The Effect of Dietary Intervention Through the Modification of Fatty Acids Composition and Antioxidant Vitamin Intake on Plasma TXB$_2$ Level in Korean Postmenopausal Women with Hypercholesterolemia

The purpose of this study was to observe the effects of dietary intervention, through the modification of dietary fatty acids composition and antioxidant vitamins, on plasma thromboxane B$_2$ (TXB$_2$) levels in postmenopausal women with hypercholesterolemia. The subjects were treated for 12 weeks with one of three methods: hormone replacement therapy (HRT group, n=8), dietary intervention (DIET group, n=8), or HRT combined with dietary intervention (HRT+DIET group, n=8). Changes in serum phospholipid fatty acids composition, serum peroxides, and plasma TXB$_2$ levels were measured at weeks 0, 4 and 12. The P/S ratio increased and the n-6/n-3 ratio decreased in the DIET and the HRT+DIET group at week 4 (p<0.05). The ratio of C20:5/C20:4 in serum phospholipid increased in the DIET (p<0.05) and the HRT+DIET groups (NS) at week 4. Plasma TXB$_2$ levels decreased in the DIET (-35%, p<0.05) and the HRT+DIET groups (-35%, NS) at week 4. Serum lipid peroxides levels significantly decreased by 10.5% and 15.2% in the DIET group at weeks 4 and 12, and by 10.8% in the HRT+DIET group only at week 12 (p<0.05). Dietary intervention may lower thrombotic risks in Korean postmenopausal women by changing the serum fatty acid composition, serum lipid peroxides levels and plasma thromboxane B$_2$ levels.

Key Words : Postmenopause; Thromboxane B$_2$; Lipid peroxides; Fatty Acids; Antioxidants

INTRODUCTION

Years of cardiovascular research focusing predominantly on males have, unfortunately, added to the impression that cardiovascular disease (CVD) is basically a male affliction. Yet, CVD is the leading cause of death among elderly women, especially postmenopausal women (1-3). Although women are largely protected from clinical coronary disease before menopause, they rapidly develop an increased risk thereafter. A lot of evidence has suggested that hormone replacement therapy (HRT) may suppress the accelerated atherosclerotic process (4). The mechanism of estrogen's protection from coronary disease appears to be partly due to the pharmacological effect of estrogen on lipoprotein levels, but we also have to consider that estrogen may reduce the risk of coronary disease by directly suppressing platelet function (4, 5). Many dietary intervention studies have also been restricted to focusing on changes in lipids and lipoproteins, without examining indices related to the thrombotic risk, e.g. platelet aggregation and thromboxane formation (6). The effects of dietary intervention, with respect to coronary disease, also need to be considered from two approaches as described by Hoover and Spector, one concerned with atherosclerosis and the other with thrombosis (7). These effects of diet are not surprising because the many nutrients which compose our diet, such as cholesterol, fatty acids, and antioxidant vitamins and minerals, have a relationship with coronary disease, with respect to either blood lipid profiles or thrombotic risks. However, much less is known about the effects of dietary intervention on thrombosis compared to the effects on blood lipid profiles.

Dietary factors considered to have a relationship with thrombosis are several fatty acids and antioxidant vitamins. Many nutritionists, to this day, continue to show interest in the fatty acids composition of lipids in humans, especially in long-chain polyunsaturated fatty acids (PUFAs) derivatives. This is due to the role of PUFAs in maintaining the fluidity and function of cell membranes and the role of PUFAs as precursors of eicosanoids (8). Many studies have shown that long-chain saturated fatty acids (SFA's) accelerate thrombus formation, whereas PUFAs do not, and the PUFA/SFA (P/S) ratio is an important determinant of thrombogenicity (9, 10). More-
over, different PUFAs have different effects: n-6 PUFAs are antiatherogenic, whereas n-3 PUFAs are antithrombogenic. Such differences have been noted since the study of Greenland Eskimos (11).

