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Estrogen worsens incipient hypertriglyceridemic glomerular injury in the obese Zucker rat

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Background. The obese Zucker rat (OZR) is a model of glomerulosclerosis and renal failure in the setting of hyperlipidemia, hyperinsulinemia, and obesity. Our prior work in OZRs has shown that ovariectomy attenuates glomerulosclerosis, while added estrogen worsens it. To investigate the mechanism of estrogen's effects on glomerular disease in this model, we evaluated the effects of ovariectomy and estrogen supplementation on seven-week peripubertal OZRs. At this time point, rats exhibit no overt histologic glomerular disease, but are just beginning to show elevated urinary albumin excretion.

Methods. Female OZRs fed ad libitum were ovariectomized at four weeks, with or without estrogen supplementation to raise estrogen levels to just below those of preoestral adults (mean 16.5 pg/mL). Sham-operated controls were included.

Results. Ovariectomy normalized albuminuria, lowered total and very low-density lipoprotein triglycerides, and reduced glomerular fibronectin expression. Estrogen supplementation worsened albuminuria and raised total/very low-density lipoprotein triglycerides and total cholesterol. Estrogen-supplemented rats exhibited enhanced glomerular deposition of apo A-IV and apo B, increased glomerular expression of desmin and type IV collagen, and increased interstitial macrophage deposition.

Conclusion. Estrogen may be permissive for the early development of renal disease in OZRs and may act by increasing triglyceride-rich lipoproteins, which then bind to glomerular cells and initiate or accelerate glomerulosclerosis.

Hyperlipidemia is increasingly accepted as a significant progression factor for renal disease. Although sex hormones are known to affect lipid levels, the relationship between this interaction and the course of renal disease remains speculative. In humans, estrogen replacement therapy has been found to be a preventative measure for the development of atherosclerosis via its antioxidant and cholesterol-lowering effects [1], but it also can induce hypertriglyceridemia [2]. The consequences of its hypertriglyceridemic actions on renal disease remain uninvestigated, and animal models of hyperlipidemia and glomerular injury are important in understanding this interaction.

We have recently reported that estrogen replacement accelerates the glomerulosclerosis seen in the obese Zucker rat (OZR), a model of hyperlipidemia, obesity, and hyperinsulinemia that has been proposed as a model of hyperlipidemic renal injury [3, 4]. In these rats, hyperlipidemia appears by three to four weeks of age, followed by macrophage deposition (4 weeks), albuminuria (6 to 7 weeks), altered production of matrix proteins (6 to 12 weeks), glomerular sclerosis (24 weeks), and early death from renal failure (48 weeks) [5]. Prior studies have shown that the glomerulosclerosis seen in OZR is both histologically and biochemically similar to that seen in other rat models of focal segmental glomerulosclerosis (FSGS) [6].

In our earlier study, ovariectomy markedly reduced albuminuria and glomerular sclerosis, while estrogen supplementation worsened both. The estrogen supplementation also markedly increased plasma lipids, especially triglycerides (TGs). Given these findings, we designed experiments to investigate the mechanism of estrogen's effects on the earliest detectable stages of renal injury, at six to seven weeks. At this time, histologic glomerulosclerosis was absent, and urinary albumin was just beginning to rise. The rats were, however, hyperlipidemic. We sought to determine how estrogen manipulation would affect serum lipoproteins and their deposition in the glomerulus and how these changes would correlate with the early development of glomerular disease in these rats.

Key words: progressive renal disease, estrogen replacement therapy, hypertriglyceridemia, glomerulosclerosis.

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METHODS

Rats

Female OZRs were obtained from the University of California, Davis, Clinical Nutrition Research Unit Animal Model Core at four weeks of age. They were housed individually in stainless steel cages with wire mesh floors and had free access to a soy-based diet and water. This soy protein-based (20%) diet was previously shown to minimize renal injury in rats [7]. Body weights were measured twice weekly. The rats were assigned to one of three experimental groups: sham operated, ovariectomized (Ovx), or Ovx and then supplemented with estrogen (Ovx + E) by subcutaneous implant. Surgery was performed at five weeks of age by dorsal approach. Animals were sacrificed at seven weeks, and the absence of ovaries was confirmed.

Estrogen treatment

17β-Estradiol (15.3 μg/24 h) was administered by subcutaneous implant at five weeks of age using time-release pellets (Innovative Research of America, Sarasota, FL, USA) [3]. Pellets were placed at the nape of the neck subcutaneously. The sham group received a placebo pellet.

Urinary albumin excretion

Urine was collected for all animals over a 24-hour period by placing rats in stainless steel metabolic cages and using flasks containing three drops of 10% sodium azide solution to inhibit bacterial proliferation. Water and moistened food were available. Urinary albumin excretion (UAE) was determined using electroimmunodiffusion using rabbit antirat albumin and purified rat albumin as previously described [3]. Urine collection was performed at seven weeks of age.

