Docosahexaenoic acid concentrations are higher in women than in men because of estrogenic effects[1–3]

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ABSTRACT

Background: During pregnancy there is a high demand for docosahexaenoic acid (DHA), which is needed for formation of the fetal brain. Women who do not consume marine foods must synthesize DHA from fatty acid precursors in vegetable foods.

Objective: We studied sex differences in DHA status and the role of sex hormones.

Design: First, DHA status was compared between 72 male and 103 female healthy volunteers who ate the same rigidly controlled diets. Second, the effects of sex hormones were studied in 56 male-to-female transsexual subjects, who were treated with cyproterone acetate alone or randomly assigned to receive oral ethinyl estradiol or transdermal 17β-estradiol combined with cyproterone acetate, and in 61 female-to-male transsexual subjects, who were treated with testosterone esters or randomly assigned for treatment with the aromatase inhibitor anastrozole or placebo in addition to the testosterone regimen.

Results: The proportion of DHA was 15 $\pm$ 4% ($\bar{x}$ $\pm$ SEM; $P <$ 0.0005) higher in the women than in the men. Among the women, those taking oral contraceptives had 10 $\pm$ 4% ($P = 0.08$) higher DHA concentrations than did those not taking oral contraceptives. Administration of oral ethinyl estradiol, but not transdermal 17β-estradiol, increased DHA by 42 $\pm$ 8% ($P < 0.0005$), whereas the antiandrogen cyproterone acetate did not affect DHA. Parenteral testosterone decreased DHA by 22 $\pm$ 4% ($P < 0.0005$) in female-to-male transsexual subjects. Anastrozole decreased estradiol concentrations significantly and DHA concentrations nonsignificantly (9 $\pm$ 6%; $P = 0.09$).

Conclusion: Estrogens cause higher DHA concentrations in women than in men, probably by upregulating synthesis of DHA from vegetable precursors. *Am J Clin Nutr* 2004;80:1167–74.

KEY WORDS n−3 Fatty acids, docosahexaenoic acid, estrogen administration, testosterone administration

INTRODUCTION

Eicosapentaenoic acid (EPA, 20:5n−3) and docosahexaenoic acid (DHA, 22:6n−3) are n−3, long-chain, highly unsaturated fatty acids (HUFAs). During pregnancy maternal DHA concentrations increase, and the fetus needs substantial quantities of DHA, especially for development of the central nervous system (1–4). There is a minor pathway of biosynthesis from the precursor α-linolenic acid (ALA, 18:3n−3) to EPA and DHA (5–9), and this pathway occurs predominantly in the liver (10). Vegetarians and persons who do not eat fish may depend on this pathway because the source of EPA and DHA is fatty fish, whereas ALA is found in vegetables, walnuts, and vegetable oils (eg, canola and soybean oils). Moreover, a high dietary intake of DHA and EPA, and maybe also of ALA, may lower the risk of fatal ischemic heart disease and sudden death (11–17).

With regard to the biosynthesis of DHA from precursors, the endogenous conversion of ^{13}C-labeled ALA into DHA seems to be greater in women than in men (9, 18), and in biopsies of subcutaneous adipose tissue, DHA concentrations were lower in men than in women (19). Previous studies in women have shown shifts in fatty acid composition—which suggest effects on elongation and desaturation—during the menstrual cycle, pregnancy, and menopause (20–22) and changes in fatty acid composition induced by several estrogenic, progestational, and antiestrogenic compounds but have not examined n−3 HUFAs (23–29). Moreover, no study has directly compared plasma n−3 HUFAs in men and women eating the same controlled diets or investigated the effects of sex hormones in a controlled trial in humans.

We chose to investigate the effects of hormones in transsexual subjects, because all are in a similar age range and are eugonadal before their sex reassignment (ie, they have sex steroid concentrations that are appropriate for their genetic sex). Estradiol and testosterone concentrations are naturally low in men and women, respectively, and cross-sex hormone administration induces subsequently profound changes in hormonal status, which provides a unique model for studying sex hormone effects on n−3 HUFAs in human subjects.

SUBJECTS AND METHODS

Comparison between men and women

This study aimed to investigate whether women have higher circulating DHA concentrations than do men who consume the

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TABLE 1
Baseline characteristics of the male-to-female transsexual subjects in the intervention studies

	Groups randomly assigned			Groups not randomly assigned	
	1 ($n = 15$)	2 ($n = 15$)	P^1	3 ($n = 10$)	4 ($n = 16$)
Intervention	Oral ethinyl estradiol plus cyproterone acetate	Transdermal 17β-estradiol plus cyproterone acetate		Cyproterone acetate alone	Oral ethinyl estradiol plus cyproterone acetate
Duration of follow-up (mo)	4	4		4	12
Age (y)	32.4 ± 6.6[2]	30.8 ± 7.5	0.55	34.2 ± 9.5	26.8 ± 6.5
BMI (kg/m^2)	22.8 ± 2.7	20.9 ± 2.6	0.06	21.7 ± 2.6	20.9 ± 2.7
Testosterone (nmol/L)	22.9 ± 6.3	20.9 ± 6.4	0.40	19.6 ± 6.2	22.9 ± 6.4
17β-Estradiol (pmol/L)	97 ± 31	81 ± 16	0.09	83 ± 18	100 ± 16
Luteinizing hormone (IU/L)	3.2 ± 1.6	2.5 ± 1.6	0.21	2.4 ± 1.4	3.2 ± 2.3
Follicle-stimulating hormone (IU/L)	3.4 ± 2.0	3.7 ± 3.8	0.76	3.7 ± 2.9	2.6 ± 2.3

[1] *t* test for comparison between the 2 randomly assigned groups.
[2] $\bar{x}$ ± SD (all such values).

same diet. We studied 175 healthy volunteers who consumed strictly controlled diets for 3-wk periods of dietary intervention. The 3 experiments ran from 1987 to 1992. Most subjects were university students, and the women and the men were similar in age. The diets contained no n−3 fatty acids from fish (ie, no EPA or DHA) (30). All the subjects were normolipemic and apparently healthy and had a median age of 23 y (range: 18–67 y) and a mean (±SD) body mass index (in kg/m^2) of 22.2 ± 2.6. We categorized the subjects into 3 groups: 72 men, 71 women not taking oral anticontraceptives (OACs), and 32 women taking OACs. The diets contained constant amounts of ALA. The compositions of other macro- and micronutrients were exactly the same for the men and the women. All diets supplied on average between 11.0 and 12.4 MJ (2630 and 2950 kcal)/d and 2.8% of total energy intake as stearic acid (18:0), 0.1% as *trans* fatty acids, 10% as palmitic acid (16:0), 10% as oleic acid (18:1n−9), and 10% as myristic acid for the different controlled diets. Energy requirements were estimated from 3-d food records, and each subject received a diet that maintained his or her body weight. Body weight was recorded twice weekly, and energy intake was adjusted when necessary. The subjects were asked to maintain their usual pattern of activity and not to change their smoking habits or use of oral contraceptives. They recorded in diaries any sign of illness, any medications or oral contraceptives taken, and any deviations from their diets. Inspection of the diaries showed that the participants followed the instructions carefully.

