# Increased Activity of Lecithin:Cholesterol Acyltransferase During Short-Term Oral Estrogen Progestin Replacement Therapy in a Group of Postmenopausal Women

N. Ulloa, C. Verdugo, M. Rios, J. Sepúlveda, S. Sepúlveda, R. Naveas, and C. Calvo

**The aim of the study was to assess the short-term effect of estrogen-progestin therapy on the plasma level of lecithin: cholesterol acyltransferase ([LCAT] EC 2.3.1.43), a key enzyme in the cholesterol reverse-transport process. The trial included 21 women with at least 6 months of menopause, which was confirmed by anamnesis, physical evaluation, and follicle-stimulating hormone (FSH) determination. Women receiving pharmacological treatment or who had any kind of endocrine disorder were excluded. In addition, we evaluated and confirmed normal Papanicolaou and mammography tests in all 21 women included in the trial. They received conjugated equine estrogen 0.625 mg daily, plus cyclic medroxyprogesterone acetate (5 mg daily) for 12 days each month. Plasma levels of LCAT, cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides, apoB, and apoAI were evaluated before and after 1 and 3 months of therapy. Pretherapy and posttherapy results were analyzed statistically by Wilcoxon's rank-sum test for paired samples. No significant changes were observed either for body mass index or for blood pressure. A significant increase in plasma LCAT activity was found at the first and third month posttherapy ($P < .005$). In addition, after 3 months of therapy, HDL-C significantly increased ($P < .005$), in contrast to the significant decrease detected in total cholesterol ($P < .025$), LDL-C ($P < .005$), cholesterol to HDL-C and LDL-C/HDL-C ratios ($P < .005$). Triglyceride levels did not show significant modification. In conclusion, our results indicate that short-term estrogen-progestin therapy produces a significant increase in plasma LCAT activity, as well as beneficial changes in the lipid profile, in postmenopausal women.**



CARDIOVASCULAR DISEASE is the major cause of death for women in the Western world. In the United States each year, 500,000 women die of cardiovascular disease.[1] In Chile in 1996, the relative mortality from circulatory disease for women aged 45 to 65 years reached 33.1%, constituting the second major cause of mortality.[2] The low incidence of vascular events in premenopausal women, with a marked increase in myocardial infarction after menopause, is well recognized and supports estrogen deficiency as an important factor. The role of estrogens is further supported by evidence from a number of epidemiological studies showing a substantial reduction in cardiovascular disease among women receiving estrogen replacement therapy.[3-6] Several biologically plausible mechanisms to explain estrogen-mediated cardioprotection have been proposed, including beneficial changes in plasmatic lipids and lipoprotein levels,[7-10] carbohydrate metabolism,[11,12] and coagulation factors and endothelial function.[13,14] One the most attractive mechanisms is the favorable effect of estrogen on lipoproteins: increasing high-density lipoprotein cholesterol (HDL-C) and decreasing low-density lipoprotein cholesterol (LDL-C).

The mechanisms that explain the modification in plasma lipids and lipoprotein levels have been elucidated to a certain extent. It has been demonstrated that 17α-ethinyl estradiol induces mRNA for the LDL receptor in rabbit liver, which can lead to an increase in LDL receptor levels and a decrease of this lipoprotein in the plasma.[15] On the other hand, it has been observed that postmenopausal estrogen replacement therapy increases the rate of production of large triglyceride-rich very-low-density lipoprotein (VLDL) and decreases plasma LDL levels as a result of increased clearance.[8] A definitive mechanism to explain the increase in HDL after estrogen administration is not available at present. An increase in plasma $HDL_2$ is due, in part, to suppression of hepatic lipase activity, because this leads to reduced HDL clearance[16] and the plasma may be enriched thereby with $HDL_2$.[17]

However, it has been reported that, together with the increase of plasma $HDL_2$, an increase of $HDL_3$ also occurs, by a mechanism not yet established.[7,9] Another enzyme that plays a key role in $HDL_1$ metabolism is lecithin:cholesterol acyltransferase ([LCAT] EC 2.3.1.43). This enzyme is secreted by the liver and catalyzes the transfer of the acyl chain from the sn-2 position in the phosphatidylcholine to the cholesterol, producing lysophosphatidylcholine and cholesterol ester. Normally, LCAT is responsible for the maturation of nascent HDLs, transforming them into $HDL_3$ and then into $HDL_2$; the removal of cholesterol and phospholipid excess from LDLs, VLDLs, and chylomicrons; and the efflux of cholesterol from cell membranes into HDLs. Thus, LCAT contributes to reverse cholesterol transport, an essentially antiatherogenic pathway.[18] The effect estrogens may have on plasma LCAT activity has not been established, and an eventual modification of LCAT activity could determine changes in $HDL_3$ and $HDL_2$ levels and subsequently in the extent of reverse cholesterol transport and cardiovascular disease risk.

