Raloxifene and hormone replacement therapy increase arachidonic acid and docosahexaenoic acid levels in postmenopausal women

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Abstract

Estrogens may affect the essential n-6 and n-3 fatty acids arachidonic acid (AA; C20:4n-6) and docosahexaenoic acid (DHA; C22:6n-3). Therefore, we investigated the long-term effects of hormone replacement therapy and raloxifene, a selective estrogen-receptor modulator, in two randomized, double-blind, placebo-controlled studies. In study I, 95 healthy, non-hysterectomized, early postmenopausal women (age range 47–59 years) received one of the following treatments: daily raloxifene 60 mg ($n=24$), daily raloxifene 150 mg ($n=23$), 0·625 mg conjugated equine estrogens (CEE) plus 2·5 mg medroxyprogesterone acetate (MPA; $n=24$), or placebo ($n=24$). In study II, 30 men (age range 60–69 years) received daily 120 mg raloxifene ($n=15$) or placebo ($n=15$). In study I, plasma cholesteryl ester fatty acids were measured at baseline and after 6, 12, and 24 months in 83 (drop out rate 13%), 73 (23%), and 70 (25%) women respectively. In study II, fatty acids were measured at baseline and after 3 months in 29 men (drop out rate 3%). In postmenopausal women, administration of 150 mg raloxifene increased AA by a mean of +6·1% ($P=0·055$, not significant). Administration of CEE plus MPA increased AA by +14·1% ($P<0·0005$). Mean changes in DHA were +22·1% ($P=0·003$) and +14·9% ($P=0·047$) respectively, as compared with placebo. In men, 120 mg raloxifene for 3 months did not significantly affect AA (–5·2%; $P=0·342$) or DHA (+4·0%; $P=0·755$), but it increased testosterone levels by +19·8% ($P=0·006$). Administration of raloxifene 150 mg/day as well as CEE plus MPA to postmenopausal women increases the proportion of AA and DHA in plasma cholesteryl esters during a follow-up of 2 years. Short term administration of raloxifene in elderly men did not affect AA or DHA. The synthesis of AA and DHA from precursors may be enhanced through an estrogen receptor-dependent pathway.

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Introduction

The n-6 (i.e. omega-6) and n-3 (i.e. omega-3) highly unsaturated fatty acids (HUFA) are essential fatty acids, and need to be supplied as such or as their polyunsaturated fatty acid precursors in the diet. Eicosapentaenoic (EPA; C20:5n-3) and docosahexaenoic acid (DHA; C22:6n-3) are n-3 HUFA and are primarily found in fatty fish. Although there is a biosynthetic pathway from the precursor α-linolenic acid (C18:3n-3) into EPA and DHA (Emken *et al.* 1994, Cho *et al.* 1999, Salem *et al.* 1999, Vermunt *et al.* 2000, Pawlosky *et al.* 2001, Burdge *et al.* 2002), the quantitative contribution of this pathway to tissue EPA and DHA levels is unclear. In contrast to EPA and DHA, α-linolenic acid is found in vegetables, walnuts and vegetable oils (e.g. canola and soybean oil). The n-6 series includes arachidonic acid (AA; C20:4n-6) and its precursor linoleic acid (LA; C18:2n-6). AA can be converted by oxygenases into prostaglandins, thromboxanes, leukotrienes, and other bioactive products (Brash 2001). The enzymes responsible for the conversion of linoleic acid into AA are the same as those responsible for the conversion of α-linolenic acid into EPA and DHA, and therefore the synthesis of n-3 and n-6 HUFA compete for the same elongases and desaturases (Fig. 1).

Recently, we have shown that men have lower DHA in plasma cholesteryl esters than women when both are on the same strictly controlled diets. Furthermore, we have found that administration of estrogens increases DHA levels in cholesteryl esters in male-to-female transsexual subjects (Giltay *et al.* 2004). Previous studies in postmenopausal women have shown shifts in the fatty acid spectrum during the menstrual cycle, pregnancy and menopause (Rosing *et al.* 1982, Mattsson *et al.* 1985,

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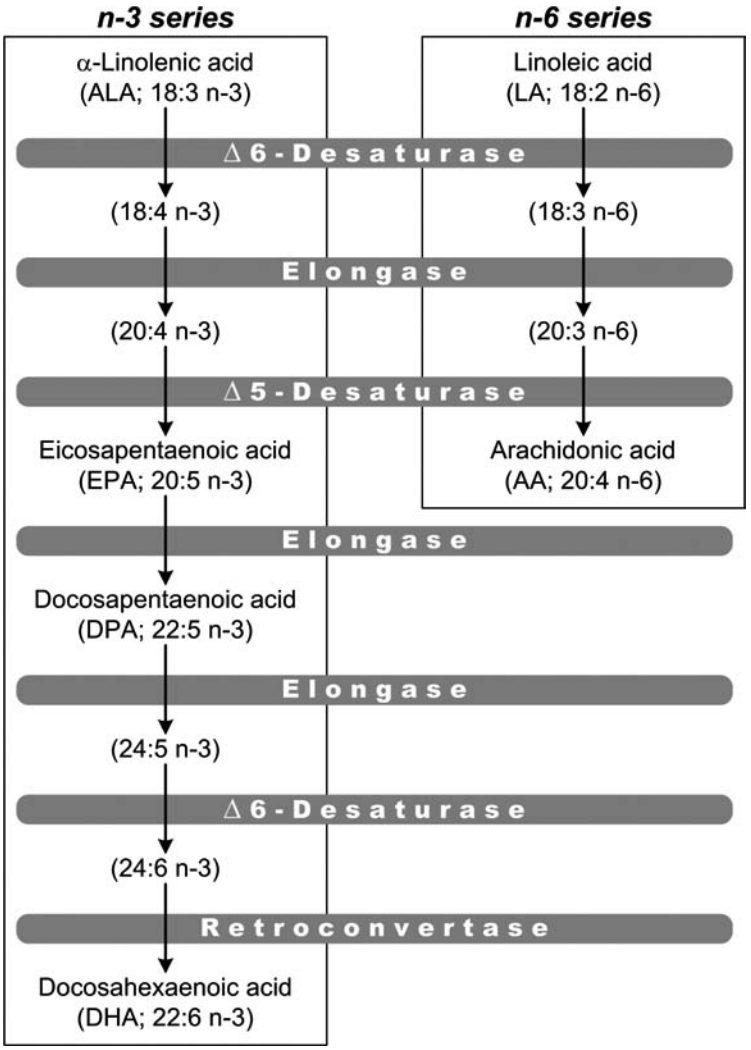

