Evidence from many large epidemiological studies indicates that postmenopausal oestrogen replacement substantially reduces the risk of cardiovascular disease (1) and it has been reported that there is also protection against death from stroke (2). The protective effect on the cardiovascular system is believed to be mediated by the effect of oral oestrogens on lipoproteins, particularly high-density lipoprotein (3). However, changes in low- and high-density lipoproteins (LDL and HDL) cannot wholly explain the reduction in risk conferred by oral oestrogens and attention is now becoming focused on the effects of oestrogens on other markers of cardiovascular risk.

Several studies have linked high levels of lipoprotein(a), a genetically determined lipoprotein variant with a lipid composition similar to that of LDL, to both cardiovascular and cerebrovascular disease (4). An increased risk of coronary atherosclerosis of at least twofold is associated with lipoprotein(a) levels of greater than 300 mg/l (5). Lipoprotein(a) has close homology to plasminogen and it is also present in atherosclerotic plaques where it appears to compete with plasminogen, reducing fibrinolytic activity within the clot. Therefore, as well as its strong association with diseases involving atherogenesis and thrombosis, lipoprotein(a) may have a possible role in coagulation and fibrinolysis (4).

To investigate whether changes in lipoprotein(a) might help to account for the reduction in cardiovascular disease and stroke associated with postmenopausal oestrogen therapy, we measured this lipoprotein before and after 4 months of treatment with oestradiol.

Patients and methods

Twenty-three women attending menopause clinics in Stobhill Hospital and the Western Infirmary, Glasgow, for treatment of climacteric symptoms were recruited to the study. All had had hysterectomy and bilateral oophorectomy for benign gynaecological disease at least 2 months prior to recruitment and had received no previous hormonal therapy. Serum samples were collected for measurement of lipoprotein(a) before and after 4 months of treatment with oestradiol valerate (2 mg/day). Lipoprotein(a) levels ranged from 35 to 720 mg/l (median 180 mg/l) before treatment and from 55 to 780 mg/l (median 130 mg/l) after oestradiol treatment and showed no consistent pattern of change. It would appear, therefore, that treatment with unopposed oestrogen in relatively low doses does not have a marked effect on lipoprotein(a), at least in the short term.
Table 1. Levels of lipoproteins and apolipoproteins in 18 postmenopausal women being treated with oestradiol valerate.*

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Month 4</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride (mmol/l)</td>
<td>1.46 ± 0.30</td>
<td>1.47 ± 0.23</td>
<td>+0.01 ± 0.19</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.73 ± 0.26</td>
<td>5.61 ± 0.23</td>
<td>−0.12 ± 0.15</td>
</tr>
<tr>
<td>VLDL cholesterol (mmol/l)</td>
<td>0.55 ± 0.14</td>
<td>0.51 ± 0.08</td>
<td>−0.04 ± 0.10</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>3.54 ± 0.25</td>
<td>3.29 ± 0.21</td>
<td>−0.25 ± 0.15</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.62 ± 0.08</td>
<td>1.81 ± 0.09**</td>
<td>+0.19 ± 0.04</td>
</tr>
<tr>
<td>HDL2 cholesterol (mmol/l)</td>
<td>0.51 ± 0.05</td>
<td>0.59 ± 0.05*</td>
<td>+0.08 ± 0.03</td>
</tr>
<tr>
<td>HDL3 cholesterol (mmol/l)</td>
<td>1.04 ± 0.03</td>
<td>1.10 ± 0.04*</td>
<td>+0.06 ± 0.02</td>
</tr>
<tr>
<td>Apolipoprotein A-I (g/l)</td>
<td>1.62 ± 0.04</td>
<td>1.84 ± 0.07**</td>
<td>+0.22 ± 0.04</td>
</tr>
<tr>
<td>Apolipoprotein B (g/l)</td>
<td>0.72 ± 0.04</td>
<td>0.70 ± 0.04</td>
<td>−0.02 ± 0.01</td>
</tr>
</tbody>
</table>

* Values are mean ± SEM. Significantly different from baseline: * p < 0.05; ** p < 0.001. LDL: low-density lipoprotein; VLDL: very-low-density lipoprotein; HDL: high-density lipoprotein.

mated enzymatically. Very-low-density lipoproteins (VLDL) were isolated from serum by ultracentrifugation in a TL 100 benchtop ultracentrifuge (Beckman, UK) using the procedure of David et al. (6) and HDL were isolated from the infranatent layer by heparin/manganes precipitation. The LDL cholesterol levels were obtained by subtracting the sum of VLDL and HDL cholesterol levels from the total serum cholesterol. We have found this method for quantitating lipoprotein fractions using the TL 100 to be quick and precise (between-batch CVs: VLDL 2.9%; LDL 1.2%; HDL, 1.8%) and to give comparable results to those obtained by preparative ultracentrifugation according to the Lipid Research Clinics protocol (TL 100 = y, preparative ultracentrifuge = x: VLDL, y = 0.96x + 0.11, r = 0.98; LDL, y = 0.94x + 0.2, r = 0.98; HDL, y = 1.04x + 0.03, r = 0.97). The HDL subfractions were quantitated using the method of Eyre et al. (7).

