Background: Fluctuations in lipid and lipoprotein levels are encountered quite often in hyperlipidemic patients. We examined the possibility that lipid and lipoprotein levels fluctuate due to the different effects of estrogen and progestogen in postmenopausal hyperlipidemic women receiving combined hormonal replacement therapy.

Methods: In an open-label study conducted during 3 consecutive hormonal cycles (3 months), levels of fasting total cholesterol, triglycerides, and low (LDLC)- and high-density lipoprotein cholesterol (HDLC) were determined in 36 postmenopausal hyperlipidemic women on day 13 of conjugated equine estrogen (1.25 mg/d) therapy and on day 25 after 12 days of receiving estrogen plus medroxyprogesterone acetate (5 mg/d).

Results: While receiving estrogen and combined therapies, means ± SD total cholesterol levels increased from 6.50 ± 0.97 mmol/L (251 ± 37 mg/dL) to 6.88 ± 1.42 mmol/L (266 ± 54 mg/dL) (P<.001); LDLC levels, from 4.05 ± 1.14 mmol/L (156 ± 44 mg/dL) to 4.62 ± 1.36 mmol/L (178 ± 52 mg/dL) (P<.001). Mean ± SD HDLC cholesterol levels decreased from 1.44 ± 0.32 mmol/L (55 ± 12 mg/dL) to 1.29 ± 0.28 mmol/L (50 ± 10 mg/dL) (P<.001); triglyceride levels, from 2.23 ± 1.03 mmol/L (197 ± 91 mg/dL) to 2.06 ± 1.04 mmol/L (182 ± 92 mg/dL) (P<.001).

Conclusions: Hyperlipidemic postmenopausal women receiving combined sequential estrogen and progestogen replacement therapy demonstrate very significant fluctuations in their lipid and lipoprotein levels. These fluctuations depend on the hormonal phase, ie, estrogen alone or combined with progestogen.

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PATIENTS AND METHODS

PATIENTS

We recruited 36 women in natural menopause for at least 2 years who were receiving combined sequential HRT and were followed up in an outpatient clinic for lipid metabolism because of dyslipidemia. Inclusion criteria included being non-smokers and in good health and having serum levels of follicle-stimulating hormone of at least 40 IU/L, luteinizing hormone of at least 30 IU/L, and estradiol of no more than 183 pmol/L. None had diabetes, renal disorders, or other metabolic or endocrine disorders. They were not receiving any medications other than hypolipidemic drugs and HRT. Subjects were referred to the clinic for lipid metabolism because they had 1 or more of the following: total serum cholesterol level of at least 5.18 mmol/L (200 mg/dL), serum triglyceride level of at least 2.26 mmol/L (200 mg/dL), serum LDL cholesterol level of at least 3.36 mmol/L (130 mg/dL), and serum HDL cholesterol level of no more than 0.9 mmol/L (35 mg/dL). Hypolipidemic treatment consisted of lovastatin (20-40 mg/d; median duration of treatment, 13 months; range, 6-17 months) or simvastatin (10-20 mg/d; median duration of treatment, 11 months; range, 8-15 months).

STUDY DESIGN

All women were receiving cyclic combined HRT (Premarin Plus MP Dexxon) consisting of 25 1.25-g tablets of conjugated equine estrogen and 12 5-mg tablets of medroxyprogesterone acetate. They took the estrogen tablet daily during the first 13 days, and 1 estrogen tablet plus 1 progestogen tablet for the last 12 days of each cycle. No hormonal therapy was administered for the next 5 days, and menses appeared in most of the women.

The hypolipidemic therapy was stopped for a washout period of at least 30 days. Thereafter, fasting blood samples for lipid profile were drawn on days 13 and 25 of each of 3 consecutive hormonal cycles (3 months). Blood was sampled between 8 AM and 10 AM after a 12-hour fast on the 13th (estrogen phase) and the 25th (combined-therapy phase) days of each cycle. The patients were asked not to make any changes in their diet. Compliance with HRT was monitored by pill count. All participants signed a written informed consent form.