Figure 1 Biosynthesis of highly unsaturated essential fatty acids. The n-3 and n-6 series of polyunsaturated FA are converted – using the same competing enzymes – to their end products by a series of sequential desaturations (adding double bonds) and elongations (adding two carbon atoms at a time). Linoleic acid is converted into arachidonic acid, and α-linolenic acid is converted into eicosapentaenoic acid and docosahexaenoic acid (Sprecher 2000).

Maynar *et al.* 2001), as well as shifts in the fatty acid spectrum induced by several estrogenic, progestational, and antiestrogenic compounds, but n-3 HUFA were not studied (Silfverstolpe *et al.* 1981, 1982, Crona *et al.* 1983, 1984, Ottosson *et al.* 1984, Enk *et al.* 1985, Mattsson *et al.* 1986). These studies found that oral estrogens increased AA in serum lecithin without influencing linoleic acid. To our knowledge, the effects of estrogens on n-3 HUFA have not previously been studied in women. An endogenous biosynthetic pathway induced by estrogens may be clinically important, since DHA may lower the risk of fatal ischemic heart disease and sudden death (Kromhout *et al.* 1985, Burr *et al.* 1989, Siscovick *et al.* 1995, Albert *et al.* 1998, 2002, Gruppo-Italiano-per-lo-Studio-della-Sopravvivenza-nell'Infarto-miocardico 1999, Oomen *et al.* 2000). Furthermore, DHA is found in high amounts in the central nervous system of the fetus (including the retina), and a non-fish eating mother may depend on the endogenous synthesis of DHA from precursors (Postle *et al.* 1995, Innis 2000, Otto *et al.* 2001).

The selective estrogen receptor modulator, raloxifene, is a benzothiophene derivative that opposes estrogen action in the breast and uterus, but acts as an estrogen agonist on the bone, liver, and some surrogate markers of cardiovascular disease. Raloxifene may be used for the prevention of postmenopausal osteoporosis, but it also

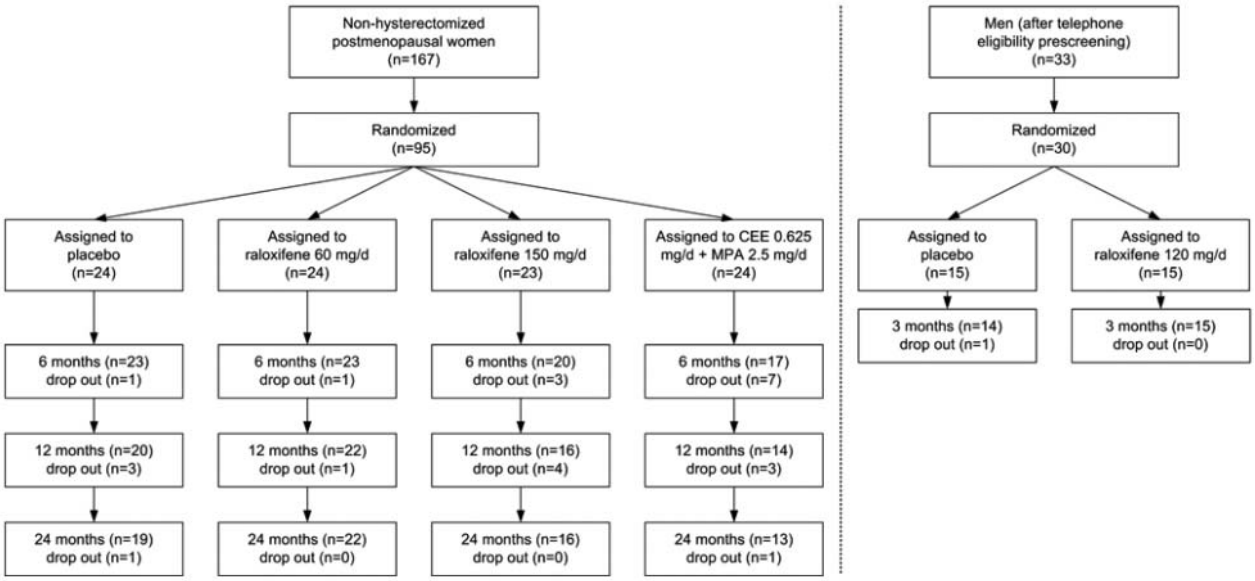

Figure 2 Randomization and follow-up of treatment groups. mg/d, mg/day; CEE, conjugated equine estrogens; MPA, medroxyprogesterone acetate.

lowers total and low-density-lipoprotein (LDL) cholesterol (de Valk-de Roo *et al.* 1999, Johnston *et al.* 2000, Walsh *et al.* 2000). Comparing raloxifene, conjugated equine estrogens (CEE) combined with 2·5 mg medroxyprogesterone acetate (MPA), and placebo in healthy early postmenopausal women and healthy elderly men may increase our understanding of their potential effects on the biosynthesis of n-3 and n-6 HUFA. We therefore investigated this as part of studies on the effects of estrogens and raloxifene on markers of cardiovascular risk and bone turnover (Smolders *et al.* 2002, Duschek *et al.* 2004).

