Effects of Hormone Replacement Therapy on Circulating Docosahexaenoic Acid and Eicosapentaenoic Acid Levels in Postmenopausal Women

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Abstract. Hormone replacement therapy (HRT) has antiatherosclerotic effects of which the mechanism remains unclear. The ingestion of fish oil or other sources of n-3 polyunsaturated fatty acids has been included in comprehensive strategies to prevent atherosclerosis. Many epidemiologic studies have shown that the dietary intake of docosahexaenoic acid and eicosapentaenoic acid has antiatherosclerotic effects. We investigated the effect of HRT on plasma docosahexaenoic acid and eicosapentaenoic acid concentrations in postmenopausal women. Fifty-nine postmenopausal women, who received conjugated estrogens (0.625 mg/day) and medroxyprogesterone (2.5 mg/day) for 12 months, and 45 control postmenopausal women, who did not receive HRT, volunteered to participate in this study. Plasma docosahexaenoic acid and eicosapentaenoic acid concentrations were measured at baseline and at 6 and 12 months after the start of HRT. HRT significantly increased the plasma docosahexaenoic acid and eicosapentaenoic acid concentrations from 134 ± 5 μg/ml and 69 ± 4 μg/ml at baseline to 156 ± 7 μg/ml and 85 ± 7 μg/ml after 12 months (both p<0.01). However, the control group showed no significant change in their plasma docosahexaenoic acid and eicosapentaenoic acid levels during the study. HRT increased plasma docosahexaenoic acid and eicosapentaenoic acid levels in postmenopausal women. We propose that the increase in docosahexaenoic acid and eicosapentaenoic acid may be partially responsible for the beneficial mechanisms by which HRT induces an antiatherosclerotic effect in postmenopausal women.

Key words: Estrogen, Docosahexaenoic acid, Eicosapentaenoic acid, Postmenopausal women


RECENTLY, the Heart and Estrogen/Progenst Replacement Study (HERS) and its follow-up study (HERS II), involving women with established coronary heart disease (CHD) as a secondary prevention measure, could not show a reduction in CHD in postmenopausal women receiving hormone replacement therapy (HRT) [1, 2]. Furthermore, no HRT is reported to decrease the progression of coronary atherosclerosis in postmenopausal women with angiographically verified coronary disease [3]. In epidemiological studies as a primary preventive measure, however, HRT reduces CHD morbidity and mortality in postmenopausal women [4, 5]. HRT also shows the reduction of progression of carotid intima-media thickness in healthy postmeno-
pausal women taking unopposed HRT [6]. HRT seems to have antiatherosclerotic effects in postmeno-
pausal women without CHD. The mechanism of the estrogen-induced antiatherosclerotic effect remains
unclear and is currently the subject of intense investiga-
tion. It has been suggested that the antiatherosclerotic effect may result from the beneficial effects of
estrogen on the concentrations of lipids [7, 8],
lipoproteins [7, 8], hemostatic factors [9], glucose
[9], nitric oxide [10], endothelin-1 [10], insulin [9],
uric acid [11], and vascular endothelial growth factor
[12]. Estrogen slows the development and limits the
adverse effects of atherosclerosis in part by alleviating
vascular endothelial dysfunction [13].

A low rate of coronary heart disease has been re-
ported in an Eskimo population that consumed a diet
which is rich in fish oil [14]. The ingestion of fish oil
or other sources of n-3 polyunsaturated fatty acids
has been included in a comprehensive strategies for
the prevention of atherosclerosis [15]. Many epide-
mio logic studies have shown that the dietary intake
of n-3 fatty acids has an antiatherosclerotic potential
[15, 16]. Furthermore, experimental [17] and clini-
cal trial data [18–20] support the protective effects
of n-3 fatty acids on atherosclerosis. The principal
n-3 fatty acids are docosahexaenoic (22: 6n-3) and eicosa-
pentaenoic acid (20: 5n-3). Since estrogen, as well
as docosahexaenoic acid and eicosapentaenoic acid,
has antiatherosclerotic effects, we hypothesized that
the antiatherosclerotic potential of estrogen may be
related to an alteration in plasma docosahexaenoic
acid and eicosapentaenoic acid concentrations.

This study was designed to investigate the effects of
administering HRT to postmenopausal women on
plasma docosahexaenoic acid and eicosapentaenoic
acid concentrations.

