Effects of Dietary Protein on Glomerular Eicosanoid Production in Rats with Bilateral Ureteral Obstruction (43812)

HIROYUKI YANAGISAWA,[1,2] JERRY MORRISSEY, NOBUTAKA KURIHARA,[2] OSAMU WADA,[2] AND SAULO KLAHR

Department of Medicine, The Jewish Hospital of St. Louis at Washington University Medical Center, 216 South Kingshighway, St. Louis, Missouri 63110

Abstract. **Greater protein intake increases glomerular eicosanoid production in rats. Bilateral ureteral obstruction (BUO) also enhances glomerular eicosanoid production in experimental animals. To examine the effects of dietary protein intake on glomerular eicosanoid production in ureteral obstruction, we measured the *in vitro* production of the vasodilatory prostaglandins, PGE_2, and 6-keto $PGF_{1\alpha}$, and the vasoconstrictor, TxB_2, and the mass of cyclooxygenase in glomeruli of sham-operated control (SOC) rats and rats with BUO of 24 hr duration fed a low- (6% casein) or a high- (40% casein) protein diet for approximately 4 weeks. The animals were pretreated or not with the angiotensin converting enzyme inhibitor, enalaprilat, prior to sham-operation or ureteral obstruction. Glomeruli from SOC rats fed a high-protein diet produced significantly greater amounts of PGE_2, 6-keto $PGF_{1\alpha}$, and TxB_2, and had substantially increased mass of cyclooxygenase when compared with glomeruli from SOC rats fed a low-protein diet. Pretreatment of animals with enalaprilat prior to sham operation prevented the increase in glomerular eicosanoid production and cyclooxygenase content in SOC rats fed a high-protein diet and the levels observed were similar to those in SOC rats fed a low-protein diet. Both eicosanoid production and cyclooxygenase mass were further increased in glomeruli from rats with BUO fed a high-protein diet when compared with glomeruli of SOC rats fed the same diet. The increased levels of these measurements in BUO rats fed a high-protein diet fell markedly when the rats were pretreated with enalaprilat *in vivo*. The values were essentially comparable to those of SOC rats fed a low-protein diet. By contrast, there was no substantial increase in the production of PGE_2, 6-keto $PGF_{1\alpha}$, and TxB_2 and in the mass of cyclooxygenase in glomeruli of BUO versus SOC rats fed a low-protein diet. Enalaprilat did not affect glomerular eicosanoid production or cyclooxygenase content in SOC and BUO rats fed a low-protein diet. Taken together, the present study indicates that dietary protein affects BUO-induced increases in glomerular eicosanoid production by altering the activity of the cyclooxygenase pathway mainly via the renin-angiotensin system. Thus, protein content in a diet may modify an alteration in renal hemodynamics caused by BUO by changing the glomerular production of eicosanoids and the activity of the renin-angiotensin system.** [P.S.E.B.M. 1994, Vol 207]

Bilateral ureteral obstruction (BUO) of 24 hr duration causes remarkable decreases in single nephron glomerular filtration rate (SNGFR) and glomerular capillary plasma flow (Q_A) (1, 2). Two potent vasoconstrictors, angiotensin II and thromboxane (Tx) A_2, have an important role in these functional changes via an increase in glomerular arteriole vasoconstriction and a decrease in the ultrafiltration coefficient (K_f) due to glomerular mesangial cell contraction (1, 2). In fact, *in vivo* inhibition of the generation of both angiotensin II and TxA_2 with the angiotensin converting enzyme (ACE) inhibitor, enalapril, and the

[1] To whom requests for reprints should be addressed at Department of Hygiene and Preventive Medicine, Faculty of Medicine, University of Tokyo, 7–3–1 Hongo, Bunkyo-ku, Tokyo 113, Japan.

[2] Present address: Department of Hygiene and Preventive Medicine, Faculty of Medicine, University of Tokyo, 7–3–1 Hongo, Bunkyo-ku, Tokyo 113, Japan.

Received October 1, 1993. [P.S.E.B.M. 1994, Vol 207]
Accepted June 14, 1994.

0037-9727/94/2072-0234$10.50/0
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thromboxane synthase inhibitor, OKY-046, prior to obstruction markedly ameliorates the decrements in GFR and renal plasma flow observed after unilateral release of BUO of 24 hr duration (3).

