Dietary Alteration of Dihomogamma-Linolenic Acid/Arachidonic Acid Ratio in a Rat 5/6-Renal-Ablation Model

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ABSTRACT

Interest in the modulation of renal diseases by polyunsaturated fatty acids (PUFA) led this group to examine the effects of borage oil (BO) and corn oil (CO) in the rat 5/6-renal-ablation model. BO is a rich source of gamma-linolenic acid (GLA; 18:3n-6), which is elongated to dihomogamma-linolenic acid (DGLA; 20:3n-6). CO is a rich source of linoleic acid (LA; 18:2n-6), a GLA and arachidonic acid (AA; 20:4n-6) precursor. The purpose of this study was to assess whether an increased DGLA:AA ratio as provided by BO would confer benefits beyond those provided by LA present in corn oil. Forty rats were used for the experiment. Seven rats were used for presurgery measurements. The remaining animals were subjected to 5/6 nephrectomy. Surviving rats (N = 30) were fed regular laboratory diet (RLD) for 7 days, at which time seven rats were used to obtain 1-wk postnephrectomy data. The remainder were then allocated to receive either RLD (N = 8), 15% BO (N = 8), or 15% CO (N = 7) diets for 20 wk. Body weight, renal phospholipid levels, renal function (proteinuria and GFR), glomerular histology, glomerular macrophage infiltration, urinary prostaglandin levels (thromboxane B2 (TxB2), 6-keto-

PGF1α), plasma lipid levels, and blood pressure were measured. Diets were well tolerated by all groups with a similar age-related gain in weight throughout the study. Efficacy of the PUFA diets was confirmed by alteration in renal tissue phospholipids; LA decreased in the RLD and BO groups, but not in the CO group. AA was higher in the BO and CO rats, but only the BO group showed a rise in GLA and DGLA incorporation. Proteinuria increased progressively in the RLD group but remained at 1-wk postsurgery levels in the BO and CO groups. Decline in GFR and mesangial expansion were significantly lessened by BO supplementation only. Both PUFA diets limited glomerulosclerosis and macrophage infiltration, but direct comparisons between BO and CO groups revealed significantly less glomerulosclerosis and macrophage infiltration in the BO group. Both BO and CO attenuated the rise in the TxB2 excretion rate and restored the 6-keto-PGF1α:TxB2 ratio to the 1-wk postsurgery level. Plasma lipid levels rose in all groups, but the rise in cholesterol level was less in the BO and CO rats, CO being the most efficacious in this regard. BP increased progressively in RLD rats, but not in the BO and CO groups, BO providing a markedly greater hypotensive effect. In summary, both CO and BO supplemented PUFA diets limited glomerular injury in the renal-ablated rats. However, BO supplementation was more effective than CO supplementation at preserving GFR, limiting mesangial expansion and glomerulosclerosis, and reducing glomerular macrophage infiltration.

Key Words: Gamma-linolenic acid, linoleic acid, glomerular injury, lipids, hypertension

The rat renal-ablation model represents renal diseases associated with glomerular scarring and progressive decline in renal function (1–4). The excision of one kidney and infarction of 2/3 of the other results in the reduction of functional nephrons below a critical number. The nephrons quickly enlarge, with an increase in single-nephron filtration rate and intraglomerular hydraulic pressure (5). The mechanisms responsible for the progression of renal failure are not known, but the changes that occur in glomerular hydraulic pressure, systemic and glomerular hypertension, lipids, prostaglandins and other autacoids have been implicated (6–8). It is likely that these
factors interact in the process of progressive scarring that ensues in remnant nephrons.

We have been interested in the effects of polyunsaturated fatty acids (PUFA) in the modulation of human and experimental renal diseases (9–11). The focus of this study was to assess the effects of borage oil (BO), rich in gamma-linolenic acid (GLA), and corn oil (CO), rich in linoleic acid (LA) in a rat 5/6-renal-ablation model of progressive glomerular injury. Dietary supplementation with GLA or LA has been shown to have antihypertensive, anti-inflammatory, and lipid-lowering effects (12–14). Both oils are known to increase arachidonic acid (AA) incorporation into cell membranes, resulting in production of vasodilatory prostacyclin and prostaglandins of the E series, which have been shown to be beneficial in renal diseases (15). LA, the major PUFA in CO, is converted to AA via GLA and DGLA, but the rate-limiting activity of the 5-6 desaturase enzyme that converts LA to GLA prevents the accumulation of the intermediates GLA and DGLA (16). In contrast, BO contains large amounts of GLA, thus bypassing the rate-limiting first step and allowing accumulation of GLA and DGLA (16).

