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ORIGINAL CONTRIBUTION

Influence of gender on DHA synthesis: the response of rat liver to low dietary α-linolenic acid evidences higher ω3 Δ4-desaturation index in females

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Abstract
Purpose The conversion rate of α-linolenic acid (ALA) into docosahexaenoic acid (DHA) is determined by dietary and non-dietary factors. Higher capacity of DHA synthesis has been evidenced in females, indicating that sex factors influence the conversion pathway. To evaluate the extent to which sexual dimorphism of DHA synthesis is subordinated to nutritional handling, we measured the ω3 Δ4-desaturation index in male and female rats receiving adequate or inadequate amounts of ALA. The ω3 Δ4-desaturation index was drawn from the DHA to docosapentaenoic acid (ω3DPA) ratio in liver phospholipids.
Methods Male and female rats born to ω3-deficient dams were fed a supplemented diet supplying low, inadequate, intermediate, or adequate ALA (5, 20, 100, or 300 mg ALA/100 g diet, respectively). Control rats from both gender received the adequate diet from fetal life.
Results Compared with control, low ALA feeding induced the ω3 Δ4-desaturation index to increase by 38 and 70% in the phosphatidylethanolamine fraction of males and females, respectively, and by 67% in phosphatidylcholine in females only. Supplementations with increased doses of ALA progressively smoothed this gender effect. Moreover, the analysis of our data from a previous study shows that ovariectomy decreased, whereas estradiol treatment increased the ω3 index to values comparable with those of diet-matched males and intact females, respectively.
Conclusion Females are more prone than males to increase their index of ω3 Δ4-desaturation, especially in response to low supplies in ALA. Estradiol supports the ω3 index, suggesting that this hormone plays a role in the effect of gender on DHA synthesis.

Keywords Docosahexaenoic acid · α-Linolenic acid · Desaturation index · Δ6-Desaturase · ω3 Fatty acid · Requirements · Gender effect · Ovariectomy · Estrogen · Liver

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Introduction

Docosahexaenoic acid (DHA, 22:6ω3) is crucial for the biogenesis of membrane phospholipids, particularly during gestation, growth, and development. This ω3 long-chain polyunsaturated fatty acid (LC-PUFA) is supplied by dietary fats, either directly from fatty fishes or under the form of its essential metabolic precursor, α-linolenic acid (ALA, 18:3ω3) from vegetable oils. The extent to which ALA is converted to DHA in the liver depends on nutritional and hormonal factors [1] and varies further in humans with polymorphisms in the gene cluster encoding the Δ5 and Δ6-desaturases [2].

Deficiency in ALA has been shown to upregulate the gene transcription of desaturases in male rat liver, indicating that low ALA intakes increase the conversion rate into DHA [3]. However, non-dietary factors such as sex and age also modulate the rate of DHA synthesis. The occurrence of sexual dimorphism in DHA synthesis in

humans is attested by higher DHA and lower ω3-docosapentaenoic acid (ω3DPA, 22:5ω3), in the blood lipids of women compared with men [4–6]. Moreover, it has been shown, in healthy adults, that an isotopic tracer dose of ALA is differently redistributed in the blood fatty acids, eicosapentaenoic acid (EPA, 20:5ω3), ω3DPA, and DHA in women [7] and only EPA and ω3DPA in men [8].

The ratio of DHA to ω3DPA in phospholipid results from the conversion of ω3DPA in DHA and from the esterification of both fatty acids to lysophospholipids. It has been demonstrated that the newly formed molecules of DHA are rapidly esterified to the lysophospholipid acceptor [9], suggesting that acyltransferases do not constitute a rate-limiting step for the deposition of DHA into phospholipids. It may be postulated that the product-to-substrate ratio, DHA/ω3DPA, will depend more on the capacity of liver to synthesize ω3DPA from dietary ALA and to convert the newly formed molecules of ω3DPA into DHA, than on the non-limiting activity of acyltransferases. However, acyltransferases can also incorporate preformed molecules of DHA and ω3DPA in phospholipids. Therefore, when DHA and ω3DPA are provided by diet or are released from body stores, the ratio of DHA to ω3DPA will reflect at least as much their proportions in the blood lipids as the capacity of liver to convert ω3DPA into DHA. The use of the DHA/ω3DPA ratio in liver phospholipids as an index of hepatic Δ4-desaturation is limited to the condition that preformed molecules of both fatty acids are not transferred from blood lipids, a condition that can be met in ω3-deficient animals.

The synthesis of DHA from ω3DPA consists of one elongation, one Δ6-desaturation, and one peroxisomal β-oxidation, which result in the net adding of one double bond in the Δ4 position [10]. Therefore, a higher ratio of DHA to ω3DPA in blood lipids and a higher production rate of ALA-derived DHA suggest higher capacity of net Δ4-desaturation in women. Data from animal studies support this hypothesis. When male and female rats born with a low DHA status and fed at weaning a diet containing no ω3 LC-PUFAs and suboptimal ALA (0.04% energy), a condition of partial repletion that stimulates the synthesis pathway, females expressed hepatic Δ6- and Δ5-desaturase at a greater level than males and their ω3 Δ4-desaturation index in liver phospholipids was 1.8 times more elevated, indicating higher capacity of DHA synthesis [11]. We therefore examined whether the levels of dietary ALA determine the extent to which sexual dimorphism impacts on the Δ4-desaturation index of the ω3 series. Likewise, the Δ4-desaturation index of the ω6 series was drawn from the ratio of 22:5ω6 to 22:4ω6, which increase in tissues reveals specific deficiency in ω3 fatty acids. In the present study, male and female rats born to ALA-deficient dams either were raised on the same deficient diet or were partly or totally repleted in ALA. In parallel, control males and females were fed from fetal life a diet containing ALA in adequate amounts. In addition, the present results on males and females were compared with our recently published data on females submitted to ovariectomy and estradiol treatment. The purpose of the present article was to propose a model for comparing the influence of diet, gender, and sex steroid hormones on DHA synthesis through the determination of one common biochemical parameter, the ω3 Δ4-desaturation index in liver.

