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Impact of combined hormone replacement therapy on serum lipid metabolism: new aspects

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ABSTRACT

The objective of this study was to evaluate the impact of combined estrogen–progestogen therapy on low density lipoprotein (LDL) particle size (determined by the LDL cholesterol/apolipoprotein B ratio). The prospective study was carried out on 139 healthy Danish early postmenopausal women. The subjects were randomized to placebo or to 2 mg estradiol valerate equivalents, either sequentially combined with 75 μg levonorgestrel, 10 mg medroxyprogesterone acetate (MPA), or 150 μg desogestrel, or continuously combined with 1 mg cyproterone acetate. LDL particle size was calculated before treatment and at nine well-defined times during the subsequent 84 days.

LDL particle size was reduced by all four treatments. This change was statistically significant for estradiol valerate combined with levonorgestrel and MPA (6.2 ± 2.7% and 5.6 ± 2.1% (mean ± SEM), respectively; $p < 0.05$ for both, placebo-corrected). Estradiol valerate combined with MPA induced cyclic (progestogen-minus estrogen-related values) decreases (−6.3 ± 2.6%; $p < 0.05$), and with levonorgestrel there were cyclic increases (5.1 ± 2.7%; $p = 0.067$) in LDL particle size (placebo-corrected).

In conclusion, combined estrogen–progestogen therapy causes a decrease in LDL particle size. A cyclic variation in LDL cholesterol/apolipoprotein B ratio was observed during sequential treatment.

INTRODUCTION

A large body of epidemiological and experimental evidence indicates that estrogen monotherapy protects against cardiovascular disease in postmenopausal women[1–10]. This beneficial effect of estrogens is partly explained by an increase in high density lipoprotein (HDL) cholesterol and a decrease in total and low density lipoprotein (LDL) cholesterol. In postmenopausal women with an intact uterus, progestogens are added to prevent endometrial hyperplasia and cancer[11,12]. However, some progestogens may partly counterbalance the estrogenic effect on the lipid metabolism. Some authors[13–15], but not all[8,16], have thus found HDL cholesterol to be reduced more by 19-nortestosterone derivatives than by 17-hydroxyprogesterone derivatives.

It has recently been suggested that increasing LDL particle size would be beneficial in terms of cardiovascular disease[17,18], and that the oxidative susceptibility of LDL increases with decreasing LDL particle size[19]. Therefore, the smaller the LDL particle size is, the more atherogenic it appears to be[20,21]. Theoretically, the LDL cholesterol/apolipoprotein B (LDL-C/apo-B) ratio can be used as a crude approximation of the LDL particle size and as an 'atherogenic index' (inverse relation)[22–24]. This quotient was originally

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employed to classify patients with familial hyperlipidemia[24].

We used data from a randomized, placebo-controlled study, originally designed to compare the effect of different postmenopausal hormone replacement therapies on bone[25] and lipid metabolism[8]. The aim of the present study was to investigate the effect of four different postmenopausal estrogen/progestogen therapies on the LDL-C/apo-B ratio, with special focus on the impact of progestogen type (using 19-nortestosterone and 17-hydroxyprogesterone derivatives).

MATERIALS AND METHODS

Patients

The patients for the original study were selected by means of a questionnaire followed by a medical and laboratory screening procedure, to be a representative sample of healthy Danish postmenopausal women, aged 45 to 55 years. All women had passed a natural menopause 6 months to 3 years previously[8]. One hundred and forty-eight women entered the study, and 139 women (94%) completed the study. For logistic reasons, the study was performed as two sub-studies (Groups A and B), and therefore there are two placebo groups. Informed consent was obtained by all participants according to the Helsinki Declaration II, and the trial was approved by the Ethical Committee of Copenhagen County.

The groups showed comparability at entry (Table 1) and at the end of the study, with respect to age, body weight, blood pressure, cigarette smoking, menopausal age, serum total cholesterol, triglycerides, HDL and LDL cholesterol, and apolipoproteins A1 and B. There was no diet restriction during the study period.