Plasma lipids, insulin, and glucose

At seven weeks of age, the animals were fasted overnight and anesthetized with sodium pentobarbital. Blood was collected from the abdominal aorta using an ethylenediaminetetraacetic acid (EDTA)-coated syringe. Plasma was obtained by centrifugation and stored at −70°C. Very low-density lipoprotein (VLDL) was isolated by centrifugation [8]. Plasma triglyceride and cholesterol were measured by enzymatic, colorimetric assays (Sigma Chemical Co., St. Louis, MO, USA). Glucose and immunoreactive insulin were determined by radioimmunoassay as previously described [3].

Estrogen levels

A tritiated estrogen tracer was added to 0.5 mL plasma, which was then extracted with diethyl ether. The ether fraction was isolated by immersion in dry ice-cooled ethyl alcohol to freeze the aqueous phase. The ether phase was then decanted, dried, and reconstituted with estrogen-free plasma. Estrogen recovery was calculated using the recovery of β counts in the reconstituted samples. A 17β-estradiol–specific radioimmunoassay (Diagnostics Systems Laboratories, Webster TX, USA) was performed on the reconstituted samples, and the results were adjusted by accounting for the recovery percentage.

Renal histology

Following exsanguination under anesthesia, rats were infused via the abdominal aorta with phosphate-buffered saline (PBS; 0.1 mol/L, pH 7.4) containing 6% sucrose and 500 U heparin/L for a sufficient time to blanch the kidneys. A 0.05% glutaraldehyde/4% paraformaldehyde in PBS fixative was then perfused for one minute or until the kidneys became rigid. The fixative was removed by reperfusion with the PBS/sucrose/heparin solution. Tissue was then embedded in paraffin for light microscopy and immunohistochemistry as described [9]. Sections were then stained by the periodic acid-Schiff technique. Glomeruli were scored by the method of Raij, Azar, and Keane as previously reported [10]. Thirty to 40 glomeruli were scored from each kidney for three parameters: FSGS, mesangial matrix expansion (MME), and mesangial cellularity (MC). Each glomerulus was scored semiquantitatively on a scale of 0 to 4 for each parameter, depending on the percentage of the glomerulus involved. FSGS was scored positive when MME, adhesion formation, and capillary obliteration were present in the same segment of the glomerulus.

Immunohistochemical localization of apolipoproteins and monocytic cells

Sections stained for apolipoproteins A-1 and A-4, B, and E are processed as described [11]. Frozen, unfixed tissue was sliced at 4 μm, dried on slides, and kept at −20°C until used. Slides were air dried and fixed for 10 minutes in acetone, rinsed in PBS, and incubated with the primary antibody diluted in PBS with 1% bovine serum albumin (BSA) for one hour (polyclonal anti–apo-A1, A4, B, or E; provided by Dr. A. van Tol, Rotterdam, The Netherlands). Slides were rinsed in PBS, and endogenous peroxidase was blocked by treatment with 0.075% H_2O_2 in PBS for 30 minutes. Following another rinse, the secondary, peroxidase-linked antibody diluted in PBS with 1% BSA and 2% normal rat serum was applied to the slides and incubated for 30 minutes. Slides were rinsed and then immersed in the peroxidase reaction solution consisting of 5% AEC stock (4 mg 3-amino-9-ethyl-carbazole per mL dimethylformamide) and 0.1% H_2O_2 (30%) in 0.1 mol/L sodium acetate buffer (pH 5.0). Slides were then rinsed and counterstained with Meyer's Haematoxilin and mounted. Total glomerular lipid deposition was assessed by Oil Red O staining [12]. Thirty to 50 glomeruli per sample were scored on a scale of 0 (no staining) to 4 (strong staining throughout all of the

glomerular visceral epithelium and mesangium of all glomeruli).

Frozen sections were stained for monocytes/macrophages using anti-ED1 (Serotec, Oxford, UK) as previously described [13].