**Materials and Methods**

**Subjects**

A total of 104 postmenopausal Japanese women
(aged 43 to 63, mean 53.4 ± 0.4 years) with climac-
teric symptoms and/or osteoporosis, volunteered to
participate in this study.

Each subject had experienced natural menopause
for at least one year or longer. Menopausal status
was confirmed by a serum FSH concentration >40
mIU/ml and a serum estradiol (E2) concentration
<20 pg/ml. None had received HRT, other steroid
hormones or any medication. Before entering the
study, each subject underwent physical and laborato-
ry examinations, including a gynecologic evaluation
and mammography, 12-lead electrocardiogram, and
echocardiogram. None of the subjects smoked, used
caffeine, or had a history of diabetes mellitus,
thyroid disease, hypertension, cardiovascular dis-
ease, venous thromboembolism, liver disorders,
unexplained vaginal bleeding, or a personal or family
history of breast cancer. No subject underwent exer-
cise or dietary therapy before the study. Subjects
were also told to maintain the same diet during the
investigation, because the reproducibility of plasma
docosahexaenoic acid and eicosapentaenoic acid
measurements is highly influenced by the consump-
tion of fish oil.

**Study Protocol**

Postmenopausal women were divided into two
groups; an HRT group (n = 59), which consisted of
those who wished to receive HRT for 12 months, and
a control group (n = 45), which consisted of those
who did not wish to receive HRT. Except for the
controls, each subject received a daily dose of HRT
(0.625 mg of conjugated equine estrogen combined
with 2.5 mg of medroxyprogesterone acetate) orally
for 12 months. Each control subject did not receive
HRT during the study. Subjects attended the HRT
clinic at the Cardiovascular Hospital of Central
Japan once a month for physical check-ups, blood
pressure and heart rate measurements, and to pro-
vide blood samples before the start of the study and
at 6 and 12 months after the start of the HRT. The
control subjects similarly attended the Cardiovascu-
lar Hospital of Central Japan. Written informed
consent was obtained from each subject before partici-
pation. The study protocol was approved by the
Ethics Committee of the Cardiovascular Hospital of
Central Japan.

Blood samples were taken in the morning after a
12-hour fast. After centrifugation, the samples were
stored at −80°C until assayed.

**Physical Examination**

Resting blood pressure and heart rate were meas-
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ured after the subject had been seated for at least 30 minutes. The blood pressure in the patient's right arm was read three times by two investigators using a mercury sphygmomanometer. The mean of the three measurements was used in the study.

**Assays**

Serum total cholesterol and triglyceride concentrations were determined using enzymatic methods (Kanto Biochemical Laboratories Co., Konosu, Japan) and an automatic analyzer (Boehringer Mannheim, Mannheim, Germany). Serum concentrations of high-density lipoprotein (HDL) cholesterol were determined by an electrophoretic method using the HDL Cholesterol Supply Kit (Helena Laboratories, Beaumont, TX, USA). The concentration of low-density lipoprotein (LDL) cholesterol was calculated according to the Friedewald formula [21]. The serum levels of FSH and estradiol were analyzed by radioimmunoassay using commercially available kits (Boehringer Mannheim, Mannheim, Germany).

Regarding the measurement of docosahexaenoic acid and eicosapentaenoic acid concentrations, immediately after the collection of venous blood (10 ml) into a heparinized glass tube, the sample was centrifuged at 400 g for 10 minutes, and the separated plasma was stored at −80°C until assayed. Fatty acid methyl esters were analyzed using gas-liquid chromatography (Shimadzu GC-14 A, Kyoto, Japan) equipped with a capillary column (0.25 mm × 50 m) packed with a liquid phase, HR-SS-10 (Shinwa Chemical Industries, Kyoto, Japan). The gas-liquid chromatography was performed using a temperature program of from 160°C to 190°C at 3°C/min, from 190°C to 200°C at 1°C/min, and from 200°C to 215°C at 0.5°C/min, with a 20-minute final hold using helium as a carrier gas. The fatty acids were identified by their retention times and quantitated by comparison with a known amount of internal standard (21:0). The within-assay and between-assay coefficients of variation were <5%. All analyses were performed by the same investigator (M. M.) who was unaware of the treatment assignment of each sample.