When PUFAs metabolism is studied, antioxidants should be measured because lipid peroxides, formed by the peroxidation of PUFAs, may be important in the development of atherosclerosis and thrombosis (12). Oxidized low-density lipoprotein (LDL) has been found in human atherosclerotic lesions. It has been demonstrated that oxidized LDL decreases the secretion of endothelium-derived relaxing factors, resulting in enhanced platelet adhesion (13, 14). Prostacyclin synthesis in the arterial endothelium was, in cell cultures, irreversibly inhibited by lipid peroxides, and intracellular lipid peroxides concentration was regulated by antioxidant enzymes such as glutathione peroxidase, superoxide dismutase, and catalase, and also by antioxidant vitamins such as alpha-tocopherol, ascorbic acid, and beta-carotene. Thus, theoretically, a deficiency in these antioxidants may lead to reduce prostacyclin synthesis, which can, in turn, promote platelet aggregability. Moreover, hypercholesterolemic patients show a higher susceptibility to LDL oxidation (15) and hyperaggregability (16, 17).

This small-scale study was done to compare the effects of dietary intervention, through the modification of both dietary fatty acids composition and antioxidant vitamins, and hormone replacement therapy (HRT), alone or in combination, on serum phospholipid fatty acids composition, on plasma TXB2 levels, and also on serum lipid peroxides levels in Korean postmenopausal women with hypercholesterolemia.

MATERIALS AND METHODS

Subjects

Thirty-six postmenopausal women, aged 50 to 61 yr, were recruited from a medical center for women in Seoul, Korea. All had hypercholesterolemia, defined as LDL cholesterol levels over 160 mg/dL, determined from the mean of two pre-study fasting samples of serum. All participants had experienced spontaneous menopause, and had not received any type of anti-platelet drug such as nonsteroidal, any anti-inflammatory agents, or any hormone treatment for at least 6 months before the study. Written, informed consent was obtained from all subjects, and this study has been performed in accordance with the principles embodied in the Helsinki Declaration.

Study design

The subjects were randomly assigned to one of three different treatments: hormone replacement therapy (HRT group, n=8), dietary intervention (DIET group, n=8) or hormone replacement therapy combined with dietary intervention (HRT+DIET group, n=8).

The HRT group and the HRT+DIET group both took, on a monthly cyclic schedule, a combined therapy of estrogen and progesterone, in which conjugated equine estrogen (CEE, Premarin®) 0.625 mg was prescribed for 30 days, and medroxy progesterone acetate (MPA, Provera®) 5 mg was combined for the first 12 days. A prescription of HRT was accomplished with office visits at weeks 0 and 4.

The balanced diet, designed on the basis of our previous studies in Korean postmenopausal women (18, 19), was provided for the DIET group and also the HRT+DIET group. The diet was modified in cholesterol, antioxidant vitamins, and fatty acid composition. The diet contained cholesterol less than 70 mg/1,000 kcal and antioxidant vitamins over 90% of the recommended dietary allowance (RDA) for Koreans. The P/S ratio of dietary fat increased to the upper limit of the recommended range, 1-1.5, and the n-6/n-3 ratio decreased to the lower limit of the recommended range, 4-10 (20). The changes in the P/S and n-6/n-3 ratio of dietary fat were achieved by making up menus with foods low in fat and cholesterol and high in antioxidant vitamins.

Energy intake was individually tailored to maintain an ideal body weight, and menus composed of 1,600 kcal, 1,700 kcal and 1,800 kcal were provided for the subjects (Table 1). Each diet consisted of a 10-day rotating menu to provide variety and maintain the acceptability of the diet. Extensive dietary counseling was given at the beginning of dietary intervention, and repeated at each subsequent visit. The subjects were given dietetic scales, measuring cups and spoons, and were also trained in estimating portion sizes with the use of uniform food models.

The study period was a total of 12 weeks. All subjects visited the clinic at weeks 0, 4 and 12 for treatment and blood sampling. At each visit, with the exception of week 0, all subjects completed three-day dietary diaries in order to monitor adherence to the treatment. Dietary intake at the baseline (week 0) was measured using a semi-quantitative food-frequency questionnaire that has been developed and validated to assess the usual food intake of Korean postmenopausal women (21, 22). Nutrient intake was calculated by using the Hanyang diet analysis program. We tried to encourage compliance in the subjects with follow-up telephone counseling once a week during the study period.

