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. The effects of the administration of exogenous hormones to humans and animals indicate 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 obtained when endogenous estrogens were low and high. Cholesterol in VLDL 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) levels 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-I 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 endogenous estrogens induce hepatic LDL receptors partly through a posttranscriptional mechanism. However, estrogens do not stimulate bile acid or cholesterol synthesis. (Arterioscler Thromb Vasc Biol. 2012;32:810-814.)

Key Words: lipids ■ lipoproteins ■ circulating PCSK9 ■ hormonal regulation ■ metabolism

Increased levels of plasma LDL-cholesterol (LDL-C) are associated with an enhanced risk of developing cardiovascular disease.1 Fertile women have lower plasma LDL-C than men.2 LDL-C increases with aging, and postmenopausal women acquire higher LDL-C than premenopausal women or men of the same age.3 Women display higher HDL cholesterol (HDL-C) levels but 30% lower bile acid and cholesterol synthesis than men throughout adult life.4 Estrogen treatment reduces LDL-C, apolipoprotein (ApoB), and lipoprotein(a) (Lp[a]), whereas HDL-C and ApoAI levels increase5–9; whether estrogens alter bile acid or cholesterol synthesis in man is unknown. The plasma level of LDL-C is largely determined by the number of hepatic LDL receptors (LDLRs),10 and treatment with estrogens at high-dose increases this number 3-fold in men.11 Estrogen treatment is the most potent way to induce hepatic LDLRs in the rat;12 this unique effect of estrogen is due to a 4- to 5-fold increased gene expression of the LDLR combined with a 50% suppression of hepatic PCSK9 gene expression.13 This occurs together with a 60% reduction of hepatic sterol regulatory element binding protein (SREBP)-2 levels,14 likely due to a pronounced (>30%) estrogen-induced increase in hepatic cholesterol.15 It is not known if the pronounced increase in hepatic LDLRs seen in human liver following estrogen treatment11 is related to reduced PCSK9.

Little data are available on the effects of endogenous estrogens on cholesterol and lipoprotein metabolism. To study this, we established a human model based on a routine clinical procedure. Women preparing for in vitro fertilization were studied by comparing 2 treatment phases: extreme suppression and strong stimulation of endogenous estrogen levels. We show that 6 days of stimulation of endogenous estrogens reduces serum levels of VLDL-C, LDL-C and ApoB, whereas triglycerides and ApoA-I increase. Circulating PCSK9 levels decrease significantly, supporting the hypothesis that estrogens also in man increase the number of hepatic LDLRs partly by reducing PCSK9. However, bile acid or cholesterol synthesis is not reduced when endogenous estrogens are high, suggesting that female sex hormones are
was approved by the Ethics Committee of Karolinska Institutet.

scanning of the ovarian follicles 9 to 10 days after the first follicle-stimulating hormone injection.

Growth hormone dose was then decreased to 300 ng/mL.

Blood samples were obtained at 8 am after overnight fast twice according to clinical history and body mass index. The response was

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>0.15 ± 0.22</td>
<td>0.15</td>
<td>(0.15–0.27)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low E2</td>
<td>5.89 ± 4.72</td>
<td>3.99</td>
<td>(1.62–19.5)</td>
<td>3720</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Growth hormone (ng/mL)</td>
<td>1.21 ± 1.72</td>
<td>0.29</td>
<td>(0.06–6.14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low E2</td>
<td>4.01 ± 4.15</td>
<td>2.23</td>
<td>(0.69–14.9)</td>
<td>232</td>
<td>0.0011</td>
</tr>
<tr>
<td>Insulin-like growth factor-1 (ng/mL)</td>
<td>182 ± 70.7</td>
<td>176</td>
<td>(83.9–386)</td>
<td>6.3 n.s.</td>
<td></td>
</tr>
<tr>
<td>Fibroblast growth factor-21 (pg/mL)</td>
<td>121 ± 122</td>
<td>63.6</td>
<td>(31.3–455)</td>
<td>14 n.s.</td>
<td></td>
</tr>
</tbody>
</table>
| Serum levels during low and high endogenous estradiol in 31 women; samples taken at 8 am after overnight fast. P values were obtained after Wilcoxon’s paired test. *All, except for one (0.27 ng/mL), had levels below the detection limit of 0.15 ng/mL.

Methods

Subjects and Study Design

Thirty-one healthy women scheduled for in vitro fertilization were studied. Their mean age was 33 ± 3.3 years, mean weight 67.0 ± 12.7 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 µg×2, and ovarian stimulation was initiated by subcutaneous administration of recombinant human follicle-stimulating hormone, either as follitropin alpha (Gonal-f, Merck Serono, Stockholm, Sweden), or follitropin beta (Puregon, Schering-Plough, Gothenburg, Sweden). The starting dose range was from 75 to 300 IU/day according to clinical history and body mass index. The response was followed up by an E2 measurement 6 days later, and by ultrasound scanning of the ovarian follicles 9 to 10 days after the first follicle-stimulating hormone injection.

All subjects gave informed consent to participate in the study that was approved by the Ethics Committee of Karolinska Institutet.

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.