All experiments used special fats developed by the Unilever Research Laboratory (Vlaardingen, Netherlands). Fasting blood samples were collected 2 or 3 times after 3 wk of each experimental diet, and EDTA plasma was stored at −80 °C. We showed previously that cholesterol ester DHA stabilizes within 2 wk (31). Plasma cholesteryl ester fatty acids were measured in serum samples and then averaged for each subject. The studies were approved by the Ethical Review Committees of Wageningen University.

Intervention studies with sex steroids

To test the role of sex hormones, the prospective effects of cross-sex hormone administration were studied in 56 male-to-female (M→F) and 61 female-to male (F→M) transsexual subjects. These studies ran from 1994 to 2003. Psychological criteria for diagnosis and treatment followed the guidelines provided by the Harry Benjamin International Sex Dysphoria Association (32). All of these subjects were eugonadal, had never taken sex steroids, and were aged between 16 and 50 y (median: 29 y). They had a mean body mass index of 22.3 ± 3.3 (33, 34). We studied 8 groups (**Tables 1** and **2**). Thirty M→F transsexual subjects were randomly assigned with open labels to receive either oral ethinyl estradiol [Lynoral, 100 μg/d; Organon, Oss, Netherlands; group 1 ($n = 15$)] or transdermal 17β-estradiol [Estraderm TTS 100, 100 μg 2 times/wk; CIBA-Geigy, Basel, Switzerland; group 2 ($n = 15$)], both in combination with cyproterone acetate (Androcur, 100 mg/d; Schering, Berlin). Cyproterone acetate is an antiandrogen with progestational properties. A control group of 10 M→F transsexual subjects received cyproterone acetate alone (group 3). Another group of 16 M→F transsexual subjects were treated with oral ethinyl estradiol plus cyproterone acetate for 12 mo (group 4).

Thirty ovariectomized, F→M transsexual subjects receiving treatment with exogenous testosterone were randomly assigned in double-blind fashion to receive the selective aromatase inhibitor anastrozole [Arimidex, 1 mg/d; Zeneca, Wilmington, DE; group 5 ($n = 16$)] or placebo [group 6 ($n = 14$)]. In ovariectomized F→M transsexual subjects, virtually all circulating testosterone originates from exogenous administration, and anastrozole inhibits the conversion of administered testosterone to 17β-estrogen (35). Thirty-one F→M transsexual subjects were treated intramuscularly with 250 mg testosterone esters (Sustanon; Organon) every 2 wk according to the standard treatment at our clinic. In 17 F→M transsexual subjects, blood was collected after 4 mo (group 7), and in 14 F→M transsexual subjects, blood was also collected after 12 mo (group 8).

In groups 4 and 8, a validated Dutch semiquantitative food-frequency questionnaire [of the European Prospective Investigation into Cancer and Nutrition (EPIC)] was used at baseline and after 1 y to estimate the daily intake of total energy and fats (36). Nutrient intakes were calculated with the use of the Dutch nutrient database. All studies were approved by the Ethical Review Committees of the VU University Medical Center.

Laboratory tests

Blood was drawn before testosterone treatment in genetic women between days 5 and 9 of the follicular phase and, during testosterone treatment, within 5–9 d after the most recent testosterone injection (in groups 6 and 7). Before and 2 and 4 mo after

TABLE 2
Baseline characteristics of the female-to-male transsexual subjects in the intervention studies

	Groups randomly assigned			Groups not randomly assigned	
	5 ($n = 16$)	6 ($n = 14$)	P^1	7 ($n = 17$)	8 ($n = 14$)
Intervention	Intramuscular testosterone esters plus anastrozole	Intramuscular testosterone esters plus placebo		Intramuscular testosterone esters	Intramuscular testosterone esters
Duration of preceding testosterone treatment (y)	7.1 ± 6.4[2]	8.3 ± 3.7	0.55	—	—
Duration of follow-up (mo)	4	4		4	12
Age (y)	36.8 ± 8.3	34.9 ± 8.4	0.53	27.1 ± 6.3	23.0 ± 4.2
BMI (kg/m^2)	—	—		23.9 ± 4.3	21.5 ± 3.2
Testosterone (nmol/L)	28.7 (6–97)[3]	30.7 (7–112)	0.85	2.0 ± 0.8	1.5 ± 0.5
17β-Estradiol (pmol/L)	117 (44–278)	85 (21–183)	0.21	189 ± 90	165 ± 63
Luteinizing hormone (IU/L)	2.2 (0–29)	3.0 (0–40)	0.64	5.6 ± 3.4	3.6 ± 1.5
Follicle-stimulating hormone (IU/L)	4.4 (0–40)	6.7 (1–71)	0.48	4.3 ± 1.0	4.7 ± 1.5

[1] *t* test for comparison between the 2 randomly assigned groups.
[2] $\bar{x}$ ± SD (all such values).
[3] Geometric $\bar{x}$; 10th–90th percentiles in parentheses (all such values).

initiation of hormone administration, blood was collected in evacuated tubes containing the calcium-chelator EDTA, which prevents not only coagulation but possibly also fatty acid oxidation. All blood was collected after a 12-h fast, immediately placed on ice, centrifuged at 3500 × *g* for 30 min at 4 °C, and stored within 1 h at −80 °C until analysis. The fatty acid composition was measured in plasma cholesteryl esters as previously described (30, 31), because n−3 fatty acids in plasma cholesteryl esters are highly correlated with dietary intake (37) and with the proportion found in skeletal muscle phospholipids (38). Plasma concentrations of HDL cholesterol and triacylglycerols were determined by using enzymatic colorimetric methods. Serum 17β-estradiol and testosterone concentrations were determined by using standardized radioimmunoassays, and luteinizing hormone and follicle-stimulating hormone concentrations were determined by using immunometric luminescence assays.

Statistical analysis

For the comparison between the men, the women not taking OACs, and the women taking OACs, an analysis of covariance (ANCOVA) for independent samples was used (with age and dietary experiment as covariates). Because the women taking OACs were significantly younger than the women not taking OACs, age was also a covariate. Post hoc paired comparisons were made by using a Sidak test to identify significant differences between groups. Data are presented as estimated marginal means (adjusted for age and dietary experiment) with 95% CIs (**Table 3**).

