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RESEARCH ARTICLES

Gender affects liver desaturase expression in a rat model of $n-3$ fatty acid repletion ☆

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Abstract

Dietary $n-3$ polyunsaturated fatty acids (PUFA) are major components of cell membranes and have beneficial effects on human health. Docosahexaenoic acid (DHA; 22:6$n-3$) is the most biologically important $n-3$ PUFA and can be synthesized from its dietary essential precursor, α-linolenic acid (ALA; 18:3$n-3$). Gender differences in the efficiency of DHA bioconversion have been reported, but underlying molecular mechanisms are unknown. We compared the capacity for DHA synthesis from ALA and the expression of related enzymes in the liver and cerebral cortex between male and female rats. Wistar rats, born with a low-DHA status, were supplied with a suboptimal amount of ALA from weaning to 8 weeks of age. Fatty acid composition was determined by gas chromatography, the mRNA expression of different genes involved in PUFA metabolism was determined by RT-PCR (low-density array) and the expression of proteins was determined by Western blot analysis. At 8 weeks, DHA content was higher (+20 to +40%) in each phospholipid class of female livers compared to male livers. The "Δ4," Δ5 and Δ6 desaturation indexes were 1.2–3 times higher in females than in males. The mRNA expression of Δ5- and Δ6-desaturase genes was 3.8 and 2.5 times greater, respectively, and the Δ5-desaturase protein was higher in female livers (+50%). No gender difference was observed in the cerebral cortex. We conclude that female rats replete their DHA status more readily than males, probably due to a higher expression of liver desaturases. Our results support the hypothesis on hormonal regulation of PUFA metabolism, which should be taken into account for specific nutritional recommendations.

Keywords: $n-3$ PUFA; Gender; Desaturases; Docosahexaenoic acid; Liver; Rats

1. Introduction

Long-chain polyunsaturated fatty acids (PUFA) are crucial for proper functioning of cell membranes, particularly in the brain. Biologically important PUFA include docosahexaenoic acid (DHA, 22:6$n-3$) and arachidonic acid (AA; 20:4$n-6$). They can be provided directly from the diet or be converted from their respective dietary essential precursors α-linolenic acid (ALA; 18:3$n-3$) and linoleic acid (LA; 18:2$n-6$). Two desaturases, Δ6-desaturase and Δ5-desaturase [coded by the fatty acid desaturase 2 (*FADS2*) and fatty acid desaturase 1 (*FADS1*) genes, respectively], located in the endoplasmic reticulum are required for the formation of the long-chain PUFA of both $n-6$ and $n-3$ series (Fig. 1). Besides, peroxisomes are required for the final steps in DHA formation by retroconversion. The main enzymes involved are thought to be acyl-coenzyme A oxidase 1 (ACOX1), multifunctional protein 2 (MFP2; 2-enoyl-CoA hydratase/D-3-hydroxyacyl-CoA dehydrogenase) and thiolase or SCPx-thiolase [1,2]. If the diet is specifically deficient in $n-3$ fatty acids, the endoreticulum and peroxisomal pathways produce $n-6$ docosapentaenoic acid ($n-6$ DPA; 22:5$n-6$) in place of DHA. However, replacement of membrane DHA with $n-6$ DPA results in functional alterations, notably in the central nervous system [3,4]. In the rat, synthesis of DHA mainly occurs in the liver [5,6], although other tissues, including the brain, have the metabolic potential to form DHA [7–9]. Tracer studies have shown that men [10] and women [11] who receive a stable isotope of ALA present different contents of newly formed DHA in their blood lipids. In men, a single dose of labeled ALA is converted into the two $n-3$

Abbreviations: AA, arachidonic acid [20:4$n-6$]; ALA, α-linolenic acid [18:3$n-3$]; ACOX1, acyl-coenzyme A oxidase 1; DHA, docosahexaenoic acid [22:6$n-3$]; $n-3$ DPA, $n-3$ docosapentaenoic acid [22:5$n-3$]; $n-6$ DPA, $n-6$ docosapentaenoic acid [22:5$n-6$]; EPA, eicosapentaenoic acid [20:5$n-3$]; EPG, ethanolamine phosphoglycerolipid; FABP, fatty acid binding protein; FADS1, fatty acid desaturase 1; FADS2, fatty acid desaturase 2; LA, linoleic acid [18:2$n-6$]; MFP1, multifunctional protein 1; MFP2, multifunctional protein 2; PPAR, peroxisome proliferator-activated receptor; PC, phosphatidylcholine; PS, phosphatidylserine; PUFA, polyunsaturated fatty acids; RIN, RNA integrity number; RQ, relative quantities; RXR, retinoid X receptor; SREBP1c, sterol-regulatory element-binding protein 1c; TLDA, *TaqMan* Low-Density Array