Materials and methods

Animals and diets

Wistar rats were reared and treated in accordance with the directive #86/609/EEC of the European Community Council. All procedures were approved by the institutional animal care and ethical committee according to the French regulation for animal experimentation (authorization no° 78–34). Pre-experimental parent dams were fed from mating to pregnancy and lactation a control diet or a low ALA diet ("deficient" diet), both containing 6.5% by weight of vegetable oils. The control diet was based on a blend of rapeseed oil and sunflower oil from the oleisol variety (54/46, wt/wt), while the deficient diet contained only sunflower oil (Table 1). The deficient and control diets contained 5 and 300 mg ALA/100 g diet, respectively, and both contained 1.2 g of linoleic acid (LA, 18:2ω6) per 100 g of diet. Newborns were randomly distributed and culled to 4 males and 4 females per litter. At weaning, pups born to control diet-fed mothers were raised on the same diet adequate for ALA, while pups born to deficient mothers were randomly assigned to a diet containing either 5 mg (follow-up of the deficient diet), 20 mg (inadequate), 100 mg (intermediate), or 300 mg (adequate diet) of ALA per 100 g (Table 1). Each group was composed of 6 males and 6 females, all issued from different litters, housed separately, and raised for 5 weeks on one experimental diet. At the age of 8 weeks, rats were killed by decapitation after an overnight fast. Liver was removed, homogenized on ice, and stored at −80 °C until the extraction of lipids.

Fatty acid analysis

The two main phospholipid classes, phosphatidylcholine (PC) and ethanolamine phosphoglycerolipid (EPG), were isolated from liver total lipids by solid-phase extraction on a 500-mg aminopropyl-bonded silica column [10] and then derivatized to fatty acid methyl esters that were separated by gas liquid chromatography and detected and quantified through a flame ionization detector [12]. The resulting peaks were automatically identified by comparison with

Table 1 Proportions of sunflower and rapeseed oils in diets

mg ALA %g diet	Low 5	Inadequate 20	Intermediate 100	Adequate 300
Oil (g/100 g of diet) Sunflower (high oleic acid)	6.5	6.3	5.4	3.0
Rapeseed	0	0.2	1.1	3.5
Fatty acids (% by wt of total FA)				
Saturated				
16:0	4.2	4.2	4.3	4.5
18:0	2.7	2.7	2.5	2.1
20:0	0.3	0.3	0.3	0.3
22:0	0.8	0.8	0.7	0.5
24:0	0.3	0.3	0.3	0.3
Total	8.3	8.3	8.1	7.7
Monounsaturated				
16:1n-7	0.1	0.1	0.1	0.2
18:1n-9	71.2	70.9	69.6	66.2
20:1n-9	0.3	0.3	0.4	0.7
Total	71.6	71.3	70.1	67.1
Polyunsaturated				
18:2n-6	19.8	19.9	20.0	20.5
18:3n-3	0.08	0.3	1.5	4.6
18:2n-6/18:3n-3	248	65	13	4.5

Each diet contained (g/100 g) total fats (6.5), casein (22), DL-methionine (0.16), cellulose (2), mineral mix (4), vitamin mix (1), corn starch (42.6), and sucrose (21.7)

standards and expressed as percentage by weight of total fatty acids on the basis of peak areas. The coefficient of variation (CV) was determined for evaluating the repeatability of quantification of the peaks. The CV ranged between 4 and 10% of the peak area value. Minor peaks (<0.05% of total area) having a CV > 20% were omitted from the sum of total identified fatty acids. The Δ4-desaturation index of each phospholipid fraction of each sample was automatically drawn from the ratio of DHA to ω3DPA.

Statistical analysis

Data are expressed as means ± SD ($n = 6$ rats/group). Comparisons between males and females from the same dietary group (gender effect in each diet group) and comparisons between ALA-supplemented rats and their gender-matched controls (diet effect within each gender) were made using 1-way ANOVA (Stat View software). When the F-test was significant, post hoc comparisons were made using Fisher's least significant difference test (PLSD). The level of significance was set at $P < 0.01$.

Results

Diet effect on the Δ4-desaturation index

All animals fed the low ALA diet had much lower DHA and much higher 22:5ω6 in their liver EPG compared with non-deficient controls, a typical consequence of ω3 deficiency (Table 2). In both males and females, the supplementation of diet with 300 mg ALA fully restored the DHA status, while the content in 22:5ω6 and the 22:5ω6/22:4ω6 ratio (ω6 Δ4-desaturation index) concurrently collapsed. Thus, the Δ4-desaturation index of both the ω3 and ω6 series was increased in the liver phospholipids of ω3-deficient rats. The replacement of 22:5ω6 by DHA in ALA-supplemented animals induced the ω6 index to fall down (by about 10 times), while the ω3 index remained high. However, the increases in DHA and ω3DPA in ALA-supplemented rats were not commensurate, resulting in a significant effect of diet on the DHA/ω3DPA ratio: it was equal to 22.9 ± 2.0 and to 16.6 ± 0.9 in the EPG fractions of males fed the low ALA diet and the control diet, respectively (Table 2). In brief, the ω3 Δ4-desaturation index was above 20 in the EPG fraction of deficient males raised on the low ALA diet, while it was equal to 15–18 in restored males (Table 2). In PC, the ω3 index value ranged from 16 to 18, either the males have been raised on the low ALA diet or on one of the three ALA-supplemented diets (Table 3). In control males that were fed the adequate diet from fetal life, both phospholipid classes had an ω3 index value of around 16–18. Thus, the chronic deficiency in ALA had increased the ω3 Δ4-desaturation index in the EPG fraction of males by around 38% compared with controls (22.9 vs. 16.6, $P < 0.01$), an effect that was, however, not observed in PC.