Dietary protein modulates renal hemodynamics in the normal state and disease in both humans and experimental animals (4–7). Micropuncture studies demonstrated that changes in dietary protein intake modulate SNGFR and Q_A in rats with BUO of 24 hr duration (8). High as compared to low protein intake caused a marked fall in SNGFR and Q_A in the setting of BUO (8). These changes in renal hemodynamics observed with high protein intake were accompanied by a significant increase in the urinary excretion of TxA_2 (measured as the stable metabolite, TxB_2) (8). Prior inhibition of the endogenous TxA_2 synthesis with the thromboxane synthase inhibitor, OKY-1581, markedly augmented SNGFR and Q_A in rats with BUO fed a high-protein diet but did not alter single nephron function in rats with BUO fed a low-protein diet (8). Similarly, in rats with BUO fed a high-protein diet blockade of the endogenous angiotensin II generation prior to obstruction substantially increased GFR and renal plasma flow after unilateral release of obstruction (8). It was most recently reported that dietary protein enhanced the expression of rat renal renin mRNA in a dose-dependent fashion (9, 10). These observations suggest that dietary protein may affect renal hemodynamics as a consequence of BUO by changing the synthetic rates and/or activities of at least two vasoconstrictors, angiotensin II and TxA_2.

We demonstrated previously enhanced production of the vasoconstrictor, TxA_2 and the vasodilatory prostaglandins, prostaglandin $(PG)E_2$ and prostacyclin (measured as the stable metabolite, 6-keto $PGF_{1\alpha}$), by glomeruli isolated from rats with BUO of 24 hr duration (11, 12). This increased production of eicosanoids by glomeruli isolated from rats with BUO is mediated by angiotensin II-induced increases in the activities of cyclooxygenase and phospholipase A_2 (PLA_2) (11, 12). The rise in the activity of cyclooxygenase is related to *de novo* synthesis of this enzyme (12). Dietary protein also causes a dose-dependent increase in the glomerular production of these eicosanoids through essentially the same mechanism as BUO (13, 14). Collectively, these observations raise the possibility that dietary protein intake modulates glomerular eicosanoid production by altering the activity of the renin-angiotensin system in the setting of BUO.

The present study was designed to examine whether or not dietary protein modulates the glomerular production of eicosanoids in the BUO model. We measured the production of PGE_2, 6-keto $PGF_{1\alpha}$, and TxB_2, and the amount of cyclooxygenase in glomeruli isolated from sham-operated control (SOC) rats and rats with BUO of 24 hr duration fed a low- (6% casein) or a high- (40% casein) protein diet for approximately 4 weeks. Moreover, a potential role of the renin-angiotensin system in the modulation of glomerular eicosanoid production by dietary protein intake was examined by pretreating SOC rats and rats with BUO fed the diet low or high in protein with the ACE inhibitor, enalaprilat, prior to sham operation or obstruction.

Materials and Methods

Chemicals and Reagents. Enalaprilat, an ACE inhibitor, was a gift of Merck, Sharp & Dohme (Rahway, NJ). PGE_2, TxB_2, 6-keto $PGF_{1\alpha}$, and goat anti-rabbit IgG (whole molecule) were purchased from Sigma Chemical Co. (St. Louis, MO). Rabbit antisera against 6-keto $PGF_{1\alpha}$ or cyclooxygenase were obtained from Cayman Chemical Co. (Ann Arbor, MI). ^{125}I sodium iodine was bought from Amersham (Arlington Heights, IL).

Dietary Regimen and Animal Models. Female Sprague-Dawley rats weighing approximately 200 g were purchased from Sasco Inc. (Omaha, NE). The animals were pair fed isocaloric diets containing either low (6% casein) or high amounts of protein (40% casein) for approximately 4 weeks. The mineral and vitamin content of the two diets was comparable (4). Details of the composition of the diets and the method of feeding were reported previously (4).

Pretreatment of animals with the ACE inhibitor, enalaprilat, prior to sham operation or obstruction was performed according to the protocol described previously (11, 12). Half of SOC rats and rats with BUO fed a low- or a high-protein diet received five ip injections of enalaprilat (5 mg/kg) dissolved in saline twice daily starting 48 hr prior to sham operation or obstruction. The rats pretreated with enalaprilat were operated 1 hr after the last ip injection of the drug. Under light ether anesthesia each group of rats were subjected to sham operation or bilateral ureteral ligation as reported previously (11, 12). After surgery the animals were given neither food nor water before sacrifice.

Preparation of Isolated Glomeruli. Isolated glomeruli were prepared as previously reported from our laboratory (11, 12, 14). Briefly, rats were anesthetized with pentobarbital (5 mg/100 g body wt) given intraperitoneally and the abdominal cavity was opened. Both kidneys were thoroughly perfused with phosphate-buffered saline through the bifurcation of the aorta. The kidneys were immediately removed and decapsulated. The cortices were dissected on ice and glomeruli were isolated by sieve techniques (mesh size 250, 150 and 75 μm). The isolated glomeruli were then washed with cold Hanks' balanced salt solution (HBSS) and placed on ice for the subsequent procedure. The preparations were confirmed to consist of greater than 90% isolated glomeruli by light microscopy.