GLA and DGLA and its metabolites may have salutary effects beyond those provided by LA or AA. Macrophages have been shown to incorporate DGLA (17) and metabolize it to 15-HETE (18), a compound with anti-inflammatory effects that also alters AA metabolism to decrease the production of the proinflammatory and vasoconstrictive leukotrienes (18). Mesangial cells can produce the same compounds (19,20).

Some beneficial effects of evening primrose oil, a GLA source, have been reported in a rat renal-ablation model (15). In the study presented here, BO was used because it provides greater amounts of GLA (six times as much GLA per unit weight as compared with evening primrose oil) (16). The purpose of this study was to compare the functional and histologic effects of these dietary maneuvers in the renal-ablated rat and assess potential mechanisms for glomerular injury.

**METHODS**

**Experimental Design**

The animal experimentation protocol was in accord with the Canadian Council on Animal Care guidelines and approved by the University Council on Animal Care, University of Western Ontario. Forty adult male Sprague-Dawley rats weighing 273 ± 6 g were used for the study. Three days before surgery, a 24-h urine sample was obtained. Just before surgery, blood pressure (BP) was recorded and blood was collected for plasma lipid measurements (N = 7). The 14C-inulin clearance rate (GFR) was also measured in these rats to obtain baseline values. These rats were then euthanized for histologic measurements. On the day of surgery, the remaining rats were anesthetized with a 1:1 mixture of ketamine (100 mg/mL):xylazine (20 mg/mL) at a dose of 0.15 mL/100 g body weight. The right kidney was removed and two of the three vessels supplying the left kidney were doubly ligated. Two anesthesia-related deaths and one postoperative death occurred within 3 days of surgery. Data from these animals were excluded. No further mortality was observed during the study.

After renal ablation, rats were maintained on regular laboratory diet (RLD) for 1 wk. Blood pressure, an 18-h urine sample, and blood for lipid level and 14C-inulin clearance rate measurements were then obtained from seven rats. These rats were then euthanized for histologic measures. The remaining rats were then randomly assigned to receive either a 15% CO (N = 7) or a 15% BO (N = 8) experimental diet or continued on the regular laboratory diet (RLD) (N = 8). The dietary compositions are listed in Table 1. To ensure that calorie intakes were identical (82 to 83 kcal/rat per day) rats were fed either 25 g/day of RLD or 24 g/day of the experimental diets. Twenty weeks after dietary assignment, all measures were repeated.

**Renal Phospholipids**

The fatty acid profiles of renal phospholipids were measured to assess the bioavailability of the specific PUFA-derived fatty acids (increased AA in response to the CO diet and increased GLA and DGLA in response to the BO diet), using a previously described method (21). In brief, at 20 wk after surgery, the collection of blood samples for lipid

