Estrogen Replacement During Hypoalbuminemia May Enhance Atherosclerotic Risk

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Abstract. Estrogen replacement therapy is considered antiatherosclerotic because it reduces LDL cholesterol and fibrinogen and increases HDL cholesterol concentrations. However, exogenous estrogen is also known to increase hepatic triglyceride production. Hyperlipidemia in the nephrotic syndrome is probably due to increased lipoprotein secretion into plasma and decreased clearance of lipoprotein cholesterol and triglycerides. Previously, lipid-lowering effects of ovariec-toomy in analbuminemic rats were observed, suggesting that in the presence of hypoalbuminemia, estrogen replacement may have adverse effects on the lipid profile. To test this hypothesis, ovariec-toomy control rats and rats with Adriamycin-induced nephrotic syndrome were treated with estradiol. In ovariec-toomized controls, estradiol reduced plasma LDL cholesterol, apolipoprotein B, and fibrinogen and increased apolipoprotein A-I and triglycerides. Nephrotic rats were characterized by a marked decrease in plasma colloid osmotic pressure, hyperfibrinogenemia, hyperlipidemia, and stimulated hepatic fatty acid synthesis. The beneficial effects of estradiol on LDL cholesterol, apolipoprotein B, and fibrinogen found in ovariec-toomized controls were not present in estradiol-treated nephrotic rats. This suggests that in hypoalbuminemia, downregulation of the LDL receptor overrides putative estradiol-induced increases in LDL receptor activity. Moreover, estrogen replacement in the nephrotic syndrome doubled fatty acid synthesis and triglyceride secretion, and markedly exacerbated hypertriglyceridemia, suggesting saturation of triglyceride clearance. Thus, severe hypoalbuminemia in rats induces an atherosclerotic metabolic response that is aggravated by estrogen replacement. These findings suggest that estrogen replacement in hypoalbuminemic subjects could be contra-indicated. (J Am Soc Nephrol 8: 1870–1876, 1997)

Estrogen replacement (ER) decreases atherosclerotic risk in the general postmenopausal female population (1,2), whereas in premenopausal women ovariec-toomy increases the incidence of coronary heart disease (3,4). Important effects of estrogens in this respect are a consistent decrease of LDL cholesterol (1,2), whereas in men severe hyperlipidemia than male NAR (18,19), and lipoprotein cholesterol levels are reduced by ovariec-toomy (20). The increased triglyceride levels and hepatic triglyceride secretion rates in female rats are corrected by ovariec-toomy and restored by ER after ovariec-toomy (20). However, the effects of ER on lipoprotein composition and triglyceride metabolism in the nephrotic syndrome have never been systematically probed.

In the current study, we tested the hypothesis that ER in the presence of hypoalbuminemia has adverse effects on atherosclerotic risk factors. Specific questions were: (1) Does ER decrease LDL cholesterol and increase HDL cholesterol in experimental nephrotic syndrome? (2) Does ER amplify VLDL HDL (11,13,14) levels would certainly be useful. However, hypertriglyceridemia is often present in patients with the nephrotic syndrome (11,15), probably due to a defect in the clearance of triglyceride-rich particles, namely chylomicrons and VLDL (16), as well as increased activity in enzymes involved in hepatic fatty acid synthesis (17). Amplifying triglyceride production on top of decreased catabolism could potentially exacerbate hyperlipidemia and therefore is a potential risk of ER in the nephrotic syndrome.