Reverse transcription-polymerase chain reaction of isolated glomeruli

Glomeruli were isolated by sieving followed by microscopic dissection to exclude tubular contamination. Total RNA was isolated from 100 glomeruli by the method of Chomczynski and Sacchi [14]. For reverse transcription (RT), glomerular RNA was treated with 1.5 U DNAse I (GIBCO, Grand Island, NY, USA). The RT reaction consisted of 3 μg of RNA, 1.5 U of RNAse-free DNAse I (GIBCO), 5 mmol/L $MgCl_2$, 2 U RNAse inhibitor (GIBCO), 2.5 mmol/L oligo$(dT)_{16}$, and 15 μL diethyl pyrocarbonate-treated water. This reaction was performed in an MJ Research ThermaCycler and was incubated for 30 minutes at 37°C, heated to 75°C for 5 minutes to inactivate the DNAse, and then cooled at 4°C. One-half microliter of 50 U/μL murine leukemia virus (MuLV) reverse transcriptase (GIBCO) was added to each tube. The reverse transcriptase reaction was run for 30 minutes at 42°C, followed by a 5-minute deactivation at 90°C, and then cooled at 4°C. For the polymerase chain reaction (PCR), 2 μL of each reaction were added to 23 μL of PCR reaction mixture, which consisted of 25 pmol each of forward and reverse primers, 2 mmol/L MgCl, 20 mmol/L dNTP, 25 mmol/L PCR buffer, 17 μL diethyl pyrocarbonate-treated water, and 1 U DNA Taq Polymerase (GIBCO). The initial denaturation step ran for three minutes at 94°C, followed by 94°C for one minute, 65°C for two minutes, and then 75°C for three minutes for 35 to 40 cycles. This was followed by an extension step at 72°C for 10 minutes. Ten microliters of each reaction was then mixed with DNA loading buffer and run on a 1.2% agarose gel and visualized by ethidium bromide staining. The primers used are listed in Table 1.

Because quantitative RT-PCR with competitive primers was not used in these studies, we only considered a result to be positive if clear gene expression was demonstrated on repeated trials with absent expression seen in other groups.

Statistical analysis

Data were analyzed using GraphPad Prism version 2.01 (San Diego, CA, USA). Data are presented as mean ± SEM. Data were first analyzed for normal distribution. They were then analyzed for differences using one-way analysis of variance (ANOVA), with a post hoc analysis using a Tukey test for multiple comparisons. UAEs and estrogen levels were transformed logarithmically prior to this analysis to normalize results. Differences were considered statistically significant at $P < 0.05$.

RESULTS

Female OZRs were ovariectomized or subjected to sham surgery at four weeks of age; some Ovx rats were also given estrogen by subcutaneous pellets. Supplemented rats with estrogen levels exceeding the reported adult OZR preoestral physiologic range of 27 to 68 pg/mL [15] were excluded from analysis to avoid possible complications of supra-pharmacologic estrogen levels. Table 2 shows the characteristics of these rats. Estrogen treated rats ate less and had lower body weights. Estrogen levels were lowest in Ovx rats and highest in the estrogen-supplemented group. All estrogen-supplemented rats had estrogen levels below those reported in adult OZRs.

Because the appearance of albuminuria is a sensitive early marker of glomerular disease, we chose to measure it in these rats at six to seven weeks, when overt histologic FSGS is not present. Figure 1 shows the amount of UAE in seven-week-old OZRs, either sham operated, Ovx, or Ovx + E. Normal levels in a lean rat are under 1 mg/day [16]. The control obese animals demonstrate abnormal albuminuria (2.3 ± 1.7 mg/24 h), which was normalized by ovariectomy (0.45 ± 0.06). Estrogen-treated rats developed severe albuminuria of up to 200 times normal (mean 97.3 ± 23.7 mg/24 h). In similar studies using control lean Zucker rats, no rats exceeded normal UAE at 5 or 10 weeks (data not shown).

Given our previous data showing elevated TGs in 20-week OZRs treated with estrogen [3], we investigated hyperlipidemia at the onset of albuminuria. VLDL represents the predominant form of lipoprotein in the Zucker rat [17]. Figure 2 shows total and VLDL TGs and total cholesterol from the same young animals as shown in Figure 1 (VLDL cholesterols were largely undetectable). Quantitatively, VLDL TGs were the most important lipoprotein measured. Ovariectomy lowered total and VLDL TG levels, whereas estrogen replacement markedly raised them (total TG: sham 298.3 ± 24.5, Ovx 172.9 ± 12.4, and Ovx + E 544.0 ± 61.6 mg/dL; VLDL TG: sham 191.9 ± 16.2, Ovx 114.2 ± 11.4, and Ovx + E 420.8 ± 52.2 mg/dL). In contrast, both ovariectomy and Ovx with added estrogen increased serum cholesterol (sham 84.2 ± 3.7, Ovx 103.7 ± 3.4, Ovx + E 174.6 ± 14.2 mg/dL). Regression analysis of UAE against lipid levels showed a strong correlation between UAE and VLDL TGs ($r^2 = 0.558$, $P < 0.0001$; Fig. 3) and between UAE and total TG ($r^2 = 0.509$, $P < 0.0001$). The relationship with total cholesterol ($r^2 = 0.441$, $P < 0.0001$) was not as strong. Notably, the correlation between UAE and estrogen levels was much weaker than with the serum TG levels ($r^2 = 0.351$, $P < 0.0001$). The increased UAE could not be attributed to elevations in insulin or in glucose levels, as shown in Table 2.