**TABLE 1. Diet formulae**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Source/Type</th>
<th>% by Weight</th>
<th>Source/Type</th>
<th>% by Weight</th>
<th>Source/Type</th>
<th>% by Weight</th>
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</thead>
<tbody>
<tr>
<td>Protein</td>
<td>Mixed animal and vegetable</td>
<td>23.40</td>
<td>Casein</td>
<td>24.00</td>
<td>Casein</td>
<td>24.00</td>
</tr>
<tr>
<td>Fat</td>
<td>Mixed animal and vegetable</td>
<td>4.50</td>
<td>Borage oil</td>
<td>15.00</td>
<td>Corn oil</td>
<td>15.00</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>Starch/sugars</td>
<td>49.00</td>
<td>Corn starch</td>
<td>36.00</td>
<td>Corn starch</td>
<td>36.00</td>
</tr>
<tr>
<td>Nondigestible</td>
<td>Mixed fiber</td>
<td>15.80</td>
<td>Cellulose</td>
<td>19.00</td>
<td>Cellulose</td>
<td>19.00</td>
</tr>
<tr>
<td>Mineral Salts and Vitamins</td>
<td>Ash: Mineral and Vitamin</td>
<td>7.30</td>
<td>USP:XIV salts</td>
<td>4.75</td>
<td>USP:XIV salts</td>
<td>4.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Vitamin fortification</td>
<td>1.00</td>
<td>Vitamin fortification</td>
<td>1.00</td>
</tr>
<tr>
<td>Physiological Fuel Value (Kcal/g)</td>
<td></td>
<td>3.30</td>
<td>Choline chloride</td>
<td>0.25</td>
<td>Choline chloride</td>
<td>0.25</td>
</tr>
</tbody>
</table>

* Regular laboratory diet (RLD) was obtained from Purina Mills Inc., St. Louis, MO. Ingredients for the experimental diets were obtained from ICN Biochemicals, Cleveland, OH. Santoquin (ICN Biochemicals, Cleveland, OH) was added to the diets (0.05% of the oil) to avoid oxidation.
and serum creatinine measurements, the animals were euthanized by CO₂ overexposure. The remnant kidneys were quickly dissected into three portions. A portion was snap-frozen in liquid nitrogen for renal phospholipid analysis, a cortical portion was immersed in OCT compound (Miles Inc., Diagnostic Division, Elkhart, IN) and frozen in liquid nitrogen for immunohistochemistry, and the remainder was fixed in 10% buffered formalin for light microscopy. For fatty acid analysis, methanol chloroform extracts of the kidney tissues were subjected to silicon-gel thin-layer chromatography. The fatty acid fractions thus isolated were subsequently separated and compared to known fatty acid standards using a Hewlett Packard Model 5890A gas chromatograph, equipped with a DB 25 megabore column (Chromatographic Specialties, Brockville, Ontario, Canada).

Renal Physiology

Glomerular Filtration Rate. The ¹⁴C-inulin clearance rate (GFR) was measured with an Alzet® mini-osmotic pump (Alza Corp., Palo Alto, CA) according to the method of Jobin and Bonjou with a slight modification as described previously (22) and expressed as ml/min per kg body weight.

Proteinuria. Proteinuria was assessed in 24-h urine samples obtained by placing the animals individually in metabolic cages (MED-TECK, Woodstock, Ontario, Canada). The proteinuria was measured by a routine sulfosalicylic precipitation method.

Glomerular Histology

Light Microscopy. Three-micrometer sections of the formalin-fixed and plastic-embedded renal tissues were cut and stained with periodic acid-silver methenamine. All tissues were evaluated by two examiners blinded to the treatment groups to which the rats belonged. Sections from preoperative control rats were used as the reference for the mesangial expansion and glomerulosclerosis scoring. Twenty-five glomeruli were examined from each tissue as previously described (21). Mesangial expansion refers to the extent by which the mesangium expanded into the glomerular capillary loops relative to the control rats. This was graded as 0 (no expansion), 1 (moderate expansion), or 2 (global expansion).

Immunohistochemistry. Four-micrometer cryostat sections were mounted on poly-L-lysine-coated slides, fixed in acetone (4°C), and air-dried. Sections were washed for 5 min in tap water and incubated overnight with a 1:250 diluted monoclonal anti-macrophage antibody (anti-ED-1; Chemicon Int., Temecula, CA). Sections were then washed with phosphate-buffered saline and incubated for 30 min at room temperature with a 1:200 diluted anti-mouse immunoglobulin G (IgG) biotinylated antibody (Vectastain Elite Kit; Vector Laboratories, Burlingame, CA). Finally, sections were washed and incubated for 30 min with a 1:50 diluted avidin-biotin-peroxidase complex. Sites of peroxidase activity were visualized by incubation in DAB solution (Zymed Laboratories, San Francisco, CA) for 1 min. Sections were counterstained with Harris’ Modified Hematoxylin with Acetic Acid (Fisher Scientific, Unionville, Ontario, Canada) for 5 s, washed with tap water, and covered with a glass slip. Negative control experiments were performed by omitting the primary antibody. Sections were scored as the number of positive-staining cells per glomerulus/total number of cells per glomerulus. Twenty-five glomeruli per section were counted.