Interaction of estrogen and hypoalbuminemia on lipoprotein metabolism was described recently in the analbuminemic rat (NAR), a model of hyperlipidemia secondary to hypoalbuminemia without proteinuria (18,19). Female NAR are more severely hyperlipidemic than male NAR (18,19), and lipoprotein cholesterol levels are reduced by ovariec-toomy (20). The increased triglyceride levels and hepatic triglyceride secretion rates in female rats are corrected by ovariec-toomy and restored by ER after ovariec-toomy (20). However, the effects of ER on lipoprotein composition and triglyceride metabolism in the nephrotic syndrome have never been systematically probed.
interference of malonyl-CoA decarboxylase and pyruvate carboxylase activities in crude homogenates and inhibition of ACC by malonyl-CoA (23). Supernatant, obtained by centrifugation at 12,000 \( \times g \) and diluted in homogenization buffer, was preincubated for 30 min at 37°C. Assays were started by adding 100 \( \mu l \) of preheated assay mixture (37°C) to 100 \( \mu l \) of the biological sample (0.37 to 0.50 mg of protein). Reactions were stopped after 1 min by adding 100 \( \mu l \) of 10 M NaOH. The total incubation mixture for the assay contained 75 mM Hepes, pH 7.5, 125 mM mannitol, 2.5 mM 2-mercaptoethanol, 3.1 mM EDTA, 4.0 mM citrate, 5.6 mM MgCl\(_2\), 2.0 mM ATP, 20 mM KClO\(_4\), 0.93% (wt/vol) bovine serum albumin (charcoal-treated and dialyzed), 0.44 mM dithioerythritol, 0.5 mM NADPH, 0.062 mM butyryl-CoA, 0.062 mM [1-\(^{14}\)C]acetate-CoA (4 Ci/mol), and 3.2 mM FAS. The rest of this procedure was performed exactly as described (23).

FAS activity was determined by measuring the incorporation of [1-\(^{14}\)C]acetate-CoA into fatty acids in the presence of malonyl-CoA. Supernatant, obtained by centrifugation at 12,000 \( \times g \) and diluted in homogenization buffer (plus 3 mM diethiothreitol), was prewarmed for 5 min at 37°C. Assays were started by adding 100 \( \mu l \) of preheated assay mixture (37°C) to 100 \( \mu l \) of the biological sample (0.075 to 0.10 mg of protein). Assays were stopped after 5 min by adding 100 \( \mu l \) of 10 M NaOH. The total incubation mixture contained 75 mM Hepes, pH 7.5, 125 mM mannitol, 2.0 mM citrate, 4.16 mM EDTA, 37.5 mM NaCl, 1.5 mM diethiothreitol, 2.5 mM 2-mercaptoethanol, 0.93% (wt/vol) bovine serum albumin (charcoal-treated and dialyzed), 0.5 mM NADPH, 0.062 mM [1-\(^{14}\)C]acetate-CoA (4 Ci/mol), and 0.195 mM malonyl-CoA. Fatty acids were extracted and quantified as described (23). Cytosolic protein was determined by the Lowry method, using bovine serum albumin as standard. Cytosolic protein concentration was similar in all groups.

**Triglyceride Secretion Rate**

Triton WR-1339 is a nonionic detergent that blocks the removal of triglyceride (TG) from the circulation, causing a linear increase of plasma TG concentration. The rate of this increase is a measure of the total TG secretion rate (24). TG secretion rate was determined under pentobarbital anaesthesia (60 mg/kg) after an overnight fast, using Triton WR-1339 (Sigma Chemical, St. Louis, MO), at a dose of 600 mg/kg injected into a tail vein. Blood was sampled from the tail artery for TG analysis just before injection of Triton (\( t = 0 \)) and at 30, 60, and 90 min after injection. The TG secretion rate was calculated as the difference between the calculated 60-min value obtained from linear regression, using the three measured samples and the measured \( t = 0 \) value.

**Lipoprotein Isolation by Density-Gradient Ultracentrifugation**

In nonfasting rats, plasma lipoproteins were separated by density-gradient ultracentrifugation (18) into six fractions (chylomicrons and VLDL, density \( d < 1.006 \) g/ml; intermediate density lipoprotein, \( d = 1.006 \) to 1.019 g/ml; low density lipoprotein, LDL1, \( d = 1.019 \) to 1.04 g/ml and LDL2, \( d = 1.04 \) to 1.063 g/ml; high density lipoprotein, HDL, \( d = 1.063 \) to 1.21 g/ml). The subdivision of LDL into LDL1 and LDL2 was performed to separate the apo B-containing lipoproteins from the other particles present in the total LDL density range of 1.019 to 1.063 g/ml (25). Lipoprotein cholesterol and triglyceride levels were measured as described below.