Materials and Methods

Female subjects

Healthy postmenopausal women were recruited through advertisements in local newspapers. In total, 167 women were screened for this two-year study, which was performed at the outpatient clinic of the Departments of Obstetrics and Gynecology and Endocrinology in the VU University Medical Center. The non-hysterectomized, early postmenopausal participants were between 47 and 59 years old, had a body mass index (BMI) between 18 and 31 kg/m^2, had had their last menstrual period between 6 and 24 months before entering the study and had no intolerable postmenopausal symptoms requiring estrogen replacement therapy. The serum follicle-stimulating hormone (FSH) concentration was above 30 IU/l in all women. Exclusion criteria were: (1) a history of cardiovascular, venous thrombotic, metabolic and endocrinological disease; (2) estrogen-dependent neoplasia; (3) excessive consumption of alcohol or abuse of drugs; (4) clinically relevant abnormalities in laboratory tests of renal and hepatic function; and (5) previous participation in any study investigating raloxifene.

Eligible women were randomly (according to a random-number table) assigned to one of the following treatments: a daily dose of 60 mg raloxifene (Eli Lilly and Company, Indianapolis, IN, USA), or of 150 mg raloxifene, or of 0·625 mg CEE (Wyeth-Ayerst, Philadelphia, PA, USA) continuously combined with 2·5 mg MPA (The Upjohn Company, Kalamazoo, MI, USA), or placebo. To maintain blinding, a double-dummy approach was used. Medication and placebo were packaged according to a random-number table and assigned to the participants in sequence.

In total, 95 women started the study of whom 70 completed the study at 2 years; 12 women dropped out between the 0- and 6-month visit, 11 women dropped out between the 6- and 12-month visit, and 2 women dropped out between the 12- and 24-month visit (Fig. 2). The 12 women who dropped out before the measurement at 6 months were excluded from the analysis. Therefore, the laboratory analyses were based on 83 women who were divided over the four groups as follows: placebo group: $n=23$; raloxifene 60 mg group: $n=23$; raloxifene 150 mg group: $n=20$; and CEE plus MPA group: $n=17$. At baseline and after 6, 12, and 24 months of follow-up, venous blood samples were collected into evacuated tubes containing the calcium chelator EDTA (ethylenediamine tetraacetic acid), between 0800 h and 1000 h after an overnight fast. Blood samples were centrifuged at 3000 ***g***

for 30 min within 1 h of collection, and plasma was divided into aliquots, snap-frozen and stored at −70 °C until analysis.

Male subjects

Male subjects were recruited through newspaper advertisements and from posters distributed in the VU University Medical Center as described previously (Duschek *et al.* 2004). The men underwent a screening questionnaire by phone. We included 30 healthy men between 60 and 70 years old in this study (Fig. 2). Exclusion criteria were: (1) psychiatric, Parkinson's, endocrine, cerebrovascular, cardiovascular, prostatic, liver or kidney disease; (2) family history of thromboembolic events; (3) use of medication for prostate, cardiovascular system, bone metabolism, cerebral function, or use of hormones of the pituitary–gonadal and -adrenal axis; and (4) complaints with respect to psychosexual disorders or the intention to father children. The subjects were randomly assigned to raloxifene hydrochloride 120 mg ($n=15$) or placebo ($n=15$) during 3 months. The medication was identically packaged so both subjects and researchers were blinded to the medication given. Smoking status (yes/no), weight, and blood pressure were assessed. At baseline and after 3 months of follow-up, venous blood samples were collected into evacuated EDTA tubes, between 0800 h and 1130 h after an overnight fast. Blood samples were centrifuged at 3500 ***g*** for 10 min within 1 h of collection, and plasma samples were stored at −70 °C until analysis. Plasma was not available for one subject using placebo. Therefore, the laboratory analyses were based on 29 men who were divided over the two groups as follows: raloxifene 120 mg group: $n=15$; placebo group: $n=14$.

Informed consent was obtained from all volunteers in both studies after oral and written information had been given. Both studies were conducted according to the principles of the Declaration of Helsinki and were approved by the medical ethical review board of the VU University Medical Center of Amsterdam.

Laboratory variables

Serum total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides were measured with enzymatic methods (Boehringer Mannheim, Mannheim, Germany), and LDL cholesterol was calculated by the Friedewald formula. Serum 17β-estradiol and total testosterone were measured using radioimmunoassays (Double Antibody, Diasorin Biomedica, Saluggia, Italy, and Coat-A-Count, Diagnostics Products Corporation, Los Angeles, CA, USA respectively). Serum luteinizing hormone (LH) and FSH were measured by immunometric assays (Fluorescense, Delfia, Wallac, Turku Finland). The fatty acid composition was measured in plasma cholesteryl esters, as previously described (Katan *et al.* 1997, Zock *et al.* 1997). Fatty acids were identified by comparison with known standards (Chrompack, Middelburg, The Netherlands). A quality control serum pool was analyzed in duplicate in each run. Ten percent of fatty acid measurements were assayed in duplicate. Intra- and interassay coefficients of variation were on average 3·3% and 2·9% respectively for fatty acids (FA) in cholesteryl esters (for $n \geqslant 30$ pairs; data not shown).