For groups 1, 2, 5, and 6, who received intervention with sex steroids, a two-factor repeated-measures analysis of variance with interaction was used to compare effects between the randomly assigned groups over time (group 1 compared with group 2, and group 5 compared with group 6). Post hoc comparisons were made by using a Sidak test to identify significant differences between time points. Data are presented as means ± SDs or SEMs or, for right-skewed data, as geometric means with 10th–90th percentiles in parentheses. For groups 3, 4, 7, and 8, results are reported by group, and repeated-measures analysis of variance or Student's *t* test for paired samples was used to analyze the effects of hormone administration over time, as appropriate. Groups 1–4 and groups 5–8 were compared at 4 mo in separate ANCOVA analyses (with baseline variables as covariates). A post hoc Sidak test was used if the ANCOVA was significant. Proportional changes were correlated by using the Pearson's correlation coefficient. A two-tailed P value < 0.05 was considered statistically significant. The software used was SPSS 10.0 (SPSS Inc, Chicago).

RESULTS

Comparison between men and women

The 72 men had a mean (±SD) age of 29.6 ± 12.9 y (range: 18–67 y), and the 103 women had a mean age of 27.4 ± 10.2 y (range: 18–67 y) ($P = 0.20$ for sex difference). DHA concentrations were 14.6 ± 3.9% ($\bar{x}$ ± SEM) higher in the 103 women than in the 72 men (mean difference: 0.070% by wt; 95% CI: 0.032, 0.108% by wt; $P < 0.0005$). Adjustment for age and dietary experiment yielded similar results (mean difference of 0.065% by wt adjusted for age and dietary experiment; 95% CI: 0.033, 0.098% by wt; $P < 0.0005$). ALA concentrations were slightly higher [5.4 ± 2.7% ($\bar{x}$ ± SEM); $P = 0.051$] in the men than in the women, and no significant sex differences were found for EPA ($P = 0.36$).

To explore the effects of OACs, we divided the women into 2 groups according to OAC usage (Table 3). The men had significantly higher concentrations of stearic acid, oleic acid, and dihomo-γ-linolenic acid (20:3n−6) than did the women who were not taking OACs (Table 3). Concentrations of palmitic acid, palmitoleic acid (16:1), 18:1n−7, γ-linolenic acid (18:3n−6), and dihomo-γ-linolenic acid were higher in the women who were not taking OACs than in the women who were, whereas concentrations of stearic acid, linolenic acid (18:2n−6), and ALA were significantly lower in the women who were not taking OACs. DHA concentrations were 10.2 ± 3.8% ($\bar{x}$ ± SEM) higher in the women who were not taking OACs than in the men (mean difference: 0.049% by wt adjusted for age and dietary experiment; 95% CI: 0.005, 0.092% by wt; $P = 0.02$ by Sidak test) and 9.8 ± 4.3% higher, though not significantly, in the 32 women who were taking OACs than in the 71 women who were not taking OACs

TABLE 3
Percentages by weight of fatty acids in cholesteryl esters in fasting serum samples from healthy men and women who consumed a controlled experimental diet for 3 wk[1]

	Group			
	Men ($n = 72$)	Women not taking OACs ($n = 71$)	Women taking OACs ($n = 32$)	P^2
		% by wt		
14:0	1.42 (1.29, 1.55)	1.36 (1.23, 1.50)	1.37 (1.17, 1.57)	0.808
15:0	0.14 (0.13, 0.15)[a]	0.14 (0.13, 0.15)	0.16 (0.15, 0.17)[b]	0.023
16:0	9.65 (9.53, 9.77)[a]	9.50 (9.38, 9.63)[a]	10.03 (9.84, 10.21)[b]	<0.0005
16:1	2.22 (2.07, 2.37)[a]	2.44 (2.29, 2.60)[a]	2.85 (2.61, 3.08)[b]	<0.0005
18:0	1.06 (1.02, 1.10)[a]	0.96 (0.92, 1.00)[b]	0.83 (0.77, 0.89)[c]	<0.0005
18:1n−7	0.99 (0.96, 1.01)[a]	1.01 (0.98, 1.04)[a]	1.11 (1.07, 1.15)[b]	<0.0005
18:1n−9	19.40 (19.12, 19.68)[a]	18.58 (18.30, 18.86)[b]	18.47 (18.05, 18.90)[b]	<0.0005
18:2n−6, LA	53.81 (53.14, 54.48)	54.90 (54.21, 55.58)[a]	53.37 (52.35, 54.39)[b]	0.024
18:3n−6	0.75 (0.69, 0.80)[a]	0.68 (0.63, 0.74)[a]	0.52 (0.44, 0.60)[b]	<0.0005
20:3n−6	0.74 (0.71, 0.77)[a]	0.65 (0.62, 0.68)[b]	0.79 (0.74, 0.83)[a]	<0.0005
20:4n−6, AA	6.21 (5.96, 6.46)	6.17 (5.92, 6.42)	6.68 (6.30, 7.06)	0.076
18:3n−3, ALA	0.41 (0.39, 0.42)	0.43 (0.42, 0.45)	0.42 (0.39, 0.44)	0.086
20:5n−3, EPA	0.43 (0.38, 0.48)	0.43 (0.38, 0.48)	0.33 (0.25, 0.41)	0.096
22:6n−3, DHA	0.48 (0.46, 0.51)[a]	0.53 (0.50, 0.55)[b]	0.58 (0.54, 0.62)[b]	<0.0005

[1] All values are estimated marginal $\bar{x}$ (adjusted for age and dietary experiment); 95% CI in parentheses. OACs, oral anticontraceptives; LA, linoleic acid; AA, arachidonic acid; ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid. Values in the same row with different superscript letters are significantly different, $P < 0.05$ (post hoc Sidak test).
[2] ANOVA test for independent measurements (with age and dietary experiment as covariates).

(mean difference: 0.052% by wt; 95% CI: −0.004, 0.108% by wt; $P = 0.08$ by Sidak test; Table 3).