The aim of this study was to determine the short-term effect of estrogen-progestin replacement therapy on plasma LCAT activity in a group of Chilean postmenopausal women.

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*From the Departamento de Bioquímica Clínica e Inmunología, Facultad de Farmacia, Departmento de Medicina Interna, Facultad de Medicina, and Departamento de Matemáticas, Facultad de Ciencias Físicas y Matemáticas, Universidad de Concepción, Concepción, Chile.*

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*Address reprint requests to N. Ulloa, MSc, Departamento de Bioquímica Clínica e Inmunología, Facultad de Farmacia, Universidad de Concepción, Casilla 237, Concepción, Chile.*



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## SUBJECTS AND METHODS

### *Patients*

This research involved the voluntary participation of 21 Chilean women (with informed consent). Women with diabetes, thyroid dysfunction, or liver or cardiovascular diseases who were using drug therapies with known effects on lipid metabolism or who were making significant changes in diet and/or life-style were excluded. Women with a minimum of 6 month's amenorrhea who were not previously on steroid treatment were included. Postmenopausal status was confirmed by medical evaluation (medical examination and anamnesis) and plasma follicle-stimulating hormone (FSH) and estradiol measurements. None of these women presented contraindications for the hormonal substitution. All of the women presented with one or more typical climacteric symptoms (hot flushes, vaginal dryness, asthenia, insomnia, etc.).

The women received a daily oral therapy consisting of conjugated equine estrogen 0.625 mg for 30 days, and during the last 12 days of each period they also received medroxyprogesterone acetate 5 mg daily. Compliance with the treatment was ensured by an educational program given by a matron. Blood samples were taken pretherapy and after 1 and 3 months of therapy, at the end of the progestative period. The mean age of the group was 54.5 ± 6.7 years (range, 45 to 70), and the mean body mass index was 29.8 ± 5.04 kg/m$^2$.

### *Methods*

Blood samples were obtained between 8 and 10 AM after an overnight fast, using EDTA 1 mg/mL.

Total cholesterol and triglyceride levels were measured by enzymatic methods. HDL-C was determined after removal of apolipoprotein (apo)-B–containing lipoproteins with sodium phosphotungstic in the presence of magnesium chloride and then measurement of the floating cholesterol. LDL-C was calculated using Friedewald's formula. Apo AI and apo B levels were measured by immunoturbidimetric methods. FSH and estradiol were determined by the enzyme-linked immunosorbent assay technique. For all of these determinations, Boehringer Mannheim (Mannheim, Germany) reactive kits were used.

LCAT activity (EC 2.3.1.43) was determined by a radioactive test using fresh discoidal artificial liposomes as substrate. Liposomes containing phosphatidylcholine 2.49 μmol/mL, cholesterol 0.125 μmol/mL, $^3$H-cholesterol $10^6$ cpm, and apoAI 0.010 μmol were prepared by the sodium cholate dispersion method described by Matz and Jonas.[19] LCAT activity was determined as described previously by Calvo et al[20] with some modifications. Briefly, 100 μL fresh human plasma was preincubated for 5 minutes at 37°C with 20 μL β-mercaptoethanol 100-mmol/L, 10 μL human albumin, free fatty acids 50 mg/mL, and 70 μL buffer with Tris 100-mmol/L, NaCl 0.14-mol/L, and EDTA 1-mmol/L, pH 7.4. The reaction was started by adding 100 μL liposomes, incubation was performed at 37°C for 90 minutes, and the reaction was stopped by addition of 500 μL methanol. Lipids were extracted from the reaction mixture with 1 mL *n*-hexane by mixing in vortex for 30 seconds at standard speed. Finally, 600 μL of the extract was chromatographed in a column with activated silica gel G (0.5 g); esterified cholesterol was selectively eluted with 3 mL diethyl ether:*n*-hexane 6:1 (vol/vol). This eluate was mixed with 5 mL liquid scintillation mixture, and radioactivity was measured in a Packard 1600 TR liquid scintillation analyzer (Packard Instrument, Meriden, CT). Each time, blanks without plasma were assayed in parallel. All determinations were performed in duplicate. LCAT activity was expressed as nanomoles of cholesterol esterified per hour per milliliter of plasma or as a percentage of activity.