Design

The women were randomized to treatment with placebo or 1.5 mg 17β-estradiol, sequentially combined with 150 μg desogestrel, or to 2 mg estradiol valerate, either continuously combined with 1 mg cyproterone acetate (CPA), or sequentially combined with 75 μg levonorgestrel (LNG) or 10 mg medroxyprogesterone acetate (MPA) (Figure 1). The study lasted for 12 weeks. The rationale for choosing these hormone replacement therapy (HRT) regimens was that they are commonly used among women after the menopause. Furthermore, using this design, we could study the influence on

Table 1 Mean (± SD) values of various baseline parameters for the active treatment and placebo groups

	Estradiol valerate/CPA	*Estradiol valerate/LNG*	*Placebo A*	*Estradiol valerate/MPA*	*Estradiol/ desogestrel*	*Placebo B*
Age (years)	51.4 ± 1.8	51.6 ± 2.1	50.0 ± 2.2	51.4 ± 1.8	50.5 ± 2.7	50.7 ± 2.2
Weight (kg)	66.2 ± 11.6	67.1 ± 10.9	64.6 ± 8.0	64.2 ± 9.9	66.3 ± 12.5	64.6 ± 8.7
Blood pressure (mmHg)	116 ± 13/ 74 ± 9	118 ± 12/ 75 ± 9	114 ± 12/ 76 ± 7	109 ± 15/ 73 ± 9	112 ± 10/ 74 ± 7	112 ± 14/ 71 ± 10
Cigarettes smoked/day	8.0 ± 7.4	6.1 ± 8.0	6.9 ± 7.8	3.7 ± 6.4	5.8 ± 9.1	7.5 ± 7.6
Time since menopause (months)	21.2 ± 9.0	22.9 ± 9.8	17.3 ± 8.6	23.4 ± 9.6	21.3 ± 8.6	24.0 ± 8.3
Serum triglycerides (mmol/l)	1.36 ± 1.54	1.11 ± 0.58	1.04 ± 0.50	1.10 ± 0.46	1.02 ± 0.32	1.08 ± 0.32
Total serum cholesterol (mmol/l)	6.34 ± 0.90	6.63 ± 1.23	6.10 ± 1.13	6.86 ± 0.95	6.58 ± 1.13	6.53 ± 1.52
HDL-cholesterol (mmol/l)	1.51 ± 0.43	1.56 ± 0.52	1.68 ± 0.35	1.58 ± 0.37	1.55 ± 0.44	1.60 ± 0.30
LDL-cholesterol (mmol/l)	4.30 ± 0.92	4.58 ± 1.27	3.96 ± 1.08	4.79 ± 1.00	4.57 ± 1.22	4.44 ± 1.47
Apolipoprotein A1 (g/l)	1.43 ± 0.21	1.45 ± 0.27	1.46 ± 0.18	1.54 ± 0.23	1.47 ± 0.22	1.47 ± 0.17
Apolipoprotein B (g/l)	1.05 ± 0.24	1.01 ± 0.29	0.96 ± 0.25	1.04 ± 0.20	1.01 ± 0.21	0.98 ± 0.25

CPA, cyproterone acetate; LNG, levonorgestrel; MPA, medroxyprogesterone acetate; HDL, high-density lipoprotein; LDL, low-density lipoprotein