Intervention studies in M→F transsexual subjects

Fifty-six M→F transsexual subjects were treated with oral ethinyl estradiol plus cyproterone acetate for 4 mo [group 1 ($n = 15$)], transdermal 17β-estradiol plus cyproterone acetate for 4 mo [group 2 ($n = 15$)], cyproterone acetate alone for 4 mo [group 3 ($n = 10$)], and oral ethinyl estradiol plus cyproterone acetate for 12 mo [group 4 ($n = 16$)] (**Table 4**). After estrogen administration, circulating sex hormones were profoundly altered (groups 1–4, Table 4). HDL-cholesterol and triacylglycerol concentrations increased after oral administration of estrogens, and HDL-cholesterol concentrations decreased after transdermal estrogen administration ($P < 0.05$ for interaction between groups 1 and 2; Table 4), which indicated different effects on hepatic lipid metabolism (39, 40). In M→F transsexual subjects, DHA increased significantly with oral ethinyl estradiol (group 1) but not with transdermal 17β-estradiol (group 2) [41.7 ± 8.4% ($\bar{x}$ ± SEM) compared with 5.2 ± 6.7%; $P < 0.0005$ for interaction; Table 4]. In the group treated with oral ethinyl estradiol for 4 mo (ie, group 1), DHA increased between 0 and 2 mo but not between 2 and 4 mo ($P = 0.004$ and $P = 0.124$, respectively, by Sidak test). Cyproterone acetate alone (group 3) did not significantly decrease DHA [−6.6 ± 6.1% ($\bar{x}$ ± SEM); $P = 0.335$; Table 4]. The ANCOVA test was significant for comparison of 4-mo DHA concentrations between groups 1–4 ($P < 0.0005$; adjusted for baseline DHA). The post hoc Sidak test showed significantly higher DHA concentrations in both groups treated with ethinyl estradiol and cyproterone acetate than in the groups treated with transdermal 17β-estradiol and cyproterone acetate alone (groups 1 and 4 compared with groups 2 and 3; all $P < 0.05$).

No further changes occurred between 4 and 12 mo (group 4) during oral administration of ethinyl estradiol [−6.3 ± 4.4% ($\bar{x}$ ± SEM); $P = 0.449$ by Sidak test; Table 4]. The M→F transsexual subjects who received transdermal estrogens showed a nonsignificant initial decrease in DHA ($P = 0.108$) and then a return to baseline concentrations after 4 mo ($P = 0.075$). Total energy intake tended to decrease after 1 y of treatment with oral ethinyl estradiol (group 4), as estimated by using the food-frequency questionnaire ($P = 0.06$; Table 4).

Intervention studies in F→M transsexual subjects

Sixty-one F→M transsexual subjects were randomly assigned to receive intramuscular testosterone esters plus anastrozole [group 5 ($n = 16$)] or intramuscular testosterone esters plus placebo [group 6 ($n = 14$)] or were treated with intramuscular testosterone esters for 4 [group 7 ($n = 17$)] or 12 [group 8 ($n = 14$)] mo (**Table 5**). In the F→M transsexual subjects who were already being treated with testosterone, anastrozole (group 5) decreased 17β-estradiol concentrations and increased luteinizing hormone and follicle-stimulating hormone concentrations in comparison with placebo (group 6) (all $P < 0.005$ for interaction; Table 5). Anastrozole had no significant effect on testosterone concentrations (group 5 compared with group 6; Table 5). DHA concentrations decreased 9.4%, though not significantly ($P = 0.089$ for interaction), when anastrozole (group 5) in comparison with placebo (group 6) was added to the testosterone regimen [−5.7 ± 6.6% ($\bar{x}$ ± SEM) for group 5 compared with 3.7 ± 4.5% for group 6; Table 5].

After 4 mo of testosterone administration, circulating testosterone concentrations increased, whereas mean 17β-estradiol, luteinizing hormone, and follicle-stimulating hormone concentrations decreased significantly (groups 7 and 8 combined; all $P < 0.05$). In the 31 F→M transsexual subjects, DHA decreased after 4 mo of testosterone administration [−21.9 ± 3.7% ($\bar{x}$ ± SEM); $P < 0.0005$; groups 7 and 8 combined; Table 5]. The proportional change in serum 17β-estradiol was significantly

TABLE 4

Hormone and lipid concentrations, percentages by weight of n−3 fatty acids in EDTA plasma cholesteryl esters, and energy and fat intakes in 30 male-to-female (M→F) transsexual subjects randomly assigned to receive ethinyl estradiol plus cyproterone acetate (CA) or 17β-estradiol plus CA ($n = 15$ in each group), in 10 M→F transsexual subjects treated with CA, and in 16 M→F transsexual subjects treated with ethinyl estradiol plus CA for 12 mo[1]