Statistical analysis

Data are given as means (S.E.M.), or geometric means (with percentiles p25 and p75) for right-skewed data, or proportional changes as compared with baseline. If 17β-estradiol values were below the lower limit of detection of 5 pmol/l, that value was used for statistical calculations. We compared baseline measurements between groups using standard parametric tests.

For each FA, the relative percentage of total fatty acids was determined (i.e. g/100 g total FA). Therefore, a major difference or change in one particular fatty acid will consequently induce a difference or change in the opposite direction in the relative amount of all other fatty acids. We calculated percentage changes versus baseline after 6, 12, and 24 months (for postmenopausal women) or 3 months (for men) for single fatty acids (i.e. $((t_1\text{-}t_0)/t_0)\bullet 100$; cf. Fig. 3). Subsequently, we calculated the differences between the active treatment group versus the placebo group, for the time points 6, 12, and 24 months (cf. Table 2) for women or 3 months (cf. Table 4) for men (i.e. $[((t_1\text{-}t_0)/t_0)\bullet 100]^{\text{active treatment}} - [((t_1\text{-}t_0)/t_0)\bullet 100]^{\text{placebo}}$) using a Student's *t*-test for independent samples. For postmenopausal women, a multivariate ANOVA (MANOVA) for repeated measurements was used to compare treatment effects of randomized groups with the placebo group. For men, an independent *t*-test was used to compare treatment effects of the raloxifene group with the placebo group. Proportional changes were correlated using the Pearson's correlation coefficient.

A two-tailed $P<0{\cdot}05$ was considered statistically significant and statistical analyses were not corrected for multiplicity. The software used was the Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) v. 10·0.

Results

Postmenopausal women

The baseline characteristics of the 83 women are presented in Table 1. Women in the raloxifene 150 mg group had a smaller BMI and women in the CEE group less often were smokers, and no significant differences were found among the mean values of other characteristics. Serum 17β-estradiol and FSH concentrations, as well as plasma

Table 1 Descriptive characteristics at baseline of postmenopausal women treated with raloxifene or hormone replacement therapy. Values are given as number (percentage), means (± S.D.), or geometric means (p25–p75) for right-skewed data

	Placebo	Raloxifene 60 mg/day	Raloxifene 150 mg/day	CEE 0·625 mg/day	*P*-value*
Number (*n* (%))	23 (27·7)	23 (27·7)	20 (24·1)	17 (20·5)	
Age (years)	51·6 ± 3·0	50·7 ± 3·1	51·3 ± 2·3	51·1 ± 2·3	0·751
Duration of amenorrhea (months)	13·0 ± 4·9	13·3 ± 5·5	13·0 ± 5·3	12·5 ± 4·9	0·977
Body mass index (kg/m^2)	26·8 ± 4·3	25·1 ± 2·9	23·7 ± 3·0	25·2 ± 2·1	0·034
Systolic blood pressure (mmHg)	126 ± 15	117 ± 15	124 ± 10	128 ± 18	0·098
Diastolic blood pressure (mmHg)	82 ± 8	77 ± 11	79 ± 7	82 ± 8	0·278
Smokers (*n* (%))	10 (43·5)	5 (21·7)	9 (45·0)	1 (5·9)	0·023
Alcohol use (*n* (%))	14 (60·9)	15 (65·2)	7 (35·0)	6 (35·3)	0·091
Total cholesterol (mmol/l)	5·8 ± 0·9	6·1 ± 1·1	6·1 ± 0·8	6·1 ± 0·9	0·704
FSH (IU/l)	94 ± 37	85 ± 30	90 ± 31	96 ± 32	0·706
17β-estradiol (pmol/l)	11·7 (5–23)	14·3 (5–28)	15·8 (5–28)	22·5 (10–44)	0·335
C12 (g/100 g)	0·89 ± 0·20	0·89 ± 0·17	0·87 ± 0·11	0·89 ± 0·18	0·944
C14 (g/100 g)	0·89 ± 0·15	0·89 ± 0·19	1·01 ± 0·25	0·92 ± 0·15	0·165
C16 (g/100 g)	10·33 ± 0·79	10·58 ± 0·75	10·82 ± 0·76	10·29 ± 0·50	0·082
C16:1 (g/100 g)	3·24 ± 0·94	3·05 ± 1·30	3·07 ± 1·02	2·84 ± 0·47	0·672
C18 (g/100 g)	0·86 ± 0·15	0·85 ± 0·17	0·93 ± 0·18	0·93 ± 0·19	0·318
C18:1n-9 (g/100 g)	16·27 ± 1·93	16·47 ± 2·22	16·98 ± 2·26	16·46 ± 1·61	0·709
C18:1n-7 (g/100 g)	1·05 ± 0·19	1·07 ± 0·17	1·01 ± 0·12	1·06 ± 0·17	0·669
C18:2n-6 (LA; g/100 g)	52·42 ± 4·27	51·47 ± 5·08	50·02 ± 4·73	52·29 ± 3·30	0·303
C20:4n-6 (AA; g/100 g)	6·14 ± 1·59	6·52 ± 1·45	6·84 ± 1·49	6·12 ± 1·25	0·351
C18:3n-3 (ALA; g/100 g)	0·61 ± 0·11	0·52 ± 0·12	0·52 ± 0·16	0·59 ± 0·16	0·074
C20:5n-3 (EPA; g/100 g)	0·95 ± 0·51	1·05 ± 0·51	0·93 ± 0·30	1·11 ± 0·59	0·630
C22:5n-3 (DPA; g/100 g)	0·09 ± 0·06	0·07 ± 0·04	0·11 ± 0·08	0·09 ± 0·06	0·154
C22:6n-3 (DHA; g/100 g)	0·63 ± 0·27	0·61 ± 0·25	0·59 ± 0·14	0·63 ± 0·23	0·908