Urinary Prostaglandins

Urine samples were subjected to liquid phase extraction. In brief, 1 mL of urine was mixed with 2 mL of acetone. The mixture was centrifuged at 2500 rpm for 10 min at 4°C. Two ml of hexane was added to the supernatant, mixed vigorously, and centrifuged as described above. The upper hexane layer was discarded. The pH of the lower level was adjusted to 3.5 by using citric acid, then 2 mL of chloroform was added and mixed for 2 min. The mixture was centrifuged as described earlier. The upper layer was discarded and the lower chloroform layer was dried under nitrogen and reconstituted to 1 mL using the assay buffer provided with the kit. Urinary thromboxane B₂ (TxB₂) and 6-keto-PGF₁α were measured using the respective RIA kits (Amersham Canada Ltd., Oakville, Ontario, Canada).

Plasma Lipids

Blood samples for plasma lipid measurements were collected by cardiac puncture into tubes containing EDTA (1.5 mg/mL). The plasma cholesterol and triglycerides were assessed using enzymatic reagents (cholesterol, CHOD-PAP; triglyceride, without free glycerol; Boehringer-Mannheim, Montreal, Quebec, Canada) as described previously (10).

Blood Pressure

Blood pressure was measured by a tail plethysmographic method utilizing the Harvard Apparatus Rat Tail Blood Pressure System (Harvard Bioscience, South Natick, MA) as described previously (10).

Statistics

Sample Size. Sample size calculations were based on GFR measurements. In previous studies, RLD-fed rats had a SD of 0.7 mL/min/kg for GFR measurements at 20 wk. A difference of 2 × SD at 20 wk was desired. This yielded a sample size of 6.5 rats/group based on α = 0.05 (Zα for two-tailed test = 1.96) with a desired power of 0.95 (Zβ = 1.64). To allow for baseline (N = 7) and 1-wk postnephrectomy (N = 7) measurements and surgery-related mortality, 40 rats were used.

Data Analysis. The changes in measurements over the study period were assessed by the analysis of variance, followed by Bonferroni’s t tests to determine the source of the difference(s). Proteinuria, which was not normally distributed, was compared using Kruskal-Wallis analysis followed by its multiple comparison technique. The value of P < 0.05 was considered significant.

RESULTS

Dietary Intake and Body Weight

Diets were totally consumed within 24 h and the weight gain was similar in all groups, rising from a mean of 275 ± 16 g before surgery to 295 ± 23 g at 1
wk after nephrectomy to 560 ± 66 g for RLD, 560 ± 70 g for CO, and 528 ± 38 g for BO at 20 wk after surgery.

Renal Phospholipids

Profiles of selected fatty acids from total phospholipid fractions from the remnant renal tissues obtained at 20 wk in the three groups are shown in Table 2. The LA (18:2n-6) content was lower in the tissue of the CO (12.6 mol%) and the BO (9.1 mol%)-fed rats compared with the RLD-fed rats (14.6 mol%). Levels of AA (20:4n-6) increased significantly in the CO (30.5 ± 0.9 mol%) and the BO (29.3 ± 0.4 mol%) groups compared with the RLD group (17.0 mol%). As expected, the GLA (18:3n-6) and DGLA (20:3n-6) levels increased in the BO group only and were significantly greater than in the CO or RLD groups.

Renal Physiology

Glomerular Filtration Rate. The inulin clearance rate (GFR) declined at 1 wk and continued to decline in the RLD and CO groups. This decline was significantly attenuated in the BO group (Table 3).

Proteinuria. The urinary protein excretion rate rose significantly at 1 wk after nephrectomy (Table 3) and continued to rise in the RLD group over the 20-wk period. Both the CO and BO diets were equally effective in reducing proteinuria.

