefore, hyperleptinemia present in obese Zucker rats may contribute to enhanced ROS formation. Leptin is a small peptide hormone involved in regulating food intake and energy expenditure, and it is primarily produced in adipose tissue. Circulating leptin concentrations directly reflect the amount of body fat. Leptin binds to a long receptor (ObRb) in the hypothalamus and regulates food intake through the release of neurotransmitters. Several leptin receptor variants secondary to alternative splicing have been identified (for review, see Ref. 49). In particular, a short leptin receptor (ObRa) is expressed in various peripheral tissues including the kidney and has been shown to be an active signaling isoform (46) that may traduce the harmful effects of leptin when concentrations of this hormone are increased (12, 32, 48). Consistent with this hypothesis, we observed that obese Zucker rats fed with casein demonstrated hyperleptinemia and a significant upregulation of ObRa mRNA levels. The soy protein diet did not reduce serum leptin levels but completely prevented the upregulation of ObRa expression.

Increased evidence of glomerulosclerosis has been described in humans suffering from massive obesity and hyperleptinemia, suggesting that leptin may contribute to the development of glomerulosclerosis (11, 43, 45). In support of this, Wolf et al. (48) reported that glomerular endothelial cells express the short leptin receptor ObRa and that leptin stimulates the expression of profibrotic cytokine TGF-β in these cells, thus inducing proliferation. The relevance of these in vitro findings was validated in vivo when rats that were infused with leptin developed glomerulosclerosis and proteinuria. Furthermore, in mesangial cells from the db/db mouse, leptin was shown to upregulate the TGF-β type II receptor and type I collagen expression (12). Consistent with these studies, we observed that obese animals fed a casein diet showed increased ObRa mRNA levels and also exhibited greater TGF-β expression (Fig. 6), whereas animals fed a soy protein diet showed a significant reduction in TGF-β mRNA levels. Thus it is possible that the renoprotective effects of soy are in part mediated by its ability to reduce the expression of this profibrotic cytokine.

Taking all of our findings together, we propose that the renoprotection conferred by soy protein in obese Zucker rats appears to be mediated by several mechanisms, as illustrated in Fig. 7. During casein protein feeding, oxidative stress is increased, and it is not only a known cause of renal injury, but it may also contribute to a reduction in NO availability. Additionally, upregulation of ObRa expression may contribute to enhanced renal TGF-β expression, which may also be upregulated by other mechanisms, such as angiotensin II signaling. Soy protein feeding effectively reduced renal lipoperoxidation, as demonstrated by enhanced catalase activity. The antioxidant effects of soy may contribute to the reestablishment of NO production and may also reduce the inactivating eNOS phosphorylation. Additionally, the soy protein diet was also associated with a reduction in TGF-β mRNA levels in the obese Zucker rat. Therefore, our study suggests that long-term soy protein intake may help prevent obesity-induced renal disease.

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**Fig. 7. Schematic representation of mechanisms involved in renal protection conferred by a soy protein diet.**
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