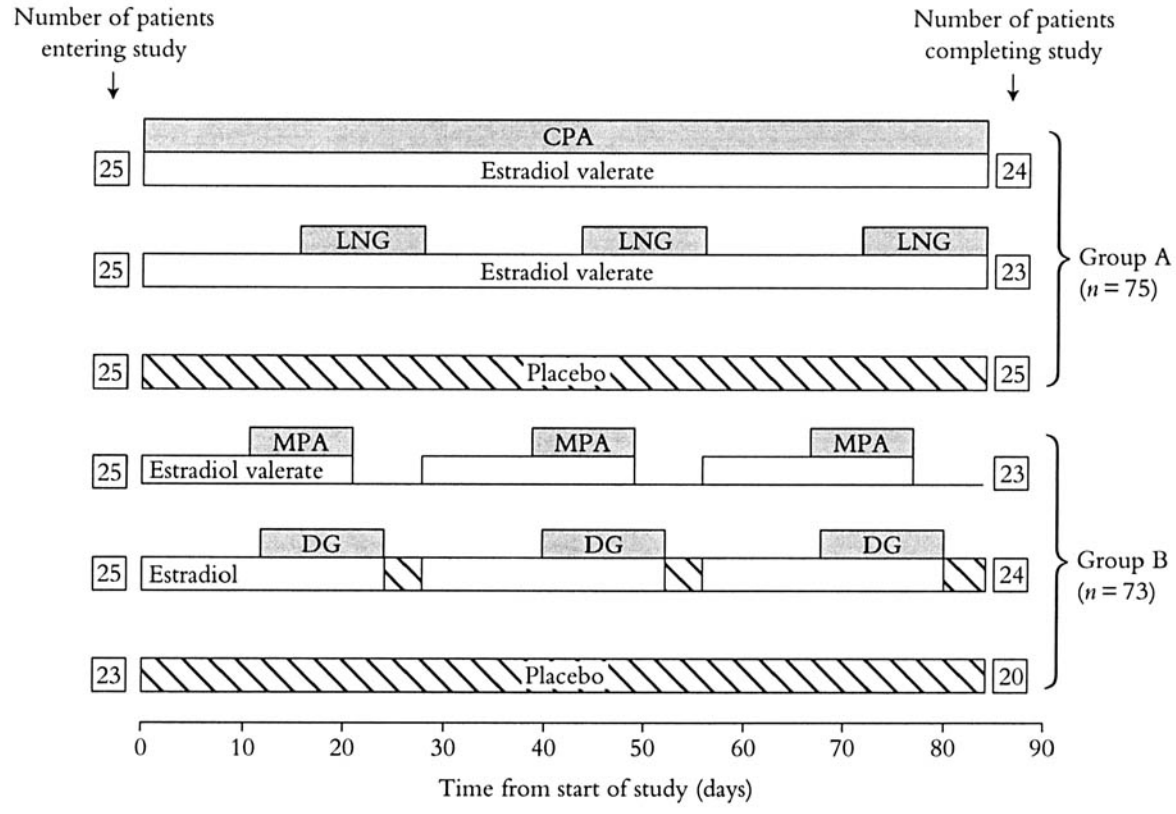

Figure 1 The drug regimens of the different study groups. Cyproterone acetate (CPA) and medroxyprogesterone acetate (MPA) are 17-hydroxyprogesterone derivatives, whereas levonorgestrel (LNG) and desogestrel (DG) are 19-nortestosterone derivatives. Groups A and B denote the two substudies

the lipid metabolism of 17-hydroxyprogesterone derivatives and 19-nortestosterone derivatives, and of continuous vs. sequential treatment. Initial serum lipids and lipoproteins in the fasting state were measured after overnight tobacco abstinence. During the following three cycles these parameters were measured at the time of maximal estradiol and progestogen influence, and 2–3 days after the progestogen period (Figure 2). The general modifications in routine lipid metabolism parameters induced by these therapies have been described previously[8].

Methods

LDL cholesterol was calculated using the Friedewald equation after enzymatic measurement of serum total cholesterol, HDL cholesterol and serum triglycerides[26]. Apolipoprotein levels were assessed by immunoturbidimetry. Details about the assessments are given elsewhere[8]. Blood pressure was measured throughout the study.

Statistics

Serial measurements were analyzed by a two-stage method, as recently described by Matthews and colleagues[27]. In the first stage, two summary measures of the fluctuations in the LDL-C/apo-B ratio induced by hormone replacement therapy were identified and calculated for each subject, i.e. one for the total response and one for the cyclic response (see below). In the second stage, the mean summary measures in the treatment groups were compared with those of the corresponding placebo group by unpaired *t*-tests.

