

Effects of Hormone Replacement Therapy on the Main Fatty Acids of Serum and Phospholipids of Postmenopausal Women

CHRISTINA PIPERI, MICHAEL TZIVRAS, CHRISTOS KALOFOUTIS,
AIKATERINI SKENDERI, VASILIKI MICHALAKI and ANASTASIOS KALOFOUTIS

Department of Biological Chemistry, University of Athens, School of Medicine, Athens, Greece

Abstract. *Background: The anti-atherosclerotic effects of hormone replacement therapy (HRT) in postmenopausal women are partly mediated by improvement of the lipid and lipoprotein profiles. The present study aimed to investigate the effects of HRT on the main fatty acids of serum and phospholipids in postmenopausal women. Patients and Methods: Serum samples of two groups of postmenopausal women, receiving either single oestrogen or in combination with progestogens, were analysed before and after a 6-month treatment period. Results: Of the main serum fatty acids, there was a significant reduction in palmitic ($p < 0.05$) and arachidonic acids ($p < 0.001$), followed by an increase in oleic ($p < 0.05$) and linoleic acids ($p < 0.05$) in postmenopausal women receiving HRT compared to single oestrogen. The main fatty acids in phospholipids showed a similar pattern in those women. Conclusion: The above results demonstrate the beneficial effects of HRT in reducing the risk of cardiovascular disease through modification of the fatty acid profiles of postmenopausal women.*

During the menopause, significant reductions in the circulating levels of oestradiol and estrone are believed to influence hepatic lipid and lipoprotein metabolism (1). Postmenopausal women usually have increased levels of the atherogenic LDL-cholesterol and decreased anti-atherogenic HDL-cholesterol, putting them at higher risk of developing cardiovascular disease (2). Oral hormone replacement therapy (HRT) has been shown to reverse these changes, acting as a primary preventive treatment for more than 35% of menopausal women, independent of their geographic location (3-5). Progestogens are commonly used

in combination with oestrogens since they reduce the risk of endometrial neoplasia which is associated with oestrogen replacement therapy in postmenopausal women (6).

The mechanism of the oestrogen-induced anti-atherosclerotic effects remains unclear and is currently the subject of extensive investigation. It has been suggested that the benefits of oestrogen therapy may result from its effects on the concentrations of lipids (7), lipoproteins (8), hemostatic factors, glucose (9), nitric oxide, endothelin-1 (10), insulin, uric acid (11) and vascular endothelial growth factor (12).

Alterations of the serum phospholipids levels, being important structural and functional components of cell membranes and mitochondria, have also been associated with cardiovascular disease. The ratio of HDL-phospholipids to HDL-cholesterol may have a predictive value in the assessment of cardiovascular risk (13). Phospholipids confer integrity to cell membranes and regulate the enzymatic activity of various metabolic processes, so that changes in their concentration can affect lipoprotein levels (14, 15). Furthermore, there is supporting evidence for the influence of hormonal factors on the fatty acid content and metabolism in women (16-18). Studies have shown that HRT reduces the proportion of polyunsaturated fatty acids in the platelet membranes of postmenopausal women (19) and that the fatty acid composition of tissues is believed to influence their responsiveness to insulin by modifying cell membrane properties (20).

In order to elucidate the mechanism of action of HRT on lipid metabolism, with particular emphasis on the main fatty acids content, serum and phospholipid samples of two groups of postmenopausal women, receiving either single oestrogen or in combination with progestogens, were analysed before and after a treatment period of 6 months.

Patients and Methods

The study involved 127 postmenopausal women, aged between 46 and 68 years (mean age 56 ± 8.2 years), who met the following eligibility criteria: (i) no hormone replacement therapy for the

Correspondence to: Prof. Anastasios Kalofoutis, Department of Biological Chemistry, University of Athens, School of Medicine, Goudi 115 27 Athens, Greece. Tel: (0030 210) 7791207/ 7771155, Fax: (0030 210) 7791207, e-mail: akalofoutis@med.uoa.gr

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preceding 6 weeks; (ii) the presence of hot flushes with or without episodes of drenching sweats; (iii) hysterectomy because of non-malignant disease at least 6 months previously; (iv) absence of diabetes mellitus, thyroid disease, ischaemic heart disease, thrombophlebitis, chronic liver, renal, cerebral or gallbladder disease, malabsorption or known lipid abnormalities; (v) elevated serum follicle stimulating hormone (FSH) to levels >30 IU/l and elevated leuteinising hormone (LH) to levels >20 IU/l; and (vi) no consumption of n-3 fatty acid supplements and no smoking.