The estrogen-associated increases in albuminuria and

Table 1. List of primers used in this study

Type	Primer
Glomerular protein primers	
Heparan sulfate proteoglycan	5′ GCGGAGTATGTCATTGGCAGTGTG3′
	5′ AGGCGTCATCTGGGCACCACATCA3′
Collagen IV-α1	5′ CTCTGGGGACAACATCCG 3′
	5′ TCTTCTCATGCACACTTGGC 3′
Desmin	5′ ACAACCTGATAGACGACC 3′
	5′ CTGAGTCAAGTCTGAAACC 3′
Laminin	5′ AACGGGTGTGCTGGGTGCCTTTGA3′
	5′ CCATCCTCATCCCGACATCCAGCA3′
Fibronectin	5′ CTCCTGTGGTCATTGATGCCTCCA 3′
	5′ GGTATGGTCTTGGCCTAAGCCTGA 3′
Collagenase 72 kD	5′ CCACGCTGCGGCAACCCAGATGTGGCCAAC 3′
	5′ GTGGCAGCCCACGAGTTTGGCCACGCC 3′
Collagenase 92 kD	5′ ACCGCTATGGTTACACTCGG 3′
	5′ GCAGGCAGAGTAGGAGTG 3′
Cytokine primers	
Interleukin (IL)-1α	5′ AGCAACATCAAACAAAGGGAAG 3′
	5′ GCAATTAACAGCTCTGGGAAAG 3′
IL-1β	5′ CGACCTTCTTTTCCTTCATC 3′
	5′ CCGCTTTTCCATCTTCTTC 3′
IL-2	5′ GTACAGGATGCAACTCCTGTCTTG3′
	5′ CAATGGTTGCTGTCTCATCAGC 3′
IL-6	5′ CCTTCTCCACAAGCGCCTTCGGTC 3′
	5′ GCCTGCAGCTTCGTCAGCAGGCTG 3′
Tumor necrosis factor-α	5′ CTCAGATCATCTTCTCAAAACTCG 3′
	5′ TGTCCCTTGAAGAGAACCTG 3′

Table 2. Biochemical characteristics of rats

	Sham operated	Ovari-ectomized	Ovx + estrogen
Body wt *g*	216 ± 3	226 ± 4	192 ± 3[ab]
Estrogen level *pg/mL*	4.09 ± 1.42	0.60 ± 0.13[a]	16.49 ± 2.61[ab]
Insulin level *pm/mL*	1206 ± 257	965 ± 73	1548 ± 166
Glucose *mg/dL*	238 ± 5	266 ± 15	213 ± 3[b]

[a] $P < 0.05$ vs. sham operated
[b] $P < 0.05$ vs. ovariectomized

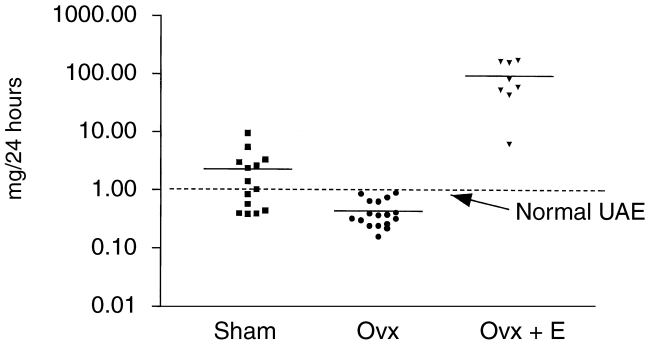

Fig. 1. Urinary albumin excretion in seven-week obese Zucker rats (OZRs). Abbreviations are: sham, sham operated; Ovx, ovariectomized; Ovx + E, ovariectomized with estrogen replacement ($N = 20$ to 30 per group).

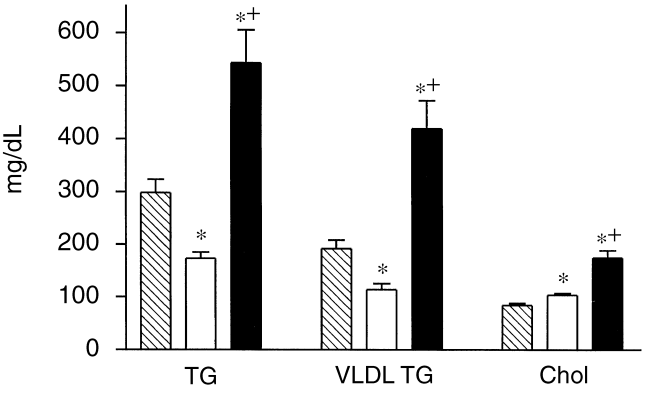

Fig. 2. Total and very low density lipoprotein (VLDL) lipids in seven-week OZRs ($N = 20$ to 30 per group). Symbols are: (▨) sham; (□) Ovx; (■) Ovx + E; $^{*}P < 0.05$ vs. sham; $^{+}P < 0.05$ vs. Ovx. Definitions are in the legend to Figure 1.