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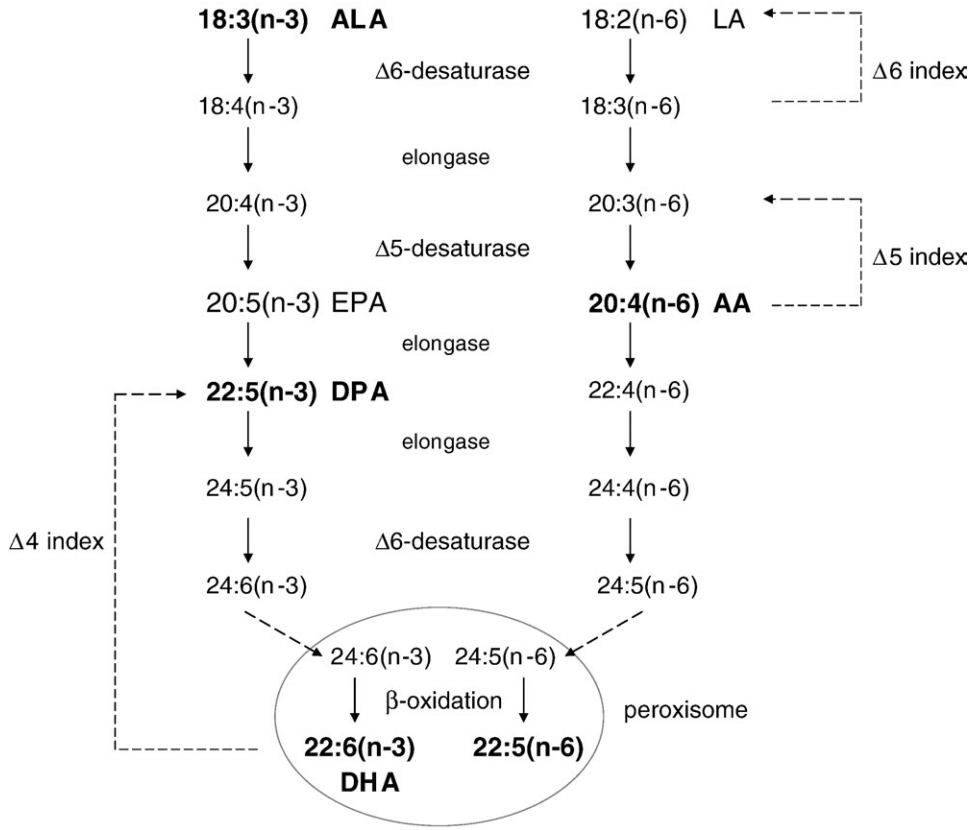

Fig. 1. Biosynthesis of $n-3$ and $n-6$ PUFA from their dietary precursors. (Reprinted with permission. [1])

pentaenoic acids — eicosapentaenoic acid (EPA; 20:5$n-3$) and $n-3$ docosapentaenoic acid ($n-3$ DPA; 22:5$n-3$) — while women produce the three long-chain derivatives EPA, DPA and DHA [12]. Higher DHA synthesis in women could be due to gender-related differences in sexual hormone status and, thereby, differential hormonal regulation of PUFA metabolism. This hypothesis is supported by clinical data, since estrogens, not androgens, have been shown to increase the concentrations of AA and DHA in the blood lipids of patients treated with sex hormone steroids [13,14]. A recent study has shown that the DHA content in the liver was higher in female rats than in male rats [15], and it has been suggested that estradiol replacement in ovariectomized rats increased the liver concentration of PUFA in total phospholipids [16]. However, the molecular mechanisms by which sexual hormones could favor the conversion of PUFA are not elucidated.

In this study, we determined whether expression of converting enzymes could be related to gender differences in tissue DHA content. For that purpose, we compared the capacities of male and female rats to replete their tissue DHA content (plasma, liver and cerebral cortex) in response to the introduction of ALA in the rearing diet. We then compared the gender difference in the replenished PUFA status with differences in the mRNA and protein expressions of the main enzymes involved in PUFA metabolism.

2. Materials and methods

2.1. Animals

Rats were reared in an air-conditioned room (20°C) that was illuminated for 12 h (0700–1900 h) and consumed food and water ad libitum. All animal experiments were carried out in accordance with the European Community Council Directive of November 24, 1986 (86/609/EEC).

2.2. Experimental design

Six female Wistar rats were fed a low-ALA-containing diet (5 mg/100 g of a semisynthetic diet containing sunflower oil as vegetable lipid source) from mating to pregnancy and lactation. Litters were culled to eight pups, with an equal number of males and females on postnatal day (P1), and weaned on P21. The pups (n=18 males and n=18 females) were supplied with a suboptimal amount of ALA (20 mg/100 g diet, provided by the addition of rapeseed oil; Bally, France) from weaning to 8 weeks of age. Each diet contained 5% fat and 870 mg LA/100 g diet. There was no other $n-3$ PUFA in the diet (Table 1). At 3, 5 or 8 weeks of age, male (n=6) and female (n=6) rats were killed by decapitation between 0900 and 1100 h after one night of fasting. Trunk blood was collected and centrifuged to isolate plasma. Cerebral cortices and livers were rapidly removed on ice and separated into two pools: one was frozen in liquid nitrogen and stored at −80°C until use for analysis of fatty acids and protein expression, and the other was stored at −20°C in RNAlater (RNA Stabilization Reagent; Qiagen, France) until extraction and quantification of mRNA.