Eicosanoid Production. Eicosanoid production by isolated glomeruli was determined as previously reported from our laboratory (11, 12, 14). In brief, isolated glomeruli were suspended in warm HBSS and preincubated at 37°C for 10 min. The preparations (approximately 100 μg protein/500 μl HBSS) were transferred into plastic centrifuge tubes and incubated under continuous shaking (80 cycles/min) at 37°C for 10 min. Incubations were terminated by centrifugation (10,000*g* for 1 min) at room temperature and the supernatants were stored at −70°C for PGE_2, 6-keto $PGF_{1\alpha}$, and TxB_2 determinations. The net production of PGE_2, 6-keto $PGF_{1\alpha}$, and TxB_2 was determined from the difference between the production of each eicosanoid in a 10-min incubation period and the production of the compound in a 10-min preincubation period. Values for the net production of eicosanoids were corrected for the protein content of the glomeruli and expressed as pg eicosanoids formed per mg protein per 10 min of incubation.

The production of PGE_2, 6-keto $PGF_{1\alpha}$, and TxB_2 by isolated glomeruli was determined by assaying them in the supernatant buffer using specific radioimmunoassays (RIAs). The cross-reactivities of antisera against PGE_2, 6-keto $PGF_{1\alpha}$, or TxB_2, and details of the RIA have been previously described (11, 15, 16). All determinations were performed in duplicate.

Preparation of Glomerular Membranes. Glomerular membranes were prepared as described previously (12, 14). Isolated glomeruli were washed twice with cold Ca^{++}- and Mg^{++}-free HBSS by centrifugation/resuspension and suspended in 250 μl of ice-cold homogenate buffer (25 m*M* Tris HCl, pH 7.5, 1 m*M* EDTA, 1 m*M* dithiothreitol, and 100 units/ml trazylol). Glomerular homogenates were prepared with 20 strokes of a teflon glass homogenizer and centrifuged at 100,000*g* for 60 min. The pellets were washed in 2 ml of ice-cold homogenate buffer and again centrifuged at 100,000*g* for 60 min. The washed pellets were resuspended in 250 μl of ice-cold homogenate buffer and stored at −70°C as membrane extracts of glomeruli.

Immunoblots for the Determination of Cyclooxygenase. Immunoblots were used to determine relative levels of cyclooxygenase in glomeruli according to the protocol described previously (12, 14). Glomerular membranes were dissolved in sodium dodecylsulfate (SDS) sample buffer and heated for 2 min at 90°C. The membrane proteins (2.5 μg protein) were subjected to 13.5% SDS-polyacrylamide gel electrophoresis (17). This protein content was within the linear range of detection. The proteins were electrophoretically transferred to a nitrocellulose membrane in cold transfer buffer consisting of 20% methanol, 25 m*M* Tris and 190 m*M* glycine. The nitrocellulose membrane was washed three times for 5 min each with 20 m*M* Tris-HCl, pH 7.5, containing 500 m*M* NaCl (A-buffer) to remove methanol and then incubated with A-buffer containing 3% gelatin and 0.02% NaN_3 to saturate nonspecific binding sites. After washing three times for 5 min each with 20 m*M* Tris-HCl, pH 7.5, containing 500 m*M* NaCl and 0.05% Tween 20 (B-buffer), the membrane was incubated for 1 hr at room temperature with rabbit antisera against cyclooxygenase (1:1000 dilution) in immunoblotting buffer (B-buffer, containing 1% gelatin and 0.02% NaN_3). The nitrocellulose sheet was washed with B-buffer three times for 5 min each and then incubated with $[^{125}I]$-labeled goat anti-rabbit IgG (4 million cpm/ml) in immunoblotting buffer for 30 min at room temperature. The sheet was washed, dried, and autoradiographed for 24 hr. Blots on the autoradiograph were scanned with a GS 300 Densitometer (Hoefer Scientific Instruments, San Francisco, CA). Values were determined from the peak height of each band in arbitrary units. All samples were run simultaneously to diminish interassay variation.

Determination of Angiotensin II and Bradykinin Concentrations and ACE Activity. Concentrations (pg/ml) of angiotensin II and bradykinin in plasma were measured with specific RIAs as described previously (18, 19). Serum ACE activity was determined by fluorometric analysis of the enzymatic cleavage of hippurate from hippuryl-histidyl-leucine as reported previously (20). The ACE activity was expressed as nmole L-histidyl-L-leucine formed per min per ml.

Calculations and Statistical Analysis. The protein content of glomerular preparations was determined by the method of Lowry *et al.* (21). Data reported are means ± SE of five or six separate preparations of each group of rats. The individual or interactive effects of protein intake and enalaprilat administration on body and kidney weight were examined using two-way analysis of variance (ANOVA). Likewise, the effects of protein intake, BUO, and enalaprilat administration on glomerular eicosanoid production and cyclooxygenase mass were evaluated with three-way ANOVA. Comparisons between groups were done by Duncan's multiple range comparison test. Differences are considered significant when $P < 0.05$.