serum lipids were also associated with changes in glomerular apolipoprotein (apo) deposition, as demonstrated by immunostaining shown and tabulated in Figure 4 and 5. Estrogen significantly increased apo B and apo A-IV glomerular staining, but no differences were seen in apo E and apo A-I. Although there were no statistically significant differences seen in Ovx animals versus control, it is notable that Ovx animals were the only group to show nondetectable levels of glomerular apo E, A-I, and A-IV. Total glomerular lipid deposition, as measured by Oil Red O staining, was not detected in any group (data not shown). No notable interstitial apolipoprotein staining was detected in any group. However, peritubular Apo A-IV was noted in most rats, independent of estrogen status or amount of glomerular deposition (Fig. 5 c, d, arrows).

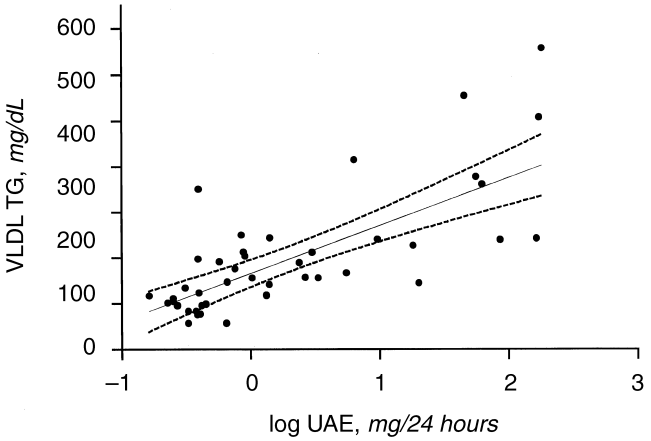

Fig. 3. Linear regression of VLDL triglycerides (TG) versus log (urinary albumin excretion, UAE) in seven-week OZRs ($r^2 = 0.604$, $P < 0.0001$).

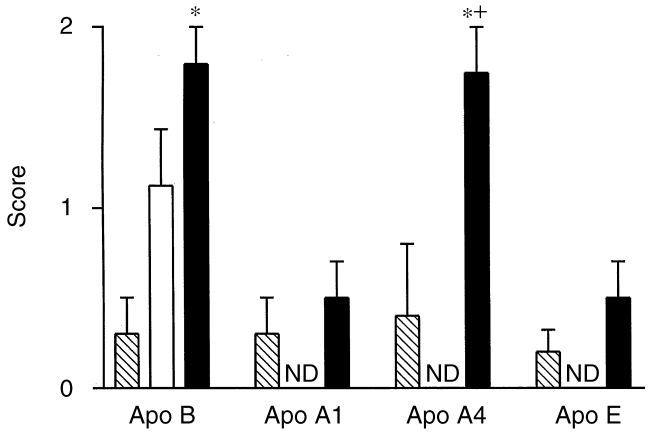

Fig. 4. Apolipoprotein scoring in seven-week OZRs. Scoring represents mean of 30 to 50 glomeruli per rat with five to six per group. Symbols are: (▨) sham; (□) Ovx; (■) Ovx + E; $*P < 0.05$ vs. sham; $^{+}P < 0.05$ vs. Ovx. Abbreviation ND is not detected.

Focal segmental glomerulosclerosis is associated with early alterations in glomerular cellular proteins involved in matrix expansion and basement membrane remodeling. To investigate this, we used RT-PCR of microdissected glomeruli to evaluate gene expression of selected matrix proteins. As shown in Figure 6, compared with sham-operated controls, ovariectomy reduced the expression of fibronectin, whereas estrogen treatment increased expression of desmin and type IV collagen. Estrogen manipulation had no effect on the expression of heparan sulfate proteoglycan, laminin, or the 72 or 92 kD collagenases (data not shown). These alterations in glomerular gene expression came before any overt histologic changes in glomerular morphology were present, as the scorings of MC and MME were low in all groups and was not affected by estrogen manipulation (data not shown).