**Statistical Analysis**

All results are expressed as the mean ± SEM. Student's t test was used to analyze the differences between the values for age, height, body weight, body mass index, alcohol consumption, blood pressure, heart rate, FSH, estradiol, total cholesterol, triglyceride, HDL cholesterol, LDL cholesterol, docosahexaenoic acid, and eicosapentaenoic acid at baseline in the HRT and control groups. A two-way analysis of variance (ANOVA) and Scheffe's F-test were used to analyze the differences between values recorded at baseline and at 6 and 12 months. All probability values are 2-tailed. A value of p<0.05 was considered to be statistically significant.

**Results**

We divided the postmenopausal women into two groups: those receiving HRT (n=59) and the controls, who did not receive HRT (n=45). The characteristics of the study population are shown in Table 1. Before treatment, there were no significant differences in age, height, body weight, body mass index, and alcohol consumption between the HRT group and the control group.

The changes in hemodynamic and sex hormone levels in the HRT group and the control group at baseline and at 6 and 12 months after the initiation.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>HRT group (n = 59)</th>
<th>Control group (n = 45)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>53.5 ± 0.5</td>
<td>53.2 ± 0.7</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>153.4 ± 0.8</td>
<td>153.8 ± 0.7</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>56.4 ± 1.1</td>
<td>56.4 ± 1.5</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.9 ± 0.4</td>
<td>23.8 ± 0.5</td>
</tr>
<tr>
<td>Alcohol consumption (g/week)</td>
<td>12.5 ± 2.2</td>
<td>12.3 ± 2.6</td>
</tr>
</tbody>
</table>

Mean ± SEM.

HRT = hormone replacement therapy.
of the study are presented in Table 2. Before treatment, there were no significant differences in blood pressure and heart rate between the HRT group and the control group. Moreover, the HRT did not affect the blood pressure and heart rate in the two groups during the study. The baseline serum levels of FSH and estradiol were consistent with a postmenopausal state. There were no significant differences in baseline FSH and estradiol values between the two groups. The serum levels of FSH were significantly reduced compared with the baseline levels at 6 (p<0.001) and 12 months (p<0.01) after the start of HRT in the HRT group but were unchanged in the control group. In contrast, the serum levels of estradiol were significantly elevated compared with baseline levels at 6 (p<0.001 and p<0.001, respectively) and 12 months (p<0.001 and p<0.001, respectively) after the start of HRT in the HRT group but were unchanged in the control group. These findings indicate the good compliance of the participants with the HRT regimen.

The lipid profiles of the subjects are shown in Table 3. HRT significantly reduced the total cholesterol and LDL cholesterol levels in the HRT group after 6 (p<0.01 and p<0.01, respectively) and 12 months (p<0.01 and p<0.01, respectively), compared with the baseline values. The administration of HRT significantly increased the HDL cholesterol levels after 6 (p<0.01) and 12 months (p<0.01) and the triglyceride levels after 12 months (p<0.05), compared with the baseline values. However, the control group did not exhibit any significant change in their total cholesterol, triglyceride, HDL cholesterol, and LDL cholesterol levels during the study. Before treatment, there were no significant differences in total cholesterol, triglyceride, HDL cholesterol, and LDL cholesterol levels between the HRT group and the control group.

The baseline values for both the plasma docosahexaenoic acid and eicosapentaenoic acid concentrations were not significantly different between the two groups. HRT significantly increased the mean plasma docosahexaenoic acid concentration from 134 ± 5 μg/ml at baseline to 156 ± 7 μg/ml after 12 months.

Table 2. Changes in hemodynamic and sex hormone levels in the HRT group and the control group at baseline and at 6 and 12 months after the initiation of the study