Results

Effects of Dietary Protein Modifications on Body and Kidney Weight. Body and kidney weight of low or high protein-fed SOC rats that were or were not pretreated with the ACE inhibitor, enalaprilat, prior to sham operation was measured at the time of sacrifice. Body weight was 253 (means) ± 3.9 (SE) g or 263 ± 2.7 g in the low or high protein-fed group (n = 10) that was not given enalaprilat and 251 ± 5.1 g or 261 ± 5.3 g in the low or high protein-fed group (n = 8) that was given enalaprilat. Similarly, kidney weight

was 0.92 ± 0.02 g or 1.06 ± 0.03 g in the low or high protein-fed group ($n = 20$) that was not given enalaprilat and 0.89 ± 0.03 g or 1.04 ± 0.03 g in the low or high protein-fed group ($n = 16$) that was given enalaprilat. Compared with low protein intake, high protein intake significantly ($P < 0.05$) increased kidney weight but did not affect body weight. Additionally, enalaprilat had no effect on either body or kidney weight of the two groups of rats.

Eicosanoid Production by Isolated Glomeruli. Table I summarizes the production of PGE_2, 6-keto $PGF_{1\alpha}$, and TxB_2 by glomeruli isolated from low or high protein-fed SOC and BUO rats that were or were not given the ACE inhibitor, enalaprilat, prior to sham operation or obstruction. The production (pg/mg protein/10 min of incubation) of eicosanoids was determined in six separate glomerular preparations of each group of rats.

The glomerular production of PGE_2, 6-keto $PGF_{1\alpha}$, and TxB_2 was significantly increased by 2.9, 1.9, and 2.2 times, respectively, in SOC rats fed a high-protein diet when compared with those fed a low-protein diet. Similarly, glomeruli from rats with BUO fed a high-protein diet produced significantly greater amounts of PGE_2, 6-keto $PGF_{1\alpha}$, and TxB_2 by approximately 1.8 times than glomeruli from SOC rats fed the same diet. By contrast, the glomerular production of the three eicosanoids was comparable in SOC and BUO rats fed a low-protein diet.

Pretreatment of rats with the ACE inhibitor, enalaprilat, prior to sham operation decreased the glomerular production of PGE_2, 6-keto $PGF_{1\alpha}$, and TxB_2 in SOC rats fed a high-protein diet to levels comparable to those observed in SOC rats fed a low-protein diet. Likewise, inhibition of the endogenous angiotensin II generation with enalaprilat prior to obstruction markedly decreased the glomerular production of PGE_2, 6-keto $PGF_{1\alpha}$, and TxB_2 by 71%, 60%, and 57%, respectively, in rats with BUO fed a high-protein diet. Eicosanoid production levels in glomeruli of rats with BUO fed a high-protein diet were not significantly different from those of SOC rats fed a low-protein diet. Enalaprilat did not affect glomerular eicosanoid production in SOC and BUO rats fed a low-protein diet. No significant difference was observed in the glomerular production of eicosanoids between low protein-fed SOC and BUO rats that received enalaprilat or between low protein-fed SOC or BUO rats that were given or not given the ACE inhibitor.

Levels of Cyclooxygenase in Glomeruli. Table II depicts the quantity of cyclooxygenase in glomerular membranes obtained from low or high protein-fed SOC and BUO rats that were or were not given the ACE inhibitor, enalaprilat, prior to sham operation or obstruction. A well-characterized antibody against cyclooxygenase (12, 14) was used for Western blotting to detect glomerular cyclooxygenase. Immunoidentifiable bands below 72 kDa in size probably reflect breakdown products of cyclooxygenase, since preadsorption of the antibody eliminates their presence and they appear in immunoprecipitates of metabolically labeled cyclooxygenase (12, 14). Detectable amounts of cyclooxygenase (72 kDa) existed in membranes of glomeruli (Fig. 1) but not in cytosolic extracts of glomeruli. The levels (arbitrary units) of cyclooxygenase were determined in five separate glomerular membranes of each group of rats.

The mass of cyclooxygenase was significantly in-

Table I. Eicosanoid Production by Glomeruli Isolated from Low or High Protein-Fed SOC and BUO Rats That Did or Did Not Receive Enalaprilat Prior to Sham Operation or Obstruction

	Rats not given enalaprilat				Rats given enalaprilat			
	Low protein		High protein		Low protein		High protein	
	SOC	BUO	SOC	BUO	SOC	BUO	SOC	BUO
PGE_2	656 ± 91	542 ± 113	1928 ± 301[a]	3530 ± 447[a,b]	584 ± 78	639 ± 115	735 ± 80[c]	1013 ± 144[c]
6-keto $PGF_{1\alpha}$	63 ± 14	72 ± 12	117 ± 17	209 ± 38[a,b]	50 ± 8	56 ± 11	60 ± 8	84 ± 19[c]
TxB_2	53 ± 11	49 ± 11	118 ± 14[a]	211 ± 37[a,b]	63 ± 7	56 ± 4	73 ± 8	90 ± 7[c]