Lavaud et al have proposed that glomerular monocyte/macrophage deposition, with consequent cytokine elaboration, is important in the development of renal disease in this model [6]. In these young rats, we investigated both glomerular and interstitial macrophage deposition as well as cytokine expression. Shown in Figure 7 is a semiquantitative depiction of ED1+ monocytes/macrophages in the renal glomerulus and interstitium. Despite evidence for incipient renal injury in control and estrogen-treated groups, there was no evidence of increased glomerular monocyte deposition. In contrast, the interstitial monocyte/macrophage number was increased in estrogen-treated rats. Two estrogen-treated rats exhibited moderate interstitial fibrosis, but interstitial fibrosis was absent in all other rats (data not shown). RT-PCR of microdissected glomeruli detected no intergroup differences in expression of interleukin-1α (IL-1α), IL-1β, IL-2, IL-6, or tumor necrosis factor-α (data not shown).

DISCUSSION

In this study, we report that estrogen manipulation of young OZR affects cellular and clinical events at the onset of renal injury. Although previous studies have investigated the characteristics of renal disease in the OZR, none have dealt with potential mechanisms of disease onset. Previous work from our laboratories documented gender specificity, with more severe glomerulosclerosis found in female OZRs [5]. Subsequent work showed that estrogen accelerated the development of glomerular damage in female OZRs at 21 weeks of age [3]. In these older rats, estrogen also raised serum lipids from control levels, which in this model were already high [3]. Estrogen also increased albuminuria. The present study examines the effect of estrogen on glomerular damage in much younger rats at the onset of glomerular injury and shows that albuminuria can occur very early, associated with a rise in serum lipids. Interestingly, ovariectomy attenuated hypertriglyceridemia and glomerular disease, lowering urinary albumin excretion to below that of control animals.

The effect of estrogen may be due to its hyperlipidemic actions. Previous studies have shown conflicting effects of estrogen administration on hyperlipidemia in OZRs, but have not evaluated its effect on renal disease. Similar to our study, Wilson, Wilson, and Eaton showed increased serum TG with estrogen supplementation in Ovx OZR [18], but little effect on cholesterol. In contrast, Liao, Angelin, and Rudling found decreases in both TG and cholesterol [19]. It is unlikely that the hyperlipidemia was secondary to albuminuria alone. While it is possible that the UAE of >100 mg/24 h seen in three estrogen supplemented rats contributed to their hypertriglyceridemia, this "nephrotic-range" albuminuria was not present in a majority of rats in the high-estrogen group. In