2.3. Fatty acid analysis

Total lipids were extracted from rat samples (cerebral cortex, liver and plasma) with 4 ml of chloroform–methanol (2:1, vol/vol). The three main phospholipid classes — phosphatidylserine (PS), phosphatidylcholine (PC) and ethanolamine phosphoglycerolipid (EPG) — were isolated by solid-phase extraction on a 500-mg aminopropyl-bonded silica column, as previously described [17]. The fatty acids were transmethylated then separated by gas chromatography [18]. The resulting peaks were identified by comparison to standards and expressed as percentage by weight of total fatty acids on the basis of peak areas in PS, PC and EPG. Desaturation indexes (product/substrate ratio) were generated from PUFA composition in phospholipids from 8-week-old rats: "Δ4" desaturation index represents the DHA/$n-3$ DPA ratio, Δ5 desaturation index represents the AA/20:3$n-6$ ratio and Δ6 desaturation index represents the 18:3$n-6$/LA ratio (Fig. 1). Minor PUFA such as 18:4$n-3$ and 20:4$n-3$ were not considered.

2.4. Real-time RT-PCR using low-density array

Total RNA was isolated from 50 mg of liver and cerebral cortex using an RNeasy Lipid Tissue Midi Kit with DNase digestion (Qiagen). RNA concentration and purity were verified by A_{260}/A_{280} and A_{260}/A_{230} ratios (Biophotometer; Eppendorf SARL, France). The quality and integrity of RNA were then checked by microcapillary

Table 1
Composition of diets

	Diet fed during pregnancy and lactation	Postweaning diet
Percentage		
Casein	22	22
dl-methionine	0.2	0.2
Cellulose	2	2
Mineral mix	4	4
Vitamin mix	1	1
High oleic sunflower oil	5	4.8
Rapeseed oil	0	0.2
Corn starch	43.9	43.9
Sucrose	21.9	21.9
Fatty acid composition (% by weight of total fatty acids)		
16:0	4.7	4.5
18:0	2.4	2.3
18:1*n*−9	68.5	69.2
18:2*n*−6	22.8	21.9
n−6 LCPUFA	ND	ND
18:3*n*−3	0.1	0.5
n−3 LCPUFA	ND	ND

The preweaning and postweaning diets supplied 5 and 20 mg of ALA per 100 g, respectively.
ND, not detected.

electrophoresis measurements on an Agilent 2100 bioanalyseur (Agilent Technologies, France) with an RNA 6000 Nano LabChip Kit (Agilent Technologies). The Agilent 2100 expert software calculates the 28S:18S ratio and the RNA integrity number (RIN). All assays were run in accordance with the manufacturer's instructions. Only samples displaying a RIN of ≥8.0 and an A_{260}/A_{280} of >1.8 were retained. cDNA was prepared from 1 μg of total RNA using a high-capacity cDNA Archive Kit (Applied Biosystems, France). We quantified mRNA levels of key genes by creating a rat PUFA metabolism *TaqMan* Low-Density Array (TLDA) (Applied Biosystems) (Table 2). In this study, the TLDA card was configured into eight identical 24-gene sets. This configuration makes possible the simultaneous analysis of 22 genes and the two reference genes *18S* and *GAPDH* in two replicates of eight different samples per run. The *TaqMan* probe and primer sets for each gene were selected from predesigned *TaqMan* Gene Expression Assay (Applied Biosystems). Thus, each 2-μl well of a 384-well card contains specific primers and a probe that is capable of detecting a single gene. cDNA samples were diluted with nuclease-free water (1:4) and mixed with 55 μl of *TaqMan* Universal PCR Master Mix. Then, 100 μl of the sample-specific PCR mixture was loaded into a sample port. Once the TLDA plate had been centrifuged twice for 1 min each at 1200 rpm to distribute the samples from the loading port into each well, the card was sealed before PCR amplification was performed with an Applied Biosystems Prism 7900 HT Sequence detection system. The thermal cycling conditions were 2 min at 50°C and 10 min at 95°C, followed by 40 cycles of 30 s at 97°C and 1 min at 59.7°C. TLDA cards were analyzed with RQ documents and the RQ Manager Software for automated data analysis. Expression values of target genes were calculated based on the comparative threshold cycle (C_t) method: they were normalized to the expression of 18S rRNA, which produced insignificant variations, to create ΔC_t values. Thereafter, they were normalized to a calibrator (8-week-old males) to generate $\Delta\Delta C_t$ values. Relative quantities (RQ) were calculated with the equation: $RQ=2^{-\Delta\Delta C_t}$.