Blood analysis

At each visit, a sample of venous blood was drawn after an overnight fast of 12 to 14 hr. Serum estradiol (E2) levels were assayed by a procedure based on the competitive binding principles of radioimmunoassay using the Biodata Estradiol Maia kit (Code 12264). Serum vitamin A and E levels were determined using high performance liquid chromatography (HPLC) as described by Bieri et al. (23). Serum β-carotene
Effect of Dietary Intervention on Plasma TXB$\textit{2}$ Levels

Table 1. Nutrients composition of menus provided to the DIET and the HRT+DIET groups

| Nutrients                  | 1,600 kcal | 1,700 kcal | 1,800 kcal | p-value  |
|----------------------------|------------|------------|------------|----------|
| Energy (kcal)              | 1595.0±6.1 | 1710.0±7.2 | 1796.9±5.6 | 0.000    |
| % of energy from protein   | 17.6±0.4   | 17.5±0.5   | 17.5±0.4   | 0.981    |
| from fat                   | 18.0±0.5   | 18.0±0.5   | 17.7±0.5   | 0.510    |
| from Carbohydrate          | 63.1±0.3   | 64.1±0.4   | 63.0±0.4   | 0.790    |
| Dietary fiber (g)          | 28.6±2.1   | 27.7±1.9   | 31.8±1.9   | 0.321    |
| Vitamin A (% RDA)          | 117.9±13.2 | 109.6±13.4 | 115.9±10.4 | 0.885    |
| Vitamin C (% RDA)          | 299.9±22.3 | 283.6±19.0 | 318.0±28.1 | 0.588    |
| Vitamin E (% RDA)          | 95.9±5.2   | 96.0±4.3   | 95.8±3.6   | 0.999    |
| Cholesterol (mg)           | 81.9±5.1   | 84.1±5.7   | 91.3±5.8   | 0.456    |
| mg/1,000 kcal              | 513.3±3.2  | 491.3±3.3  | 509.3±3.3  | 0.882    |
| SFA (% of energy)          | 4.5±0.2    | 4.4±0.2    | 4.3±0.1    | 0.590    |
| MUFAs (% of energy)        | 4.5±0.2    | 4.4±0.2    | 4.5±0.2    | 0.951    |
| PUFA (% of energy)         | 6.0±0.4    | 5.9±0.3    | 6.3±0.3    | 0.711    |
| n-6 (% of energy)          | 10.8±0.5   | 11.0±0.5   | 11.2±0.5   | 0.915    |
| n-3 (% of energy)          | 2.4±0.2    | 2.5±0.2    | 2.4±0.2    | 0.822    |
| P/S                        | 1.3±0.1    | 1.4±0.1    | 1.4±0.1    | 0.524    |
| n-6/n-3                    | 4.7±0.4    | 4.5±0.4    | 4.8±0.3    | 0.873    |

mean±SE; Values with different alphabets within the same row are significantly different among groups at $p<0.05$ by Duncan’s multiple range test; p-value by one-way ANOVA. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; P/S, PUFA/SFA; n-6, n-6 fatty acids; n-3, n-3 fatty acids.

levels were determined according to the method used by K im (24) and vitamin C levels were determined according to the method used by Otsuka et al. (25) using H PLC. Serum lipid peroxides were measured fluorometrically using thiobarbituric acid reaction (26). Thromboxane B2 (TXB2), the stable hydrolysis product of TXA2, was measured in plasma using an enzymeimmunoassy system kit (Code RPN 220, A mersham U.K.).

For analyzing fatty acids composition in serum phospholipid, the serum phospholipid was separated using thin-layer chromatography on silica gel G-60 (Merch, 20 × 20 cm) after extracting serum lipid according to the method used by Folch (27). Methylation of fatty acids was immediately carried out according to the procedure of Morrison and Smith (28). Fatty acid methyl esters were quantified using gas chromatography (Shimadzu GC-17A) utilizing a 30 m wide-bore column (0.52 mm internal diameter, Supelco-wax). Fatty acids in lipid fractions were obtained as percent of distribution.

Table 2. The general characteristics of the subjects

| Variables | HRT (n=8) | DIET (n=8) | HRT+DIET (n=8) | p-value |
|-----------|-----------|------------|----------------|---------|
| Age (yr)  | 51.2±1.2  | 52.0±2.4   | 52.0±2.0       | 0.865   |
| Height (cm)| 156.1±1.8 | 156.9±2.6  | 159.8±2.0     | 0.512   |
| Weight (kg)| 59.0±1.6  | 61.3±2.9   | 61.7±0.2       | 0.600   |
| BMI (kg/m²)| 24.4±0.6  | 25.8±1.2   | 25.4±0.8       | 0.872   |
| FBS (mg/dL)| 83.7±2.6  | 84.5±3.6   | 86.3±1.8       | 0.830   |
| TC (mg/dL)| 254.2±6.7 | 252.5±4.0  | 253.6±8.0       | 0.936   |
| TG (mg/dL)| 111.2±8.0 | 110.3±11.6 | 114.0±2.7       | 0.965   |
| LDL-C (mg/dL)| 166.6±7.7 | 165.2±6.7  | 165.9±8.6       | 0.992   |
| HDL-C (mg/dL)| 65.4±2.4  | 65.3±3.6   | 67.7±5.8       | 0.889   |