					P		
	Baseline	2 mo	4 mo	12 mo	Time (subgroup)[2]	Time × treatment group[3]	Time[4]
OEE and CA compared with T17βE and CA							
Testosterone (nmol/L)							
OEE and CA	22.9 ± 6.3[5]	1.1 ± 0.1	1.0 ± 0.1	—	—	0.40	<0.0005
T17βE and CA	20.9 ± 6.4	1.5 ± 1.7	1.0 ± 0.3	—	—		
17β-Estradiol (pmol/L)							
OEE and CA	97 ± 31	—[6]	—[6]	—	—	—	—
T17βE and CA	81 ± 16[a]	163 ± 116[a,b]	183 ± 131[b]	—	0.008		
Luteinizing hormone (IU/L)							
OEE and CA	3.2 ± 1.6	0.3 ± 0.0	0.3 ± 0.0	—	—	0.12	<0.0005
T17βE and CA	2.5 ± 1.6	2.8 ± 8.5	0.5 ± 0.5	—	—		
Follicle-stimulating hormone (IU/L)							
OEE and CA	3.4 ± 2.0	0.5 ± 0.0	0.5 ± 0.0	—	—	0.62	<0.0005
T17βE and CA	3.7 ± 3.8	2.2 ± 6.0	0.5 ± 0.1	—	—		
HDL cholesterol (mmol/L)							
OEE and CA	1.02 (0.91–1.17)[7]	—	1.09 (1.00–1.18)	—	0.041	0.001	—
T17βE and CA	1.08 (0.95–1.25)	—	1.03 (0.91–1.18)	—	0.004		
Triacylglycerols (mmol/L)							
OEE and CA	1.09 (0.74–1.69)	—	1.20 (0.88–1.75)	—	0.023	0.013	—
T17βE and CA	0.91 (0.69–1.22)	—	0.82 (0.67–1.00)	—	0.26		
18:3n−3, ALA (% by wt)							
OEE and CA	0.57 ± 0.39	0.61 ± 0.20	0.64 ± 0.25	—	—	0.42	0.186
T17βE and CA	0.61 ± 0.16	0.63 ± 0.12	0.62 ± 0.17	—	—		
20:5n−3, EPA (% by wt)							
OEE and CA	0.83 ± 0.31[a]	0.58 ± 0.30[b]	0.98 ± 0.93[a,b]	—	0.001	0.010	—
T17βE and CA	0.75 ± 0.31	0.76 ± 0.23	0.76 ± 0.35	—	0.99		
22:5n−3, DPA (% by wt)							
OEE and CA	0.06 ± 0.03	0.06 ± 0.03	0.07 ± 0.04	—	—	0.98	0.172
T17βE and CA	0.10 ± 0.02	0.11 ± 0.04	0.11 ± 0.03	—	—		
22:6n−3, DHA (% by wt)							
OEE and CA	0.52 ± 0.15[a]	0.63 ± 0.16[b]	0.72 ± 0.18[b]	—	< 0.0005	< 0.0005	—
T17βE and CA	0.54 ± 0.16	0.49 ± 0.13	0.55 ± 0.16	—	0.06		
CA alone							
Testosterone (nmol/L)	19.6 ± 6.2	—	8.8 ± 6.1	—	0.001		
17β-Estradiol (pmol/L)	83 ± 17	—	41 ± 14	—	< 0.001		
Luteinizing hormone (IU/L)	2.4 ± 1.4	—	2.4 ± 1.3	—	0.93		
Follicle-stimulating hormone (IU/L)	3.7 ± 2.9	—	2.4 ± 2.0	—	0.07		
18:3n−3, ALA (% by wt)	0.58 ± 0.13	—	0.60 ± 0.14	—	0.58		
20:5n−3, EPA (% by wt)	0.58 ± 0.16	—	0.60 ± 0.18	—	0.80		
22:5n−3, DPA (% by wt)	0.07 ± 0.01	—	0.09 ± 0.02	—	0.040		
22:6n−3, DHA (% by wt)	0.45 ± 0.12	—	0.42 ± 0.14	—	0.34		
OEE and CA							
Testosterone (nmol/L)	22.9 ± 6.4[a]	—	1.0 ± 0.1[b]	1.0 ± 0.2[b]	< 0.0005		
17β-Estradiol (pmol/L)	100 ± 16	—	—[6]	—[6]	—		
Luteinizing hormone (IU/L)	3.2 ± 2.3[a]	—	0.3 ± 0.1[b]	0.3 ± 0.0[b]	< 0.0005		
Follicle-stimulating hormone (IU/L)	2.6 ± 2.3[a]	—	0.5 ± 0.1[b]	0.6 ± 0.1[b]	0.002		
Total energy intake (MJ/d)	13.7 ± 4.7	—	—	11.4 ± 5.0	0.06		
Total fat intake (g/d)	126.2 ± 49.3	—	—	107.8 ± 56.0	0.23		
Saturated fat intake (g/d)	49.1 ± 21.9	—	—	41.3 ± 21.8	0.21		
Monounsaturated fat intake (g/d)	48.2 ± 19.4	—	—	41.4 ± 21.3	0.27		
Polyunsaturated fat intake (g/d)	22.7 ± 10.1	—	—	20.2 ± 12.1	0.38		
18:3n−3, ALA (% by wt)	0.45 ± 0.11[a]	—	0.59 ± 0.17[b]	0.62 ± 0.18[b]	0.001		
20:5n−3, EPA (% by wt)	0.57 ± 0.29	—	0.67 ± 0.47	0.56 ± 0.23	0.51		
22:5n−3, DPA (% by wt)	0.08 ± 0.13	—	0.02 ± 0.03	0.03 ± 0.05	0.29		
22:6n−3, DHA (% by wt)	0.52 ± 0.19[a]	—	0.68 ± 0.22[b]	0.64 ± 0.20[b]	< 0.0005		

[1] OEE, oral ethinyl estradiol; T17βE, transdermal 17β-estradiol; ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid. Fatty acids are *cis* isomers. Values in the same row with different superscript letters are significantly different, $P < 0.05$ (post hoc Sidak test).

[2] ANOVA for repeated measurements for the effect of time within a subgroup or *t* test for paired samples.

[3] ANOVA for repeated measurements for the interaction term between treatment group and time.

[4] ANOVA for repeated measurements for the main effect of time (for the 2 randomly assigned groups combined).

[5] $\bar{x} \pm$ SD (all such values).

[6] Ethinyl estradiol cannot be detected in conventional 17β-estradiol assays.

[7] Geometric $\bar{x}$; 10th–90th percentiles in parentheses (all such values).

TABLE 5

Hormone concentrations, percentages by weight of n−3 fatty acids in EDTA plasma cholesteryl esters, and energy and fat intakes in 30 female-to-male (F→M) transsexual subjects who were already receiving testosterone treatment and who were randomly assigned to receive anastrozole ($n = 16$) or placebo ($n = 14$) and in 17 and 14 F→M transsexual subjects starting treatment with intramuscular testosterone esters for 4 and 12 mo, respectively[1]