Table 2 Fatty acid composition and Δ4-desaturation indices in the liver EPG of males (M) and females (F) (means ± SD)

EPG	5 mg		20 mg		100 mg		300 mg		Control 300 mg	
	M	F	M	F	M	F	M	F	M	F
% of total FA in EPG										
16:0	15.3 ± 0.5	12.0 ± 1.0¶	15.3 ± 0.7	12.5 ± 0.3¶	15.6 ± 0.4	12.9 ± 0.6¶	16.5 ± 0.5	12.4 ± 0.5¶	15.8 ± 0.6	12.1 ± 1.5¶
18:0	20.6 ± 1.5	30.0 ± 0.8¶	23.5 ± 1.2‡	27.8 ± 1.2¶	24.1± 0.6‡	28.4 ± 0.8¶	22.2 ± 1.5	28.2 ± 0.6¶	20.1 ± 1.1	27.3 ± 2.2¶
18:1 n-9	4.7 ± 0.9	3.7 ± 0.6¶	4.2 ± 0.7	3.6 ± 0.5	3.7 ± 0.2	3.3 ± 0.3	4.2 ± 0.9	2.7 ± 0.4¶	4.1 ± 0.4	3.3 ± 0.4
18:1 n-7	1.8 ± 0.3	0.9 ± 0.04¶	1.7 ± 0.2	1.0 ± 0.15¶	2.0 ± 0.1	1.2 ± 0.1¶	2.1 ± 0.4	0.8 ± 0.05¶‡	2.3 ± 0.2	1.1 ± 0.1¶
18:2 n-6	2.8 ± 0.5	2.3 ± 0.4	2.1 ± 0.2‡	2.2 ± 0.2‡	2.5 ± 0.2	2.5 ± 0.3	2.8 ± 0.6	2.2 ± 0.3‡	2.9 ± 0.2	3.0 ± 0.2
20:4 n-6	34.4 ± 1.5‡	31.6 ± 1.7‡	32.6 ± 1.0‡	31.1 ± 0.9‡	30.4 ± 0.8	30.2 ± 0.4‡	27.7 ± 1.1	26.8 ± 1.1	29.9 ± 0.9	25.6 ± 1.6
22:4 n-6	1.5 ± 0.1‡	1.3 ± 0.2‡	1.35 ± 0.1‡	1.0 ± 0.1¶‡	0.67 ± 0.06‡	0.65 ± 0.05‡	0.36 ± 0.05	0.38 ± 0.02	0.4 ± 0.1	0.36 ± 0.03
22:5 n-6	11.5 ± 1.8‡	10.6 ± 1.9‡	9.2 ± 0.7‡	9.7 ± 1.1‡	1.67 ± 0.32‡	1.47 ± 0.25‡	0.29 ± 0.1	0.35 ± 0.1	0.3 ± 0.05	0.23 ± 0.05
20:5 n-3	0.0 ± 0.0‡	0.0 ± 0.0‡	0.05 ± 0.03‡	0.05 ± 0.03‡	0.32 ± 0.05	0.3 ± 0.05‡	0.45 ± 0.1	0.35 ± 0.1‡	0.5 ± 0.1	0.8 ± 0.2
22:5 n-3	0.14 ± 0.04‡	0.15 ± 0.02‡	0.35 ± 0.06‡	0.23 ± 0.04¶‡	0.87 ± 0.1	0.6 ± 0.05¶‡	1.25 ± 0.05	0.93 ± 0.1¶	1.11 ± 0.1	1.0 ± 0.1
22:6 n-3	3.2 ± 0.16‡	4.46 ± 0.35¶‡	5.62 ± 0.38‡	6.87 ± 0.4¶‡	15.6 ± 0.6‡	16.2 ± 0.8	18.2 ± 1.9	21.0 ± 1.9‡	18.5 ± 1.1	17.1 ± 0.9
Δ4-desat. index										
ω3	22.9 ± 2.0‡	29.7 ± 1.0¶‡	16.1 ± 2.8	29.9 ± 2.6¶‡	17.9 ± 2.8	27.2 ± 2.1¶‡	14.6 ± 1.6	22.8 ± 2.6¶‡	16.6 ± 0.9	17.1 ± 2.6
ω6	7.5 ± 0.9‡	8.1 ± 0.8‡	6.8 ± 0.6‡	9.6 ± 1.2¶‡	2.5 ± 0.3‡	2.3 ± 0.2‡	0.75 ± 0.1	0.9 ± 0.2	0.7 ± 0.1	0.6 ± 0.1

Signification of symbols: ¶ the gender effect (F vs. M) was tested by comparing males and females of the same diet group; ‡ the diet effect was tested in each gender by comparing supplemented rats (fed the 5, 20, 100, or 300 mg ALA diet) and the non-deficient control rats of the same gender (1-way ANOVA and PLSD test in both comparisons, $P < 0.01$)

Table 3 Fatty acid composition and Δ4-desaturation indices in the liver PC of males (M) and females (F) (means ± SD)