mean±SEM; p-value by ANOVA; BMI, body mass index; FBS, fasting blood sugar; TC, total cholesterol; TG, triglyceride; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; HRT, hormone replacement therapy group; DIET, dietary intervention group.

RESULTS

Of the thirty-six subjects who entered the study, 24 completed the study period. Twelve subjects failed to complete the study; four subjects did not complete it for personal reasons, two were lost to follow-up, and six had problems following the dietary intervention program. There was no significant difference in the general characteristics of the subjects among the three groups (Table 2).

Serum estradiol (E2) levels significantly increased in the two groups taking hormone replacement therapy (H RT), and there were small but significant increases in the serum estradiol (E2) levels in the DIET group (Fig. 1). There was a significant difference in the degree of changes in the serum E2 levels among the groups ($p<0.05$); serum E2 levels based on the initial value were significantly higher, at weeks 4 and 12, in the HRT and HRT+DIET groups than in the DIET group.

Changes in daily mean nutrient intake during the study period are shown in Table 3. Nutrient intake of the HRT group did not change, but there were significant changes in the nutrient intake of both the DIET group and the HRT+DIET group, at weeks 4 and 12 ($p<0.05$). Antioxidant vitamin A and E intake, which was under 75% of the recommended dietary allowance (RDA) for Koreans at the baseline, increased to over 90% of the RDA. Cholesterol intake decreased to less than 70 mg/1,000 kcal. The P/S ratio increased from less than 1.0 to 1.3 in the DIET group ($p<0.05$), and decreased from 5.9 to 4.0-5.5 in the HRT+DIET group ($p<0.05$). The ratio of n-6/n-3 decreased from 6.9 to 3.5-4.0 in the DIET group ($p<0.05$) and decreased from 5.9 to 4.0-5.5 in the HRT+DIET group (NS). According to an analysis of the standardized, quantitative dietary records, the balanced diet actually consumed by the subjects during the study period was close to the principles for dietary changes specified in the study design, with substantial differences between the HRT and the dietary
Table 3. Changes in nutrients intake during the study period

| Nutrients | HRT | DIET | HRT+DIET |
|-----------|-----|------|----------|
| Energy (kcal) | 2,053.8±108.6 | 1,975.7±69.0 | 2,013.2±103.2 |
| % of energy | 2,052.4±136.8 | 1,744.2±50.4 | 1,772.4±26.7 |
| from protein | 2,043.1±139.9 | 1,747.2±38.9 | 1,811.9±6.8 |
| % | 15.4±0.7 | 17.1±0.7 | 16.5±1.0 |
| from fat | 15.7±1.0 | 18.2±0.4 | 18.1±0.6 |
| % | 24.3±1.2 | 22.9±1.2 | 21.7±0.1 |
| from carbohydrate | 59.5±1.4 | 60.5±0.7 | 63.8±0.9 |
| % | 55.3±7.1 | 54.7±7.9 | 57.0±4.9 |
| Dietary fiber (g) | 22±3.9 | 21.9±2.1 | 22.0±3.9 |
| % | 20.6±1.1 | 15.7±1.5 | 15.8±0.9 |
| Vitamin A (% RDA) | 23.5±5.4 | 30.6±4.6 | 27.1±1.0 |
| % | 22.3±4.8 | 27.9±0.3 | 33.7±5.1 |
| Vitamin C (% RDA) | 16.8±0.9 | 17.3±1.2 | 18.2±0.7 |
| % | 24.1±2.2 | 18.0±0.6 | 17.9±0.6 |
| HRT DIET HRT | 56.5±2.6 | 63.5±1.2 | 62.5±1.1 |
| SFA | 50.3±8.4 | 95.4±13.6 | 102.5±18.3 |
| mg/1,000 kcal | 216±1.19 | 257.5±39.7 | 288.6±26.7 |
| % | 194±1.29 | 281.5±23.2 | 274.7±50.2 |
| Cholesterol (mg) | 71.5±3.1 | 96.5±7.4 | 102.5±1.8 |
| % | 11.6±2.0 | 18.0±2.2 | 20.8±1.6 |
| PUFA | 226±1.88 | 92.1±8.1 | 87.5±8.5 |
| % | 233±8.0 | 73.8±12.0 | 91.7±7.4 |

Values are mean ± SE; *Significantly different from the initial value in the same group at p<0.05 by repeated measure design.