Fatty acids are proportions (g/100 g total FA) in plasma cholesteryl esters. AA, arachidonic acid; ALA, α-linolenic acid; CEE, conjugated equine estrogens combined with 2·5 mg medroxyprogesterone acetate; DHA, docosahexaenoic acid; DPA docosapentaenoic acid; EPA, eicosapentaenoic acid; FSH, follicle-stimulating hormone; LA, linoleic acid; LH, luteinizing hormone.
P-value by one-way ANOVA or chi-squared-test for between-group differences.

concentrations of total cholesterol decreased during treatment with CEE combined with 2·5 mg MPA ($P<0·005$ for all). Serum 17β-estradiol and FSH remained unchanged in the other three groups (data not shown), and plasma concentrations of total cholesterol decreased during raloxifene 150 mg treatment ($P=0·002$). There were no statistically significant baseline differences for any of the individual fatty acids between the treatment groups.

Treatment with high-dose, but not low-dose, raloxifene resulted in higher levels of AA (by +5·6%, +8·3%, and +4·5% at 6, 12 and 24 months respectively) and DHA (by +21·1%, +24·5%, and +20·6% at 6, 12 and 24 months respectively) relative to those in the placebo group (Fig. 3 and Table 2). Therefore, the mean increases over 2 years for AA and DHA were +6·1% and +22·1% respectively, upon treatment with 150 mg raloxifene relative to placebo. Treatment with CEE plus MPA also resulted in higher levels of AA (by +13·2%, +18·2%, and +11·0% at 6, 12, and 24 months respectively) and DHA (by +14·4%, +20·9%, and +9·4% at 6, 12, and 24 months respectively) relative to those in the placebo group (Fig. 3 and Table 2). Therefore, the mean increases over 2 years for AA and DHA were +14·1% and +14·9% respectively, upon treatment with CEE plus MPA relative to placebo.

Proportional changes of DHA and α-linolenic acid were inversely associated (at 6 months: $r=–0·26$; $P=0·048$). Proportional changes of DHA and AA were positively associated (at 6 months: $r=0·45$; $P<0·0005$), as were DHA and EPA (at 6 months: $r=0·34$; $P=0·008$; Table 3).

Men

At baseline, men using placebo and men using raloxifene were of a similar age (63·9 (S.D. 2·4) vs 63·1 (S.D. 2·5) years; $P=0·35$). Furthermore, there were no differences in weight, smoking status or blood pressure between the treatment groups. There were also no statistically significant baseline differences for any of the individual fatty acids between both groups (data not shown).

The raloxifene-treated men increased more in weight than the placebo-treated men (2·5 kg with raloxifene vs 1·4 kg with placebo; $P=0·02$). Raloxifene 120 mg as compared with placebo for 3 months did not affect AA (–5·2%; $P=0·342$) or DHA (+4·0%; $P=0·755$; Fig. 3 and Table 4). Raloxifene, however, increased LH and testosterone concentrations (by +26·2%; $P=0·023$ and +19·8%; $P=0·006$ respectively; Table 4), and decreased HDL-cholesterol concentrations (by –11·3; $P=0·016$).