ANOVA P value	PGE_2	6-keto $PGF_{1\alpha}$	TxB_2
Protein	0.0001	0.0001	0.0001
BUO	0.0001	0.0001	0.0001
Enalaprilat	0.0001	0.0001	0.0001
Protein × BUO	0.0001	0.0001	0.0001
Protein × enalaprilat	0.0001	0.0001	0.0001
BUO × enalaprilat	0.0001	0.0017	0.0001
Protein × BUO × enalaprilat	0.0001	0.0038	0.0003

Note. Data reported represent means (pg/mg protein/10 min of incubation) ± SE of values obtained from six separate preparations. SOC, sham-operated control; BUO, bilateral ureteral obstruction; PGE_2, prostaglandin E_2; 6-keto $PGF_{1\alpha}$, 6-keto prostaglandin $F_{1\alpha}$; TxB_2, thromboxane B_2.

[a] $P < 0.05$ compared with the value of the low protein-fed SOC group that was or was not pretreated with enalaprilat.

[b] $P < 0.05$ when comparing the value of the high protein-fed BUO versus SOC group that was or was not pretreated with enalaprilat.

[c] $P < 0.05$ compared with the value of each group that was not pretreated with enalaprilat.

Table II. Cyclooxygenase Mass in Glomeruli Isolated from Low or High Protein-Fed SOC and BUO Rats That Did or Did Not Receive Enalaprilat Prior to Sham Operation or Obstruction

	Rats not given enalaprilat				Rats given enalaprilat			
	Low protein		High protein		Low protein		High protein	
	SOC	BUO	SOC	BUO	SOC	BUO	SOC	BUO
Cyclooxygenase	3.38 ± 0.75	3.53 ± 0.17	6.25 ± 0.63[a]	8.28 ± 0.65[a,b]	3.38 ± 0.54	4.13 ± 0.85	4.50 ± 0.53[c]	5.03 ± 0.38[c]

ANOVA *P* value	Cyclooxygenase
Protein	0.0001
BUO	0.0001
Enalaprilat	0.0001
Protein × BUO	0.0350
Protein × enalaprilat	0.0001
BUO × enalaprilat	NS
Protein × BUO × enalaprilat	0.0089

Note. Data reported are in arbitrary units per 2.5 μg of glomerular membrane protein. Values are means ± SE obtained from five separate glomerular preparations. SOC, sham-operated control; BUO, bilateral ureteral obstruction; NS, not significant.
[a] $P < 0.05$ compared with the value of the low protein-fed SOC group that was or was not pretreated with enalaprilat.
[b] $P < 0.05$ when comparing the value of the high protein-fed BUO versus SOC group that was or was not pretreated with enalaprilat.
[c] $P < 0.05$ compared with the value of each group that was not pretreated with enalaprilat.

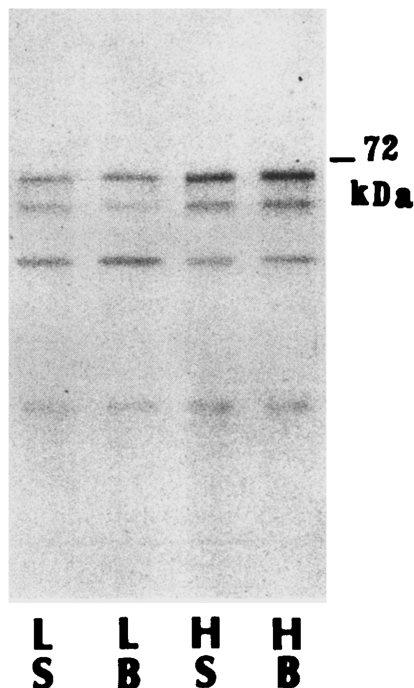

Figure 1. Western blots of cyclooxygenase (72 kDa) in glomerular membranes (2.5 μg protein) obtained from SOC and BUO rats fed a low- or a high-protein diet. SOC, sham-operated control; BUO, bilateral ureteral obstruction; LS, low protein-fed SOC rats; LB, low protein-fed BUO rats; HS, high protein-fed SOC rats; HB, high protein-fed BUO rats.

creased by 1.8 times in SOC rats fed a high-protein diet when compared with those fed a low-protein diet. Similarly, rats with BUO given a high-protein diet had significantly increased amounts of cyclooxygenase by 1.3 times when compared with SOC rats fed the same diet. By contrast, the mass of cyclooxygenase in rats with BUO fed a low-protein diet was not significantly different from that of SOC rats fed the same diet.