Glomerular Histology

Light Microscopy. Mesangial expansion was significantly increased in the RLD and CO groups at 20 wk compared with the 1-wk data. This increase in expansion was not seen in the BO group (Table 4). Glomerulosclerosis was also increased at 20 wk in the RLD and CO groups but not the BO group.

Immunohistochemistry. The ED1-immunohistochemistry staining pattern for the intraglomerular macrophages is depicted in Figure 1 (A and B). The glomerular macrophage counts are shown in Figure 2.

Table 2. Renal phospholipid levels (mol% of total phospholipid levels)

<table>
<thead>
<tr>
<th>Phospholipid</th>
<th>Diet</th>
<th>Week 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>18:2n6</td>
<td>RLD</td>
<td>14.6 ± 0.1</td>
</tr>
<tr>
<td>(LA)</td>
<td>Corn oil</td>
<td>12.6 ± 0.4a</td>
</tr>
<tr>
<td></td>
<td>Borage oil</td>
<td>9.1 ± 0.7ab</td>
</tr>
<tr>
<td>20:4n6</td>
<td>RLD</td>
<td>17.0 ± 0.8</td>
</tr>
<tr>
<td>(AA)</td>
<td>Corn oil</td>
<td>30.5 ± 0.9a</td>
</tr>
<tr>
<td></td>
<td>Borage oil</td>
<td>29.3 ± 1.2a</td>
</tr>
<tr>
<td>18:3n6</td>
<td>RLD</td>
<td>Not detected</td>
</tr>
<tr>
<td>(GLA)</td>
<td>Corn oil</td>
<td>Not detected</td>
</tr>
<tr>
<td></td>
<td>Borage oil</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td>20:3n6</td>
<td>RLD</td>
<td>0.9 ± 0.0</td>
</tr>
<tr>
<td>(DGLA)</td>
<td>Corn oil</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Borage oil</td>
<td>2.5 ± 0.0ab</td>
</tr>
</tbody>
</table>

a Significantly different compared with RLD.
b Significantly different compared with corn oil.

Macrophage infiltration was observed in all experimental groups at 20 wk, but this was significantly lower in the BO and CO groups, the BO diet being significantly more protective than the CO diet in this regard.

Urinary Prostaglandins

The urinary prostaglandin levels are shown in Table 5. The TxB2 levels rose progressively to Week 20 in the RLD group. This rise was significantly attenuated by both the CO and BO diets, with 20-wk values being similar to the 1-wk values. The 6-keto-PGF1α values remained similar in all groups throughout the study, resulting in an increased 6-keto-PGF1α:TxB2 ratio in both the BO and CO groups versus the RLD group.

Plasma Lipids

Plasma lipid levels are shown in Table 6. Plasma cholesterol levels increased significantly in the RLD (264%) and the BO (78%) groups at 20 wk but remained similar to the 1-wk level in the CO group. The plasma triglyceride level rose significantly in all groups, but the rise was attenuated in the CO rats.

Blood Pressure

Blood pressure rose significantly at 1 wk after surgery (171 ± 19 mm Hg) from the presurgery value of 143 ± 14 mm Hg, and continued to rise in the RLD group through to 20 wk (176 ± 22 mm Hg), but declined in both the CO group (136 ± 19 mm Hg) and the BO group (89 ± 12 mm Hg) at 20 wk. Twenty-week values in CO were significantly lower than RLD at 20 wk or 1-wk values. Twenty-week BO values were significantly lower than all other time points.

DISCUSSION

Ablation of 5/6 of the renal mass in rats leads to the development of systemic and glomerular hypertension, progressive proteinuria, declining glomerular filtration rate (GFR), and glomerulosclerosis (1–3). The remnant nephron hypertrophies and values for single-nephron GFR and glomerular capillary hydraulic pressure rise (5), and a process of progressive glomerular injury ensues. A number of mechanisms have been implicated in the development of this injury aside from the changes in glomerular and systemic hemodynamics, including dyslipidemia (5,6) and increased generation of vasoconstrictive and prothrombotic mediators such as TxA2 and leukotriene B4 (7,8).