The scheme was approved by the institutional review board and informed consent was obtained from the subjects, who were divided into two groups by randomization using a computer program. Sixty-five women (Group A) received 0.625 mg of conjugated equine oestrogens (CEO) (Premarin, Wyeth Laboratory) daily for 21 days, followed by 7-day tablet-free interval. Sixty-two women (Group B) received the same dosage of conjugated oestrogens (CIO) continuously, along with 5 mg metrogestone (6, 17-dimethylpregna-4,6-diene-3,20-dione) (MG) (Colprone, Wyeth Laboratories) for the last 10 days of the 21-day treatment regimen. For the same period (*i.e.* the last 10 days) patients in Group A received an identical placebo. After the completion of 6 treatment cycles, blood samples were taken for further examination. Baseline values were taken at the start of the experimental schedule.

The blood samples were obtained before the beginning of the regimen (baseline values), after an overnight fast of 14 hours, by venipuncture in tubes containing 2.5 mmol/l potassium ethylenediaminetetra acetic acid and were analysed for lipids and lipoproteins. Similar samples were collected in each group on day 21 of the 6 treatment cycles. The collection on week 23, day 21 ensured that the values represented treatment for 10 days with MG or placebo.

The total lipid extraction from each followed the method of Folch *et al.* (21), using a 2:1 v/v mixture of CHCl_3 : CH_3OH in a nitrogen atmosphere. The separation of phospholipids by column and thin layer chromatography, as well as phosphorus determination, were performed as described previously (22).

The main fatty acids were determined by gas-chromatography in the serum phospholipid fraction. The methyl esters of the phospholipid fraction were prepared by conversion of fatty acids to methyl esters, as described by Dodge and Phillips (23). The methyl esters were analysed by gas chromatography using a flame ionization detector (Perkin-Elmer 900 Gas-Chromatography) on a column packed with 3% EGS (ethylene glycol succinate) on Chromosorb W. The chromatography was isothermal at 180°C . High purity nitrogen was used as a carrier gas. Peaks were identified against commercially available reference methyl esters and the areas of the peaks were measured by triangulation. All the solvents used were redistilled and all experiments were performed in triplicate.

Statistical analysis. Statistical analysis of the results was performed with the Student's *t*-test between baseline and experimental values in each group. The mean value \pm SD (percentage concentration of phospholipids) and \pm SEM (for each group) was calculated. The probability measures for all the calculations are also provided.

Results

There was no significant difference between the mean age of the examined subjects (53.6 ± 6.8 years in Group A and

52.9 ± 7.2 years in Group B). Sixty-five women were assigned to receive conjugated oestrogens (CEO) plus placebo (Group A), while sixty-two received CEO plus MG (Group B).

Table I shows the percentage concentration of the main fatty acids in the serum of the two treatment groups at the end of 6 months. No differences were found in the baseline values between the two groups. Patients treated with CEO plus placebo (Group A) displayed a significant increase in oleic acid ($p < 0.05$) and linoleic acid ($p < 0.05$), followed by a reduction in arachidonic acid ($p < 0.01$). Group B patients showed significant reduction in palmitic acid ($p < 0.05$), followed by an increase in oleic ($p < 0.05$) and linoleic acids ($p < 0.05$) and a statistically significant reduction in arachidonic acid ($p < 0.001$).

Table II presents the percentage concentration of the main fatty acids in the serum phospholipids of the same patient groups at the end of 6 months. No differences were found in baseline values between the two groups. In patients treated with CEO plus placebo (Group A), a reduction in palmitic acid ($p < 0.01$) was observed, followed by an increase in oleic ($p < 0.001$) and arachidonic acids ($p < 0.01$). A similar pattern was observed for Group B patients, although the changes were more profound, with a striking reduction in palmitic acid ($p < 0.001$), followed by a significant increase in oleic ($p < 0.001$) and arachidonic acids ($p < 0.001$).

