Endogenous Estrogens Lower Plasma PCSK9 and LDL Cholesterol But Not Lp(a) or Bile Acid Synthesis in Women

Lena Persson, Peter Henriksson, Eli Westerlund, Outi Hovatta, Bo Angelin, Mats Rudling

Objective—Cholesterol and lipoprotein metabolism display pronounced gender differences. Premenopausal women have lower LDL and higher HDL cholesterol, whereas men display higher synthetic rates of bile acids and cholesterol. Administration of exogenous hormones to humans and animals suggest that these gender differences can often be explained by estrogens. We evaluated how increased levels of endogenous estrogens modulate cholesterol and lipoprotein metabolism in women.

Methods and Results—We studied healthy women during initiation of in vitro fertilization using blood samples when endogenous estrogens were low and high. Cholesterol in very LDL and LDL, but not in HDL, was reduced 20% when estrogens were high. Apolipoprotein B levels decreased 13%. Apolipoprotein A-I and triglyceride levels increased 8% and 37%, respectively, whereas lipoprotein(a) were unchanged. Circulating PCSK9, a suppressor of LDL receptors, was reduced 14% when estrogens were high. Serum markers of bile acid and cholesterol synthesis were unaltered. Growth hormone levels increased 3-fold when estrogens were high, whereas insulin-like growth factor-1 and fibroblast growth factor-21 concentrations were unaltered.

Conclusion—In women, Apolipoprotein B-containing particles and circulating PCSK9 are reduced when endogenous estrogens are high, indicating that estrogens induce hepatic LDL receptors partly through a posttranscriptional mechanism. However, estrogens do not stimulate bile acid or cholesterol synthesis.

Key Words: lipids ■ lipoproteins ■ circulating PCSK9 ■ hormonal regulation ■ metabolism
Thirty-one healthy women scheduled for in vitro fertilization were studied. Their mean age was 33.3 (SD) years, mean weight 67.0 ± 13.3 kg, and mean body mass index 24.1 ± 3.6. To induce controlled ovarian hyperstimulation, endogenous estrogens were first suppressed using a gonadotropin releasing hormone agonist, buserelin (Suprecur, Aventis Pharma, Frankfurt, Germany). Starting on the 21st day of the menstrual cycle, all patients received 300 μg × 3 of buserelin as nasal spray. After the menstrual bleeding, 2 weeks later, an estradiol (E₂) measurement was carried out to verify suppression. The gonadotropin releasing hormone dose was then decreased to 300 ng/mL.

Blood Sampling
Blood samples were obtained at 8 am after overnight fast twice during the in vitro fertilization procedure: at suppression and after 6 days stimulation of estrogen synthesis (Table 1). Serum was isolated and samples stored at −80°C.

Table 1. Hormonal Levels

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
<th>Median</th>
<th>Range</th>
<th>% Change</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol (E₂) (ng/mL)</td>
<td>Low E2</td>
<td>0.15 ± 0.22</td>
<td>0.15</td>
<td>(0.15–0.27)*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>High E2</td>
<td>5.89 ± 4.72</td>
<td>3.99</td>
<td>(1.62–19.5)</td>
<td>3720</td>
</tr>
<tr>
<td>Growth hormone (ng/mL)</td>
<td>Low E2</td>
<td>1.21 ± 1.72</td>
<td>0.29</td>
<td>(0.06–6.14)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High E2</td>
<td>4.01 ± 4.15</td>
<td>2.23</td>
<td>(0.69–14.9)</td>
<td>232</td>
</tr>
<tr>
<td>Insulin-like growth factor-1 (ng/mL)</td>
<td>Low E2</td>
<td>182 ± 70.7</td>
<td>176</td>
<td>(83.9–386)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High E2</td>
<td>170 ± 70.7</td>
<td>175</td>
<td>(72.7–337)</td>
<td>−6.3 n.s.</td>
</tr>
<tr>
<td>Fibroblast growth factor-21 (pg/mL)</td>
<td>Low E2</td>
<td>142 ± 118</td>
<td>101</td>
<td>(33.2–445)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High E2</td>
<td>121 ± 122</td>
<td>63.6</td>
<td>(31.3–455)</td>
<td>−14 n.s.</td>
</tr>
</tbody>
</table>

Serum levels during low and high endogenous estradiol in 31 women; samples taken at 8 am after overnight fast.