PC	5 mg		20 mg		100 mg		300 mg		Control 300 mg	
	M	F	M	F	M	F	M	F	M	F
% of total FA in PC										
16:0	22.1 ± 1.1	16.7 ± 0.9[¶‡]	22.8 ± 0.4	16.5 ± 0.5[¶‡]	24.7 ± 1.2	21.0 ± 1.2[¶]	22.9 ± 0.5	16.3 ± 0.8[¶‡]	23.6 ± 1.5	19.3 ± 0.8[¶]
18:0	19.8 ± 1.5	25.8 ± 1.6[¶]	19.7 ± 1.5	27.6 ± 0.4[¶]	19.3 ± 0.9	30.5 ± 2.3[¶‡]	19.0 ± 0.7	28.7 ± 0.9[¶]	17.8 ± 0.9	25.9 ± 1.6[¶]
18:1 n-9	6.8 ± 0.6	7.9 ± 1.1	6.8 ± 0.4	6.7 ± 0.4	9.5 ± 1.0[‡]	9.1 ± 0.6	7.1 ± 0.5	6.3 ± 0.3[‡]	6.5 ± 0.6	8.0 ± 0.9
18:1 n-7	2.7 ± 0.2	1.3 ± 0.1[¶]	2.5 ± 0.2	1.5 ± 0.2[¶]	3.0 ± 0.2	2.0 ± 0.2[¶]	3.0 ± 0.3	1.4 ± 0.1[¶]	3.3 ± 0.3	1.7 ± 0.1[¶]
18:2 n-6	5.0 ± 0.2[‡]	5.2 ± 0.7[‡]	4.5 ± 0.4[‡]	4.5 ± 0.2[‡]	6.3 ± 0.7	6.0 ± 0.3	6.4 ± 0.9	5.5 ± 0.2[‡]	6.2 ± 0.3	7.3 ± 0.8
20:4 n-6	33.5 ± 1.3	33.7 ± 1.8[‡]	33.3 ± 1.4	32.2 ± 0.9[‡]	27.0 ± 2.8	23.6 ± 2.1	29.4 ± 1.2	29.2 ± 0.9[‡]	30.7 ± 1.7	26.9 ± 0.7[¶]
22:4 n-6	0.5 ± 0.05[‡]	0.44 ± 0.1[‡]	0.47 ± 0.05[‡]	0.35 ± 0.05[‡]	0.16 ± 0.05	0.14 ± 0.02	0.1 ± 0.02	0.1 ± 0.01	0.1 ± 0.05	0.1 ± 0.05
22:5 n-6	5.5 ± 0.7[‡]	5.3 ± 1.1[‡]	4.6 ± 0.5[‡]	5.0 ± 0.4[‡]	0.66 ± 0.1[‡]	0.5 ± 0.1[‡]	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.02	0.12 ± 0.03
20:5 n-3	0.0 ± 0.0[‡]	0.0 ± 0.0[‡]	0.0 ± 0.0[‡]	0.0 ± 0.0[‡]	0.1 ± 0.05[‡]	0.1 ± 0.05[‡]	0.2 ± 0.05	0.2 ± 0.05	0.25 ± 0.05	0.4 ± 0.1
22:5 n-3	0.08 ± 0.01[‡]	0.06 ± 0.01[‡]	0.14 ± 0.02[‡]	0.1 ± 0.02[‡]	0.3 ± 0.01[‡]	0.17 ± 0.03[¶‡]	0.45 ± 0.02	0.4 ± 0.04	0.42 ± 0.04	0.36 ± 0.1
22:6 n-3	1.28 ± 0.1[‡]	1.96 ± 0.2[¶‡]	2.4 ± 0.1[‡]	3.1 ± 0.1[¶‡]	5.3 ± 0.6[‡]	4.44 ± 0.7[‡]	7.9 ± 0.8	9.2 ± 0.8[‡]	7.55 ± 0.8	7.07 ± 0.3
Δ4-desat. index										
ω3	16.0 ± 1.7	32.7 ± 2.6[¶‡]	17.1 ± 0.6	30.6 ± 3.0[¶‡]	17.7 ± 1.7	26.1 ± 1.1[¶‡]	17.6 ± 1.3	23.0 ± 2.6[¶]	17.9 ± 1.5	19.6 ± 3.9
ω6	10.9 ± 1.1[‡]	12.0 ± 1.2[‡]	9.8 ± 0.7[‡]	14.3 ± 1.2[¶‡]	4.1 ± 0.3[‡]	3.5 ± 0.3[‡]	1.8 ± 0.2	1.5 ± 0.2	1.4 ± 0.3	1.2 ± 0.1

Signification of symbols: [¶] the gender effect (F vs. M) was tested by comparing males and females of the same diet group; [‡] the diet effect was tested in each gender by comparing supplemented rats (fed the 5, 20, 100, or 300 mg ALA diet) and the non-deficient control rats of the same gender (1-way ANOVA and PLSD test in both comparisons, $P < 0.01$)

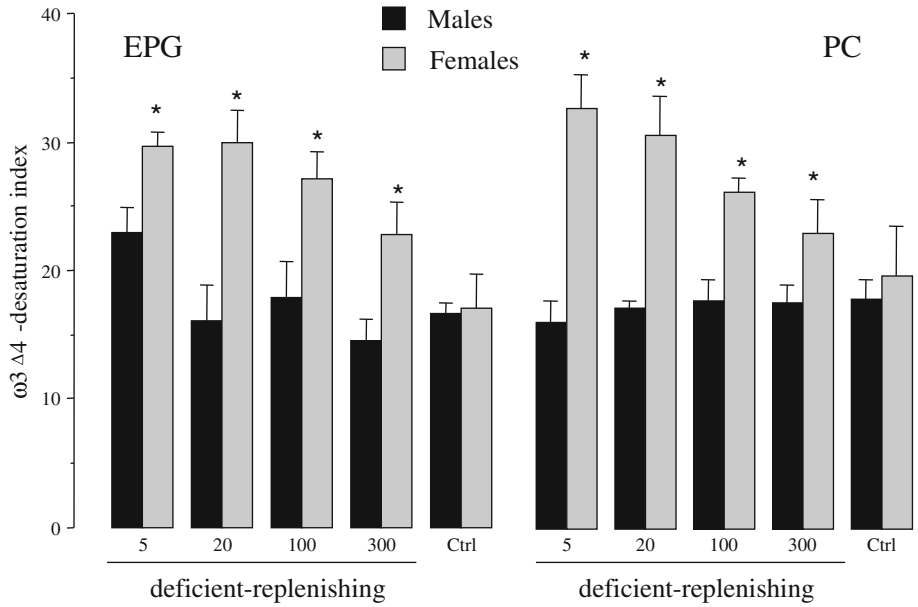

Fig. 1 Changes in the hepatic $\omega 3$ Δ4-desaturation index in response to dietary ALA (mg per 100 g diet), in the EPG (*left*) and PC (*right*) fractions of males (*black*) and females (*grey*). The data are drawn from values in Tables 2 and 3. Animals were born to deficient dams fed the 5-mg ALA diet or to non-deficient mothers fed the control diet (300 mg ALA). At weaning, the deficient rats were fed for 5 weeks the 5-mg ALA diet or a diet supplemented with 20 (inadequate), 100 (intermediate), or 300 mg (adequate) ALA. The non-deficient rats continued to receive the control diet. Values are means ± SD ($n = 6$ per group). The (*asterisk*) symbol indicates that the index value in females is significantly different compared with males of the same diet group. (1-way ANOVA and PLSD test, $P < 0.01$) (the diet effect within each gender is not indicated in this figure)