Discussion

The beneficial effects of HRT in circulating lipid concentrations have been investigated previously (22), but inadequate data exist regarding the overall effects of HRT on fatty acid metabolism and, especially, on the levels of serum and phospholipid fatty acids.

The composition of fatty acids represents an additional factor reflecting hormonal fluctuations and there is some evidence that menopausal status influences their metabolism (24). Furthermore, HRT alone and supplemented with dietary fatty acids has been shown to alter the fatty acid profile of postmenopausal women by reducing the proportion of polyunsaturated fatty acids in their platelet membranes (19, 25).

In the present study, both the main fatty acids in serum as well as the main fatty acids in phospholipids of postmenopausal women receiving HRT were analysed. The fatty acid content of tissues is believed to influence their responsiveness to various hormones, including insulin, by modifying cell membrane properties (26, 27). Our data showed that although oestrogen alone elevated the oleic (18:1) and linoleic acid (18:2) levels and reduced the arachidonic acid (20:4) concentration, its combination with progestones potentiated these effects and further

Table I. Percentage concentration of the main serum fatty acids in the examined subjects (mean±SD).

Fatty acid	Group A (N=65)			Group B (N=62)		
	Baseline	CEO +placebo	p<	Baseline	CEO +MG	p<
Palmitic 16:0	17.14±1.80	16.72±1.66	NS	17.66±1.83	16.19±1.64	0.05
Palmitoleic 16:1	0.86±0.09	0.80±0.09	NS	0.82±0.08	0.84±0.08	NS
Stearic 18:0	21.24±2.04	22.10±2.09	NS	21.56±2.06	22.13±2.05	NS
Oleic 18:1	17.47±1.80	18.64±1.9	0.05	17.72±1.82	18.84±1.88	0.05
Linoleic 18:2	7.16±1.1	8.08±1.14	0.05	7.10±1.08	8.53±1.18	0.05
di-homo-γ-linoleic, DGLA 20:3	2.08±0.62	1.98±0.6	NS	1.99±0.61	2.04±0.61	NS
Arachidonic 20:4	25.13±3.92	23.78±3.72	0.01	25.28±3.93	22.92±3.84	0.001
Docosapentaenoic, DPA 22:5	2.14±0.64	2.09±0.61	NS	2.12±0.64	2.08±0.6	NS
Docohexaenoic, DHA 22:6	1.74±0.52	1.8±0.53	NS	1.78±0.53	1.96±0.54	NS

Table II. Percentage concentration of the main fatty acids in the phospholipids of the examined subjects (mean±SD).

Fatty acid	Group A (N=65)			Group B (N=62)		
	Baseline	CEO +placebo	p<	Baseline	CEO +MG	p<
Palmitic 16:0	23.36±2.4	21.73±2.1	0.01	22.89±2.25	20.12±1.79	0.001
Palmitoleic 16:1	2.38±0.61	2.10±0.52	NS	2.86±0.64	2.64±0.62	NS
Stearic 18:0	21.68±2.06	22.10±2.10	NS	21.72±2.08	22.24±2.19	NS
Oleic 18:1	18.94±1.74	20.98±1.8	0.001	19.36±1.78	21.33±2.06	0.001
di-homo-γ-linoleic, DGLA 20:3	2.42±0.66	2.26±0.62	NS	2.31±0.64	2.26±0.63	NS
Arachidonic 20:4	21.38±2.04	22.54±2.36	0.01	21.97±2.06	23.18±2.29	0.001
Docosapentaenoic, DPA 22:5	2.54±0.65	2.4±0.63	NS	2.44±0.62	2.38±0.64	NS
Docohexaenoic, DHA 22:6	1.88±0.54	1.96±0.56	NS	1.92±0.55	2.10±0.63	NS

significantly decreased the palmitic acid (16:0) levels. Previous studies have shown that the serum cholesteryl ester proportions of palmitic (16:0), palmitoleic (16:1) and di-homo-γ-linolenic (20:3) acids are inversely related, while the proportions of linoleic acid (18:2) are positively related to insulin sensitivity (26). Although no mechanism has been suggested for the HRT reduction on arachidonic acid (20:4), other studies (19) have also observed decreased proportions of polyunsaturated fatty acids, mainly arachidonic acid (20:4), in the platelet membranes of postmenopausal women. On the contrary, in a study of postmenopausal women where the sequential estradiol-norethisterone acetate regimen was given for a 3-month period, no effect was observed on non-esterified fatty acids or the levels of lipid peroxidation products (28).