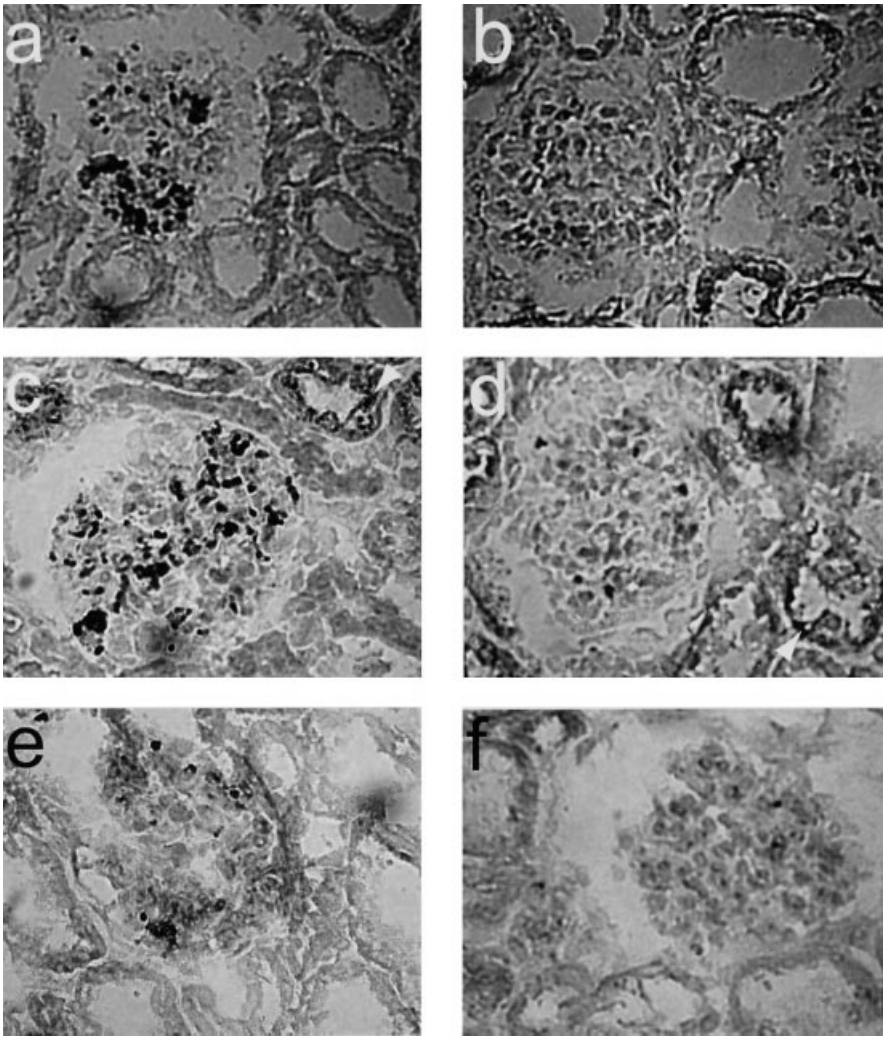

Fig. 5. Representative glomerular apolipoprotein immunohistochemistry in seven-week OZRs. Sections represent staining in estrogen-supplemented (*a, c,* and *e*) and sham-operated (*b, d,* and *f*) rats. Specific antibodies against apo A-I (a and b), apo A-IV (c and d), and apo B (e and f) were used. The arrow denotes tubular apo A-IV staining (magnification ×40).

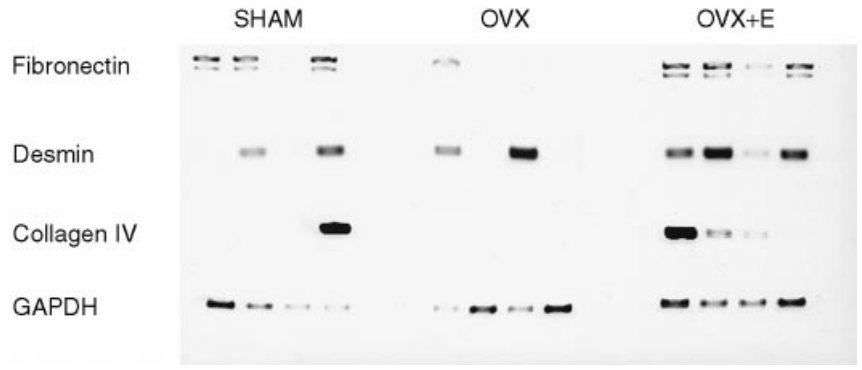

Fig. 6. Glomerular gene expression in seven-week OZRs by RT-PCR. The results were determined in duplicate ($N = 4$ in each group).

the control and Ovx rats, none had UAE over 10 mg, yet there were significant differences in lipid levels. It is highly unlikely that differences in albuminuria of the magnitude seen in these rats can explain the reduction in serum lipids seen in the Ovx animals.

Rats given exogenous estrogen developed significant albuminuria, glomerular deposition of apo A-IV and apo B, and markedly elevated total and VLDL TGs. VLDL and total TG levels correlated better with albuminuria than did estrogen levels. Total cholesterol was modestly increased, and serum glucose and insulin levels were not elevated. Added estrogen may therefore be acting via

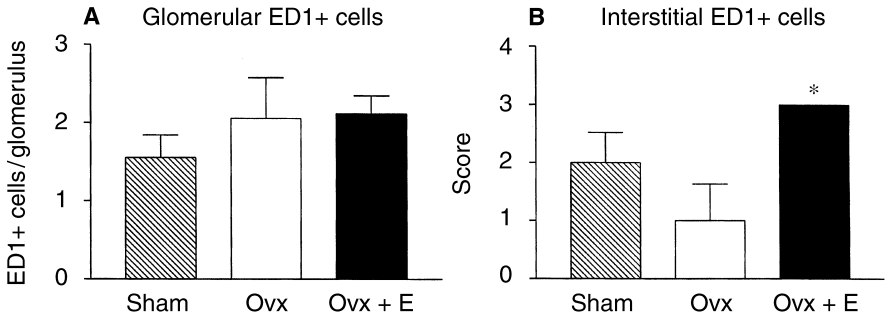

Fig. 7. Glomerular (*A*) and interstitial (*B*) monocyte/macrophage deposition by ED1 staining. Means represent 20 scores from four to five rats per group. Symbols are: (▨) sham; (□) Ovx; (■) Ovx + E; $*P < 0.05$ vs. Ovx.

elevations in serum TG-rich lipoproteins and/or deposition of specific lipoproteins in the glomerulus. In contrast, ovariectomy in these prepubertal animals reduced albuminuria and reduced serum and VLDL TGs, although the TG reductions were much less marked than the elevations induced by added estrogen. Ovariectomy did not change total cholesterol, insulin levels, or serum glucose. While Ovx rats showed no statistically significant changes in glomerular lipoprotein deposition, this was the only group to show nondetectable glomerular apo A-I, A-IV, and E.