Pretreating rats with the ACE inhibitor, enalaprilat, prior to sham operation decreased the mass of cyclooxygenase in SOC rats fed a high-protein diet to levels comparable to that observed in SOC rats fed a low-protein diet. Again, the amount of cyclooxygenase was significantly reduced by 39% in rats with BUO fed a high-protein diet when the rats were pretreated with enalaprilat prior to obstruction. As with eicosanoid production, the mass of cyclooxygenase in rats with BUO fed a high-protein diet was essentially comparable to that of SOC rats fed a low-protein diet. By contrast, enalaprilat did not affect cyclooxygenase content in SOC and BUO rats fed a low-protein diet. There was no significant difference in the mass of cyclooxygenase between low protein-fed SOC and BUO rats that received enalaprilat or between low protein-fed SOC or BUO rats that were pretreated or not with the ACE inhibitor.

Role of Cyclooxygenase Mass in Glomerular Eicosanoid Production. We found previously a progressive linear increase in the activity and mass of glomerular cyclooxygenase and in the production of glomerular eicosanoids as protein intake was increased (14). The measure of cyclooxygenase activity essentially extrapolated to zero activity of the enzyme with zero protein in a diet (14). Immunoblots, however, measured more amounts of cyclooxygenase in glomerular membranes of a low-protein diet group than could be accounted for by the activity, suggesting that a pool of immunologically identifiable cyclooxygenase enzyme exists that is inactive (14).

Figure 2 shows a relationship between PGE_2 production and cyclooxygenase mass in glomeruli isolated from low or high protein-fed SOC and BUO rats that did or did not receive the ACE inhibitor, enalaprilat, prior to sham operation or obstruction. This relation-

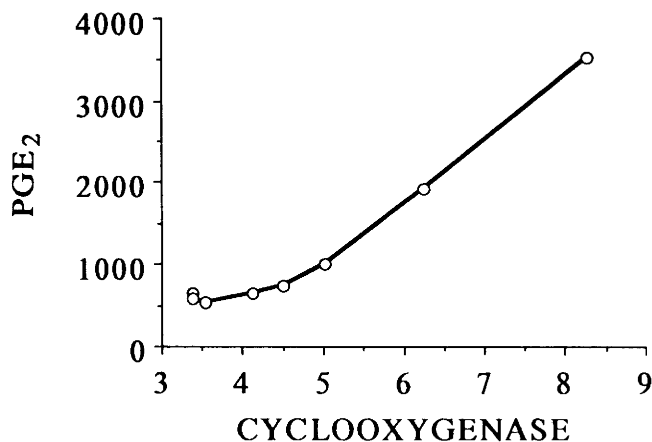

Figure 2. Relationship between cyclooxygenase mass (arbitrary units) and PGE_2 production (pg/mg protein/10 min of incubation) in glomeruli isolated from SOC and BUO rats fed a low- or a high-protein diet and given or not given enalaprilat prior to sham operation or obstruction. PGE_2, prostaglandin E_2; SOC, sham-operated control; BUO, bilateral ureteral obstruction.

ship was determined from the mean values of PGE_2 and cyclooxygenase shown in Tables I and II. The production of PGE_2, a major eicosanoid synthesized by rat glomeruli, was progressively enhanced with increases in the amount of cyclooxygenase, which indicates that changes in cyclooxygenase mass are directly linked to the regulation of glomerular eicosanoid production in this model. Accordingly, in this study we determined the quantity of glomerular cyclooxygenase instead of the activity of this enzyme.

Plasma Levels of Angiotensin II and Bradykinin and Serum ACE Activity. Table III presents plasma levels (pg/ml) of angiotensin II and bradykinin and serum ACE activity (nmole/min/ml) in low or high protein-fed SOC and BUO rats that were given or not given the ACE inhibitor, enalaprilat, prior to sham operation or obstruction. These values were determined in five separate blood samples of each group of rats.

Levels of angiotensin II and ACE activity were significantly greater by 1.9 and 1.3 times, respectively, in high protein-fed SOC rats than in low protein-fed SOC rats. Also, ACE activity was significantly (1.7 times) greater in high protein-fed BUO rats than in high protein-fed SOC rats. This finding is similar to that of rats with unilateral ureteral ligation fed a standard (23% protein) diet (22). However, levels of angiotensin II were substantially decreased in high protein-fed BUO versus SOC rats and the value was not significantly different from that of low protein-fed SOC rats while the renin-angiotensin system is well known to be enhanced shortly after the onset of obstruction (1, 2, 22), suggesting the rapid turnover of angiotensin II at 24 hr after the induction of BUO in rats fed a high protein diet. On the other hand, the altered levels of angiotensin II in high protein-fed SOC and BUO rats were restored to levels comparable to those of low protein-fed SOC rats when the rats were pretreated with enalaprilat. Again, prior administration of enalaprilat prevented the increase in ACE activity in high protein-fed SOC and BUO rats. The value of high protein-fed SOC rats was comparable to that of low protein-fed SOC rats. The value of high protein-fed BUO rats was markedly decreased by 91% when compared with that of low protein-fed SOC rats. Additionally, angiotensin II levels and ACE activity were not significantly different between low protein-fed SOC rats that were or were not pretreated with enalaprilat.