**Biochemical Analyses**

Plasma and urinary protein levels were determined by the Bradford method. Plasma and urinary creatinine were determined colorimetric-
Statistical Analyses

Hepatic enzyme activities were calculated per whole organ and then normalized for body weight. Plasma triglycerides and proteinuria were log-normalized to achieve a normal distribution. Results are expressed as arithmetic means ± SEM. Data were tested by two-way ANOVA and Student-Neuman-Keuls test for multiple comparisons if the variance ratio (F) reached statistical significance (P < 0.05).

Results

Body weight increased steadily in OVX and OVX + NS rats, so that final body weight in these OVX animals was approximately 300 g (Table 1). E2 treatment prevented the increase in body weight in both OVX and OVX + NS rats. E2 had no effect on proteinuria in OVX + NS rats. Plasma creatinine was slightly increased in OVX + NS rats. In normal rats, E2 treatment increased plasma albumin and decreased fibrinogen (Table 1). In OVX + NS rats, in which albumin was low and fibrinogen high, E2 did not change these proteins. Thus, E2 had marked and diametrically opposite effects on two major plasma proteins in OVX rats, but not in OVX + NS rats.

The effects of E2 in the normal OVX rats consisted of increases in plasma triglycerides and apo A-I, and a decrease in both LDL1 cholesterol and apo B. No changes occurred in other apolipoproteins (Table 2). Note that LDL2 cholesterol and HDL cholesterol were not affected by E2 treatment. In OVX + NS rats, the effects of E2 were dramatically different. OVX + NS rats had much higher triglyceride levels (5.1 ± 1.5 mmol/L; P < 0.01 versus OVX) that were markedly exacerbated in the OVX + NS + E2 group (23.6 ± 6.4 mmol/L; P < 0.05 versus OVX + NS and P < 0.01 versus OVX + E2). VLDL cholesterol and intermediate density lipoprotein cholesterol, which were not detectable in the OVX and OVX + E2 groups, were increased in OVX + NS rats, and VLDL cholesterol was even higher in OVX + NS + E2 rats (Table 2). However, E2 had no effect on the very high LDL1 and apo B levels in OVX + NS + E2 rats, and E2 also had no effect on the high HDL2 and apo E levels in OVX + NS rats (Table 2). Both HDL cholesterol and apo A-I were increased in OVX + NS rats (P < 0.01), and, again, E2 did not affect HDL cholesterol in OVX + NS rats. However, E2 increased apo A-I, as well as apo A-IV, in OVX + NS + E2 rats (Table 2).

Liver weight, factored for body weight, was markedly increased by approximately 60% in OVX + NS and OVX + NS + E2 rats (P < 0.01), and was increased by approximately 18% by E2 in the OVX rats (unpaired t test: P < 0.01). However, the post hoc test was not significant for either the OVX versus OVX + E2 or OVX + NS versus OVX + E2 groups (Table 3). ACC and FAS activities in the liver, factored for body weight, were significantly increased in OVX + NS rats (P < 0.01). Compared with OVX rats, the ACC and FAS activities in OVX + E2 rats were increased. This difference was only significant for ACC (unpaired t test: P < 0.05). ACC and FAS activities were markedly higher in the OVX + NS + E2 group than in the OVX + NS group (P < 0.05). Thus, activity of these key enzymes in fatty acid synthesis was more susceptible to E2 stimulation in OVX + NS than in OVX livers.

Triglyceride secretion was studied in the fasting state in a second cohort. These rats showed the same characteristic differences as had been observed in the first cohort. There were E2-related differences in body weight, whereas the OVX + NS rats were severely proteinuric (OVX + NS: 885 ± 85 and OVX + NS + E2: 1012 ± 160 mg/d, respectively) and hyperlipidemic. Fasting triglyceride levels were also increased in the OVX-E2 group (0.95 ± 0.16 mmol/L versus 0.55 ± 0.10 mmol/L in the OVX group; P < 0.05) and markedly exacer-

<table>
<thead>
<tr>
<th>Table 1. Effects of estrogen on body weight, renal function, and plasma proteins in ovariecotomized nephrotic (OVX + NS) rats</th>
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</thead>
<tbody>
<tr>
<td>Category</td>
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<tr>
<td>-----------</td>
</tr>
<tr>
<td>Body weight (g)</td>
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<tr>
<td>UprotV (mg/d)</td>
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<tr>
<td>Creatinine (µmol/L)</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
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<tr>
<td>Fibrinogen (g/L)</td>
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<td>π (mmHg)</td>
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</table>