This study was designed to assess whether dietary supplementation of GLA provided as BO conferred any benefit beyond that which might be derived from LA provided as CO. A third group of animals, fed an RLD, was included to provide a contemporaneous comparison of the course of glomerular injury as well as usual renal phospholipid composition. The RLD diet also allowed us to ensure that the PUFA diets were not detrimental.

Renal phospholipid analysis confirmed the bioavail-
Alteration of DGLA/AA Ratio in Renal Ablation

### TABLE 3. Renal function

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Diet</th>
<th>Presurgery</th>
<th>Week 1</th>
<th>Week 20</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RLD</td>
<td>9.8 ± 1.7</td>
<td>5.7 ± 1.6(^a)</td>
<td>1.7 ± 0.8(^{a,b,d})</td>
</tr>
<tr>
<td>GFR (mL/min per kg)</td>
<td>Corn oil</td>
<td>5.7 ± 1.6(^a)</td>
<td>2.6 ± 0.8(^{a,b})</td>
<td>3.7 ± 2.3(^a)</td>
</tr>
<tr>
<td></td>
<td>Borage oil</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proteinuria (mg/24h)</td>
<td>RLD</td>
<td>4 (range, 2 to 6)</td>
<td>32(^a) (range, 11 to 134)</td>
<td>170(^{a,b,c,d}) (range, 82 to 352)</td>
</tr>
<tr>
<td></td>
<td>Corn oil</td>
<td></td>
<td></td>
<td>37(^a) (range, 8 to 80)</td>
</tr>
<tr>
<td></td>
<td>Borage oil</td>
<td></td>
<td></td>
<td>27(^a) (range, 9 to 51)</td>
</tr>
</tbody>
</table>

\(^a\) Significantly different compared with presurgery.
\(^b\) Significantly different compared with Week 1.
\(^c\) Significantly different compared with corn oil.
\(^d\) Significantly different compared with borage oil.

### TABLE 4. Glomerular histology

<table>
<thead>
<tr>
<th>Score/Diet</th>
<th>Week 1</th>
<th>Week 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesangial Expansion Score (mean ± SD)</td>
<td>RLD 55 ± 14</td>
<td>124 ± 25(^a)</td>
</tr>
<tr>
<td></td>
<td>Corn Oil 99 ± 24</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Borage Oil 83 ± 23(^b)</td>
<td></td>
</tr>
<tr>
<td>Glomerular Sclerosis Score (mean ± SD)</td>
<td>RLD 48 ± 18</td>
<td>166 ± 49(^a)</td>
</tr>
<tr>
<td></td>
<td>Corn Oil 109 ± 21(^{a,b})</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Borage Oil 78 ± 14(^{b,c})</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Significantly greater than RLD at 1 wk.
\(^b\) Significantly lower than RLD at 20 wk.
\(^c\) A direct comparison between CO and BO by t test: \(P = 0.005\).

ability of the specific PUFA diet-derived fatty acids in that both the CO and BO diets had much greater levels of AA tissue incorporation compared with RLD. The BO-fed rats were alone in showing an increased incorporation of GLA and DGLA. LA, enriched in corn oil, is metabolized to AA through the intermediates GLA and DGLA, but the properties of the 6-6 desaturase enzyme are such that LA entry into the pathway is rate-limiting, and GLA and DGLA do not tend to accumulate (16). BO, on the other hand, provides an excess of GLA and DGLA, exceeding the ability of the 6-6 desaturase enzyme to metabolize DGLA to AA (23). Therefore, the DGLA:AA ratio rises, as we observed in our BO-fed rats.

We found that these changes in membrane fatty acid composition were accompanied by significant reductions in urinary protein excretion rates in both groups of PUFA-fed rats compared with the untreated rats. The mean value for GFR was also significantly greater in rats receiving BO (3.7 ± 2.3) compared with untreated rats (1.7 ± 0.8). Although GFR values tended to be higher in the CO group (2.6 ± 0.8) than in the untreated rats, the difference did not reach statistical significance. In accord with the effect on GFR, we found that the RLD and CO groups had significantly greater mesangial expansion scores at 20 wk compared with values measured 1 wk after renal ablation, whereas this rise was not significant in the BO group.