Gender effect on LC-PUFAs

All replenishing females had an $\omega 3$ Δ4-desaturation index above 22 in both phospholipid classes (Tables 2 and 3), indicating that males and females differently responded to ALA supplementations. Moreover, the $\omega 3$ Δ4-desaturation index value peaked at about 30 in females raised on the low ALA diet, corresponding to an increase of around 70% compared with non-deficient control females. In comparison with the deficient males, the DHA/ω3DPA ratio of the deficient females was 1.3 and 2 times more elevated, in EPG and PC, respectively. These data indicate that females were more prone than males to increase their $\omega 3$ Δ4-desaturation index in response to ALA deficiency. The supplementations in ALA decreased the $\omega 3$ Δ4-desaturation index in both phospholipid classes of females, which remained, however, higher compared with the diet-matched males (Fig. 1). Under adequate supplementation (300 mg ALA), the difference between females and males decreased but remained significant. However, in control animals, the $\omega 3$ Δ4-desaturation index was comprised between 16 and 18, in both genders and in both phospholipid classes (Fig. 1). Thus, the higher ALA intakes smoothed the effect of gender on the $\omega 3$ Δ4-desaturation index.

Gender effect on saturated and monounsaturated fatty acids

The contents in 16:0, 18:0 (saturated fatty acids, SFA) and 18:1ω7 (monounsaturated fatty acid, MUFA) of both phospholipid classes were significantly different in males and females (Tables 2 and 3). Indeed, in the five groups of diet, 16:0 and 18:1ω7 were significantly higher in the EPG and PC fractions of males, while 18:0 was significantly higher in females. Moreover, oleic acid (18:1ω9) tended to increase in the EPG fraction of males, by approximately 20% compared with females, though the difference in means within each group of diet did not reach the level of statistical significance (Table 2). However, the gender difference was significant when it was tested regardless of diet, by comparing the means of the thirty individual values of males and females (4.2 ± 0.4 vs. 3.3 ± 0.4, data not shown).

Globally, the ALA content in diets had no effect on the SFA and MUFA levels in the liver phospholipids of both genders. On the other hand, the occurrence of a sex effect on SFA was well illustrated by the ratio of 18:0 to 16:0, which was clearly gender- and phospholipid class-specific (Fig. 2). Thus, a gender-specific value of the 18:0/16:0 ratio was drawn from the ratios in the two phospholipid classes of all gender-matched animals. The average values

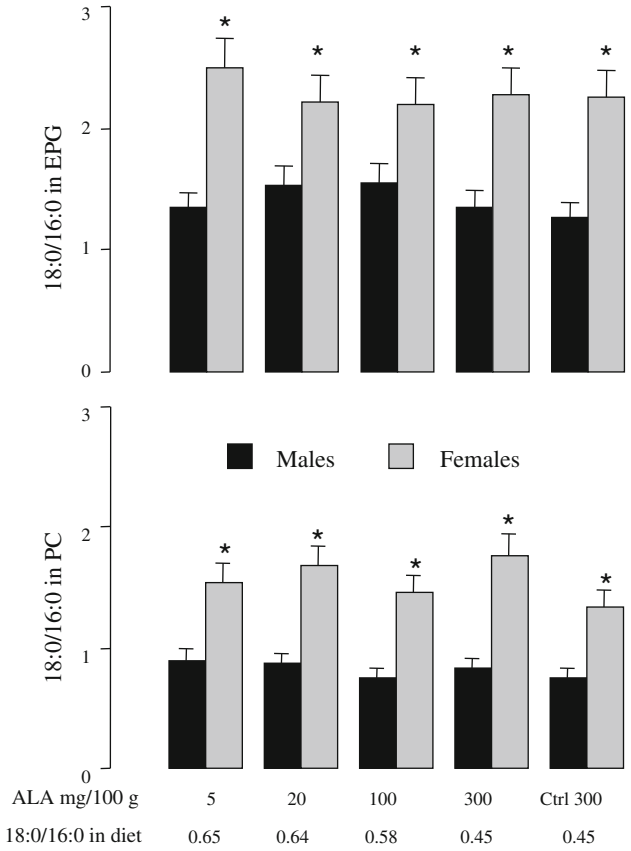

Fig. 2 Histograms of the 18:0/16:0 ratio in the EPG and PC fractions of males (*black*) and females (*grey*) fed the different diets (see Fig. 1 for details). The (*asterisk*) symbol indicates that the ratio value in females is significantly different compared with the diet-matched males. (1-way ANOVA and PLSD test, $P < 0.01$). The average values of the five diet groups were 44 and 75% higher in the EPG and PC fractions of females compared with those of males

were, respectively, equal to 2.3 ± 0.1 and 1.4 ± 0.1 in the EPG and PC fractions of females ($n = 30$), versus 1.6 ± 0.1 and 0.8 ± 0.07 in the EPG and PC of males ($n = 30$). In summary, the 18:0/16:0 ratio was 44 and 75% higher in the EPG and PC fractions of female liver phospholipids compared with male counterparts, whatever the ALA content in diet. Therefore, the $\omega 3$ Δ4-desaturation index, depending on gender and diet, and the 18:0/16:0 ratio, depending on gender, were not correlated (Fig. 3).