### *Quality Control*

The samples were drawn by venipuncture, and the plasma was obtained immediately. All determinations were made in fresh plasma stored at 4°C prior to the reaction. The determinations of lipoprotein parameters were controlled using a control serum (Precinorm L; Boehringer). Control charts were devised for each parameter and analyzed following Shewart's multirules.[21] For determination of LCAT activity, the plasma samples were kept at 4°C for 1 hour maximum and the assay was performed at the same time for all samples. The interassay variation for LCAT activity was 9%.

### *Statistical Analysis*

Results are expressed as the mean ± SD. Observed variations before and after therapy were assessed by Wilcoxon's rank-sum test for paired samples.

## RESULTS

The mean basal level of FSH and estradiol was 60 ± 18 mUI/mL and 42 ± 15 pg/mL, respectively. After 1 month of therapy, FSH decreased to 38 ± 10 mU/mL, and at 3 months to 29 ± 8 mU/mL.

The mean body mass index was 29.8 ± 5.0, 30.7 ± 5.9, and 32.2 ± 7.8 kg/m$^2$ before and after 1 and 3 months of therapy, respectively. Systolic blood pressure was 128 ± 22, 129 ± 22, and 127 ± 23 mm Hg before and after 1 and 3 months of therapy, respectively. Diastolic blood pressure was 82 ± 13, 78 ± 10, and 78 ± 10 mmHg before and after 1 and 3 months of therapy, respectively. The changes observed for either body mass index or blood pressure were not significant.

Evaluation of the lipid profile (Table 1) showed a significant decrease in total cholesterol ($P < .025$) and LDL-C ($P < .005$), as well as a significant increase in HDL-C ($P < .005$) at the third month of therapy. On average, triglycerides showed a tendency to increase. However, because it was found that this process was not uniform within the group (some women

**Table 1. Effect of Estrogen-Progestin Replacement Therapy on the Lipid Profile**

| Time of Therapy | Total Cholesterol (mmol/L) | HDL-C (mmol/L) | LDL-C (mmol/L) | Triglycerides (mmol/L) | Apo AI (mg/dL) | Apo B (mg/dL) |
|---|---|---|---|---|---|---|
| Basal | 6.05 ± 1.16 | 1.23 ± 0.31 | 4.00 ± 1.14 | 2.00 ± 0.13 | 154 ± 26 | 113 ± 32 |
| First month | 5.51 ± 0.91 | 1.44 ± 0.31 | 3.23 ± 0.70 | 2.24 ± 0.11 | 162 ± 26 | 105 ± 34 |
| Third month | 5.61 ± 0.88 | 1.44 ± 0.35 | 3.23 ± 0.70 | 2.24 ± 0.14 | 160 ± 26 | 109 ± 27 |
| Variation at third month (%)* | −7.3 | +17.0 | −19.3 | +12.0 | +3.9 | −3.5 |
| *P*† | <.025 | <.005 | <.005 | NS | NS | NS |

Abbreviation: NS, not significant.

*Calculated between the mean values basally and at the third month after therapy.

†Calculated by Wilcoxon's rank-sum test.

showed an increase and others showed a decrease), and according to the statistical test used, this difference was not significant. Plasma concentrations of apoAI and apoB did not show significant changes.

Also, beneficial modifications expressed as a significant decrease in the total cholesterol to HDL-C ratio ($P < .005$) and LDL-C/HDL-C ratio ($P < .005$) were observed at the third month of treatment with respect to basal values (data not shown).

Figure 1 shows individual changes in plasma LCAT activity observed during the study. With the exception of three patients, an increase of LCAT activity was observed at the first month posttreatment. Consistently, all of these women showed an increase of enzyme activity after 3 months of therapy. Mean LCAT activity increased significantly from 8.81 ± 1.83 nmol cholesterol ester (h/mL (95% confidence interval, 7.96 to 9.67) to 12.6 ± 2.76 nmol cholesterol ester/h/mL (95% confidence interval, 11.24 to 13.80) after the first month of therapy. At the third month of treatment, a similar tendency was detected, reaching a mean of 14.4 ± 3.78 nmol cholesterol ester/h/mL (95% confidence interval, 12.72 to 16.24). The posttherapy mean LCAT activity was 42% ($P < .005$) and 64% ($P < .005$), significantly higher than the basal mean value at the first and third month, respectively. Figure 1 shows the relative changes (percentages) in LCAT activity, considering 100% as the basal mean value.