*OVX controls and Adriamycin-induced NS rats were treated with E2 or sham implants. OVX, ovariecotomized; E2, estradiol; NS, nephrotic syndrome; UprotV, proteinuria; π, colloid osmotic pressure.

b P < 0.05 NS versus non-NS.

c P < 0.05 E2 versus non-E2.
Table 2. Effects of estrogen on plasma lipids, lipoproteins, and apolipoproteins in ovariectomized nephrotic (OVX + NS) ratsa

<table>
<thead>
<tr>
<th>Category</th>
<th>OVX (n = 6)</th>
<th>OVX + E2 (n = 6)</th>
<th>OVX + NS (n = 8)</th>
<th>OVX + NS + E2 (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH (mM)</td>
<td>2.3 ± 0.10</td>
<td>2.4 ± 0.18</td>
<td>15.3 ± 1.8b</td>
<td>16.7 ± 1.2b</td>
</tr>
<tr>
<td>TG (mM)</td>
<td>0.4 ± 0.05</td>
<td>0.8 ± 0.04c</td>
<td>6.3 ± 1.3b</td>
<td>23.0 ± 5.3bc</td>
</tr>
<tr>
<td>VLDL CH (mM)</td>
<td>ND</td>
<td>ND</td>
<td>0.77 ± 0.69b</td>
<td>2.61 ± 1.17bc</td>
</tr>
<tr>
<td>IDL CH (mM)</td>
<td>ND</td>
<td>ND</td>
<td>0.86 ± 0.33b</td>
<td>1.17 ± 0.31b</td>
</tr>
<tr>
<td>LDL1 CH (mM)</td>
<td>0.37 ± 0.06</td>
<td>0.11 ± 0.05c</td>
<td>3.02 ± 0.70b</td>
<td>3.49 ± 0.40b</td>
</tr>
<tr>
<td>LDL2 CH (mM)</td>
<td>1.02 ± 0.08</td>
<td>0.91 ± 0.10</td>
<td>2.84 ± 0.34b</td>
<td>3.11 ± 0.31b</td>
</tr>
<tr>
<td>HDL CH (mM)</td>
<td>0.87 ± 0.08</td>
<td>0.97 ± 0.07</td>
<td>2.33 ± 0.27b</td>
<td>2.03 ± 0.25b</td>
</tr>
<tr>
<td>apo A1 (mg/dl)</td>
<td>36.0 ± 3.4</td>
<td>49.5 ± 3.0d</td>
<td>172 ± 20b</td>
<td>228 ± 13bc</td>
</tr>
<tr>
<td>apo AIV (mg/dl)</td>
<td>18.1 ± 2.4</td>
<td>22.0 ± 1.0</td>
<td>15.2 ± 1.0</td>
<td>32.2 ± 2.9bc</td>
</tr>
<tr>
<td>apo B (U)</td>
<td>100 ± 7</td>
<td>48 ± 2e</td>
<td>318 ± 31b</td>
<td>306 ± 38b</td>
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<tr>
<td>apo E (mg/dl)</td>
<td>25.4 ± 2.4</td>
<td>25.5 ± 1.6</td>
<td>43.1 ± 3.1b</td>
<td>44.2 ± 1.8b</td>
</tr>
</tbody>
</table>

a OVX controls and Adriamycin-induced NS rats were treated with E2 or sham implants. Plasma cholesterol, triglycerides, lipoproteins, and apolipoproteins were measured in the nonfasted state. CH, cholesterol; TG, triglycerides; VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; ND, not detectable. Other abbreviations as in Table 1.

b P < 0.05 NS versus non-NS.

c P < 0.05 E2 versus non-E2.