The total response in the LDL-C/apo-B ratio to various HRT regimens was calculated as the difference between the average value during treatment and the initial value:

$$\sum_{i=1}^{3} \frac{(A_i + B_i)}{6} - 100\%$$

where A and B are values of the ratio at the time of blood sampling (Figure 2).

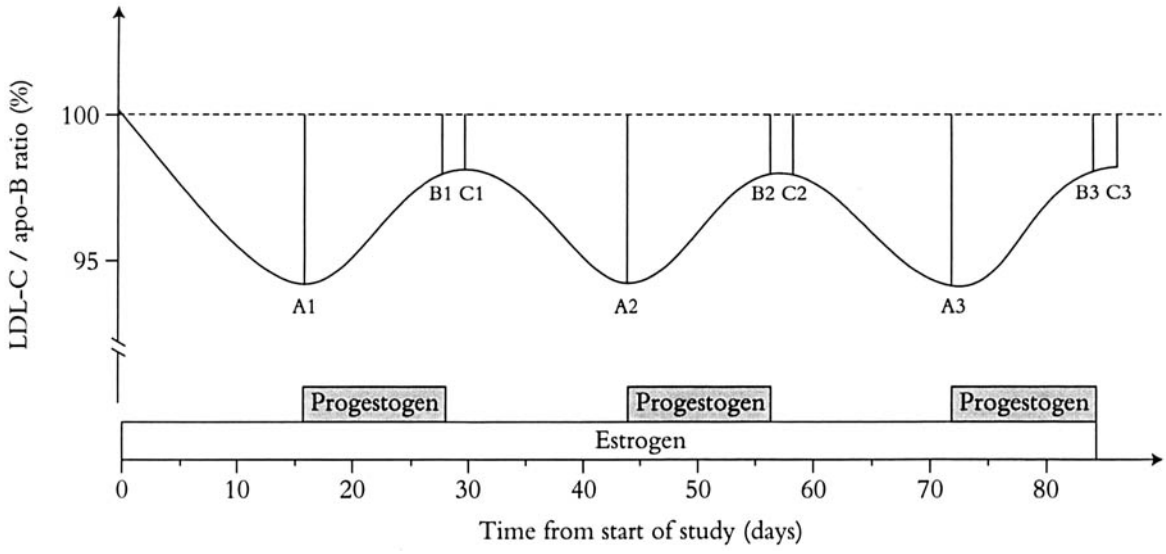

Figure 2 Diagram showing the fluctuations (cyclic changes) in the LDL-cholesterol/apolipoprotein B ratio (LDL-C/apo-B) during three estrogen–progestogen cycles. Definitions of the times of blood sampling are: A, at the end of the estrogen period; B, at the end of the progestogen period; and C, 2 or 3 days after the progestogen period

Cyclic variations were calculated as the average of the three maximal differences between progestogen- and estrogen-related values:

$$\sum_{i=1}^{3} \frac{\max(B_i - A_i, C_i - A_i)}{3}$$

The contribution from a systematic change to the cyclic variations was negligible and for simplicity not eliminated. For active treatments and placebo groups, baseline values (mean ± SD) of age, age at menopause, serum total cholesterol, serum triglycerides, HDL cholesterol, LDL cholesterol and apolipoprotein B were determined. Analysis of covariance was carried out to determine the relation between cyclic changes in the LDL-C/apo-B ratio and treatment, after adjustment for simultaneous changes in serum triglycerides. All procedures were performed using the Statistical Analysis System[28] with a significance level of 5%.

RESULTS

For the total response, all groups showed a decrease in the LDL-C/apo-B ratio during the 84 days. These changes were, after correction for the respective placebo groups, statistically significant: for treatment with estradiol valerate and LNG, 6.2 ± 2.7%, and for treatment with estradiol valerate and MPA, 5.6 ± 2.1% (mean ± SEM; $p < 0.05$ for both). Regarding estradiol valerate with CPA, and estradiol with desogestrel, the corresponding decreases were 3.5 ± 2.7% and 1.1 ± 2.6%, respectively, after adjustment for corresponding placebo groups. There was no significant change in serum triglycerides (total response) between the hormonal and the respective placebo groups (data not shown).