Ovarian hormone alterations

We compared, in a recent study, ovariectomized (OVX) females treated with or not with 17β-estradiol (E2) and sham-operated control (SOC) females, which were all born to deficient mothers and fed the 20-mg ALA inadequate diet up to the age of 8 weeks [13]. Re-examination of our data on liver fatty acid compositions indicates that ovariectomy had decreased the liver $\omega 3$ Δ4-desaturation index by 50% compared with SOC and furthermore that this index in OVX females was identical to that of the diet-matched males from this study (Fig. 4). Moreover, the treatment of OVX females with E2 prevented the Δ4 index to decrease in comparison with SOC or with the diet-matched females from this study.

Discussion

We described a model of DHA recovery in male and female rats, which received low ALA supplies (lower than needs) from fetal life and were supplemented with ALA from weaning to 8 weeks of age. Males and females were compared by using the DHA/ω3DPA ratio in liver phospholipids as an indicator of hepatic net Δ4-desaturation.

Utility and limits of the $\omega 3$ Δ4-desaturation index

When the $\omega 3$ status is low at birth and when $\omega 3$ LC-PUFAs are not directly provided by an external source, DHA must be synthesized from its upstream metabolic precursors of the $\omega 3$ series. In this condition, the product/substrate ratio of DHA to ω3DPA in liver phospholipids may be taken as a relevant indicator of the hepatic Δ4-desaturation step. However, it must be taken into account that acyltransferases control the extent to which the newly formed molecules of DHA and ω3DPA are esterified into different classes of phospholipids. Therefore, the DHA/ω3DPA ratio of each phospholipid class is generated and modulated by the enzymatic processes of Δ4-desaturation on the one hand and of acylation/deacylation on the other hand. In the absence of $\omega 3$ LC-PUFAs in diet, ω3DPA derives from EPA, the Δ5-desaturated metabolite of ALA. The newly formed molecules of ω3DPA may be esterified into lysophospholipids or elongated to 24:5$\omega 3$, which is a substrate of the Δ6-desaturase [9]. The Δ6-desaturation of 24:5$\omega 3$ produces 24:6$\omega 3$ which is rapidly shortened to DHA through one cycle of β-oxidation [9]. Thus, the extent to which ω3DPA is either esterified or converted determines the ratio of ω3DPA incorporation into phospholipids relative to DHA, i.e., the $\omega 3$ Δ4-desaturation index. On the other hand, when DHA and ω3DPA are directly ingested, these preformed molecules are potential substrates for hepatic acyltransferases and the DHA/ω3DPA ratio in liver phospholipids will reflect, at least in part, the relative proportions of DHA and ω3DPA in the blood lipids. Therefore, when $\omega 3$ LC-PUFAs are present in diet, the ratio of DHA to ω3DPA in liver phospholipids becomes a much more distant reflection of the hepatic $\omega 3$ Δ4-desaturation step. Therefore, we determined the $\omega 3$ index in the two major phospholipid classes of liver, EPG and PC, in male and female rats born with a low DHA status and

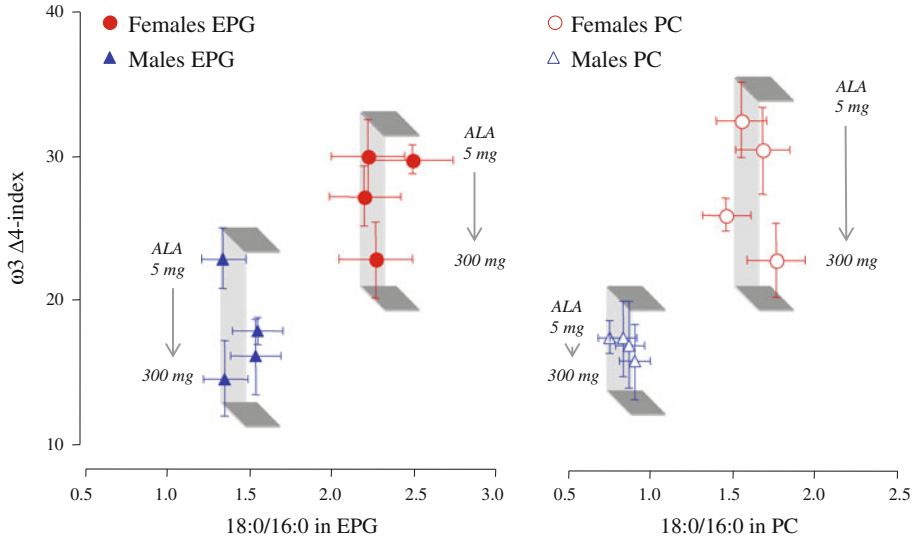

Fig. 3 Plot of the DHA/ω3DPA and 18:0/16:0 ratios illustrating the gender and diet effects in replenishing males and females. The changes in the ω3 Δ4-desaturation index (ordinates) and in the 18:0/16:0 ratio (*x*-axis) were not correlated. The vertical *shadowed bars* show that the ω3 Δ4-desaturation index decreased with ALA replenishment (from 5 to 300 mg ALA) in both genders, and illustrate the shift in the 18:0/16:0 ratio that exists between females and males independently of the ALA content in diets