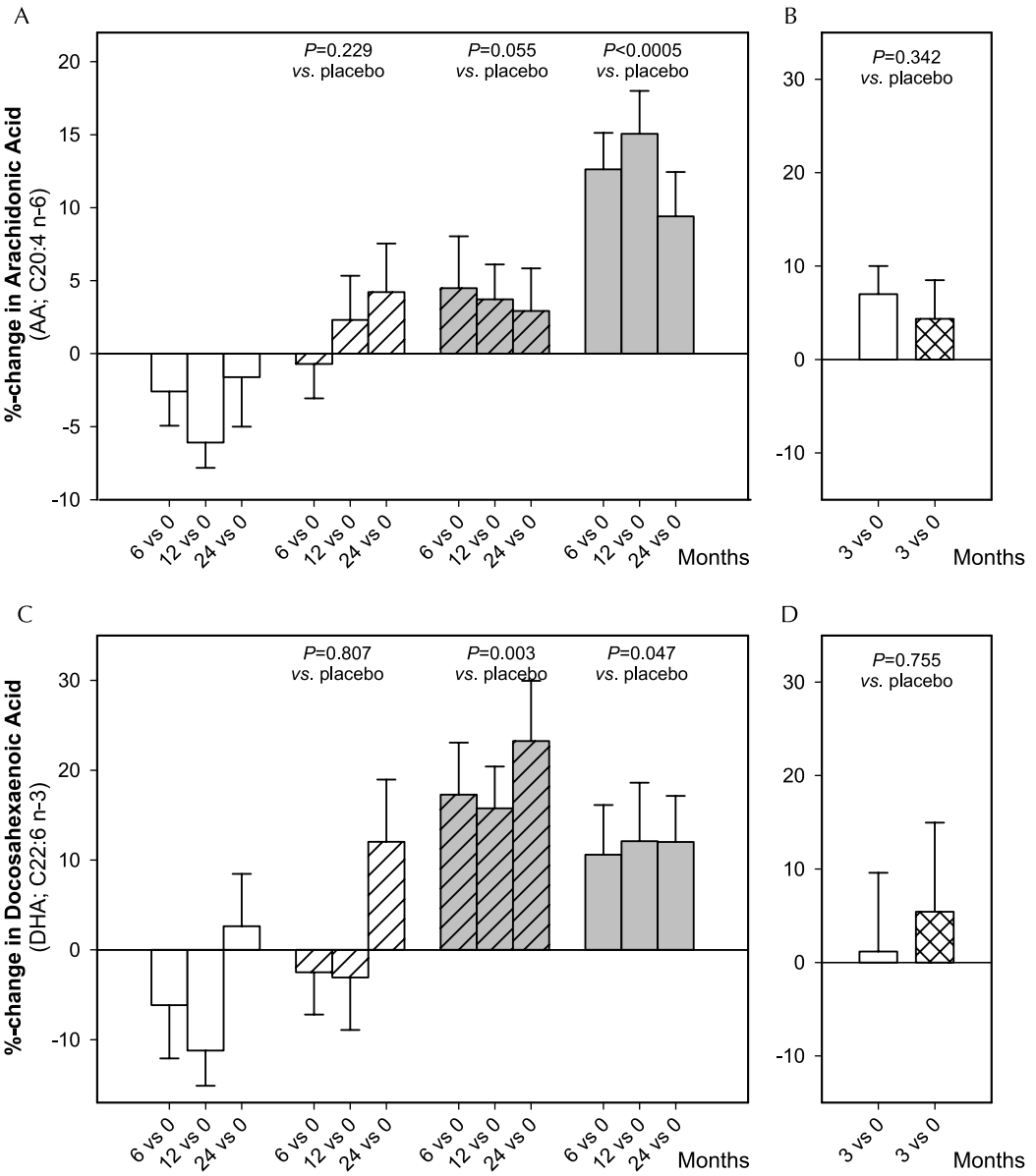

Figure 3 Mean percentage change in proportions of arachidonic acid (AA; C20:4n-6) (A and B) and docosahexaenoic acid (DHA; C22:6n-3) (C and D) in plasma cholesteryl esters in (A and C) non-hysterectomized postmenopausal women ($n=71$) and (B and D) men ($n=29$). *P*-values were assessed by MANOVA (A and C) or by *t*-test for independent samples (B and D). (A and C) Open bars, placebo; hatched bars, 60 mg/day raloxifene; shaded and hatched bars, 150 mg/day raloxifene; shaded bars, CEE and MPA. (B and D) Open bars, placebo; cross-hatched bars, 120 mg/day raloxifene.

Discussion

Our data show that raloxifene at a dose of 150 mg/day and hormone replacement therapy increase DHA levels in plasma cholesteryl esters. In line with this 15% increase are the observations that administration of high dose ethinyl estradiol to male-to-female transsexuals increases DHA levels by about 40% and, conversely, that testosterone administration decreases DHA by about 20% in female-to-male transsexuals (Giltay *et al.* 2004). The present groups of women and men were considerably older than the group of transsexuals studied previously, who had a median age of 29 years (Giltay *et al.* 2004). Our present findings are also consistent with our previous observation that men had 15% lower DHA levels than women, when everyone consumed the same controlled diets (Giltay *et al.*

Table 2 Percentage change in fatty acids (FA) in cholesteryl esters in fasting EDTA blood samples in non-hysterectomized postmenopausal women receiving different treatments, corrected for change in the placebo group. Data are mean change (S.E.M.) from baseline for active treatment relative to placebo