Serum Analyses

Free estradiol was measured using routine clinical assays (detection limit, 0.15 ng/mL). Total cholesterol and triglycerides (TG) were determined using colorimetric techniques with reagents from Roche Diagnostics GmbH (Mannheim, Germany). Lipoprotein cholesterol profiles were obtained by fast performance liquid chromatography using kits from Roche Diagnostics GmbH (Mannheim, Germany). Cholesterol in lipoproteins were calculated from areas under the curve. Apos A-I, B, and Lp(a) were determined using immunoturbidimetric techniques with reagents from Kamiya Biomedical Company (KAI-002 and KAI-004, Seattle, WA) and Diasys Diagnostic system GmbH (Lp[a] 21 FS, Holzheim, Germany). Cholesterol ester transfer protein activity was measured using a fluorescence assay kit from BioVision (K601-100, Mountain View, CA).

Unesterified lathosterol (reflecting cholesterol synthesis) was extracted with the following procedure. We mixed 25 µL serum from each individual with 0.5 mL isotonic NaCl and 3 mL Folch and incubated overnight, and the CHCl3 phase was collected and dried at 60°C under nitrogen. The samples were diluted in 0.5 mL MeOH/H2O 4:1 and isolated by Isolute MF-C18 0.1 g columns. The samples were derivatized with trimethylsilyl reagent (pyridine:hexamethyldisilazane:trimethylchlorosilane 3:2:1), dried at 60°C under nitrogen and dissolved in hexan prior to gas chromatography-mass spectrometry analysis. Δ7-Lathosterol was used as internal standard (25 µL sample). Lathosterol levels were normalized for total cholesterol as previously outlined. Serum levels of 7α-hydroxy-4-cholesten-3-one (C4) (marker for bile acid synthesis) were analyzed by high-pressure liquid chromatography and normalized for total serum cholesterol. Serum sitosterol and campesterol (reflecting cholesterol absorption) were extracted in duplicate with the following procedure. 20 µL serum from each individual, 250 µL 33% KOH and 1 mL 99.5% EtOH were thoroughly mixed for 2 hours, for extraction 1 mL isotonic NaCl and 2 mL CHCl3 were added and vortexed whereafter the CHCl3 phase was collected and dried at 40°C under nitrogen. The samples were derivatized with trimethylsilyl reagent, dried at 60°C under nitrogen and dissolved in hexane prior to gas chromatography-mass spectrometry analysis. Δ7-Campesterol and Δ7-Sitosterol were used as internal standards (25 µL/sample). Campesterol and sitosterol were normalized for total cholesterol.

Commercially available ELISA kits were used to measure PCSK9 (Cat. No. Circlex 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 (FGF)-21 (Cat. No. DF2100). All kits were used according to manufacturer’s instructions.

Statistics

Significances of differences were tested by Wilcoxon’s paired test and correlations were tested using Pearson’s correlation constant r, using computer software (GraphPad Prism).

Results

All participants responded to treatment, and after suppression of estrogen synthesis all but one 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 by 14% (Figure B). There was a positive correlation between the changes in ApoB and PCSK9 (R=0.38, P<0.05, not shown).
We evaluated whether estrogen status influenced aspects of cholesterol metabolism. However, neither bile acid synthesis determined from the plasma marker C4/c, cholesterol synthesis as reflected by lathosterol/c levels, nor intestinal cholesterol absorption, assessed from plasma plant sterol levels or CETP activity were altered in response to endogenous estrogens (Table 3). In accordance with stable HDL-C levels, the activity of cholesteryl ester transfer protein in serum was also unaltered (Table 3).

Estrogen is known to enhance GH secretion. In this work, we used a clinical standard procedure to evaluate metabolic responses to variations in endogenous estrogens in healthy women. By comparing a situation of estrogen depletion with a state of estrogen excess, major effects on lipoprotein and cholesterol metabolism could be established within 6 days of elevated estrogens. Several of these mimicked those observed after short-term treatment with exogenous estrogens.9 The exposure time is likely important and prolonged elevation of endogenous estrogens may cause stronger effects similar to those observed during long-term treatment.5–9 Our results also clearly demonstrate some important species differences with regard to how estrogens influence lipid metabolism.

Discussion

In this work, we used a clinical standard procedure to evaluate metabolic responses to variations in endogenous estrogens in healthy women. By comparing a situation of estrogen depletion with a state of estrogen excess, major effects on lipoprotein and cholesterol metabolism could be established within 6 days of elevated estrogens. Several of these mimicked those observed after short-term treatment with exogenous estrogens.9 The exposure time is likely important and prolonged elevation of endogenous estrogens may cause stronger effects similar to those observed during long-term treatment.5–9 Our results also clearly demonstrate some important species differences with regard to how estrogens influence lipid metabolism.
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 LDLRs 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 SREBP-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 SREBP-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 SREBP-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 TG levels but does not affect the synthesis of bile acids or cholesterol in mammals. 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 were high, supporting the concept that GH and estrogens have opposite effects on IGF-1 and Lp(a) levels. The metabolic regulator FGF-21 is not influenced by estrogen status, and in accordance there seems to be no gender difference in FGF-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

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