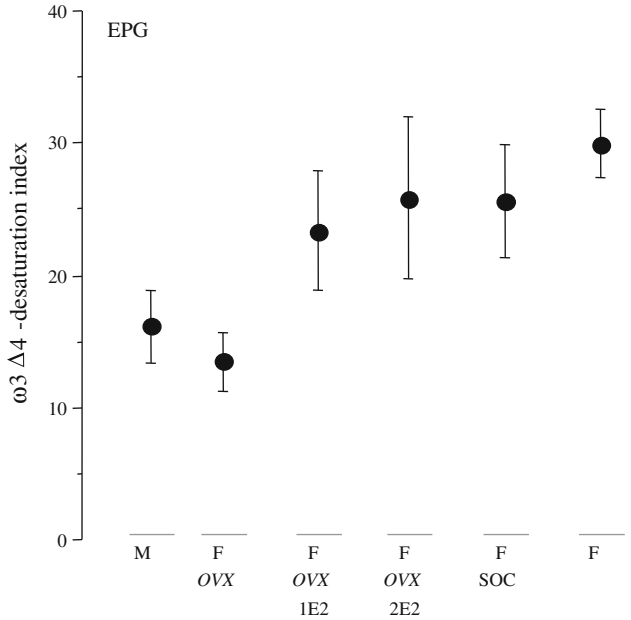

Fig. 4 Comparison of the ω3 Δ4-desaturation index mean values ($n = 6$ per group) in the liver EPG of intact males (*M*), intact females (*F*), sham-operated control females (*SOC*), ovariectomized (*OVX*) females treated with 8 or 16 µg 17β-estradiol per day (OVX+1E2 and OVX+2E2, respectively), or OVX females without treatment. Values for males and females are drawn from the 20-mg ALA group (Table 2), and values for groups SOC and OVX are drawn from our previous study [13]. *Vertical bars* correspond to the 99% confidence interval on each value. All animals from both studies were born to deficient dams fed the 5-mg ALA diet and were supplemented for 5 weeks with the 20-mg ALA diet. In that condition, males and OVX females have an ω3 Δ4-desaturation index of 15–16, while intact females, SOC females, and OVX females treated with E2 have an ω3 Δ4-desaturation index of 25–30

supplemented with low, inadequate, intermediate, or adequate doses of ALA, in the absence of ω3 LC-PUFA in diet.

Gender effect on the ω3 Δ4-desaturation index

We found that both males and females increased their ω3 Δ4-desaturation index in response to low or inadequate ALA diets. However, the amplitude of this compensatory metabolic response depended on gender: in comparison with gender-matched controls, the ω3 Δ4-desaturation index increased in deficient females by about 70% in both phospholipid classes, while it increased in deficient males by 38% in EPG only. Therefore, females were more prone than males to increase their hepatic ω3 Δ4-desaturation index in response to ALA deficiency. Moreover, the difference between males and females decreased with adequate supplementation, and it was not significant in control animals that had received adequate ALA supplies (300 mg/100 g diet) from fetal life. It is likely that animals that were born to control dams and raised on the adequate diet had higher body stores of DHA and ω3DPA than those that were born to deficient mothers and raised on an inadequate diet. In non-deficient animals, substantial amounts of LC-PUFAs may be released from body stores and transferred through blood lipids to liver phospholipids. Therefore, the DHA/ω3DPA ratio could be less representative of DHA synthesis in non-deficient animals, and thus, this index of the ω3 Δ4-desaturation step would be less dependent on the gender effect.

The question remains whether the effect of gender on the ω3 index may be ascribed to Δ4-desaturation and/or acylation. It appeared that DHA and ω3DPA were incorporated in the liver phospholipids of males and females according to a ratio which was about identical in EPG and PC, while their absolute levels in both classes were markedly different. Thus, the DHA/ω3DPA ratio values varied with gender and ALA supplementation, regardless

of the particular fatty acid distribution in each class of phospholipid acceptor. These data suggest that, in both genders, the esterification process does not prevail on that of Δ4-desaturation in the final setting of the DHA/ω3DPA ratio. A major condition is that preformed DHA and ω3DPA are not directly acylated. Therefore, DHA and ω3DPA must not be stored in adipose tissues (deficiency status) and not provided from an external source (no ω3 LC-PUFAs in diet). Otherwise, their ratio in phospholipids could be much more determined by the esterification process than by the Δ4-desaturation step.

Gender effect on saturated and monounsaturated fatty acids

In the five groups of diet, the liver phospholipids of females contained more stearic acid (18:0), less palmitic acid (16:0), and less MUFA than those of males. In particular, the average value of the 18:0/16:0 ratio was significantly higher in both phospholipid classes of females (+44% in EPG and +75% in PC compared with males). The hypothesis could be made that the elongation rate of 16:0 to 18:0 is higher in females, while the rate of Δ9-desaturation of 16:0 and 18:0 to 16:1ω7 and 18:1ω9 is higher in males. Both pathways, elongation and desaturation, might take account for this gender-specific lipid pattern. However, it must be considered that SFA and MUFA were present in substantial amounts in diets (especially 18:1ω9) and that these fatty acids are susceptible to be stored in body fats or to be catabolized through β-oxidation. Therefore, we cannot evaluate which pathway, uptake by liver from blood lipids, storage in triacylglycerols, hepatic de novo synthesis, desaturation, elongation, and deposition in phospholipids, or degradation through β-oxidation, was more sustained in each gender. These data essentially indicate that SFA and MUFA were differently metabolized in males and females, either the animals were ω3 deficient, restoring or restored. Clearly, the deposition of SFA and MUFA in each class of liver phospholipids depended on gender but not on the ALA contents in diets.

The gender effect on the ω3 Δ4-desaturation index is subordinated to ALA intakes

The plot of the ω3 Δ4-indices against the 18:0/16:0 ratios (Fig. 3) illustrates our finding that gender and ALA supplementation differently impacted on LC-PUFA and on SFA. This representation shows that the highest doses of ALA smooth the effect of gender on the ω3 Δ4-index, whereas the 18:0/16:0 ratio is class and gender specific without being influenced by the level of ALA in diet. Dietary ALA may be stored in triacylglycerols, degraded through β-oxidation, or converted into LC-PUFA. The hypothesis has been made that a gender-specific partitioning of ALA among these metabolic routes determines the extent to which ALA enters the DHA synthesis pathways [14]. The oxidative pathway being more active in males, less ALA would be available to be converted into LC-PUFA, explaining the lower rate of DHA synthesis in males compared with females [14]. However, DHA and ω3DPA both derive from the conversion of ALA, especially in ω3-replenishing animals, and the difference in their ratio in the liver phospholipids of males and females cannot be explained by the upstream partitioning of ALA. In males, higher ω3DPA and lower DHA may take account for a metabolic limitation that would occur downstream of the conversion pathway, i.e., at the Δ4-desaturation step.