	Months	Raloxifene 60 mg/day	*P*-value	Raloxifene 150 mg/day	*P*-value	CEE 0·625 mg/day	*P*-value
FA							
C12	6	0·1 (4·3)		9·0 (4·7)		6·0 (4·9)	
	12	4·6 (5·4)		11·5 (6·7)		3·7 (5·8)	
	24	8·7 (4·9)	0·239	8·1 (5·7)	0·072	3·7 (6·5)	0·346
C14	6	12·2 (6·8)		7·1 (9·5)		−3·0 (7·7)	
	12	2·8 (10·0)		−5·1 (10·8)		−12·0 (11·1)	
	24	7·0 (9·7)	0·405	−3·8 (9·4)	0·849	−8·4 (10·5)	0·653
C16	6	−0·6 (2·1)		0·3 (2·5)		4·0 (3·0)	
	12	−1·0 (2·5)		0·6 (2·2)		0·5 (2·5)	
	24	−1·0 (2·7)	0·943	−0·2 (2·9)	0·561	1·9 (3·6)	0·155
C16:1	6	15·8 (6·1)		17·8 (9·3)		16·8 (6·0)	
	12	18·8 (9·2)		14·2 (9·3)		8·7 (9·1)	
	24	18·8 (9·2)	0·201	14·6 (8·8)	0·304	9·5 (9·9)	0·421
C18	6	−2·2 (4·8)		−5·6 (5·6)		−13·6 (6·1)	
	12	−4·0 (5·6)		0·7 (5·1)		−18·1 (5·9)	
	24	−10·8 (7·1)	0·611	−6·0 (6·8)	0·597	−11·7 (8·9)	0·012
C18:1n-9	6	2·8 (2·8)		0·4 (3·5)		1·9 (3·0)	
	12	2·6 (3·6)		2·4 (3·4)		−2·7 (3·4)	
	24	−0·7 (3·7)	0·656	−0·6 (3·2)	0·759	−1·0 (3·9)	0·956
C18:1n-7	6	−5·5 (4·3)		1·1 (4·3)		4·2 (4·9)	
	12	−3·3 (4·4)		5·8 (4·1)		3·0 (4·5)	
	24	−3·5 (4·8)	0·812	1·7 (4·0)	0·213	0·1 (5·0)	0·216
C18:2n-6 (LA)	6	−1·2 (1·6)		−2·4 (1·9)		−3·7 (2·0)	
	12	−1·7 (2·0)		−3·6 (1·8)		−2·0 (2·0)	
	24	−0·5 (2·3)	0·653	−2·1 (2·1)	0·210	−2·0 (2·7)	0·386
C20:4n-6 (AA)	6	−0·1 (3·3)		5·6 (3·9)		13·2 (3·6)	
	12	6·9 (3·8)		8·3 (3·3)		18·2 (3·7)	
	24	5·8 (4·8)	0·229	4·5 (4·6)	0·055	11·0 (4·8)	<0·0005
C18:3n-3 (ALA)	6	−2·8 (7·2)		−6·3 (9·0)		−7·9 (7·2)	
	12	−7·4 (7·7)		−12·7 (7·4)		−11·6 (7·4)	
	24	0·3 (8·8)	0·688	−11·6 (8·2)	0·329	9·4 (8·7)	0·052
C20:5n-3 (EPA)	6	−15·8 (11·8)		11·9 (16·1)		−33·9 (11·8)	
	12	−16·5 (15·2)		−8·0 (17·3)		−18·0 (18·1)	
	24	−23·0 (19·7)	0·283	−9·1 (24·5)	0·833	−42·8 (23·1)	0·190
C22:5n-3 (DPA)	6	−1·2 (16·3)		−3·5 (17·3)		2·4 (21·8)	
	12	−26·5 (17·2)		−21·2 (21·5)		−35·3 (21·8)	
	24	−27·7 (16·4)	0·272	−19·5 (23·8)	0·616	−19·5 (21·9)	0·485
C22:6n-3 (DHA)	6	1·3 (7·3)		21·1 (8·0)		14·4 (8·0)	
	12	5·7 (7·4)		24·5 (6·5)		20·9 (7·6)	
	24	9·4 (9·2)	0·807	20·6 (8·8)	0·003	9·4 (8·2)	0·047

CEE, conjugated equine estrogens combined with 2·5 mg MPA.
P-values by MANOVA test for repeated measurements, compared with placebo.

Table 3 Correlation coefficients between the percentage change during 6 months of treatment in FA in 60 women using raloxifene (60 or 150 mg) or CEE plus MPA. Data are Pearson's correlation coefficient (*P*-values)

	C18:2n-6 (LA)	C20:4n-6 (AA)	C18:3n-3 (ALA)	C20:5n-3 (EPA)	C22:5n-3 (DPA)
C20:4n-6 (AA)	−0·128 (0·331)	—	−0·456 (0·000)	−0·215 (0·099)	−0·019 (0·886)
C18:3n-3 (ALA)	0·035 (0·792)	−0·456 (0·000)	—	0·169 (0·197)	−0·256 (0·048)
C20:5n-3 (EPA)	−0·275 (0·034)	−0·215 (0·099)	0·169 (0·197)	—	0·050 (0·702)
C22:5n-3 (DPA)	−0·335 (0·009)	−0·019 (0·886)	0·171 (0·191)	0·050 (0·702)	—
C22:6n-3 (DHA)	−0·184 (0·159)	0·453 (0·000)	−0·256 (0·048)	0·340 (0·008)	0·172 (0·188)

Table 4 Percentage change during 3 months of treatment in endocrine, lipid and FA in cholesteryl esters in fasting EDTA blood samples in 15 men using raloxifene 120 mg corrected for change in 14 men using placebo. Data are mean changes (S.E.M.) from baseline for active treatment relative to placebo

	Raloxifene *vs.* placebo	*P*-value
17β-estradiol	11·7 (10·1)	0·257
Testosterone	19·8 (6·7)	0·006
LH	26·2 (10·8)	0·023
FSH	8·6 (9·0)	0·346
Total cholesterol	−7·1 (4·5)	0·124
HDL-cholesterol	−11·3 (4·4)	0·016
LDL-cholesterol	−10·3 (6·8)	0·141
Triglycerides	11·8 (10·5)	0·273
C12	−10·2 (24·4)	0·680
C14	−2·7 (8·3)	0·749
C16	−0·3 (2·9)	0·909
C16:1	3·3 (7·6)	0·667
C18	−1·5 (8·4)	0·856
C18:1n-9	−2·2 (3·8)	0·568
C18:1n-7	−12·1 (6·4)	0·070
C18:2n-6 (LA)	0·8 (2·7)	0·766
C20:4n-6 (AA)	−5·2 (5·4)	0·342
C18:3n-3 (ALA)	−6·6 (6·7)	0·337
C20:5n-3 (EPA)	−33·3 (30·3)	0·281
C22:5n-3 (DPA)	14·0 (10·6)	0·198
C22:6n-3 (DHA)	4·0 (12·8)	0·755

P-values by *t*-test for independent samples, compared with placebo.