Table 3. Effects of estrogen on liver weight (g/100 g body wt), and the activities of ACC and FAS expressed as μmol/min per 100 g body wt in ovariectomized nephrotic (OVX + NS) ratsa

<table>
<thead>
<tr>
<th>Category</th>
<th>OVX (n = 6)</th>
<th>OVX + E2 (n = 6)</th>
<th>OVX + NS (n = 8)</th>
<th>OVX + NS + E2 (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver weight</td>
<td>2.7 ± 0.7</td>
<td>3.2 ± 0.1</td>
<td>4.5 ± 0.3b</td>
<td>5.0 ± 0.2b</td>
</tr>
<tr>
<td>ACC</td>
<td>0.31 ± 0.02</td>
<td>0.42 ± 0.04</td>
<td>0.58 ± 0.07b</td>
<td>0.99 ± 0.13bc</td>
</tr>
<tr>
<td>FAS</td>
<td>1.87 ± 0.13</td>
<td>2.15 ± 0.11</td>
<td>3.17 ± 0.35b</td>
<td>4.84 ± 0.45bc</td>
</tr>
</tbody>
</table>

a OVX controls and Adriamycin-induced NS rats were treated with E2 or sham implants. ACC, acetyl CoA carboxylase; FAS, fatty acid synthase. Other abbreviations as in Table 1.

b P < 0.05 NS versus non-NS.

c P < 0.05 E2 versus non-E2.

bated by E2 in the OVX + NS + E2 group (33.0 ± 8.3 versus 8.5 ± 2.2 mmol/L in the OVX + NS group; P < 0.05). Triglyceride secretion rates were virtually identical in OVX, OVX + E2, and OVX + NS rats, but were increased twofold in OVX + NS + E2 rats (P < 0.05 versus both OVX + NS and OVX + E2 groups) (Figure 1).

Discussion

Although ER is known to decrease atherosclerotic risk in postmenopausal women in general, the data in the present study clearly show that estrogen may have adverse effects in the presence of hypoalbuminemia. In our rat model, we found that the well known effects of ER after ovariectomy, i.e., a reduction of LDL and fibrinogen, were lost in rats with OVX + NS. Moreover, in these markedly hypoalbuminemic NS rats, estrogen also doubled VLDL triglyceride production and strongly exacerbated hypertriglyceridemia.

ER reduces LDL cholesterol (5) and apo B (2) in humans, as well as female rats (present study). The reduction in LDL cholesterol is probably due to an increase in the number of

Figure 1. Effects of estrogen on triglyceride secretion rate in ovariectomized nephrotic (OVX + NS) rats. Ovariectomized control rats (OVX) and Adriamycin-induced NS rats were treated with estradiol (E2) or sham implants. Triglyceride secretion rate was measured after an overnight fast. *P < 0.05 NS versus non-NS; #P < 0.05 E2 versus non-E2.
LDL (apo B/E) receptors, an effect observed in rats (27,28), hamsters, and rabbits (29), as well as humans (30). Hypoalbuminemia in the nephrotic syndrome is associated with an increase in LDL cholesterol (9–11). Turnover studies in patients indicate that this may be at least partly due to a decrease in receptor-related clearance (31), and in rats with experimental nephrotic syndrome, downregulation of hepatic LDL receptor expression has recently been documented (32). Our data suggest that in the presence of impaired receptor-related clearance, ER cannot upregulate clearance of LDL.

ER increases HDL cholesterol in women (33–36). Both turnover studies in patients and molecular biological analysis of the livers of rodents with experimental nephrosis indicate that the increase in HDL cholesterol is probably due to an increase in the synthesis of apo A-I (34,37), as well as a reduction in the production (28) and activity of hepatic lipase (28,34–36). HDL cholesterol remained unchanged by E2 treatment in our OVX rats, whereas apo A-I increased. This is in sharp contrast to the effects of pharmacological doses of E2 in male rats (27,38), which are known to cause severe lowering of HDL cholesterol. Earlier studies, using physiological doses of E2 (1 to 5 μg/kg per d) in female rats, resulted in effects quite similar to ours, e.g., increase in apo A-I concentration, without a significant effect on HDL cholesterol (39).