Apolipoproteins A-I and B were estimated by an immuno-turbidimetric method using commercial antisera and reference standards (Incstar Ltd.) on a Roche Cobas Fara (between-batch CV of 2.5% for A-I and 1.4% for B).

Lipoprotein(a) levels were determined by an enzyme-linked immunosorbent assay (ELISA) using a commercial kit (Immuno Ltd.) that has been shown to be precise and robust (8). In our hands, the assay gave a between-batch CV of 11%.

Plasma fibrinogen was assayed according to Clauss (9). Levels of tissue plasminogen activator antigen (tPA) and plasminogen activator antigen inhibitor activity (PAI) were assayed using commercial ELISA kits supplied by Biopool, Porton Products Ltd. and Kabi Vitrum, respectively.

Levels of all analytes at 4 months were compared with baseline levels using Wilcoxon's matched-pairs signed rank test.

Results

The results showed an increase in HDL cholesterol and apoprotein A-I by 12% and 14%, respectively (p < 0.001). The HDL2 and HDL3 cholesterol levels increased by 16% and 6%, respectively (p < 0.05). There were no other significant changes in lipoprotein levels (Table 1). Pretreatment lipoprotein(a) levels ranged from 35 to 720 mg/l (median 180 mg/l) and after 4 months from 55 to 780 mg/l (median 130 mg/l) and showed no consistent pattern of change. Of the five patients with high initial values (> 300 mg/l), three showed an increase and two a decrease (Fig. 1).

There were no significant changes in any of the

Fig. 1. Individual serum lipoprotein(a) concentrations in 18 postmenopausal women before and after 4 months of treatment with oestradiol valerate (2 mg/day).
indices of coagulation and fibrinolysis. Mean fibrinogen levels were 2.45 ± 0.49 (SD) g/l pretreatment and 2.65 ± 0.46 g/l at 4 months; mean tPA levels were 4.39 ± 1.56 µg/l at zero time and 3.72 ± 1.64 µg/l at 4 months; mean PAI levels were 133.7 ± 56.5% at zero time and 127.2 ± 72.3% at 4 months.

Discussion

We conclude that treatment with unopposed oestrogen in relatively low doses does not have a marked effect on lipoprotein(a), at least in the short term, and therefore it would seem unlikely that changes in this risk factor contribute to the decrease in cardiovascular risk associated with oestrogen treatment. The significant increase in HDL cholesterol and apolipoprotein A-I concentrations found in this study are in agreement with those reported previously for this preparation (10). The HDL subfractions also increased significantly, with the greatest increase occurring in the HDL₂ cholesterol, as found previously (11). Although mean levels of LDL cholesterol were 7% lower after treatment, the change was not statistically significant. However, the dose of oestrogen used was low and, with one exception, the women in the study were normolipaemic on recruitment. Previous work has indicated that oestrogen-induced reductions in LDL concentrations are dose dependent and are much less marked in women who do not have hypercholesterolaemia than in those who do (12, 13).

There are few data on the influence of gonadal hormones, either endogenous or exogenous, on lipoprotein(a). Levels have been shown to increase during pregnancy (14). A significant positive correlation between lipoprotein(a) concentration and age has been demonstrated in women but not in men, with postmenopausal women exhibiting a significantly higher mean concentration than premenopausal women (15). In the latter study, a corresponding increase in plasma fibrinogen after the onset of menopause was found. Treatment of postmenopausal women with high doses of the androgenic progestogen norethisterone has been found to reduce substantially lipoprotein(a) levels (16), as has treatment with stanozolol, an anabolic steroid structurally related to testosterone (17). Lipoprotein(a) concentrations have also been measured in premenopausal women being treated with the anabolic steroid danazol, a derivative of norethisterone. A significant decrease in lipoprotein(a) was found and, interestingly, danazol has been reported to reduce plasma levels of fibrinogen and increase antithrombin III and plasminogen levels (18). There would appear to be a parallel between concentrations of lipoprotein(a) and clotting factors.

In this study, we found that after treatment with oestradiol there was no consistent trend in lipoprotein(a) concentrations and no change in fibrinogen, tPA and PAI. Lipoprotein(a) levels remained high in the five patients whose baseline levels were in the high-risk category (i.e. > 300 mg/l). This is in contrast to the effects reported for norethisterone (16) and danazol (18), both of which treatments brought all lipoprotein(a) concentrations into the low-risk category irrespective of their baseline levels.

To our knowledge, there is only one other report on the effects of postmenopausal oestrogens on lipoprotein(a). It was found that after 6 months of treatment with conjugated equine oestrogens and transdermal oestradiol there was a decrease in lipoprotein(a) levels (19). However, the changes failed to achieve statistical significance, and the greatest decreases in concentration were observed in women with low baseline levels.

Nowadays, most hormone replacement therapy prescribed is combined oestrogen/progestogen. A substantial reduction in lipoprotein(a) levels has been reported in 10 patients being treated with high-dose conjugated equine oestrogens combined with the cyclical addition of medroxyprogesterone acetate (20). The decreases in lipoprotein(a) observed after treatment with norethisterone suggest that the combined preparations currently used in Europe, employing androgenic progestogens, may have a favourable effect on this risk factor. Larger, longer term studies are necessary to clarify fully the effects of single or combined preparations of hormone replacement therapy on lipoprotein(a).

References


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