Table 4. Changes in fatty acids composition in serum phospholipid during the study period

| Fatty acids | HRT | DIET | HRT+DIET |
|-------------|-----|------|----------|
| C14:0 | 0.7±0.4 | 0.94±0.08 | 0.66±0.05 |
| % | 0.60±0.03 | 0.56±0.01 | 0.55±0.02 |
| C16:0 | 49.09±1.30 | 55.67±2.28 | 50.99±1.45 |
| % | 53.02±1.94 | 46.60±1.07 | 50.78±1.11 |
| C18:0 | 22.87±0.51 | 22.87±0.93 | 21.26±0.32 |
| % | 25.77±0.55 | 22.66±0.48 | 23.69±0.49 |
| C18:1 | 6.71±0.67 | 5.14±0.90 | 7.15±0.48 |
| % | 3.92±0.51 | 7.11±0.16 | 6.96±0.47 |
| C18:2 (n-6) | 14.71±0.90 | 11.76±1.02 | 14.77±1.52 |
| % | 12.38±1.27 | 15.67±0.32 | 12.90±0.87 |
| C18:3 (n-3) | 0.25±0.04 | 0.23±0.05 | 0.27±0.05 |
| % | 0.27±0.02 | 0.26±0.01 | 0.29±0.06 |
| C20:4 (n-6) | 3.83±0.45 | 2.34±0.61 | 3.15±0.63 |
| % | 2.51±0.06 | 3.12±0.05 | 3.59±0.05 |
| C20:5 (n-3) | 0.44±0.07 | 0.20±0.03 | 0.28±0.08 |
| % | 0.42±0.03 | 0.61±0.02 | 0.36±0.29 |
| C22:6 (n-3) | 1.41±0.04 | 0.86±0.29 | 1.48±0.40 |
| % | 1.11±0.16 | 2.44±0.12 | 1.35±0.06 |
| SFA | 72.66±1.01 | 79.49±2.24 | 72.91±1.70 |
| % | 79.39±1.95 | 69.62±3.88 | 75.02±1.13 |
| MUFA | 6.71±0.67 | 5.14±0.90 | 7.15±0.48 |
| % | 3.92±0.51 | 7.11±0.16 | 6.96±0.47 |
| PUFA | 20.64±0.97 | 15.39±1.62 | 19.25±0.99 |
| % | 16.69±1.45 | 22.47±0.24 | 19.02±1.16 |
| n-6 | 18.54±0.91 | 14.03±1.40 | 17.92±1.84 |
| % | 14.89±1.32 | 19.26±0.31 | 16.02±0.06 |
| n-3 | 2.10±0.13 | 1.29±0.31 | 2.03±0.49 |
| % | 1.80±0.19 | 3.21±0.14 | 2.00±0.13 |
| P/S | 0.29±0.02 | 0.20±0.03 | 0.28±0.03 |
| % | 0.21±0.02 | 0.32±0.01 | 0.24±0.02 |
| n-6/n-3 | 8.94±0.58 | 12.69±1.72 | 11.44±2.21 |
| % | 8.81±0.90 | 6.10±0.32 | 8.07±0.37 |

Values are mean ± SE; *Significantly different from the initial value in the same group at p<0.05 by repeated measure design.

intervention groups.

The changes in the fatty acids composition of serum phospholipid (PL) reflected, to some extent, the fatty acid composition of the modified diet (Table 4). During dietary intervention, the relative % of C14:0, C16:0 and C18:0 decreased, whereas C18:1, C18:2 (significant only in the HRT+DIET group), C20:4, C20:5 and C22:6 elevated, at week 4 (p<0.05) and, to a lower extent, at week 12 in the DIET and the HRT + DIET groups. With these changes in individual fatty acids composition, the P/S ratio increased and the n-6/n-3 ratio decreased at week 4 (p<0.05), but both tended to return to the initial values at week 12 after dietary intervention. The ratio of C20:5/20:4 in serum PL did not significantly change in the HRT group but elevated in the DIET group (p<0.05) and HRT+DIET group (NS) at week 4 (Fig. 2).