2.5. Protein expression by Western blot analysis

Samples of liver homogenates were lysed in CHAPS buffer (20 mmol/L Tris–HCl, 0.4 mol/L CHAPS, benzonase and a protease inhibitor cocktail; Calbiochem, France) and sonicated. Protein contents were then determined by the Bio-Rad DC Protein Assay. Samples were diluted in Tris–HCl buffer containing 20 mmol/L sodium dodecyl sulfate and 4 mmol/L dithiothreitol, and Western blot analysis was performed using the commercial NUPAGE system (NOVEX Bis-Tris Mini Gels; Invitrogen, France): 30 μg of liver proteins was separated by 4–12% acrylamide gels (pH 7.0) and transferred to a nitrocellulose membrane (2 h, 30 V, 4°C). Membranes were incubated at 4°C overnight with Tris–HCl buffer containing 0.1% Tween 20 (vol/vol) and 5% milk (wt/vol) (TBSTm). Then the membranes were incubated with goat polyclonal anti Δ5-desaturase antibodies (diluted 1:200 in TBSTm; Novus Biological) or rabbit polyclonal anti-rat MFP2 (prepared as described in Dieuaide-Noubhani et al. [19]; diluted 1:200 in TBSTm) for 2 h at 4°C. The membranes were washed three times in Tris–HCl buffer containing 0.1% Tween 20 (vol/vol) (TBST), incubated with peroxidase-conjugated anti-goat or anti-rabbit secondary antibody (diluted 1:10000) for 2 h at 4°C and then washed three times in TBST. Enhanced chemiluminescence (Amersham ECL Plus) with a Fujifilm LAS-1000 Plus Camera system was used to detect immunopositive areas. Staining intensity was measured with Advanced Image Data Analyzer software v. 3.22 (Raytest).The densitometry for Δ5-desaturase or MFP2 was normalized with reference to β-actin. The membranes were washed with TBST, incubated in TBSTm at 4°C overnight and then incubated with mouse monoclonal anti β-actin antibody (diluted 1:10000; Sigma) and peroxidase-conjugated anti-mouse secondary antibody using the same procedure as described for Δ5-desaturase and MFP2.

Table 2
Gene names and GenBank accession numbers

Function	Gene name	GenBank accession no.	Usual name
Fatty acid transport	Fatty acid binding protein 3	NM_024162	FABP3
	Fatty acid binding protein 5	NM_145878	FABP5
	Fatty acid binding protein 7	NM_030832	FABP7
Fatty acid mitochondrial oxidation	Carnitine palmitoyltransferase 1a	NM_031559	CPT1-a
	Carnitine palmitoyltransferase 1b	NM_013200	CPT1-b
PUFA desaturation	Fatty acid desaturase 1	NM_053445	Δ5 desaturase
	Fatty acid desaturase 2	NM_031344	Δ6 desaturase
Fatty acid peroxisomal oxidation	Multifunctional protein 1	NM_017008	MFP1
	Multifunctional protein 2	NM_024392	MFP2
	Acyl-coenzyme A oxidase 1	NM_017340	ACOX1
Fatty acid incorporation into phospholipids	Acetyl-coenzyme A acyltransferase 1	NM_012489	ACAA1
	Acetyl-coenzyme A acyltransferase 2	NM_130433	ACAA2
Transcription factors	Peroxisome proliferator-activated receptor α	NM_013196	PPARα
	Peroxisome proliferator-activated receptor δ	NM_013141	PPARδ
	Peroxisome proliferator-activated receptor γ	NM_013124	PPARγ
	Retinoic acid receptor α	NM_031528	RARα
	Retinoid X receptor α	NM_012805	RXRα
	Estrogen receptor 1	NM_012689	ERα
	Estrogen receptor 2	NM_012754	ERβ

2.6. Statistical analysis

Data are expressed as mean±SD. Data were analyzed for significant effects by two-way analysis of variance (ANOVA; SigmaStat 3.1 software, advisory statistics for scientists) with factors Age (3, 5 and 8 weeks) and Gender (male and female). When the normality test of ANOVA was passed, post hoc comparisons between means within each group were performed using Bonferroni's test. We tested the age effect within each gender group and the gender effect within each age group. A nonparametric permutation test (StatXact 8 software) was used for Western blot analysis (repeated experimentation). Statistical significance was set at $P<.01$ or $P<.05$.

3. Results

3.1. Weight gain

There was no difference in weight between the two groups at the beginning of the study: male and female rats at weaning (3 weeks old) had an average weight of 60 g (61.1±7.7 vs. 62.0±6.9 g). The weight gain from weaning to 8 weeks of age was 1.5-fold higher in males (278±26 vs. 184±18 g).

3.2. PUFA composition in plasma, liver and cerebral cortex

3.2.1. DHA content increased during ALA supplementation

Male and female rats aged 3 weeks had low and identical DHA status in the plasma, liver and cerebral cortex. Maximum DHA content accounted for 5.9% (males) and 5.7% (females) of the total fatty acids in cortex PS, 4.9% of the total fatty acids in cortex EPG, 2.7–2.8% of the total fatty acids in liver EPG and 1.2–1.3% of the total fatty acids in plasma PC (Table 3). High incorporation of AA and *n*−6 DPA was observed in all tissues in both male and female rats. DHA content significantly increased in plasma, liver and cerebral cortex phospholipids in response to the introduction of rapeseed oil in the rearing

Table 3
Effect of age and gender on phospholipid PUFA composition in the plasma, liver and cerebral cortex from male and female rats