Results
All participants responded to treatment, and after suppression of estrogen synthesis all but 1 had E₂ levels below the detection limit of 0.15 ng/mL. Six days stimulation of estrogen synthesis by follicle-stimulating hormone increased endogenous E₂ levels >30-fold (Table 1). Total cholesterol levels were reduced 14% in response to elevated estrogen levels (Table 2). This was due to reductions in LDL and VLDL, whereas HDL cholesterol was unaltered (Figure A). The retention time of the LDL-C peak was significantly longer during high estrogen levels (Figure A), indicating smaller particles. ApoB levels were reduced 13% when estrogens were high, whereas ApoA-I increased 8% (Table 2), resulting in a reduced ApoB/ApoA-I ratio. Concomitantly, serum total triglycerides were significantly increased by 37%. There was no change in Lp(a) levels (Table 2) whereas PCSK9 levels were significantly reduced 14% (Figure B). There was a positive correlation between the changes in ApoB and PCSK9 (R = 0.38, P < 0.05, not shown).

Methods
Subjects and Study Design
Commercially available ELISA kits were used to measure PCSK9 (Cat. No. Circulex CY-8079; CycLex, Japan), and (all from R&D Systems, Minneapolis, MN) growth hormone (GH; Cat. No. DGH00); insulin-like growth factor (IGF)-1 (Cat. No. DG100); and fibroblast growth factor-21 (Cat. No. DF2100). All kits were used according to manufacturer’s instructions.

Statistics
Significances of differences were tested by Wilcoxon’s paired test using computer software (GraphPad Prism).

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Table 2. Lipids and Apoproteins

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
<th>Median</th>
<th>Range</th>
<th>% Change</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td></td>
<td></td>
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<tr>
<td>Low E2</td>
<td>6.3 ± 1.4</td>
<td>6.2</td>
<td>(3.3–9.2)</td>
<td></td>
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</tr>
<tr>
<td>High E2</td>
<td>5.4 ± 1.2</td>
<td>5.4</td>
<td>(3.0–8.8)</td>
<td>−14</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total TG (mM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Low E2</td>
<td>0.63 ± 0.60</td>
<td>0.51</td>
<td>(0.10–3.6)</td>
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<tr>
<td>High E2</td>
<td>0.86 ± 0.56</td>
<td>0.86</td>
<td>(0.10–2.8)</td>
<td>37</td>
<td>0.012</td>
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</table>

Table 3. Serum Markers of Cholesterol Metabolism

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
<th>Median</th>
<th>Range</th>
<th>% Change</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lathosterol/c (mg/mole)</td>
<td></td>
<td></td>
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<tr>
<td>Low E2</td>
<td>327 ± 121</td>
<td>323</td>
<td>(169–652)</td>
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<tr>
<td>High E2</td>
<td>375 ± 185</td>
<td>309</td>
<td>(104–807)</td>
<td>15</td>
<td>NS</td>
</tr>
<tr>
<td>C4/c (mg/mole)</td>
<td></td>
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<tr>
<td>Low E2</td>
<td>2.98 ± 2.03</td>
<td>2.25</td>
<td>(0.90–8.70)</td>
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</tr>
<tr>
<td>High E2</td>
<td>3.47 ± 3.04</td>
<td>3.05</td>
<td>(0.70–16.8)</td>
<td>17</td>
<td>NS</td>
</tr>
<tr>
<td>Sitosterol/c (mg/mole)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Low E2</td>
<td>524 ± 202</td>
<td>499</td>
<td>(187–966)</td>
<td></td>
<td></td>
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<tr>
<td>High E2</td>
<td>507 ± 188</td>
<td>506</td>
<td>(199–856)</td>
<td>−3.4</td>
<td>NS</td>
</tr>
<tr>
<td>Campesterol/c (mg/mole)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Low E2</td>
<td>752 ± 262</td>
<td>699</td>
<td>(346–1490)</td>
<td></td>
<td></td>
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<tr>
<td>High E2</td>
<td>755 ± 257</td>
<td>761</td>
<td>(278–1248)</td>
<td>0.4</td>
<td>NS</td>
</tr>
<tr>
<td>Lp(a) (mM)</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Low E2</td>
<td>24.3 ± 23.3</td>
<td>16.6</td>
<td>(2.53–87.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High E2</td>
<td>23.6 ± 24.2</td>
<td>15.0</td>
<td>(1.71–102)</td>
<td>13</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

We evaluated whether estrogen status influenced aspects of cholesterol metabolism. However, neither bile acid synthesis determined from the plasma marker C4/c, cholesteryl synthesis as reflected by lathosterol/c levels, nor intestinal cholesterol absorption, assessed from plasma plant sterol levels or CETP activity were altered (Table 3) in accordance with stable HDL-C levels, the activity of cholesteryl ester transfer protein in serum was also unaltered.