				P		
	Baseline	4 mo	12 mo	Time (subgroup)[2]	Time × treatment group[3]	Time[4]
TE and A compared with TE and P						
Testosterone (nmol/L)						
TE and A	28.7 (6–97)[5]	26.8 (4–101)	—	—	0.36	0.31
TE and P	30.7 (7–112)	20.0 (3–66)	—	—		
17β-Estradiol (pmol/L)						
TE and A	117 (44–278)	40 (18–301)	—	0.001	0.003	
TE and P	85 (21–183)	88 (29–207)	—	0.85		
Luteinizing hormone (IU/L)						
TE and A	2.2 (0–29)	19.5 (1–60)	—	<0.0005	<0.0005	
TE and P	3.0 (0–40)	2.6 (0–51)	—	0.36		
Follicle-stimulating hormone (IU/L)						
TE and A	4.4 (0–40)	29.4 (3–83)	—	<0.0005	<0.0005	
TE and P	6.7 (1–71)	4.4 (0–77)	—	0.02		
18:3n−3, ALA (% by wt)						
TE and A	0.50 ± 0.16[6]	0.54 ± 0.12	—	0.34	0.040	
TE and P	0.58 ± 0.13	0.51 ± 0.07	—	0.01		
20:5n−3, EPA (% by wt)						
TE and A	0.77 ± 0.41	0.70 ± 0.26	—	—	0.31	0.90
TE and P	0.64 ± 0.23	0.70 ± 0.34	—	—		
22:5n−3, DPA (% by wt)						
TE and A	0.04 ± 0.04	0.04 ± 0.04	—	—	0.57	0.65
TE and P	0.03 ± 0.04	0.03 ± 0.04	—	—		
22:6n−3, DHA (% by wt)						
TE and A	0.42 ± 0.17	0.38 ± 0.15	—	—	0.089	0.51
TE and P	0.40 ± 0.11	0.42 ± 0.15	—	—		
Intramuscular TE, 4 mo						
Testosterone (nmol/L)	2.0 ± 0.8	33 ± 9.1	—	<0.0005		
17β-Estradiol (pmol/L)	189 ± 90	130 ± 33	—	0.01		
Luteinizing hormone (IU/L)	5.6 ± 3.5	2.4 ± 2.1	—	0.005		
Follicle-stimulating hormone (IU/L)	4.3 ± 1.0	2.8 ± 1.1	—	0.002		
18:3n−3, ALA (% by wt)	0.48 ± 0.11	0.45 ± 0.1	—	0.28		
20:5n−3, EPA (% by wt)	0.55 ± 0.26	0.42 ± 0.17	—	0.01		
22:5n−3, DPA (% by wt)	0.02 ± 0.03	0.02 ± 0.04	—	0.73		
22:6n−3, DHA (% by wt)	0.46 ± 0.12	0.35 ± 0.12	—	0.008		
Intramuscular TE, 12 mo						
Testosterone (nmol/L)	1.5 ± 0.5[a]	29.4 ± 10.2[b]	32.9 ± 11.4[b]	<0.0005		
17β-Estradiol (pmol/L)	165 ± 63	125 ± 32	131 ± 34	0.09		
Luteinizing hormone (IU/L)	3.6 ± 1.5	2.7 ± 3	2.5 ± 2.1	0.32		
Follicle-stimulating hormone (IU/L)	4.7 ± 1.5	3.9 ± 1.9	3.4 ± 2.2	0.26		
Total energy intake (MJ/d)	10.1 ± 3.9	—	10.2 ± 4.3	0.89		
Total fat intake (g/d)	95.5 ± 45.2	—	97.1 ± 52.1	0.79		
Saturated fat intake (g/d)	34.8 ± 17.1	—	36.4 ± 20.4	0.52		
Monounsaturated fat intake (g/d)	37.5 ± 19.1	—	38.2 ± 20.7	0.77		
Polyunsaturated fat intake (g/d)	18.7 ± 9.6	—	17.9 ± 10.4	0.53		
18:3n−3, ALA (% by wt)	0.49 ± 0.11	0.46 ± 0.10	0.46 ± 0.13	0.48		
20:5n−3, EPA (% by wt)	0.69 ± 0.51	0.43 ± 0.12	0.55 ± 0.28	0.03		
22:5n−3, DPA (% by wt)	0.07 ± 0.07	0.05 ± 0.04	0.04 ± 0.04	0.40		
22:6n−3, DHA (% by wt)	0.51 ± 0.11[a]	0.39 ± 0.07[b]	0.39 ± 0.10[b]	<0.0005		

[1] TE, testosterone esters; A, anastrozole; P, placebo; ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid. Fatty acids are *cis* isomers. Values in the same row with different superscript letters are significantly different, $P < 0.05$ (post hoc Sidak test).

[2] ANOVA for repeated measurements for the effect of time within a subgroup or *t* test for paired samples.

[3] ANOVA for repeated measurements for the interaction term between treatment group and time.

[4] ANOVA for repeated measurements for the main effect of time (for the 2 randomly assigned groups combined).

[5] Geometric $\bar{x}$; 10th–90th percentiles in parentheses (all such values).

[6] $\bar{x} \pm$ SD (all such values).

correlated with the proportional change in DHA ($r = 0.40$, $P = 0.03$; groups 7 and 8 combined). The ANCOVA test was significant for comparison of 4-mo DHA concentrations between groups 5–8 ($P = 0.029$; adjusted for baseline DHA), and the post hoc Sidak test showed a significant difference between groups 6 and 7 ($P = 0.03$).

No further changes occurred between 4 and 12 mo (group 8) during testosterone administration [−0.5 ± 4.5% ($\bar{x} \pm$ SEM);

$P = 0.999$ by Sidak test; Table 5). As estimated by using the food-frequency questionnaire, the intakes of total energy and fat did not change significantly after 1 y of testosterone treatment (group 8; Table 5).

DISCUSSION

We found that DHA concentrations in plasma cholesteryl esters were higher in the women than in the men and that this difference was independent of dietary differences. The difference in DHA is equal to what can be achieved by an extra intake of 62 mg DHA/d (31), which is provided by ≈1 regular fish-oil capsule once every other day or 1–2 fatty fish meals/mo. Such differences may be relevant with respect to pregnancy, which is associated with high circulating concentrations of both estrogens and DHA, and the fetus depends on the DHA supply from the pregnant mother (1–4). Furthermore, the inclusion of fatty fish meals in the diet is recommended for the prevention of cardiovascular disease (11–17).

Previous studies suggesting sex differences in n−3 fatty acid status are difficult to interpret because the diets were not kept the same for men and women; thus, any observed differences in n−3 fatty acid status may have reflected differences in dietary intake (19, 41). However, the sex difference in DHA observed in our study was not caused by sex differences in dietary intake, because subjects received the same controlled diets and maintained their body weight. The men and the women, therefore, consumed comparable amounts of n−3 HUFAs within the same meal context. Notably, we also found differences between the sexes and between the women who were or were not taking OACs in the proportions of several saturated and monounsaturated fatty acids. These findings are in line with those from previous intervention studies in (oophorectomized) postmenopausal women that showed that several estrogenic compounds decreased concentrations of stearic acid and oleic acid and increased palmitic acid in serum lecithin or cholesteryl esters (23–29).

Treatment with oral ethinyl estradiol, but not with transdermal 17β-estradiol, increased DHA concentrations. This supports the finding that women of reproductive age seem to have a greater capacity to convert ALA to DHA than do men (9, 18). Conversely, testosterone administration decreased DHA. This may be an effect of testosterone itself or of the decrease in plasma 17β-estradiol associated with the administration of testosterone. Our findings support the idea that estrogen is the responsible hormone. This is in line with the positive correlation between the proportional changes in plasma DHA and serum 17β-estradiol and with our finding that the aromatase inhibitor anastrozole—which blocks the conversion of androgens to estrogens and decreases 17β-estradiol concentrations—further decreased DHA, although the decrease was of borderline significance. Moreover, in the group treated with transdermal 17β-estradiol, plasma testosterone decreased to almost nil, whereas no effect of this reduction in testosterone was observed on DHA.