LIPID AND LIPOPROTEIN LEVEL DETERMINATION

Cholesterol levels were assayed using the enzymatic cholesterol oxidase para-aminophenazone peroxidase method (kit 236691; Boehringer Mannheim, Indianapolis, Ind). Serum triglyceride levels were determined by measuring glycerol levels after enzymatic hydrolysis with lipase (kit 701912; Boehringer Mannheim). High-density lipoprotein cholesterol was isolated using the Northwest Lipid Research Clinic heparin-manganese procedure,18 and cholesterol content was determined using the enzymatic method previously mentioned. Levels of LDL cholesterol were calculated using the formula of Friedewald et al.19 Between-batch coefficient of variation for cholesterol was 0.6%; for triglycerides, 3.2%. Within-batch coefficient of variation for cholesterol was 0.86%; for triglycerides, 1.05%.

STATISTICAL ANALYSIS

The lipid and lipoprotein values that were determined during 3 successive months were averaged for each patient. The Wilcoxon matched-pairs signed rank test was used to evaluate significance between fasting lipid and lipoprotein levels in the estrogen-alone and combined-therapy phases. The relationship between fasting lipid and lipoprotein levels and the amount of change between phases was tested using the Spearman test. Unless otherwise indicated, data are given as mean ± SD.

RESULTS

The mean age of the study population was 54.6 ± 3.6 years (median age, 55 years; range, 49-64 years). The mean fasting levels of lipids and lipoproteins during the 3 consecutive hormonal cycles are shown in the Table. Highly significant differences in fasting levels of lipids and lipoproteins were found between the estrogen and combined-therapy phases. For the entire study period, changes from the estrogen phase to the combined-therapy phase were as follows: Mean total cholesterol levels increased by 6%, from 6.50 ± 0.97 mmol/L (251 ± 37 mg/dL) to 6.88 ± 1.42 mmol/L (266 ± 54 mg/dL) (P < .001). Mean LDL cholesterol levels increased by 13.8%, from 4.05 ± 1.14 mmol/L (156 ± 44 mg/dL) to 4.62 ± 1.36 mmol/L (178 ± 52 mg/dL) (P < .001). The addition of progestogen decreased mean HDL cholesterol levels by 10.4%, from 1.44 ± 0.32 mmol/L (55 ± 12 mg/dL) to 1.29 ± 0.28 mmol/L (50 ± 10 mg/dL) (P < .001). It also decreased the mean fasting triglyceride levels by 5.5%, from 2.23 ± 1.03 mmol/L (197 ± 91 mg/dL) to 2.06 ± 1.04 mmol/L (182 ± 92 mg/dL) (P < .001).

By examining the individual response (Figure), we found that LDL cholesterol changes for all participants ranged from −0.44 to 1.76 mmol/L (−17 to 68 mg/dL). Levels of LDL cholesterol increased during the combined-therapy phase by more than 0.55 mmol/L (21.3 mg/dL) in 50% (18) of the subjects and by more than 0.67 mmol/L (26 mg/dL) in 25% (9) of the subjects. The changes in HDL cholesterol levels ranged from −0.31 to 0.44 mmol/L (−12 to 17 mg/dL). Levels of HDL cholesterol decreased in the progestogen phase by more than 0.18 mmol/L (7 mg/dL) in 50% (18) of the women and by more than 0.2 mmol/L (8 mg/dL) in 25% (9) of the women.

Significant positive correlations were found: between mean fasting LDL cholesterol levels during the estrogen phase and the mean increase of LDL cholesterol levels during the combined-therapy phase.
bined-therapy phase (R = 0.35; P = .04), and between the mean fasting triglyceride levels during the estrogen phase and the mean decrease of HDLC levels during the combined-therapy phase (R = 0.35; P = .04).