Levels of bradykinin were comparable in low or high protein-fed SOC rats. Bradykinin levels were significantly increased by 1.8 times in high protein-fed BUO rats when compared with high protein-fed SOC rats. Pretreatment of rats with enalaprilat further increased levels of bradykinin by 1.6 times in high protein-fed SOC rats and by 1.5 times in high protein-fed BUO rats. However, enalaprilat did not affect bradykinin levels in low protein-fed SOC rats.

Discussion

SOC rats fed a high-protein (40% casein) diet had significantly greater production of glomerular PGE_2,

Table III. Plasma Concentrations of Angiotensin II and Bradykinin and Serum ACE Activity in Low or High Protein-Fed SOC or BUO Rats That Did or Did Not Receive Enalaprilat Prior to Sham Operation or Obstruction

	Rats not given enalaprilat			Rats given enalaprilat		
	Low protein	High protein		Low protein	High protein	
	SOC	SOC	BUO	SOC	SOC	BUO
ANG II	31.0 ± 3.5	58.4 ± 5.8[a]	20.8 ± 3.2[b]	36.6 ± 4.5	29.2 ± 2.9[c]	33.0 ± 6.2
ACE	20.0 ± 1.6	26.7 ± 1.5[a]	44.5 ± 1.2[a,b]	15.9 ± 1.1	15.5 ± 1.8[c]	3.9 ± 0.5[a,b,c]
BK	9.7 ± 1.0	12.1 ± 1.3	21.7 ± 1.4[a,b]	11.8 ± 1.2	18.8 ± 1.9[a,c]	32.7 ± 2.9[a,b,c]

Note. Levels (pg/ml) of ANG II and BK and ACE activity (nmole/min/ml) are means ± SE of values obtained from five rats, SOC, sham-operated control; BUO, bilateral ureteral obstruction; ANG II, angiotensin II; ACE, angiotensin converting enzyme; BK, bradykinin.

[a] $P < 0.05$ compared with the value of the low protein-fed SOC group that was pretreated or not with enalaprilat.
[b] $P < 0.05$ when comparing the value of the high protein-fed BUO versus SOC group that was or was not pretreated with enalaprilat.
[c] $P < 0.05$ compared with the value of each group that was not pretreated with enalaprilat.

6-keto $PGF_{1\alpha}$, and TxB_2 than SOC rats fed a low-protein (6% casein) diet. There was a significant increase in the mass of glomerular cyclooxygenase in SOC rats fed a high versus a low protein diet. Blockade of the endogenous angiotensin II generation with the ACE inhibitor, enalaprilat, prior to sham operation returned the increase in eicosanoid production and cyclooxygenase mass in glomeruli isolated from SOC rats fed a high-protein diet to levels comparable to those seen in glomeruli obtained from SOC rats fed a low-protein diet. These measurements were paralleled by both plasma levels of angiotensin II and serum ACE activity. As reported by Don *et al.* (13), these results provide evidence that greater protein intake increases glomerular eicosanoid production via activation of the cyclooxygenase pathway due to enhanced activity of the renin-angiotensin system in SOC rats.

Glomeruli isolated from rats with BUO fed a high-protein diet produced significantly more PGE_2, 6-keto $PGF_{1\alpha}$, and TxB_2, and had substantially increased amounts of cyclooxygenase when compared with glomeruli obtained from SOC rats fed the same diet. Prior inhibition of the endogenous angiotensin II synthesis with enalaprilat prevented the increases in eicosanoid production and cyclooxygenase content of glomeruli obtained from high protein-fed BUO rats. The levels of eicosanoids and cyclooxygenase were less than those observed in glomeruli isolated from high protein-fed SOC rats and were not essentially different from the levels seen in glomeruli obtained from low protein-fed SOC rats. These results indicate that the ACE inhibitor, enalaprilat, prevented activation of the cyclooxygenase pathway by blocking a further increase in the activity of the renin-angiotensin system caused by BUO versus SOC in rats ingesting a high-protein diet. Indeed, serum ACE activity was significantly increased in high protein-fed BUO rats than in high protein-fed SOC rats although plasma levels of angiotensin II could not be evaluated probably because of its altered turnover caused by 24 hr after the onset of BUO in rats fed a high-protein diet. Moreover, enalaprilat significantly inhibited the increased ACE activity in high protein-fed BUO rats and the value was markedly lower than that of low protein-fed SOC rats. Thus, under the condition of high protein intake the renin-angiotensin system may have a major role in enhancing the cyclooxygenase pathway not only in glomeruli of SOC rats but also in glomeruli of BUO rats.