Figure 3 gives the mean summary measures of cyclic variations after correction for changes in the corresponding placebo groups. Treatment with estradiol valerate and LNG or MPA demonstrated a cyclic variation in the LDL-C/apo-B ratio after correction for placebo. For estradiol valerate with LNG, the cyclic response was 5.1 ± 2.7% (mean ± SEM; $p = 0.067$), i.e. a relative increase in the ratio during phases with combined estradiol valerate and LNG as compared to the estrogen monotherapy phase. For estradiol valerate and MPA, a statistically significant ($p < 0.05$) cyclic variation of −6.3 ± 2.6% in the opposite direction was observed. Correction for the non-significant cyclic variation in serum triglycerides (by analysis of covariance) attenuated the cyclic changes in the LDL-C/apo-B ratio (data not shown). Since treatment with estradiol valerate and CPA was continuous, no cyclic change was expected or found. The absolute baseline values in the LDL-C/apo-B ratio (mmol/g) are given in Table 2. During the 84 days,

blood pressure remained unchanged from baseline in all groups (data not shown).

DISCUSSION

Subjects with a predominance of large LDL particles have recently been found to have a lower risk of coronary heart disease as compared to subjects with smaller and denser LDL particles[20,21]. This is supported by accumulating evidence suggesting that the smaller and denser LDL particles bind more easily to the arterial wall than do larger and less dense LDL particles, and subsequently have increased susceptibility to oxidation in the arterial wall[19,29].

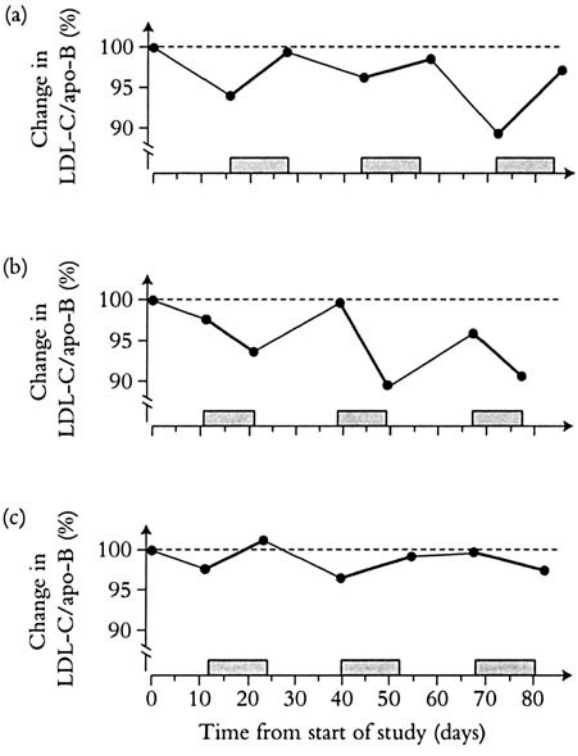

Figure 3 The placebo-corrected cyclic variations in the LDL-cholesterol/apolipoprotein B ratio (LDL-C/apo-B) for (a) estradiol valerate and levonorgestrel, (b) estradiol valerate and medroxyprogesterone acetate, and (c) estradiol and desogestrel. Periods with progestogen are shown as shaded bars; changes during progestogen periods are in bold

Table 2 Absolute baseline values (mean ± SD) of the LDL-cholesterol/apolipoprotein B ratio (LDL-C/apo-B)

	LDL-C/apo-B (mmol/g)
Estradiol valerate/CPA	4.34 ± 0.75
Estradiol valerate/LNG	4.56 ± 0.50
Placebo A	4.32 ± 0.51
Estradiol valerate/MPA	4.67 ± 0.50
Estradiol/desogestrel	4.47 ± 0.61
Placebo B	4.48 ± 0.52