## DISCUSSION

Postmenopausal hormone therapy has been considered a legitimate component of preventive health care for older women,[1,9] with many benefits such as relief of climacteric symptoms and prevention of osteoporosis and cardiovascular disease.[22] To prevent endometrial hyperplasia in postmenopausal women, the combined use of estrogen and progestins has been recommended. However, estrogen-induced increases in HDL-C are attenuated by concomitant administration of progestins, particularly those with androgen activity.[9,23,24] Recent epidemiological research[25] has proven that the effect of progestins on the lipid profile depends on the type of progestin used, the amount, and the administration mode (cyclical or continuous). These investigators have reported that conjugated equine estrogen with cyclic micronized progesterone had the most favorable effect on HDL-C levels. They also found that a combination with cyclic medroxyprogesterone induced a beneficial effect on this lipoprotein. The present short-term study shows that in this group of postmenopausal women, estrogen-progestin substitution (daily conjugated equine estrogen 0.625 mg for 30 days and medroxyprogesterone acetate 5 mg during the last 12 days of each period) produces as much beneficial modification of the lipoprotein profile as found in other previous epidemiological research.[7-10,17,25] It is encouraging to confirm that a sample of our population responds similarly to other populations studied.

The evaluation of LCAT activity in this group of women during therapy showed a significant increase ($P < .005$) in the plasma activity after 1 month of therapy and a tendency for even higher values during the third month. Considering the mechanism of action of steroid hormones, the observed increase could be related to induction of LCAT synthesis. However, to confirm this hypothesis, it is necessary to evaluate the mass of LCAT. The magnitude of the effect can be expected to be significant due to the high hormone levels in the portal circulation attained with the oral therapy.

Francone et al[26] have provided evidence that a pre-$\beta_3$–migrating fraction of HDL contains the major proportion of LCAT and plays a key role in the esterification and transfer of cell-derived cholesterol to α-migrating HDL ($HDL_3$ and $HDL_2$). Therefore, the increase of LCAT activity could explain the elevation of $HDL_3$ and $HDL_2$, which has been reported with estrogen replacement therapy.[8-10,17] No other trial on the effect

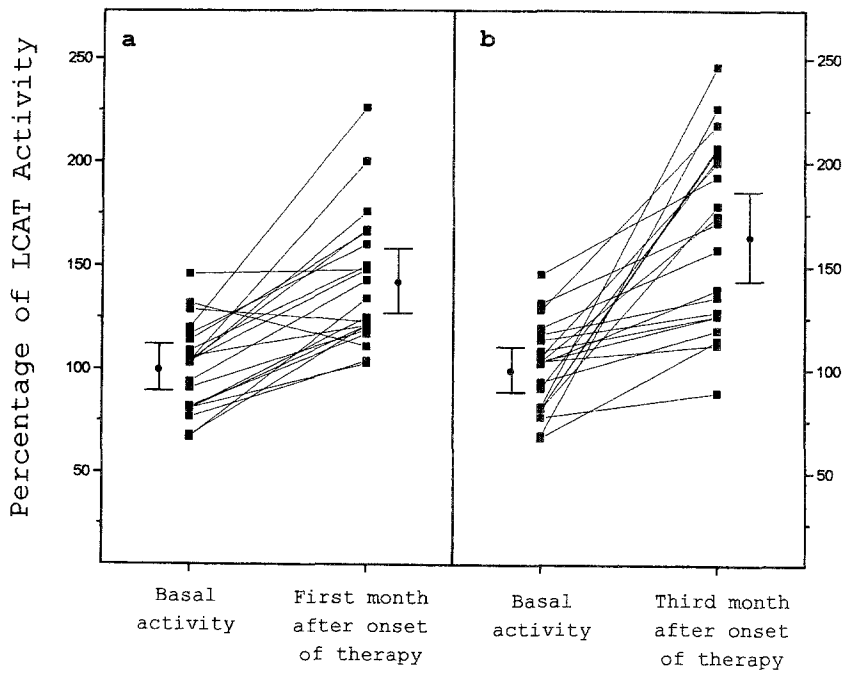


**Fig 1. Individual LCAT activity modifications at the first month (a) and third month (b) after onset of therapy. Data are expressed as the relative change (percentage) in LCAT activity, considering 100% as the basal mean value. The point on the vertical lines indicates the mean value and 95% confidence interval at different times (basal or first or third month after onset of therapy).**

of estrogen-progestin substitution on plasma LCAT activity using these dosages has been previously reported.

LCAT activity within HDL provides a driving force for the net transfer of cholesterol from peripheral cells into HDL particles. If the key role of this enzyme in reverse cholesterol transport is considered, an increase of its plasma activity should be beneficial. Indeed, recent research has shown that expression of the human gene for LCAT in transgenic mice alters the lipoprotein levels toward a less atherogenic profile.[27]

It would be interesting to study the effect of other hormone substitution schemes on plasma levels of this enzyme, as well as the effect of long-term replacement therapy. It would also be beneficial to evaluate the effect of different sex hormones individually (estrogens and progestins) on LCAT activity.

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