Increased production of apo A-I and HDL are hallmarks of the experimental nephrotic syndrome (40–43), resulting in increases in plasma apo A-I (42,44). OVX analbuminemic rats show high HDL levels (20) similar to those observed in OVX nephrotic rats in the present study. These rats were free of proteinuria, and therefore the changes in apo A-I in nephrotic rats can probably be ascribed to low albumin. In the nephrotic rats in the present study, ER failed to cause a further increase in HDL cholesterol, whereas apo A-I levels again were elevated. Whether the further increase in plasma apo A-I in the nephrotic rats with ER was due to enhancement of hepatic synthesis is not clear, although this would be parallel to what was observed for fatty acid synthesis (see below). Although not determined directly in the present study, it can be concluded that ER had a profound effect on HDL composition, resulting in apo A-I-rich particles relatively poor in cholesterol.

Plasma apo A-IV did not increase in OVX rats with ER, which is in accordance with the observations by Staels et al. in rats at a low dosage of estradiol (≤0.2 μg/d) (45). In that study, higher doses of estradiol caused a marked decrease of apo A-IV (45). Although apo A-IV was unchanged in untreated nephrotic rats (44), ER more than doubled apo A-IV levels. Apo A-IV is partially cleared by the kidneys (46). It is possible that ER affects this process in the nephrotic kidney. Nephrotic syndrome was associated with an increase in apo E and LDL2, conforming with previous observations in male rats (17,44).

Chronic ER is associated with hypertriglycerideremia and increased hepatic triglyceride secretion (6) due to an increase in VLDL triglyceride (6) and apo B (5) production. Triglyceride removal depends on a saturable enzymatic process, primarily involving lipoprotein lipase (7). Thus, increasing VLDL triglyceride production is likely to lead to hypertriglycerideremia, and the plasma triglyceride concentration will depend on the activity of the lipolytic system. This could cause a problem in the nephrotic syndrome, in which lipolytic activity is known to be impaired (16,47,48). In primary hypertriglycerideremia due to low lipoprotein lipase activity, hyperestrogenism secondary to pregnancy (49,50), ER (51), or estrogen in contraceptive agents (52,53) can result in excessively high triglyceride levels. Enhanced hypertriglycerideremia has been reported in patients with secondary hyperlipidemia who were treated with ER (54).

In the present study, we observed exacerbation of hypertriglycerideremia, as well as increased VLDL cholesterol in hypoalbuminemic rats when subjected to ER. This is probably due to an increase in fatty acid synthesis in the nephrotic syndrome (see Table 3). We have also previously observed such increases by ER in albuminemic rats (20). Thus, hypertriglycerideremia in a variety of conditions may be susceptible to amplification by ER. It is therefore attractive to postulate a common derangement in triglyceride metabolism in all such conditions. Apparently, either superimposing an increase in triglyceride production upon a derangement in triglyceride removal or further enhancement of a priori increased triglyceride synthesis, or both, will lead to saturation of the lipolytic system and severe hypertriglycerideremia.

ER decreases fibrinogen (1,2). The mechanism of this effect is not clear, but one possibility is by increasing fibrinolysis (55). In the nephrotic syndrome, fibrinogen synthesis is increased (56), fractional catabolism is decreased (56), and fibrinolysis is severely disturbed (12). Our data indicate that for fibrinogen, as is the case for LDL, the disturbance induced by the nephrotic syndrome is such that ER becomes ineffective. In contrast to what we observed for fibrinogen, ER increased plasma albumin levels by nearly 40%. Surprisingly, we were unable to find data in the literature on this effect of ER on plasma albumin concentration in OVX animals. Albumin levels found in the untreated OVX control rats were somewhat low, as were the values for plasma colloid osmotic pressure, compared with what we have observed previously in intact female Sprague Dawley rats (57). These relatively low values appeared to be normalized by ER. Possibly, hepatic albumin synthesis is supported by estrogen. Our data suggest that at a dietary protein intake of approximately 20% (present study), the stimulus of nephrotic hypoalbuminemia on hepatic albumin synthesis is maximal, so that no further stimulation by ER was possible. This does not seem to be the case for the hepatic triglyceride secretion rate, which is doubled by ER in NS, leading to extreme hypertriglycerideremia.

In summary, severe hypoalbuminemia, characteristic of the nephrotic syndrome, induces a metabolic response that is aggravated by ER. These findings suggest that ER in hypoalbuminemic subjects may be contra-indicated.

Acknowledgments

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