Accordingly, at 20 wk after renal ablation, only the BO group showed significantly lower mesangial expansion compared with RLD group. Both PUFA diets were effective in limiting the glomerular sclerosis, compared with the RLD group. A direct comparison between the BO group and the CO group revealed that
The BO diet was significantly more effective in reducing glomerulosclerosis (78 ± 14) than the CO diet was (109 ± 21; P = 0.006 on t test analysis), in keeping with the observation of GFR and mesangial expansion.

The beneficial effects of the PUFA diets could not be attributed to differences in calorie or protein intake, because the diets were isocaloric and contained similar amounts of protein. Moreover, the beneficial effects of the PUFA diets occurred despite the fact that the PUFA diets were higher in fat content (15%) than the RLD (4.5%). High fat intake has been associated with deterioration of renal function in hyperfiltration injury (6).

The marked increase in renal-tissue AA incorporation seen in the PUFA-fed rats suggests that AA-derived prostaglandin metabolites might play a role in modulating the course of renal injury in the BO and CO groups. Metabolites of AA include cyclooxygenase products such as the 1 and 2 series prostaglandin E and thromboxanes (24), as well as lipoxygenase products such as the 5-lipoxygenase sulfidopeptide leukotrienes (25) and the 15-lipoxygenase 15-Hydroxyeicosatetraenoic acid (26). An increase in the ratio of thromboxane to prostacyclin has been shown to be associated with progressive renal failure (9), and the inhibition of thromboxane synthesis ameliorates injury in this model in concert with a reduction in glomerular capillary pressure and decreases in thrombotic events (7). Therefore, we sought to relate the attenuation in glomerular injury with the PUFA diets to changes in the urinary excretion rate of TxB2, the stable metabolite of TxA2, and 6-keto PGF1α, the metabolic end-product of prostacyclin. We observed a progressive rise in the urinary excretion rate of TxB2 in the renal-ablated rats on RLD, but this rise was significantly reduced by both of the PUFA diets. We also found that the urinary excretion rate of 6-keto PGF1α was unchanged by the PUFA diets, despite an increased availability of AA. Recent studies have shown that TxB2 can increase the production of extracellular matrix by mesangial cells in vitro (27), so a diet-induced decrease in renal TxB2 synthesis might be expected to limit mesangial expansion and glomerulosclerosis in the remnant glomerulus. In this regard, pharmacologic inhibition of TxA2 synthetase has been shown to reduce mesangial expansion in rats with experimental Type 1 diabetes mellitus (28).

Taken together, the studies alluded to earlier and data presented here suggest that one of the mechanisms responsible for attenuation of glomerular injury in the renal-ablated rats maintained on the CO and BO diets is the reduction in TxA2 production. It should be noted that we did not measure 2,3-dinor TxB2, so we cannot be sure that all of the urinary TxB2 measured is of renal origin (29). Moreover, although BO attenuated glomerular injury more than CO, we did not identify differences in the effects of these two interventions on the urinary excretion rates of TxB2 or 6-keto PGF1α.

It is established that spontaneous or diet-induced hypercholesterolemia predisposes to glomerulosclerosis (6,30,31). Although both PUFA diets were efficacious in lowering cholesterol, CO was more effective. Therefore, although the effects on lipids may have contributed to the lessening of glomerulosclerosis seen with the PUFA diets, especially CO, it does not explain the additional protection seen with BO. Consequently, the beneficial histologic and functional effects seen in the BO group must be the result of other mechanisms.

Treatment of systemic hypertension has also been shown to limit glomerular injury in renal-ablated rats, and we observed significant effects of the PUFA diets on blood pressure. Although both of the diets led to a reduction in blood pressure, the BO diet appeared to be much more effective. We did not perform micropuncture studies in these rats, but a decline in glomerular capillary pressure, along with a decline in systemic blood pressure, may have been partially responsible for the amelioration of glomerular injury in the BO-fed rats. It must be noted that although decrements in systemic blood pressure may reflect declines in intraglomerular pressures, this is not always the case (32).