Endogenous synthesis of DHA from ALA via EPA requires elongases and desaturases, and isotope studies in adults (6, 9, 18, 42, 43) and infants (44) show that humans can indeed convert ALA to DHA in vivo, predominantly in the liver (but also in the lung, heart, and skeletal muscle) (10). These data also suggest that the rate of conversion of ALA to longer chain n−3 HUFAs is too low to affect health, yet most of these studies were done in men. We found that oral ethinyl estradiol administration increased DHA, whereas administration of transdermal 17β-estradiol had no effect on DHA. The hepatic effect of synthetic ethinyl estradiol—because of its first pass through the liver (45) and compound-specific effects (46)—may be greater than that of transdermally absorbed 17β-estradiol (47). Therefore, we speculate that hepatic synthesis of DHA represents the major source of the increase in DHA in women compared with men (10). The initial decrease in EPA, an intermediate in DHA synthesis, also suggests an increased conversion of EPA to DHA after initiation of ethinyl estradiol treatment. The alternative explanation, ie, that the increase in DHA is the result of a specific decrease in DHA clearance, which leads to high DHA concentrations in estrogenic milieus, seems less likely. From an evolutionary perspective, it seems plausible that an increase in endogenous maternal DHA biosynthesis during pregnancy and lactation enabled optimal fetal and neonatal growth and brain development (1–3). Vegetarian and non-fish-eating mothers may depend totally on this biosynthetic pathway to acquire DHA from ALA (1, 2).

The interpretation of the effects of cross-sex hormone administration is limited by the inclusion of a relatively small number of subjects, the open-label design, and the lack of a true placebo group, which was due to the nature of the study population and the treatment indication. Therefore, we do not know if the effects of sex steroids are similar to those found in eugonadal subjects who take sex hormones appropriate to their biological sex. Yet, the effects of cross-sex hormone administration were strong and consistent with the sex difference found in the healthy male and female volunteers.

In summary, our data suggest that biosynthesis of DHA is greater in women than in men because of the effects of sex hormones, presumably estrogens. A strong stimulus with estrogens induced an increase in DHA status, whereas a testosterone stimulus induced a decrease in DHA. Epidemiologic studies pointed toward a protective effect of 1–2 weekly servings of fish, especially fatty fish (11–15), against ischemic heart disease and sudden death. These protective effects were confirmed in the Diet and Reinfarction Trial (DART; 16) and the GISSI (Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico)-Prevenzione trial (17) in coronary patients who received either dietary advice to consume fish or fish-oil capsules, respectively. Because DHA status is lower in men, who also happen to have a higher risk of heart disease, dietary guidelines may need to recommend higher fish consumption for men than for women (48, 49). Moreover, these findings also suggest that maternal synthesis of DHA is under estrogenic control, which may contribute to the physiologic increase in maternal DHA concentrations during pregnancy (1, 2, 4). Placental transfer of DHA may subsequently provide the fetus with adequate amounts of DHA (1–3).

EJG and LJGG participated in the planning, design, and execution of the intervention studies in transsexual subjects, and AWFTT participated in the planning, design, and execution of the study on anastrozole. MBK and PLZ participated in the design and execution of the cross-sectional part of the study in which men and women were compared. All authors participated in the overall analysis and the writing of the paper. None of the authors had any conflicts of interest.

REFERENCES

1. Burdge GC, Postle AD. Hepatic phospholipid molecular species in the guinea pig. Adaptations to pregnancy. Lipids 1994;29:259–64.