2004). Burdge and colleagues also observed that women of reproductive age seem to have a greater capacity to convert α-linolenic acid into DHA than men (Burdge & Wootton 2002, Burdge *et al.* 2002). The increase in DHA levels upon estrogen and raloxifene administration suggests an enhanced endogenous biosynthesis of DHA through an estrogen receptor-dependent pathway. This is supported by our finding that the proportional decrease in α-linolenic acid – the precursor of DHA – was correlated with the proportional increase in DHA, and that the proportional increase in AA – synthesized through the same enzymes as DHA (Fig. 1) – was associated with the proportional increase in DHA. These effects may be clinically relevant, because during pregnancy the fetus needs substantial quantities of AA and DHA for development of the brain and retina (Postle *et al.* 1995, Innis 2000, Otto *et al.* 2001) and there is a preferential transport of AA and DHA over the placental membrane (Dutta-Roy 2000). Vegetarian and non-fish eating mothers may depend on this biosynthetic pathway to synthesize DHA from α-linolenic acid (Burdge & Postle 1994, Postle *et al.* 1995). In addition, the protective effect of dietary α-linolenic acid against cardiovascular disease (de Lorgeril *et al.* 1994, Singh *et al.* 1997, 2002) might require its conversion to EPA and DHA. In the perspective of DHA and AA, raloxifene seems to be the preferable compound in postmenopausal women when weighed against CEE plus MPA. Raloxifene has beneficial effects on bone density and lipids of raloxifene (de Valk-de Roo *et al.* 1999, Johnston *et al.* 2000, Walsh *et al.* 2000), and in the present study increased DHA levels more strongly than AA (when given in a dosage of 150 mg per day), while n-3 HUFA are relatively deficient compared with n-6 HUFA in the dietary pattern in industrialized countries. The lack of effect on DHA and AA in men, combined with the negative effect on HDL-cholesterol and a significant increase in serum prostate specific antigen (PSA) levels (Duschek *et al.* 2004), would lead us not to recommend raloxifene administration in men.

In women treated with medroxyprogesterone acetate a decrease in serum lecithin AA was found (Enk *et al.* 1985), whereas estrogens were found to increase AA (Silfverstolpe *et al.* 1981, 1982, Crona *et al.* 1983, 1984, Ottosson *et al.* 1984, Enk *et al.* 1985, Mattsson *et al.* 1986). We found that raloxifene and hormone replacement therapy in postmenopausal women caused increases in AA and DHA, both fatty acids with important bioactivities. AA serves as a substrate for cyclooxygenases, lipoxygenases and cytochrome P450 epoxygenases, and is converted into many active prostanoids, whereas DHA inhibits the synthesis of these bioactive prostanoids (Corey *et al.* 1983, Smith 1989). As reported in studies by others on the potential effects of steroidal hormones on fatty acid metabolism (Silfverstolpe *et al.* 1981, 1982, Crona *et al.* 1983, 1984, Ottosson *et al.* 1984, Enk *et al.* 1985, Mattsson *et al.* 1986), we also found that estrogens (plus MPA) increased the saturated fatty acid, palmitic acid (C16:0), and decreased stearic acid (C18:0) in cholesteryl esters.

It is not totally clear why we found increases in AA and DHA in postmenopausal women but not in men treated with raloxifene. In part, this may be due to the shorter duration of follow-up of 3 months and the older mean age of the participating men as compared with the women (mean age 63 vs 51 years old respectively). Moreover, this may be due to the dissimilar endocrine and metabolic effects of raloxifene in women and men. There may be a differential distribution among the sexes of the two types of intracellular estrogen receptor that have now been identified, estrogen receptor α (on chromosome 6) and estrogen receptor β (on chromosome 14). Moreover, we found that raloxifene in men increased LH concentrations and consequently testosterone, and that may have antagonized the potential estrogenic effects on fatty acid metabolism. In contrast, the male-to-female transsexuals treated with estrogens showed a profound suppression of both testosterone and LH upon estrogen treatment, with a subsequent increase in DHA (Giltay *et al.* 2004).

The increase in the levels of AA and DHA in postmenopausal women is likely due to increased activity of elongases and desaturases in the liver (Fig. 1). First, isotope studies in adults (Emken *et al.* 1987, el Boustani *et al.* 1989, Salem *et al.* 1999, Burdge & Wootton 2002, Burdge *et al.* 2002) and infants (Salem *et al.* 1996) have shown that endogenous synthesis of DHA from α-linolenic acid and of AA via linoleic acid occurs predominantly in the

liver (besides the lung, heart, and skeletal muscle) (Cho *et al.* 1999). Secondly, we previously (Giltay *et al.* 2004) found that oral ethinyl estradiol – which has a first-pass hepatic impact (Goebelsmann *et al.* 1985, Goldzieher 1989) – induced larger increases in DHA than transdermal 17β-estradiol – which lacks such a hepatic impact.

The potential limitations of our study merit consideration. Relatively, there were many women in the hormone replacement group who withdrew from the study due to bleeding problems (Fig. 2). Our data were somewhat limited by the fact that we had no data on dietary (fish) consumption, therefore some confounding effects from dietary factors cannot be excluded. Furthermore, an alternative explanation for the increase in DHA may be a specific decrease in DHA clearance. The number of smokers and the mean body mass index differed significantly between the randomized groups of postmenopausal women at baseline, which may have affected the response of AA and DHA towards raloxifene and hormone replacement therapy.

In summary, we found a long-term effect of raloxifene and hormone replacement therapy on the levels of AA and DHA in postmenopausal women. No effects on AA and DHA levels, however, were found in middle-aged men treated with short-term raloxifene. The data in women are consistent with our previous observation in transsexual subjects (Giltay *et al.* 2004). It supports the idea that estrogenic effects can explain the sex difference of higher DHA levels in women as compared with men, and agrees with the unique need of women to provide AA and DHA to the fetal brain (Postle *et al.* 1995, Innis 2000, Otto *et al.* 2001). Further experimental (animal) studies are required to enhance our understanding of the potential role of estrogens in regulating hepatic HUFA metabolism.

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