The changes in plasma TXB2 levels are shown in Fig. 3. In the HRT group, plasma TXB2 levels changed from 36.33±3.49 pg/mL to 36.17±2.40 pg/mL at week 4, and to 39.33±9.66 pg/mL at week 12, but this change was not statistically significant. In the DIET group, at week 4, TXB2 levels had decreased from 35.00±8.57 pg/mL to 23.10±3.74 pg/mL (-35.0%, p<0.05). In the HRT+DIET group, at week 4, TXB2 levels had decreased from 28.79±3.91 pg/mL to 23.40±5.33 pg/mL (-18.8%, NS). Thus, at week
Effect of Dietary Intervention on Plasma TXB2 of this study, was ingested at a level higher than the RDA, group (the DIET group and the HRT group) also observed; serum vitamin E levels were higher in both min E levels, at week 12, a difference due to treatment was observed; plasma TXB2 levels in the DIET and the HRT group (p<0.05). In the HRT group significantly decreased by 10.5% at week 4 and decreased by 15.2% at week 12 (p<0.05). In the HRT group, the serum MDA levels decreased by 10.8% only at week 12 (p<0.05) (Fig. 4).

The changes in serum antioxidant are shown in Table 5. In the DIET group, serum vitamin A level increased by 10.9% at week 4 and increased by 27.3% at week 12 (p<0.05). In the HRT+DIET group, serum vitamin A level increased by 11.9% only at week 12 (p<0.05). In the HRT+DIET group, serum- carotene levels increased by 3.6% at week 4 and increased by 5.2% at week 12, whereas in the DIET group, the serum vitamin E level increased by 14.3% at week 4 and increased by 22.7% at week 12 (p<0.05). As for serum vitamin E levels, at week 12, a difference due to treatment was also observed; serum vitamin E levels were higher in both the DIET group and the HRT+DIET group than in the HRT group (p<0.05). Serum vitamin C, which, at the beginning of this study, was ingested at a level higher than the RDA, did not significantly change throughout the study period. With changes in serum antioxidant vitamin A, vitamin E and -carotene levels, the serum MDA levels in the DIET group significantly decreased by 10.5% at week 4 and decreased by 15.2% at week 12 (p<0.05). In the HRT+DIET group, the serum MDA levels decreased by 10.8% only at week 12 (p<0.05) (Fig. 4).

Fig. 1. The changes in serum estradiol (E2) level. 'a', significantly different from the initial value in the same group at p<0.05 by a repeated measure design; Values with different alpha-bets within the same week are significantly different among groups based on the initial value at p<0.05 as analyzed by a repeated measure design.

DISCUSSION

It is well established that Greenland Eskimos have a low incidence of cardiovascular disorders and they also have a bleeding tendency that may result from reduced platelet aggregation. It has been shown that their intake of n-3 fatty acid, C20:5 is high and, conversely, their intake of n-6 fatty acids, C18:2 and C20:4 is low. Also, both their plasma and platelet lipids have a similar fatty acid pattern to that found in their diet (29). Therefore, it has been suggested that a shift in dietary fatty acids composition towards the Greenland Eskimo pattern may prevent certain cardiovascular disorders, especially by reducing

Table 5. Changes in serum antioxidant vitamins during the study period

| Nutrients | HRT | DIET | HRT+DIET |
|-----------|-----|------|----------|
|           | 0 week | 4 week | 12 week | 0 week | 4 week | 12 week | 0 week | 4 week | 12 week |
| Vitamin A (µg/mL) | 0.55±0.03 | 0.58±0.02 | 0.57±0.04 | 0.55±0.05 | 0.61±0.07 | 0.70±0.10 | 0.59±0.03 | 0.70±0.12 | 0.66±0.03 |
| Vitamin C (µg/mL) | 11.58±1.7 | 12.60±2.33 | 20.76±1.86 | 20.81±2.15 | 21.49±2.61 | 22.86±2.05 | 21.25±1.15 | 22.35±2.85 | 22.01±0.80 |
| Vitamin E (µg/mL) | 9.66±0.17 | 9.10±0.46 | 10.40±0.67 | 11.17±0.27 | 9.19±0.61 | 8.71±0.48 | 10.43±0.28 |

Values are mean±SE; 'a' significantly different from the initial value in the same group at p<0.05 by repeated measure design; Values with different alphabets within the same week are significantly different among groups based on the initial value at p<0.05 as analyzed by repeated measure design.
the risk of thrombosis (30).