2. Postle AD, Al MD, Burdge GC, Hornstra G. The composition of individual molecular species of plasma phosphatidylcholine in human pregnancy. Early Hum Dev 1995;43:47–58.
3. Innis SM. The role of dietary n−6 and n−3 fatty acids in the developing brain. Dev Neurosci 2000;22:474–80.
4. Otto SJ, van Houwelingen AC, Badart-Smook A, Hornstra G. Changes in the maternal essential fatty acid profile during early pregnancy and the relation of the profile to diet. Am J Clin Nutr 2001;73:302–7.
5. Emken EA, Adlof RO, Gulley RM. Dietary linoleic acid influences desaturation and acylation of deuterium-labeled linoleic and linolenic acids in young adult males. Biochim Biophys Acta 1994;1213:277–88.
6. Salem N Jr, Pawlosky R, Wegher B, Hibbeln J. In vivo conversion of linoleic acid to arachidonic acid in human adults. Prostaglandins Leukot Essent Fatty Acids 1999;60:407–10.
7. Vermunt SH, Mensink RP, Simonis MM, Hornstra G. Effects of dietary alpha-linolenic acid on the conversion and oxidation of ^{13}C-alpha-linolenic acid. Lipids 2000;35:137–42.
8. Pawlosky RJ, Hibbeln JR, Novotny JA, Salem N Jr. Physiological compartmental analysis of alpha-linolenic acid metabolism in adult humans. J Lipid Res 2001;42:1257–65.
9. Burdge GC, Jones AE, Wootton SA. Eicosapentaenoic and docosapentaenoic acids are the principal products of alpha-linolenic acid metabolism in young men. Br J Nutr 2002;88:355–63.
10. Cho HP, Nakamura M, Clarke SD. Cloning, expression, and fatty acid regulation of the human delta-5 desaturase. J Biol Chem 1999;274:37335–9.
11. Kromhout D, Bosschieter EB, de Lezenne Coulander C. The inverse relation between fish consumption and 20-year mortality from coronary heart disease. N Engl J Med 1985;312:1205–9.
12. Oomen CM, Feskens EJ, Rasanen L, et al. Fish consumption and coronary heart disease mortality in Finland, Italy, and The Netherlands. Am J Epidemiol 2000;151:999–1006.
13. Siscovick DS, Raghunathan TE, King I, et al. Dietary intake and cell membrane levels of long-chain n−3 polyunsaturated fatty acids and the risk of primary cardiac arrest. JAMA 1995;274:1363–7.
14. Albert CM, Hennekens CH, O'Donnell CJ, et al. Fish consumption and risk of sudden cardiac death. JAMA 1998;279:23–8.
15. Albert CM, Campos H, Stampfer MJ, et al. Blood levels of long-chain n−3 fatty acids and the risk of sudden death. N Engl J Med 2002;346:1113–8.
16. Burr ML, Fehily AM, Gilbert JF, et al. Effects of changes in fat, fish, and fibre intakes on death and myocardial reinfarction: diet and reinfarction trial (DART). Lancet 1989;2:757–61.
17. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico. Dietary supplementation with n−3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Lancet 1999;354:447–55.
18. Burdge GC, Wootton SA. Conversion of alpha-linolenic acid to eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in young women. Br J Nutr 2002;88:411–20.
19. Tavendale R, Lee AJ, Smith WC, Tunstall-Pedoe H. Adipose tissue fatty acids in Scottish men and women: results from the Scottish Heart Health Study. Atherosclerosis 1992;94:161–9.
20. Rosing U, Johnson P, Olund A, Samsioe G. Relative fatty acid composition of serum lecithin in the second half of the normal pregnancy. Gynecol Obstet Invest 1982;14:225–35.
21. Mattsson LA, Silfverstolpe G, Samsioe G. Fatty acid composition of serum lecithin and cholesterol ester in the normal menstrual cycle. Horm Metab Res 1985;17:414–7.
22. Maynar M, Mahedero G, Maynar I, Maynar JI, Tuya IR, Caballero MJ. Menopause-induced changes in lipid fractions and total fatty acids in plasma. Endocr Res 2001;27:357–65.
23. Silfverstolpe G, Johnson P, Samsice G, Svanborg A, Gustafson A. Effects induced by two different estrogens on serum individual phospholipids and serum lecithin fatty acid composition. Horm Metab Res 1981;13:141–5.
24. Silfverstolpe G, Samsioe G, Svanborg A, Gustafson A. Lipid metabolic studies in oophorectomized women: effects of synthetic progestogens on individual serum phospholipids and serum lecithin fatty acid composition. Gynecol Obstet Invest 1982;13:117–26.
25. Crona N, Silfverstolpe G, Samsioe G. A double blind cross-over study on the effects of ORG OD14 compared to estradiol valerate and placebo on the fatty acid composition of serum lecithin and cholesterol ester in oophorectomized women. J Clin Endocrinol Metab 1983;56:1116–9.
26. Crona N, Enk L, Samsioe G, Silfverstolpe G. Changes in relative fatty acid composition of serum lecithin and cholesterol ester after treatment with two gonane progestins administered alone and in combination with ethinyl estradiol. Arch Gynecol 1984;236:35–40.
27. Ottosson UB, Lagrelius A, Rosing U, von Schoultz B. Relative fatty acid composition of lecithin during postmenopausal replacement therapy—a comparison between ethinyl estradiol and estradiol valerate. Gynecol Obstet Invest 1984;18:296–302.
28. Enk L, Crona N, Friberg LG, Samsioe G, Silfverstolpe G. High-dose depot-medroxyprogesterone acetate—effects on the fatty acid composition of serum lecithin and cholesterol ester. Gynecol Oncol 1985;22:317–23.
29. Mattsson LA, Cullberg G, Samsioe G. The relative fatty acid composition of serum lecithin and cholesterol ester: influence of an estrogen-progestogen regimen in climacteric women. Am J Obstet Gynecol 1986; 155:174–7.
30. Zock PL, Mensink RP, Harryvan J, de Vries JH, Katan MB. Fatty acids in serum cholesteryl esters as quantitative biomarkers of dietary intake in humans. Am J Epidemiol 1997;145:1114–22.
31. Katan MB, Deslypere JP, van Birgelen AP, Penders M, Zegwaard M. Kinetics of the incorporation of dietary fatty acids into serum cholesteryl esters, erythrocyte membranes, and adipose tissue: an 18-month controlled study. J Lipid Res 1997;38:2012–22.
32. Walker PA, Berger JC, Green R, Laub DR, Reynolds CL, Wollman L. Standards of care: the hormonal and surgical sex reassignment of gender dysphoric persons. Arch Sex Behav 1985;14:79–90.
33. Giltay EJ, Verhoef P, Gooren LJ, Geleijnse JM, Schouten EG, Stehouwer CD. Oral and transdermal estrogens both lower plasma total homocysteine in male-to-female transsexuals. Atherosclerosis 2003;168:139–46.
34. Elbers JMH, Giltay EJ, Teerlink T, et al. Effects of sex steroids on components of the insulin resistance syndrome in transsexual subjects. Clin Endocrinol 2003;58:562–71.
35. Buzdar AU, Robertson JF, Eiermann W, Nabholtz JM. An overview of the pharmacology and pharmacokinetics of the newer generation aromatase inhibitors anastrozole, letrozole, and exemestane. Cancer 2002; 95:2006–16.
36. Ocke MC, Bueno-de-Mesquita HB, Pols MA, Smit HA, van Staveren WA, Kromhout D. The Dutch EPIC food frequency questionnaire. II. Relative validity and reproducibility for nutrients. Int J Epidemiol 1997; 26:S49–58.
37. Nikkari T, Luukkainen P, Pietinen P, Puska P. Fatty acid composition of serum lipid fractions in relation to gender and quality of dietary fat. Ann Med 1995;27:491–8.
38. Andersson A, Nalsen C, Tengblad S, Vessby B. Fatty acid composition of skeletal muscle reflects dietary fat composition in humans. Am J Clin Nutr 2002;76:1222–9.
39. Chetkowski RJ, Meldrum DR, Steingold KA, et al. Biologic effects of transdermal estradiol. N Engl J Med 1986;314:1615–20.
40. De Lignieres B, Basdevant A, Thomas G, et al. Biological effects of estradiol-17β in postmenopausal women: oral versus percutaneous administration. J Clin Endocrinol Metab 1986;62:536–41.
41. Bolton-Smith C, Woodward M, Tavendale R. Evidence for age-related differences in the fatty acid composition of human adipose tissue, independent of diet. Eur J Clin Nutr 1997;51:619–24.
42. Emken EA, Rohwedder WK, Adlof RO, Rakoff H, Gulley RM. Metabolism in humans of *cis*-12,*trans*-15-octadecadienoic acid relative to palmitic, stearic, oleic and linoleic acids. Lipids 1987;22:495–504.
43. el Boustani S, Causse JE, Descomps B, Monnier L, Mendy F, Crastes de Paulet A. Direct in vivo characterization of delta 5 desaturase activity in humans by deuterium labeling: effect of insulin. Metabolism 1989;38:315–21.
44. Salem N Jr, Wegher B, Mena P, Uauy R. Arachidonic and docosahexaenoic acids are biosynthesized from their 18-carbon precursors in human infants. Proc Natl Acad Sci U S A 1996;93:49–54.
45. Goldzieher JW. Pharmacology of contraceptive steroids: a brief review. Am J Obstet Gynecol 1989;160:1260–4.
46. Goebelsmann U, Mashchak CA, Mishell DR. Comparison of hepatic impact of oral and vaginal administration of ethinyl estradiol. Am J Obstet Gynecol 1985;151:868–77.
47. Judd H. Efficacy of transdermal estradiol. Am J Obstet Gynecol 1987; 156:1326–31.
48. Krauss RM, Eckel RH, Howard B, et al. AHA Dietary Guidelines: revision 2000: a statement for healthcare professionals from the Nutrition Committee of the American Heart Association. Circulation 2000; 102:2284–99.
49. Kris-Etherton PM, Harris WS, Appel LJ. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Arterioscler Thromb Vasc Biol 2003;23:e20–30.