		Males			Females		
		3 weeks (n=6)	5 weeks (n=6)	8 weeks (n=6)	3 weeks (n=6)	5 weeks (n=6)	8 weeks (n=6)
DHA							
Plasma	PC	1.2±0.1[a]	1.9±0.07[b]	2.2±0.1[c]	1.3±0.1[a]	2.1±0.2[b]	2.7±0.2[c,*]
Liver	EPG	2.7±0.2[a]	5.4±0.2[b]	6.7±0.6[c]	2.8±0.2[a]	6.2±0.3[b,*]	7.8±0.5[c,*]
	PC	1.5±0.2[a]	2.4±0.2[b]	2.6±0.1[b]	1.5±0.1[a]	2.4±0.1[b]	3.1±0.2[c,*]
	PS	1.6±0.2[a]	1.8±0.2[a]	2.3±0.4[b]	1.6±0.2[a]	2.1±0.2[a]	3.2±0.2[b,*]
Cortex	EPG	4.9±0.2[a]	6.1±0.4[a]	8.9±0.7[b]	4.9±0.3[a]	6.3±0.4[b]	9.5±0.5[c]
	PC	0.7±0.1[a]	1.0±0.1[b]	1.6±0.1[c]	0.6±0.06[a]	1.2±0.2[b]	1.6±0.2[c]
	PS	5.9±0.4[a]	6.2±0.4[a]	9.1±0.6[b]	5.7±0.3[a]	6.4±0.4[a]	8.9±0.9[b]
AA							
Plasma	PC	28.6±1.7[a]	27.4±0.7[a]	30.0±1.0[b]	25.1±1.0[a]	28.1±1.4[a]	31.5±0.9[b]
Liver	EPG	26.3±1.7[a]	30.2±1.2[b]	31.8±1.5[b]	27.7±2.4[a]	30.9±0.8[b]	31.9±0.9[b]
	PC	25.8±1.1[a]	28.7±1.4[b]	31.0±0.6[c]	27.4±1.2[a]	28.3±1.2[a]	31.4±0.9[b]
	PS	23.1±2.1[a]	31.2±2.1[b]	30.2±0.9[b]	26.1±2.1[a]	30.4±1.2[b]	31.8±1.0[b]
Cortex	EPG	18.1±0.6[a]	15.1±1.3[b]	14.3±1.6[b]	18.2±0.6[a]	15.2±1.1[b]	14.6±1.1[b]
	PC	9.9±0.3[a]	7.2±0.4[b]	7.1±0.5[b]	9.4±0.2[a]	7.3±0.4[b]	6.8±0.3[b]
	PS	10.3±0.7[a,b]	10.9±1.1[a]	9.3±0.7[b]	10.2±0.7[a b]	11.2±1.5[a]	8.9±0.8[b]
n−6 DPA							
Plasma	PC	8.8±0.8[a]	4.9±0.7[b]	3.8±0.8[b]	8.2±0.9[a]	4.5±1.0[b]	3.7±0.5[b]
Liver	EPG	15.7±1.1[a]	11.9±1.6[b]	8.7±2.5[c]	14.7±1.3[a]	10.1±1.3[b]	8.2±0.7[b]
	PC	9.1±0.6[a]	5.7±0.7[b]	3.5±0.6[c]	8.5±0.9[a]	5.1±0.7[b]	3.9±0.6[b]
	PS	9.6±1.4[a]	5.5±0.9[b]	4.6±0.5[b]	9.6±1.4[a]	5.5±0.7[b]	5.0±0.2[b]
Cortex	EPG	18.3±0.3[a]	17.3±0.6[a,b]	16.2±1.6[b]	18.2±0.5[a]	18.0±1.4[a,b]	16.2±0.9[b]
	PC	3.0±0.1[a]	3.3±0.4[b]	3.4±0.3[b]	2.8±0.1[a]	3.3±0.2[b]	3.2±0.4[b]
	PS	22.8±1.4[a]	23.1±1.7[a]	21.8±0.9[a]	22.1±0.6[a]	22.6±0.9[d]	21.1±0.9[a]

Data are expressed as percentage by weight of total fatty acids and mean±SD (n=6).
The effect of age was significant (P<.01; two-way ANOVA), except for n−6 DPA in cortex PS. The effect of gender was significant (P<.01; two-way ANOVA) for DHA in plasma and liver phospholipids, except for liver PC.
[a,b,c]Significantly different values within males or females from weaning to 8 weeks of age (P<.01).
[*] Significant differences between age-matched males and females (P<.01).

diet from weaning (Table 3). This substantial DHA gain occurred in each phospholipid class at the detriment of n−6 DPA. AA content increased in the liver and plasma, and decreased in the cerebral cortex during ALA supplementation. No significant change in other major fatty acids was observed during the time course study.

3.2.2. DHA content was higher in female liver and plasma

A significant effect of gender was observed at 8 weeks of age (Table 3): the DHA content in each phospholipid class was higher in the plasma and liver from female rats compared to males (+20% in liver EPG to +40% in liver PS). At 5 weeks of age, the DHA content

Table 4
Effect of gender on desaturation indexes in the liver of male and female rats