In our study, we investigated the possibility that lipoprotein levels in hyperlipidemic postmenopausal women receiving combined sequential HRT fluctuate significantly throughout each cycle of treatment due to the different effect of estrogen and progestogen on plasma lipoproteins. Our results clearly demonstrated that serum levels of total cholesterol, triglycerides, LDLC, and HDLC fluctuated vastly during the different hormonal phases of this treatment, ie, the addition of progestogen to the estrogen significantly increased levels of total cholesterol and LDLc and decreased levels of HDLC.

Our findings emphasize an important point that is characteristically overlooked in investigative studies and in the routine follow-up of hyperlipidemic women receiving HRT: the importance of the timing of blood sampling for determination of lipid and lipoprotein levels.

Our results might be meaningful because of the following 3 reasons: (1) They raise the possibility that the maximal protective effect of HRT is not maintained at a constant level throughout the treatment cycle. (2) They show that fluctuations in lipoprotein levels can be detected as early as 12 to 13 days after treatment with estrogen alone or combined with progestogen has begun. (3) Insofar as antihyperlipidemic therapy is given and its dose is defined according to the individual lipid and lipoprotein levels, the timing of blood sampling for determination of these levels in hyperlipidemic postmenopausal women receiving HRT emerges as a definitive factor. We contend that this point is not sufficiently recognized by clinicians who treat postmenopausal women in general and hyperlipidemic postmenopausal women in particular.

To our knowledge, only 1 study concentrated on the same aspects as ours. Lemay et al13 demonstrated cyclical excursions in lipid and lipoprotein levels in women receiving conjugated equine estrogen (0.625 mg/d) from day 1 to day 25 and medroxyprogesterone acetate (5 mg/d) from days 14 to 25. These authors illustrated that the changes in LDLC and HDLC levels induced by both phases of treatment were repeated at each monthly interval during 2 years, and that these changes were detectable after short periods of treatment, ie, 5 to 14 days. This, in keeping with our findings, implies a rapid pharmacological effect of the hormones on the different lipoprotein fractions.

In the study by Lemay et al,13 LDLC levels decreased and levels of triglycerides and HDLC increased following 24 days of estrogen administration. The sequential addition of progestogen attenuated the eleva-

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**COMMENT**

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**Table:**

<table>
<thead>
<tr>
<th>Cycle</th>
<th>Total Cholesterol, mmol/L (mg/dL)</th>
<th>Triglycerides, mmol/L (mg/dL)</th>
<th>LDLC, mmol/L (mg/dL)</th>
<th>HDLC, mmol/L (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.51 ± 0.97 7.00 ± 1.27 7.5</td>
<td>.001 2.20 ± 1.09 2.03 ± 1.03 7.7</td>
<td>.002 4.13 ± 1.18 4.77 ± 1.44 15.4</td>
<td>.001 1.43 ± 0.32 1.28 ± 0.27 10.5</td>
</tr>
<tr>
<td></td>
<td>(251 ± 37) (270 ± 49) (180 ± 91)</td>
<td>(195 ± 96) (180 ± 91) (190 ± 91)</td>
<td>(198 ± 143) (148 ± 55)</td>
<td>(55 ± 12) (49 ± 10)</td>
</tr>
<tr>
<td>2</td>
<td>6.44 ± 0.99 6.89 ± 1.72 6.8</td>
<td>.001 2.22 ± 1.06 2.11 ± 1.13 4.9</td>
<td>.03 3.96 ± 1.14 4.53 ± 1.32 14.1</td>
<td>.001 1.45 ± 0.30 1.29 ± 0.28 10.3</td>
</tr>
<tr>
<td></td>
<td>(248 ± 38) (266 ± 66)</td>
<td>(197 ± 94) (187 ± 100)</td>
<td>(153 ± 44) (175 ± 51)</td>
<td>(55 ± 11) (49 ± 10)</td>
</tr>
<tr>
<td>3</td>
<td>6.53 ± 0.96 6.79 ± 1.24 3.8</td>
<td>.01 2.28 ± 0.98 2.05 ± 1.00 9.6</td>
<td>.001 4.02 ± 1.11 4.52 ± 1.34 12.4</td>
<td>.001 1.43 ± 0.33 1.30 ± 0.28 8.3</td>
</tr>
<tr>
<td></td>
<td>(252 ± 37) (262 ± 49)</td>
<td>(202 ± 87) (182 ± 88)</td>
<td>(155 ± 43) (174 ± 52)</td>
<td>(55 ± 13) (50 ± 10)</td>
</tr>
<tr>
<td>Overall</td>
<td>6.50 ± 0.97 6.90 ± 1.42 6.1</td>
<td>.001 2.23 ± 1.03 2.06 ± 1.04 7.6</td>
<td>.001 4.05 ± 1.14 4.62 ± 1.36 14.0</td>
<td>.001 1.44 ± 0.32 1.29 ± 0.28 9.8</td>
</tr>
<tr>
<td></td>
<td>(251 ± 37) (266 ± 54)</td>
<td>(198 ± 91) (182 ± 92)</td>
<td>(156 ± 44) (178 ± 52)</td>
<td>(55 ± 12) (50 ± 10)</td>
</tr>
</tbody>
</table>