In the present study, at week 4, with the changes in the dietary fat composition after the dietary fat modification, serum phospholipid (PL) P/S ratios increased by 52.4% in the DIET group, and increased by 85.0% in the HRT + DIET group ($p < 0.05$). Also at week 4, n-6/n-3 ratios decreased by 30.7% in the DIET group and decreased by 30.8% in the HRT + DIET group ($p < 0.05$). Despite the fact that fatty acid changes in dietary fat in the two groups were maintained until week 12, ratios in serum PL tended to return to their initial values at week 12. The reason for this finding remains uncertain, but in some cases, poor responses can be explained by poor adherence to diet. Many studies have indicated that changes in fatty acids of dietary fat led to changes in fatty acids of serum and cell lipids, and, consequently, said studies pointed out the importance of dietary habits on the blood fatty acid's composition (30-32). Therefore, we can suppose that the subjects of the HRT group and the HRT + DIET group may have progressively departed from the prescribed balanced diet, but failed to report these behavioral changes in their dietary diaries. On the other hand, in the HRT group, a few fatty acids of serum PL also changed, even though there were no significant changes in their fatty acid intakes. Several studies have suggested that estrogen can affect blood fatty acid composition, but we cannot conclude whether those changes are due to estrogen, or not (33, 34). Even now, the effect of estrogen on serum fatty acid composition is controversial, and, as such, requires further research.

Platelet aggregation is modulated by the production of TXA$_2$ and PGI$_2$. An optimal balance of TXA$_2$-PGI$_2$ may be important in the prevention of thrombotic conditions. Evidence indicates that dietary fatty acids can alter this balance (35, 36). According to Kinsella et al. (35), the only dietary fatty acid change shown to affect thrombosis in humans is an increased intake of n-3 fatty acids. When this occurs, the platelets accumulate n-3 fatty acids, especially eicosapentaenoic acid. The n-3 fatty acids replace some of the arachidonic acid in the platelet membranes, decreasing its availability for eicosanoid formation. As a result, less TXA$_2$ is produced in response to activation, and the tendency for the platelets to aggregate is reduced (30, 38). In this present study, at week 4, the ratios of C20:5/C20:4 in serum PL increased, but plasma TXB$_2$ levels, metabolites of TXA$_2$ (39), decreased by 35.0% in the DIET group ($p < 0.05$) and decreased by 18.8% in the HRT + DIET group (NS). Recently, Mantzioris et al. reported that an increase of dietary C20:5 intake led to an average 3-fold increase in C20:5 in plasma, platelet, and mononuclear cell phospholipids, and they also reported TXB$_2$ synthesis decreased by 36% (39). These findings, taken in conjunction with previous epidemiological studies in Eskimos, suggest that a change in fatty acids consumption, such that a diet with a high C20:5/C20:4 ratio could reduce platelet aggregability by decreasing plasma TXB$_2$ levels, would exert a beneficial influence on certain cardiovascular disorders.

Many studies have suggested that oxidative modification of low-density lipoprotein (LDL) promotes atherosclerosis, which, in turn, has brought about considerable interest in the role of dietary antioxidant vitamins in preventing CVD (40-42). When PUFA’s metabolism is studied, antioxidants should be measured because lipid peroxides, formed by the peroxidation of PUFA’s, may be important in the development of atherosclerosis and thrombosis (12, 43, 44). It has also been suggested...
Effect of Dietary Intervention on Plasma TXB₂ Levels

The present study is on ‘what diet is good’ rather than ‘what nutrient is good’ for the prevention of cardiovascular disease. The dietary modifications needed to prevent certain diseases may probably be much larger than those usually occurring in the human diet, making it unlikely that these processes would come into play under ordinary conditions. In fact, this study devoted considerable effort to encourage dietary compliance, which is not easy in routine clinical practice. Nevertheless, it is very stimulating to note that postmenopausal women, if they are highly motivated, can decrease their thrombotic risks by way of dietary intervention which can be achieved by replacing SFAs with PUFA, eating less fat- and cholesterol-rich foods, eating more fish and lean meat, and eating more fresh fruits and vegetables.

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