Lipid deposition is an early marker of glomerular disease in these rats, and lowering lipids has previously been shown to reduce renal disease in OZR. Lipid-lowering drugs, for example, reduce renal injury in OZRs in the absence of glomerular hemodynamic changes [4], primarily from a reduction in serum cholesterol [20]. However, the hyperlipidemia of the OZR is characterized by hypertriglyceridemia and VLDL elevations that are quantitatively much more severe than hypercholesterolemia [17]. Michel et al reduced proteinuria and mesangial expansion in OZRs by the administration of acarbose, which lowers intestinal absorption of carbohydrates and lowered TGs but not cholesterol [21]. We have recently achieved comparable attenuation of renal disease and hypertriglyceridemia by preventing the hyperphagia characteristic of these animals [22].

This report demonstrates increased glomerular deposition of apo B and apo A-IV in estrogen-treated rats, without significant changes in apo A-I, apo E, or total lipid staining. Studies on the apolipoprotein composition of hyperlipidemic OZR serum show varying patterns of distribution and quantity, highly dependent on age and dietary modification [23]. Apo A and E can circulate in either lipid-poor fractions or freely, unbound to lipids [24]. In contrast, apo B is totally insoluble in the absence of lipids, so its appearance in the mesangium is most likely as part of an intact lipoprotein. These data suggest that there may be specific deposition of intact lipoproteins or free apolipoproteins in the glomeruli of estrogen-treated rats. Since LDL and VLDL can induce proliferation in OZR mesangial cells [25], this glomerular lipoprotein deposition may be important in the initiation of disease in this model.

Although these data suggest that estrogen manipulation affected glomerular disease by changing lipid fractions, we cannot exclude a direct action of estrogen on glomerular cells or an indirect action via an uninvestigated hormone, growth factor, or glomerular hemodynamic effect. The latter is unlikely, as there is no evidence that estrogen increases either systemic blood pressure or transglomerular capillary pressure in strains of rats not sensitive to salt. Additionally, at 9 to 13 weeks of age, transglomerular capillary pressures do not differ in obese versus lean Zucker rats and are not changed by pharmacologic reduction of serum lipids [26]. While glomerular cells contain estrogen receptors and bind and proliferate in response to estrogen [27], it has not been demonstrated that estrogen alone mediates the acceleration of glomerular disease. The interaction of estrogen and lipids may be critical, as estrogen accelerates glomerular disease in OZRs and other hyperlipidemic rat models [28].

We document several early estrogen-related changes in the glomerular gene expression in OZRs. Ovariectomy reduced glomerular fibronectin expression, while estrogen treatment increased expression of desmin (produced mainly by podocytes in glomerular injury) [29] and type IV collagen. Increased desmin could reflect podocyte damage from lipoprotein binding, with consequent reduced glomerular basement membrane permselectivity or simply a general mesangial phenotypic switch (also manifested here by increased collagen IV) [30–32]. Other matrix proteins such as heparan sulfate proteoglycans, laminin, and the 72 and 92 kD collagenases were expressed in all groups and were not affected by estrogen manipulation, perhaps reflecting the early stage of injury in these rats. In the only other study evaluating glomerular gene expression in young OZR, Lavaud et al noted marked glomerular expression of collagen IV and fibronectin in 4- and 12-week male rats [6], but their data are not comparable to ours, since time points and sex of the OZRs differed.

Our work does not support a significant role for infil-

trating glomerular monocyte/macrophages and consequent cytokine production in the early glomerular disease of OZRs, with or without added estrogen. No differences in glomerular monocyte/macrophage deposition were noted. Similar to the data of Lavaud et al [6], there were few monocytes detected in glomeruli in any group. In contrast, estrogen treatment significantly increased renal interstitial monocyte/macrophages and was associated with moderated interstitial fibrosis in two rats. This effect may be due to the exposure of the interstitium to increased filtered protein, an association supported by prior studies in OZR and other rat models [33]. However, since some rats did exhibit tubular apo A-IV staining, we cannot exclude a direct effect of increased or altered lipoproteins inducing interstitial inflammation, a mechanism proposed in hypercholesterolemic rats [34].

Taken together, our data support the hypothesis that estrogen has specific lipoprotein-mediated effects on the glomeruli of young OZRs, which may accelerate or initiate glomerulosclerosis. Deposition of specific apolipoproteins in the glomerulus may lead to glomerular cell proliferation and alteration of matrix protein production early in the renal disease. When considered together with the prosclerotic effects of estrogen in other hyperlipidemic rat models, our data prompt reconsideration of estrogen's effects in humans, in whom estrogen replacement therapy is known to raise serum TG levels [2]. Select patients with hypertriglyceridemia and incipient renal disease could be at risk for progression with estrogen replacement therapy. Significantly, in humans no studies have addressed the interrelationship of estrogen, hyperlipidemia, and proteinuric renal disease. Further studies on these interactions are warranted.

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