*All values are expressed as mean ± SD. E indicates conjugated equine estrogen alone; EP, estrogen and medroxyprogesterone acetate; Δ, difference between lipid and lipoprotein levels in EP phase and in E phase expressed as percentage of the levels in the E phase; LDLC, low-density lipoprotein cholesterol; and HDLC, high-density lipoprotein cholesterol."
tion of triglyceride and HDLC levels, as was also shown in our study; however, in contrast to our findings, they reported that progestogen further reduced, although not significantly, the LDLC levels. This effect on LDLC levels was also reported by others."}

Two main differences in study design between the study by Lemay et al. and ours may explain this disagreement. First, we examined the combination of conjugated equine estrogen, 1.25 mg/d, plus cyclic medroxyprogesterone acetate, 5 mg/d, whereas Lemay et al. used a different dosage of estrogen. Moreover, a number of studies showed an inconsistent effect on LDLC levels of adding progestogen to estrogen treatment. This may be unfavorable, neutral, or favorable in terms of risk for coronary artery disease. To date, there is no means for predicting the extent and direction of the effect the addition of a progestogen to estrogen treatment has on blood lipid and lipoprotein levels. In addition, normolipidemic women were studied by Lemay et al., whereas the women in our study were hyperlipidemic. It is possible that defective synthesis or catabolism of cholesterol and lipoprotein receptors in hyperlipidemic individuals will result in different responses to HRT than in normolipidemic individuals regarding the direction and the magnitude of the response.

We showed a positive correlation between the mean LDLC level during the estrogen phase and the mean increase of LDLC during the combined-therapy phase, and between the mean triglyceride levels during the estrogen phase and the mean decrease in HDLC levels during the combined-therapy phase. This provides a means of predicting the magnitude of changes in LDLC and triglyceride levels when progestogen is added to estrogen therapy.

Our study demonstrated that significant fluctuations in lipid and lipoprotein levels exist during the different hormonal phases of hyperlipidemic women receiving estrogen plus cyclic sequential progestogen replacement therapy. The awareness of these fluctuations may be important in the planning and evaluation of clinical studies on the metabolic effects of these hormones and perhaps even more so in the regular follow-up of these women. We suggest that an individual treatment protocol be tailored for the patient’s own pattern of fluctuations of lipid and lipoprotein levels during the HRT cycle. To do this, blood for a lipid profile should initially be sampled at least once at each hormonal phase in hyperlipidemic women receiving combined sequential HRT. When significant differences are found between the phases, these women should be instructed to consistently undergo their follow-up blood test within the same hormonal phase, ie, the one that shows the poorest results, and therapeutic decisions should be based on these values.

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