		Males			Females		
		3 weeks (n=6)	5 weeks (n=6)	8 weeks (n=6)	3 weeks (n=6)	5 weeks (n=6)	8 weeks (n=6)
Δ4 desaturation index							
n−3	EPG	8.4±0.6[a]	15.7±2.0[b]	15.1±1.6[b]	8.6±1.4[a]	20.9±2.4[b*]	25.1±2.7[b*]
	PC	7.4±0.5[a]	12.9±1.6[b]	15.1±0.9[b]	7.9±1.6[a]	16.9±2.2[b*]	20.1±1.4[b*]
	PS	6.6±0.9[a]	5.7±1.0[a]	13.5±2.1[b]	8.2±1.6[a*]	8.1±0.8[a*]	21.5±2.6[b*]
Δ5 desaturation index							
n−6	EPG	133.2±21[a]	68.5±21[b]	85.2±13[b]	139.1±12[a]	87.1±16[b]	188.9±41[a*]
	PC	70.2±6.6[a]	146.3±34[b]	134.9±27[b]	77.3±6.1[a]	160.6±43[b]	159.1±16[b]
	PS	18.3±3.0[a]	29.7±6.7[b]	32.7±4.7[b]	20.6±1.8[a]	34.6±5.8[b]	44.2±5.4[b*]
Δ6 desaturation index							
n−6	EPG	0.03±0.01[a]	0.07±0.03[a]	0.05±0.02[a]	0.04±0.01[a]	0.09±0.03[b]	0.05±0.02[ab]
	PC	0.04±0.01[ab]	0.05±0.01[a]	0.02±0.01[b]	0.05±0.02[a]	0.09±0.01[a]	0.05±0.01[a*]
	PS	0.02±0.01[a]	0.04±0.02[a]	0.02±0.01[a]	0.02±0.01[a]	0.04±0.01[b]	0.04±0.01[b*]
Δ9 desaturation index							
16:1n−7/16:0	PC	0.03±0.01[a]	0.04±0.01[b]	0.03±0.01[a]	0.03±0.01[a]	0.04±0.01[b]	0.03±0.01[a]
18:1n−9/18:0	PC	0.4±0.01[a]	0.5±0.06[a]	0.5±0.07[a]	0.4±0.03[a]	0.4±0.1[a]	0.3±0.05[a*]

Desaturation indexes were generated from fatty acid content in phospholipids. "Δ4" desaturation index represents the DHA/n−3 DPA ratio, Δ5 desaturation index represents the AA/20:3n−6 ratio, Δ6 desaturation index represents the 18:3n−6/LA ratio, and Δ9 desaturation index represents the 16:1n−7/16:0 and 18:1n−9/18:0 ratios.
Values are expressed as mean±SD (n=6).
The effect of age was significant (P<.01; two-way ANOVA) for "Δ4" desaturation index.
The effect of gender was significant (P<.01; two-way ANOVA) for "Δ4" desaturation index.
[a,b]Significantly different values within males or females from weaning to 8 weeks of age (P<.01).
[*] Significant differences between age-matched males and females (P<.01).

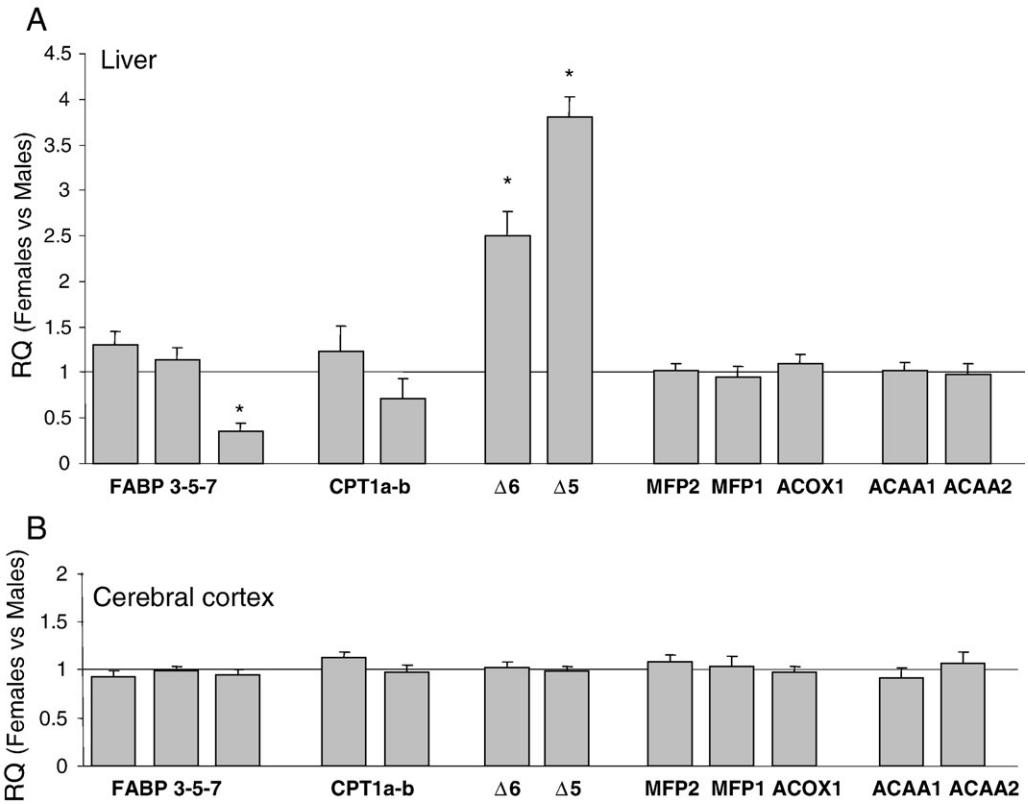

Fig. 2. Effect on gender on the mRNA expression of different genes involved in PUFA metabolism in the liver (A) and cerebral cortex (B) of 8-week-old male and female rats. Expression values of target genes are normalized to that of 18S mRNA and calibrated to that of 8-week-old males (RQ). Values are expressed as mean±SD (n=6). *Significant gender effect (P<.01).

was also higher (+10%) in female liver EPG. Gender did not affect the $n-6$ PUFA content. We considered the hypothesis that a higher DHA content in the female liver might impact on the brain where DHA is abundant. However, no effect of gender was observed in the DHA content of the cerebral cortex. No further difference in cerebral cortex fatty acids composition was observed between male and female rats.