Examination of the main fatty acid levels in serum phospholipids revealed a different pattern. Women receiving HRT presented decreased levels of palmitic acid (16:0) and increased levels of oleic acid (18:1), suggesting a possible role of oestrogens in the biosynthesis of these fatty acids.

This observation is contradictory to the study of Ottosson and colleagues (18), who showed increased palmitic acid (16:0) and decreased stearic acid (18:0) concentrations in serum phosphatidylcholine in an oral oestrogen intervention in postmenopausal women. Also, the study of Stark *et al.* (24) proposed that the increased palmitic acid (16:0) in phosphatidylcholine from oestrogen use is a result of increased phosphatidylcholine biosynthesis from cytidine diphosphocholine and reduced biosynthesis *via* methylation of phosphatidyl ethanolamine.

It is possible that the differences in fatty acid compositions found in these studies may be a result of varying dietary intakes of selected fatty acids. Since we observed similar effects with oestrogen, it is possible that the mechanism involved may be a hormonal effect of oestrogen or a result of potential liver toxicity and ¹⁷C-alkylated oestrogen products.

In addition, the discrepancies in fatty acid concentrations between ours and other studies may be due to a decreased effect of systemic endogenous, as compared to oral exogenous, oestrogens. The oral administration of

oestrogen results in a greater hepatic oestrogen concentration because of enterohepatic blood flow which may, therefore, alter the hepatic fatty acid metabolism to a greater extent.

Furthermore, the present study indicated that women receiving HRT tend to have increased arachidonic acid (20:4) levels in their phospholipids. While no specific mechanism for increased arachidonic acid (20:4) concentrations with oral oestrogen use is known, it has been suggested that oestrogen may increase the biosynthesis of arachidonic acid from linoleic acid. This is supported by the study of Stark *et al.* (24), who observed significant increases in $\Delta 6$ -desaturase, the enzyme that inserts a double bond at the ninth carbon from the fatty acid carboxy terminal, and of the total desaturase potential in women receiving HRT compared with those who did not. From studies with castrated rats, it has been proposed that $\Delta 6$ -desaturase activity is stimulated by oestradiol, whereas $\Delta 5$ -desaturase activity is inhibited by oestradiol; HRT intervention in women caused no significant changes in the $\Delta 5$ -desaturase potential derived from platelet membrane fatty acids. An increase in $\Delta 6$ -desaturase activity would result in a higher rate of oleic acid (18:1) and arachidonic acid (20:4) being produced from linoleic acid, accounting for the increased concentrations of arachidonic acid.

Other studies with oral HRT administration also support this observation of increased synthesis of arachidonic acid (20:4), which is the substrate for the production of eicosanoids (*e.g.* thromboxane A₂ and prostacyclin), which have important cardiovascular functions. It has been suggested that the increased synthesis and levels of arachidonic acid with oral oestrogen use, and the observation of increased thrombotic events with oral HRT intervention in the Heart and Estrogen/Progestin Replacement Study and the Women's Health Initiative trial, may possibly be related since arachidonic acid is a precursor of thromboxane A₂, which is a potent platelet aggregator (29).

However, prostacyclin is another by-product of the eicosanoids pathway with inhibitory effects on platelet aggregation. Administration of transdermal oestrogen without progestin has been shown to increase the capacity of plasma and serum to stimulate prostacyclin production in cultured human vascular endothelial cells (30). Oestrogens can also regulate NO production by increasing its concentration, further adding to the inhibitory effect on platelet aggregation.

Additional studies show the positive effects of HRT on plasma docosahexaenoic and eicosapentanoic acid levels in postmenopausal women with their known anti-atherosclerotic effects (24, 25). Our data support these observations, though without statistical significance.

In conclusion, the present study supports the beneficial effects of HRT on lipid structures and functions, with particular emphasis on the fatty acids composition both in serum and in phospholipids, further suggesting the existence of a complex regulatory mechanism. Other external parameters, such as dietary intervention in parallel with HRT (by replacing intake of saturated fatty acids with polyunsaturated fatty acids) are recommended, in order to avoid possible thrombotic risks.

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