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>HRT group (n = 59)</th>
<th>Control group (n = 45)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
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<tr>
<td>Baseline</td>
<td>128.5 ± 2.1</td>
<td>128.0 ± 2.5</td>
</tr>
<tr>
<td>6 months</td>
<td>129.4 ± 2.0</td>
<td>128.6 ± 2.4</td>
</tr>
<tr>
<td>12 months</td>
<td>128.0 ± 2.0</td>
<td>128.1 ± 2.6</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>82.1 ± 1.2</td>
<td>81.4 ± 1.6</td>
</tr>
<tr>
<td>6 months</td>
<td>82.0 ± 1.4</td>
<td>82.0 ± 1.6</td>
</tr>
<tr>
<td>12 months</td>
<td>80.4 ± 1.1</td>
<td>82.0 ± 1.7</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>68.7 ± 1.0</td>
<td>68.8 ± 1.7</td>
</tr>
<tr>
<td>6 months</td>
<td>67.5 ± 1.0</td>
<td>68.1 ± 1.1</td>
</tr>
<tr>
<td>12 months</td>
<td>68.4 ± 1.0</td>
<td>68.7 ± 1.3</td>
</tr>
<tr>
<td>FSH (mIU/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>73.7 ± 3.4</td>
<td>74.4 ± 2.7</td>
</tr>
<tr>
<td>6 months</td>
<td>28.9 ± 2.1*</td>
<td>72.8 ± 2.7</td>
</tr>
<tr>
<td>12 months</td>
<td>28.3 ± 2.1*</td>
<td>73.6 ± 2.9</td>
</tr>
<tr>
<td>Estradiol (pg/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>12.3 ± 1.0</td>
<td>13.8 ± 0.8</td>
</tr>
<tr>
<td>6 months</td>
<td>69.0 ± 5.7*</td>
<td>12.9 ± 0.5</td>
</tr>
<tr>
<td>12 months</td>
<td>73.1 ± 3.8*</td>
<td>13.1 ± 0.6</td>
</tr>
</tbody>
</table>

Mean ± SEM. *p<0.001 compared with baseline value (ANOVA).
HRT = hormone replacement therapy.
Table 3. Changes in lipids levels in the HRT group and the control group at baseline and at 6 and 12 months after the initiation of the study

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>HRT group (n = 59)</th>
<th>Control group (n = 45)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>198 ± 5</td>
<td>195 ± 5</td>
</tr>
<tr>
<td>6 months</td>
<td>187 ± 4*</td>
<td>202 ± 6</td>
</tr>
<tr>
<td>12 months</td>
<td>184 ± 4*</td>
<td>197 ± 6</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>110 ± 8</td>
<td>111 ± 9</td>
</tr>
<tr>
<td>6 months</td>
<td>122 ± 8</td>
<td>116 ± 12</td>
</tr>
<tr>
<td>12 months</td>
<td>126 ± 9*</td>
<td>116 ± 12</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>52.2 ± 1.9</td>
<td>51.4 ± 2.0</td>
</tr>
<tr>
<td>6 months</td>
<td>59.2 ± 2.1\†</td>
<td>50.9 ± 2.0</td>
</tr>
<tr>
<td>12 months</td>
<td>59.1 ± 1.9\†</td>
<td>50.1 ± 1.7</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>123.6 ± 4.0</td>
<td>121.9 ± 5.1</td>
</tr>
<tr>
<td>6 months</td>
<td>103.2 ± 3.4\†</td>
<td>127.6 ± 6.6</td>
</tr>
<tr>
<td>12 months</td>
<td>99.8 ± 3.6\†</td>
<td>124.0 ± 6.1</td>
</tr>
</tbody>
</table>

Mean ± SEM. \*p<0.05, \†p<0.01 compared with baseline value (ANOVA). HRT = hormone replacement therapy; HDL = high-density lipoprotein; LDL = low-density lipoprotein.

(p<0.01; Fig. 1). On the other hand, the plasma docosahexaenoic acid concentration showed no significant change in the control group throughout the 12-month study period. The mean plasma eicosapentaenoic acid concentration in the HRT group increased significantly from 69 ± 4 µg/ml at baseline to 85 ± 7 µg/ml at 12 months after the start of HRT (p<0.01; Fig. 2). However, the plasma eicosapentaenoic acid concentration did not change in the control group throughout the study.

Discussion

The present study showed that HRT increased plasma docosahexaenoic acid and eicosapentaenoic acid concentrations in postmenopausal women. In the lipid profiles of these postmenopausal women, HRT decreased the serum total cholesterol and LDL cholesterol levels and increased the serum triglyceride and HDL cholesterol levels.

The n-3 fatty acids are relatively rich in both docosahexaenoic acid and eicosapentaenoic acid and demonstrate several biologic effects that could be antiatherogenic, including arterial vasodilation [20], reduced thromboxane and enhanced prostacyclin synthesis [22, 23] the inhibition of platelet aggregation [22, 23], suppression of cardiac arrhythmia [24], decreased blood pressure [25], and the reduction of plasma triglyceride levels [32].