3.3. Desaturation indexes in the liver were affected by gender

Gender significantly affected the desaturation indexes at 8 weeks of age: the "Δ4" desaturation index (DHA/$n-3$ DPA ratio) was higher (+30% in liver PC to +70% in liver EPG) in the female liver (Table 4). The Δ5 desaturation index (20:4$n-6$/20:3$n-6$ ratio) was greater in female liver EPG and PS (+120% and +35%, respectively). It was also higher in female liver PC, but the difference with males was not significant (P=.09). Similarly, the Δ6 desaturation index (18:3$n-6$/18:2$n-6$ ratio) was greater in female liver PC and PS (+150% and +100%, respectively). The Δ9 desaturation indexes (16:1$n-7$/16:0 and 18:1$n-9$/18:0 ratio) were not influenced by gender in EPG and PS (data not shown). In PC, the 18:1$n-9$/18:0 ratio was higher in the male liver.

In 5-week-old rats, the "Δ4" desaturation index was higher in female liver phospholipids (+32% in PC to +37% in PS), and no difference was noted in 3-week-old rats. No gender effect on desaturation indexes was observed in the cerebral cortex.

3.4. The mRNA expression of Δ5- and Δ6-desaturases was higher in the female liver

The mRNA expression of key genes involved in PUFA metabolism was analyzed in 8-week-old male and female rats. The main gender-related difference concerned the *FADS1* (Δ5-desaturase) and *FADS2* (Δ6-desaturase) genes: their mRNA expression was 3.8 and 2.5 times higher, respectively, in females than in males (Fig. 2A). Besides, the mRNA expression of fatty acid binding protein (FABP) 7 was 2.9 times lower in females than in males. The expression of peroxisome proliferator-activated receptor (PPAR) α was 1.8 times lower in females than in males (Fig. 3). No significant difference in other transcription factors was observed in the liver.

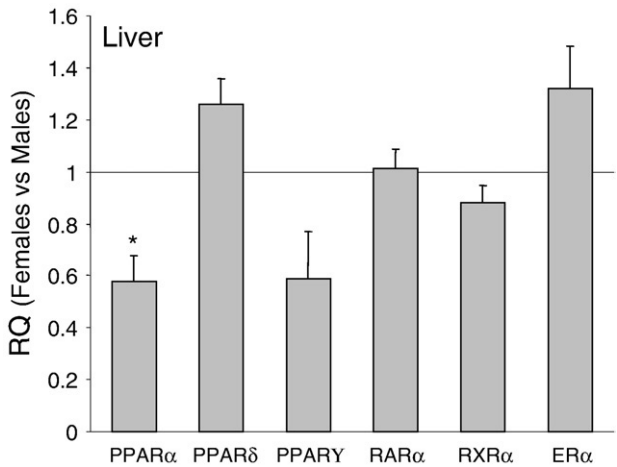

Fig. 3. Effect on gender on mRNA expression in different transcription factors in the liver of 8-week-old male and female rats. Expression values of target genes are normalized to that of 18S mRNA and calibrated to that of 8-week-old males (RQ). The mRNA expression of ERβ was not detected. Values are expressed as mean±SD (n=6). *Significant gender effect (P<.01).

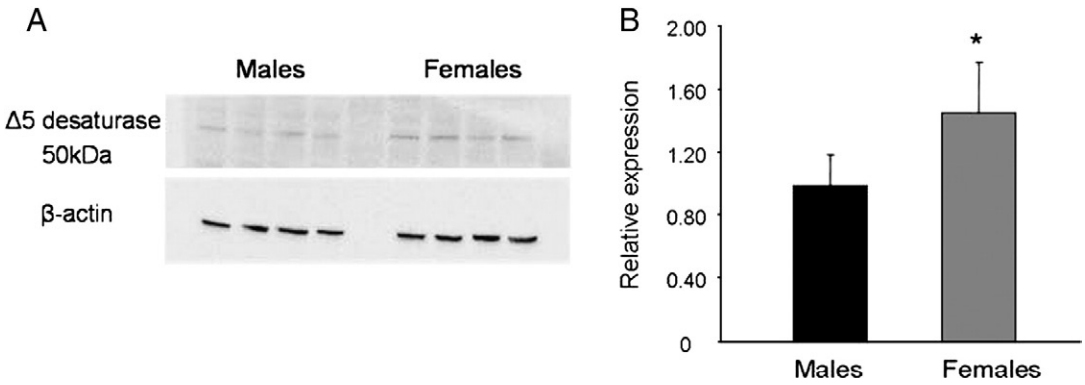

Fig. 4. Immunoreactivity of Δ5-desaturase in the liver of 8-week-old male and female rats. (A) Immunoblot of 50-kDa Δ5-desaturase. Four male and four female samples were assayed on the same gel, and this experimentation was repeated three times to have each sample in duplicate (n=6 for each group). (B) Normalization of the densitometry for 50-kDa Δ5-desaturase with β-actin. The normalized values for females are expressed as a percentage of the normalized values for males. Values are expressed as mean±SD (n=6 for each group). *Significant gender difference (nonparametric permutation test; StatXact 8 software), P<.02.