Elongase, Δ6-desaturase and peroxisomal β-oxidation enzymes are successively implied in the conversion of ω3DPA into DHA, the Δ4-desaturation step. We previously showed that the gene expression of hepatic Δ6-desaturase was 2.5 times higher in females fed the 20-mg ALA diet (inadequate) than that of diet-matched males, whereas there was no sex-related difference in the expression of peroxisomal enzymes [10]. Other works have also reported higher level of Δ6-desaturase transcripts in female liver [15], although the difference with males was of much less amplitude. In that study, the Δ4-desaturation index in the liver PC of rats aged 13 weeks was comprised, depending on diets, between 1.7 and 4.5 in males and between 3.7 and 8.0 in females [15]. These lower values compared with ours can be explained by the different nutritional handling. We used a nutritional sequence of prenatal deficiency and replenishment at weaning that stimulated the hepatic DHA synthesis, resulting in high values of ω3 Δ4-desaturation index. In the previous study [15], rats were not ω3 deficient at birth and they were adult at the beginning of the experiment (10 weeks of age vs. 3 weeks in our study) and were initially fed a low fat diet based on soja oil (40 mg ALA/100 g diet) and then a high fat diet containing 1.2 or 6 g ALA/100 g (vs. 20–300 mg/100 g herein). High supplies in ALA resulted in accretion of its downstream metabolites in liver phospholipids, EPA and ω3DPA, without a commensurate increase in DHA. Consequently, the values of liver Δ4-desaturation index in males and females were lower than those from the deficient/replenishing rats of our study. It must be taken into account that substantial increases in ALA-derived EPA in phospholipids may announce possible alteration in DHA esterification, which would render less relevant the use of the DHA/ω3DPA ratio as an indicator of the ω3 Δ4-desaturation step. Overall, both studies show that the liver ω3 Δ4-desaturation index may vary from 1.5 to 20 in males and from 3.5 to 35 in females, reflecting, respectively, higher and lower ALA intakes than needs. Difference in ω3 Δ4-desaturation index is thus a reliable indicator of sexual

dimorphism in DHA synthesis, being understood that index values in both genders will be determined by nutritional handling and ALA intakes, i.e., the lower the intakes, the higher the Δ4-desaturation index. There are two major conditions: preformed molecules must not be directly acylated and the acylation of downstream metabolites of ALA other than ω3DPA (mainly EPA) must not prevent that of DHA.

Whether sex factors influence gene expression and/or activity of Δ6-desaturase is not demonstrated. Potential mechanisms of the regulation of DHA-synthesizing enzyme transcription through estrogen signaling of PPARα activation have been hypothesized [reviewed in 16]. Moreover, it has been suggested that gender-specific epigenetic events that occur during development may affect, specifically in the liver, the transcription of PPARα-target genes [17]. Be that as it may, the PPARα signaling has been shown to be much more active or more activated in males [17, 18] and that of SREBP-1c, a lipogenic transcription factor, to be more active in females [19]. It is remarkable that PPARα and SREBP-1c, which are, nevertheless, involved in opposite metabolic pathways, are both susceptible to activate the transcription of the hepatic Δ6- and Δ5-desaturases [20, 21]. A gender-specific balance between PPARα and SREBP-1c could underlie the effect of gender on the liver capacity of DHA synthesis [reviewed in 22]. However, our present study shows that the effect of gender on the ω3 Δ4-desaturation index is much more pronounced in deficient/replenishing animals compared with those having received adequate ALA from fetal life. It may be supposed that supplying ALA in amounts lower than the needs should enhance the influence of sex factors on the gene expression of DHA-synthesizing enzymes. At the opposite, high intakes of PUFA are susceptible to downregulate the expression of desaturases [23], which may reduce or suppress the transcriptional effects of non-nutritional factors. The amplitude of sex differences in lipid gene expression will strongly depend on the nutritional history of both genders and essentially on their ALA intakes.

Ovarian steroid hormones influence the ω3 index

Re-examination of our previously published data on the impact of ovariectomy on the expression of lipid-metabolizing genes indicated that the ω3 Δ4-desaturation index in the liver of 20 mg ALA-supplemented females was influenced by the ovarian hormone status. The emergent point of these comparisons between males and intact females, on the one hand, and between OVX females with or without E2 treatment, on the other hand, is that OVX had decreased the ω3 Δ4-desaturation index in liver, whereas OVX females treated with E2 had the same ω3 index as that of intact females. Finally, it appears that the hepatic ω3 Δ4-desaturation index is enhanced in all deficient/replenishing animals, with an amplitude that depends on both the gender and the status in ovarian steroid hormones.

Δ6-Desaturase expression and ω3 Δ4-desaturation index in females

We previously reported that ovariectomy produces a lipogenic response of liver, with a marked increase in the transcripts of lipid-metabolizing genes, including the Δ6- and Δ5-desaturases, an effect totally prevented with E2 treatment [13]. Thus, OVX females treated with E2 had both their ω3 Δ4-desaturation index and level of Δ6-desaturase transcripts preserved, indicating that E2 sustains the liver capacity of DHA synthesis at the same level as that of intact females. Paradoxically, OVX females without E2 treatment exhibited an increased expression of hepatic desaturases [13], but had their index of ω3 Δ4-desaturation, the phenotype output, decreased (Fig. 2). Thus, an increase in hepatic Δ6-desaturase transcripts does not seem to be sufficient by itself to produce an increased ω3 Δ4-desaturation index, at least in this model of ovarian hormone alteration. Traductional and/or post-traductional regulation processes may be upset by ovariectomy, which could result in alterations in fatty acid trafficking or enzyme activities regardless of transcriptional effects.

We conclude that sex factors play a role in the control of the DHA synthesis terminal step, especially in case of ALA deficiency. In young females reaching reproductive age, the underpinning of DHA synthesis through ovarian steroid hormone signaling could constitute an adaptative response to intakes of ω3 fatty acids which are lower than needs. This is a metabolic advantage, compared with males, that would be reduced when physiological requirements for ω3 fatty acids are fully covered.

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