Many studies indicate that docosahexaenoic acid has various beneficial effects on cardiovascular disease [27–29]. The docosahexaenoic acid levels was associated with the risk of coronary heart disease in a case-control study of men with or without incident coronary heart disease [27]. In adipose-tissue biopsies and segments of coronary arteries sampled from human autopsies, the group with the highest degree of coronary artery disease had a lower concentration of docosahexaenoic acid in their adipose tissue than the group with the lowest degree of coronary artery disease [28]. Docosahexaenoic acid, but not eicosapentaenoic acid, inhibited ischemia-induced cardiac arrhythmia and was more effective than eicosapentaenoic acid at retarding the development of hypertension in spontaneously hypertensive rats and inhibiting thromboxane-like vasoconstrictor responses in aortas from spontaneously hypertensive rats [29]. Thus, docosahexaenoic acid may offer protective benefits against cardiovascular disease, suggesting that the increase in plasma docosahexaenoic acid concentrations as a result of HRT may be, in part,
associated with the antiatherosclerotic effects of HRT on cardiovascular disease in postmenopausal women. Eicosapentaenoic acid is available for metabolic conversion to prostacyclin [30]. Furthermore, eicosapentaenoic acid inhibits platelet aggregation [31], increases bleeding time [31], reduces whole-blood viscosity and red-cell deformability [31, 32], increases the cross-sectional area of the dorsalis pedis artery in patients with type II diabetes mellitus [33], and reduced the levels of triglycerides and cholesterol in a clinical trial of patients with thrombotic cardiovascular disease [32]. In the present study, HRT increased the plasma eicosapentaenoic acid concentrations in postmenopausal women. Therefore, the increase in plasma eicosapentaenoic acid concentrations as a result of HRT may be one example of the beneficial effects of estrogen.

The present study did not clarify the mechanisms by which HRT increased the plasma concentrations of docosahexaenoic acid and eicosapentaenoic acid in postmenopausal women. Three possibilities may be suggested. First, estradiol treatment increases the docosahexaenoic acid concentration in quail oviducts [34]. Secondly, normal pregnant women with high estrogen and progesterone levels exhibit significantly higher levels of docosahexaenoic acid and eicosapentaenoic acid, compared with nonpregnant women, suggesting that the increase in plasma docosahex-
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omega-3 acid levels that occurs during pregnancy may alter the storage and mobilization of lipid pools [35]. Finally, estrogen may increase docosahexaenoic acid and eicosapentaenoic acid absorption from the gastrointestinal tract.

Favorable changes in lipids and lipoprotein levels have been demonstrated in a clinical study evaluating the effects of exogenous estrogen on cardiovascular risk factors in women [7]. These favorable changes include an increase in HDL cholesterol and a decrease in LDL cholesterol. In the Postmenopausal Estrogen/Progestin Interventions Trial [36], the women who were randomly selected to receive conjugated equine estrogen and continuous or cyclical medroxyprogesterone acetate had smaller increases in HDL cholesterol levels, compared to the baseline value, than the women who received unopposed estrogen. Walsh et al. [7] have demonstrated an increase in HDL cholesterol and triglyceride levels and a decrease in LDL cholesterol and total cholesterol levels in postmenopausal women following treatment with equine estrogen. In the present study, similar effects on lipid and lipoprotein levels were observed in postmenopausal women. Estrogen induces changes in lipids and lipoproteins through a variety of mechanisms. For example, estrogen causes a decrease in serum total cholesterol and LDL cholesterol and an increase in HDL cholesterol by increasing hepatic LDL-receptor activity and suppressing hepatic triglyceride lipase activity; estrogen also causes an increase in triglycerides by suppressing lipoprotein lipase [7].

There are several limitations inherent to the present study. First, the present study is limited by the relatively small number of subjects and its non-randomized nature. A double-blind, randomized study with a large number of subjects is required. Secondly, we could not directly assess the antiatherosclerotic effects of HRT on tissues. A further study would ideally clarify the association of estrogen, docosahexaenoic acid, eicosapentaenoic acid, and atherosclerosis.

In conclusion, HRT increased plasma docosahexaenoic acid and eicosapentaenoic acid levels in postmenopausal women. The increase in plasma docosahexaenoic acid and eicosapentaenoic acid levels produced by HRT could be associated with the anti-atherosclerotic effects of HRT that have been observed in postmenopausal women. We propose that the increases in docosahexaenoic acid and eicosapentaenoic acid levels are partially responsible for the beneficial mechanisms by which HRT exerts an anti-atherosclerotic effect on cardiovascular disease in postmenopausal women.

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References