LA has been shown to lower blood pressure in various models of spontaneous or stress-related hy-
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TABLE 5. Urinary prostaglandin levels

<table>
<thead>
<tr>
<th>Time Point</th>
<th>Diet</th>
<th>TxB₂ (µg/h)</th>
<th>6-Keto PGF₁α (µg/h)</th>
<th>Ratio: PGF₁α/TxB₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presurgery (Week 0)</td>
<td>RLD</td>
<td>346 ± 64</td>
<td>338 ± 108</td>
<td>0.99 ± 0.32</td>
</tr>
<tr>
<td>Week 1 (Post-surgery)</td>
<td>RLD</td>
<td>607 ± 135ᵃ</td>
<td>365 ± 221</td>
<td>0.58 ± 0.29ᵇ</td>
</tr>
<tr>
<td>Week 20 (Post-surgery)</td>
<td>RLD</td>
<td>930 ± 260ᵃᵇᶜᵈ</td>
<td>231 ± 58</td>
<td>0.26 ± 0.02ᵃᵇᶜᵈ</td>
</tr>
<tr>
<td></td>
<td>Corn oil</td>
<td>509 ± 125</td>
<td>334 ± 115</td>
<td>0.65 ± 0.14ᵃ</td>
</tr>
<tr>
<td></td>
<td>Borage oil</td>
<td>575 ± 196</td>
<td>324 ± 113</td>
<td>0.61 ± 0.29ᵃ</td>
</tr>
</tbody>
</table>

ᵃ Significantly different compared with presurgery.
b Significantly different compared with Week 1.
c Significantly different compared with corn oil.
d Significantly different compared with borage oil.

TABLE 6. Plasma lipid levels

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Diet</th>
<th>Presurgery</th>
<th>Week 1</th>
<th>Week 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>RLD</td>
<td>1.1 ± 0.3</td>
<td>1.4 ± 0.4</td>
<td>5.1 ± 1.2ᵃᵇᶜᵈ</td>
</tr>
<tr>
<td>(mmol/L)</td>
<td>Corn oil</td>
<td>1.9 ± 0.2ᵃ</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Borage oil</td>
<td>2.5 ± 0.7ᵇ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides</td>
<td>RLD</td>
<td>0.5 ± 0.1</td>
<td>0.5 ± 0.2</td>
<td>1.7 ± 1.1ᵇᵇ</td>
</tr>
<tr>
<td>(mmol/L)</td>
<td>Corn oil</td>
<td>1.2 ± 0.7ᵇᵇ</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Borage oil</td>
<td>1.8 ± 0.8ᵃᵇ</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ᵃ Significantly different compared with presurgery.
b Significantly different compared with Week 1.
c Significantly different compared with corn oil.
d Significantly different compared with borage oil.

pertension, but GLA is consistently more effective (33,34). This is compatible with our observations of significantly lowered blood pressures in BO-fed rats; in fact, the hypotensive effect is greater than we have seen hitherto with any other PUFA (safflower oil [9], fish oil [10], flax oil [11]). The mechanism(s) through which BO exerts this antihypertensive effect is not clear. It cannot be explained on the basis of alterations in the TxB₂:6-keto-PGF₁α ratio, which was similar in the BO and CO groups. In spontaneously hypertensive rats, St. Louis et al. (34) showed that the borage oil-derived hypotensive effect was abolished by acetylsalicylic acid, but acetylsalicylic acid did not affect blood pressure in olive oil-fed animals. This would suggest involvement of cyclo-oxygenase metabolites of PUFA, other than AA derivatives, whose accumulation is unique to GLA supplementation. From our observations of renal tissue fatty acids, DGLA would appear to be the obvious candidate. DGLA may serve as a precursor for PGE₃ and the 15-lipoxygenase-derived 15-hydroxy-8,11,13 eicosatetraenoic acid (15-HETre) consequently, its metabolism tends to yield compounds with vasodilatory and anti-inflammatory effects.

Although DGLA may serve as a precursor for PGE₃ and the 15-lipoxygenase derived 15-HETre, it is not a candidate for the 5-lipoxygenase enzyme (17), an effect that might serve to limit the glomerular produc-