In a previous study (12) we found that in rats fed a standard (23% protein) diet BUO enhanced glomerular eicosanoid production via angiotensin II-induced increases in the activities of phosphatidylethanolamine (PE)-specific PLA_2 and cyclooxygenase. The activities of phosphatidylcholine (PC)-specific and PE-specific PLA_2 and cyclooxygenase were significantly increased in membranes of glomeruli from rats with BUO compared with SOC rats (12). The activities of PE-specific PLA_2 and cyclooxygenase in membranes of glomeruli from rats with BUO pretreated with enalaprilat prior to obstruction were decreased and comparable to those in membranes of glomeruli from SOC rats pretreated or not with the drug (12). By contrast, PC-specific PLA_2 activities were further increased in glomerular membranes of SOC and BUO rats when the rats were pretreated with enalaprilat (12). As shown in Table III, plasma levels of bradykinin in high protein-fed SOC and BUO rats that were pretreated or not with enalaprilat paralleled the activities of PC-specific PLA_2 in glomerular membranes of standard chow-fed SOC and BUO rats that were given or not given the drug. This suggests but does not prove that further activation of PC-specific PLA_2 in glomerular membranes of SOC and BUO rats after administration of enalaprilat is due to an increase in bradykinin levels as a consequence of inhibition of kininase II. Again, in the present study prior administration of enalaprilat increased plasma levels of bradykinin and decreased both the glomerular production of eicosanoids and the mass of cyclooxygenase in high protein-fed SOC and BUO rats. Thus, bradykinin does not appear to be a key hormone responsible for enhancing glomerular eicosanoid production in high protein-fed SOC and BUO rats.

Glomeruli of BUO rats fed a low-protein diet had no increase in the production of PGE_2, 6-keto $PGF_{1\alpha}$, and TxB_2 and the amounts of cyclooxygenase. Both eicosanoid production and cyclooxygenase mass were comparable in glomeruli of SOC and BUO rats fed a low-protein diet. Moreover, the ACE inhibitor, enalaprilat, had no effect on the production of eicosanoids and the mass of cyclooxygenase in glomeruli in both groups of rats fed a low-protein diet. These observations indicate that in the BUO model a low-protein diet prevents activation of the cyclooxygenase pathway by inhibiting an increase in the activity of the renin-angiotensin system which occurs after the onset of BUO. This may be the primary mechanism by which a low-protein diet suppresses the rise in glomerular eicosanoid production in rats with BUO.

Previous micropuncture studies (8) reported that changes in protein intake modified the renal hemodynamics observed after BUO of 24 hr duration. There was a markedly greater fall in SNGFR and Q_A in rats with BUO fed a high-protein (40% casein) diet for approximately 4 weeks than in those fed a low-protein (6% casein) diet for the same period of time (8). The changes in renal hemodynamics seen in high protein-fed BUO rats appear to be the result of severe renal vasoconstriction and a decrease in K_f as a consequence of the combined action of at least two potent vasoconstrictors, angiotensin II and TxA_2. On the other hand, the higher values for SNGFR and Q_A ob-

served in low protein-fed BUO rats may be due in part to inhibition of the action of the two vasoconstrictors as a consequence of dietary protein restriction. Accordingly, it is suggested that the amount of dietary protein ingested determines the degree of renal vasoconstriction by affecting the production rates and/or activities of vasoconstrictive hormones such as angiotensin II and TxA_2. The inhibitory effects of low protein intake on the production of glomerular TxA_2 and on the activity of the renin-angiotensin system may be one of the mechanisms by which dietary protein restriction prevents the fall in SNGFR and Q_A observed after the onset of BUO.

In summary, this is the first study that evaluates the effects of dietary protein manipulation on glomerular eicosanoid production in rats with BUO of 24 hr duration. High protein intake augmented glomerular eicosanoid production by enhancing the cyclooxygenase pathway in SOC rats. This enhanced cyclooxygenase pathway may be the consequence of activation of the renin-angiotensin system. High protein intake also caused a further increment in glomerular eicosanoid production in rats with BUO when compared with SOC rats. This is due to further activation of the cyclooxygenase pathway primarily via further increased levels of the renin-angiotensin system caused by BUO versus SOC. Low protein intake completely abolished a rise in glomerular eicosanoid production seen after the induction of BUO, which comes from no activation of the cyclooxygenase pathway presumably due to suppressing the renin-angiotensin system. Dietary protein-induced modulations of the glomerular production of eicosanoids and the activity of the renin-angiotensin system may influence altered renal hemodynamics as a consequence of BUO by changing the degree of renal vasoconstriction.

This work was supported by U.S.P.H.S. NIDDK Grants DK-09976, DK-07126, DK-40321, and DK-30178, and by Grants (No. 05454217) for Scientific Research from the Ministry of Education, Science, and Culture of Japan.

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