Figure A. Cholesterol profiles as assayed by fast performance liquid chromatography during low- and high-endogenous estradiol in 31 women. Gray lines indicate low estradiol (E2), where thick line represents mean, and thin line mean ± 1 SD, black lines indicate high E2, where thick line represents mean and thin line mean ± 1 SD. Samples were obtained at 8 am after overnight fast. Cholesterol fractions in the various lipoproteins were calculated from the areas under the curves, as previously described.16

Figure B. Individual serum levels of PCSK9 during low and high endogenous estradiol in 31 women; samples taken at 8AM after overnight fast. Black lines indicate mean values. Significances of differences were calculated using Wilcoxon’s paired test. **P<0.01, ***P<0.001.
As seen during estrogen treatment of both males and females, increased endogenous estrogens reduce circulating VLDL-C, LDL-C, and ApoB levels. This is in line with the concept that the plasma clearance of ApoB-containing particles is stimulated because of increased hepatic LDLRs. The number of LDLRs is presumably in part enhanced by an estrogen-induced gene expression. Our finding that high levels of endogenous estrogens reduce serum PCSK9 indicates that estrogens also increase hepatic LDLR numbers by a posttranscriptional mechanism, as has been shown in the rat. Thus, the 13% reduction in PCSK9 observed should lead to increased LDLR numbers and further reduced LDL-C levels. The finding of an estrogen-induced reduction of circulating PCSK9 is also in line with previous findings of increased PCSK9 and LDL-C levels in plasma of postmenopausal women.

In animals, hepatic HMG-CoA reductase and PCSK9 are both transcriptionally regulated by cholesterol via sterol regulatory element binding protein-2. Accordingly, circulating levels of PCSK9 and lathosterol/c levels correlate under basal conditions in humans. However, in the present study lathosterol/c levels were not influenced by estrogen status and did not correlate with PCSK9. This lack of coregulation may indicate that estrogens elicit sterol regulatory element binding protein-2-independent responses in humans. This possibility is supported by the observation that liver microsomal cholesterol is reduced in estrogen-treated men, a finding that is in contrast to what is seen in estrogen-treated rats where liver cholesterol is increased and sterol regulatory element binding protein-2 gene expression is decreased. Further investigation of these interesting species differences should be important.

Somewhat unexpectedly, HDL-C levels were not influenced by high estrogen levels. In contrast, ApoA-I was increased, presumably reflecting an increased hepatic production of this protein. This may represent an early response when estrogen levels increase. The composition of both LDL and HDL particles seems to be altered, with a small but distinct shift in the retention time of the LDL particles, indicating smaller LDL particles during high estrogen levels. This may be the result of a faster clearance of larger LDLs due to the increased LDLR expression in this situation. If this has any relevance for a potentially increased atherogenicity cannot be decided, but the reduction of the ApoB/ApoA-I ratio when estrogens were high could be taken as a sign of the opposite.

The lipid modulating effects of high endogenous estrogens appeared without any changes in bile acid synthesis or cholesterol absorption. We have recently shown that adult women have lower bile acid and cholesterol synthesis than men throughout life. Our present finding that bile acid synthesis is not influenced by high endogenous estrogens in females speaks strongly against that this hormone is involved in that gender-related difference. This is again in contrast to the situation in rodents where estrogen treatment stimulates bile acid synthesis.

As expected from previous studies of estrogen treatment, serum GH levels increased considerably when estrogens were high. Treatment of humans with GH elicits several responses in cholesterol metabolism that are similar to those of estrogen. Thus, the clearance of plasma LDL is enhanced and the number of hepatic LDLRs increased. Further, GH treatment also increases triglyceride levels but does not affect the synthesis of bile acids or cholesterol in man. Another species difference between rodents and humans is that while treatment with GH leads to increased hepatic gene expression of PCSK9 in the rat, circulating PCSK9 levels are reduced in response to GH administration in humans. Thus, it is tempting to speculate that several of the responses observed in response to high endogenous estrogen may actually be mediated by GH.

Somewhat surprisingly, high levels of endogenous estrogens did not influence Lp(a) levels. This may be another indication of the metabolic complexity in this situation. GH treatment increases the secretion of IGF-1, an important mediator of GH effects, and Lp(a) whereas estrogen treatment decreases IGF-1 and Lp(a) levels. IGF-1 and Lp(a) levels were both unaltered when estrogens where high, supporting the concept that GH and estrogens have opposite effects on IGF-1 and Lp(a) levels. The metabolic regulator fibroblast growth factor-21 is not influenced by estrogen status, and in accordance there seems to be no gender difference in fibroblast growth factor-21 serum levels.

In conclusion, endogenous estrogens exert several specific effects on cholesterol metabolism in females. Among the early effects are reduced circulating levels of PCSK9, LDL-C, and ApoB. There is previous experimental evidence supporting that some of these effects on cholesterol metabolism may be mediated by increased GH secretion. The possibility to further explore hormone-induced, nonsterol-mediated lowering of PCSK9 as a new principle of improved therapy of dyslipidemias should be of interest.

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Disclosures
None.

References


13. Temu et al Effects of Endogenous Estrogens on PCSK9 in Humans


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