CPA, cyproterone acetate; LNG, levonorgestrel; MPA, medroxyprogesterone acetate

The golden standard for assessing LDL subfractions is ultracentrifugation[18,19], but the LDL-C/apo-B ratio may be regarded as an approximation of LDL particle size or as an 'atherogenic index'[22–24]. The present placebo-controlled study investigated the LDL particle size, as estimated by this ratio, during treatment with different estrogen/progestogen combinations. We found that estradiol combined with either continuous CPA or sequential LNG, desogestrel or MPA, resulted in a reduction in particle size. This potentially adverse effect was not explained by changes in serum triglycerides. Some epidemiological studies have previously suggested that LDL particle size is not influenced by estrogen therapy[30,31]. More recent studies have, however, demonstrated that unopposed estrogen treatment induces a shift in LDL particle size toward smaller and denser particles, mainly by a reduction in the large LDL particles[32,33]. The clinical significance of a hormone-lowering effect on LDL particle size found in the more recent studies, including the present, may, considered separately, seem unfavorable with regard to coronary heart disease. It should, however, be emphasized that an increase in the number of dense, atherogenic LDL particles has not been demonstrated and that all these estrogen/progestogen therapies reduce both LDL cholesterol and apolipoprotein B significantly, which in women is also beneficial concerning coronary heart disease[34]. The potentially adverse changes in LDL particle size suggest merely caution in over-estimating the beneficial hormonal effect on LDL and HDL cholesterol in terms of cardiovascular disease. Estrogens seem thus to have several additional beneficial effects on the cardiovascular system[35]. These effects have not been fully elucidated, but experimental studies have demonstrated that several mechanisms are involved. A direct cytoprotective effect of estrogens on epithelial cells[36], decreased cholesterol ester hydrolysis[4], reduced cholesterol metabolism in the arterial wall[37], and improved regeneration of the intimal endothelium

in atherosclerotic arteries by HRT[38], have thus been described. The seemingly opposite effect of 17β-estradiol on the ratio in the different treatment groups is explained by withdrawal of the estradiol/progestogen therapy followed by treatment with either 17β-estradiol alone, placebo, or no treatment. Therefore, during the latter phase, the LDL particle size tends to shift back towards the decrease in size found during 17β-estradiol monotherapy (Figure 1). The cyclic variations in the 'atherogenic index' or LDL particle size during treatment with a 19-nortestosterone derivative (LNG) and a 17-hydroxyprogesterone derivative (MPA), were of opposite direction (Figure 3), i.e. an increase in the LDL-C/apo-B ratio during phases of 19-nortestosterone derivatives, and *vice versa* for the 17-hydroxyprogesterone derivative. These cyclic variations were attenuated but not eliminated after adjustment for cyclic changes in serum triglycerides. These findings do not support the hypothesis that 19-nortestosterone derivatives are more adverse than 17-hydroxyprogesterone derivatives with respect to cardiovascular disease. This conception primarily originates from early studies showing a marked HDL cholesterol lowering effect of very large doses of 19-nortestosterone derivatives as compared to lower doses of 17-hydroxyprogesterone derivatives[13–15]. Moreover, 19-nortestosterone derivatives have recently in animal experimental studies been found to have a neutral influence on atherosclerosis[39,40]. These results are strongly supported by recent epidemiological data[41] showing that the relative risk of developing acute myocardial infarction tends to be even lower in postmenopausal women treated with a combination of estrogen and levonorgestrel as compared to those receiving estrogen monotherapy. The adverse effects of 19-nortestosterone derivatives as judged by their influence primarily on HDL cholesterol may thus have been somewhat overestimated. One possible mechanism may, at least theoretically, have a favorable impact on the LDL particle size.

In conclusion, estrogen combined either continuously with cyproterone acetate, or sequentially with levonorgestrel, medroxyprogesterone acetate or desogestrel, seems to induce a slight general decrease in the LDL particle size. Progestogens added sequentially may have different effects on LDL particle size: levonorgestrel seems to increase and medroxyprogesterone acetate to decrease particle size.

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