3.5. mRNA expression in the cerebral cortex was not affected by gender

The mRNA expression of key genes involved in PUFA metabolism in the cerebral cortex was not affected by gender (Fig. 2B), and neither was the mRNA expression of transcription factors in the cerebral cortex (data not shown).

3.6. Δ5 protein expression was higher in the female liver

Protein expression was determined in the liver of 8-week-old male and females rats. Gender significantly affected the immunoreactivity of Δ5-desaturase, indicating that the Δ5 protein content was higher (+50%) in female livers than in male livers (Fig. 4A and B).

With the "Δ4" index (i.e., the DHA/n−3 DPA ratio) being greater in female liver, we also focused on the peroxisomal system of β-oxidation, which is required for the terminal step of DHA formation. Fig. 5 shows the immunoreactivity of MFP2 in liver homogenates from 8-week-old rats. As described before [18], in addition to the full-length 79-kDa protein, two proteolytic products — the enoyl-CoA hydratase component of MFP2 (45 kDa) and the 3-hydroxyacyl-CoA dehydrogenase component (35 kDa) — are immunodecorated. No difference in MFP2 protein expression was detected between male and female rats.

4. Discussion

Human studies have shown gender-related differences in n−3 PUFA metabolism resulting in higher DHA circulating plasma concentration in women [20]. It has been suggested that women could have a greater capacity for ALA conversion into DHA, but the underlying mechanisms are unknown [21]. We have previously shown that estradiol modulates the conversion of ALA into long-chain derivatives in neuroblastoma cells, probably at the endoplasmic reticulum level [22]. Our present study aimed at comparing the capacity of DHA synthesis from ALA and the expression of related enzymes between male and female rats.

It has been previously shown in male rats that dietary n−3 deprivation up-regulates the activity of desaturases and elongases, and that this regulation occurs in the liver, not in the brain [23,24]. In our study, we compared the stimulation of the n−3 PUFA conversion pathway in the liver and brain of male and female rats born with a low-DHA status. For that purpose, mothers were deprived of n−3 PUFA from mating, and their pups were raised for 5 weeks on an n−3 PUFA repletion diet containing a suboptimal amount of ALA.

We found that males and females, which have the same low-DHA status at weaning, differently respond to this ALA supplementation. At the age of 8 weeks, the DHA content in each class of phospholipids was higher in the plasma and liver of females. DHA/n−3 DPA, the so-

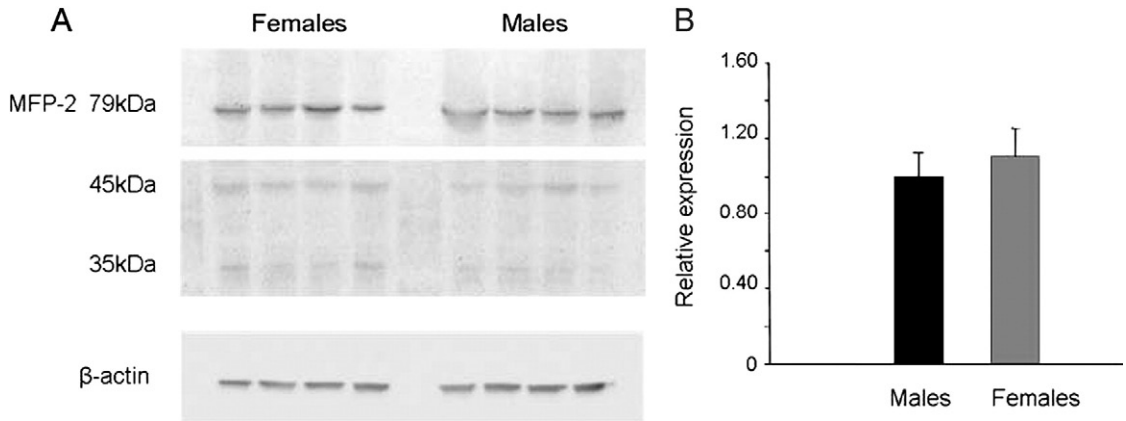

Fig. 5. Immunoreactivities of MFP2 in the liver of 8-week-old male and female rats. (A) Immunoblot of MFP2. Four male and four female samples were assayed on the same gel, and this experimentation was repeated three times to have each sample in duplicate (n=6 for each group). (B) Normalization of the densitometry for MFP2 (79 kDa) with β-actin. The normalized values for females are expressed as percentage of the normalized values for males. Values are expressed as mean±SD (n=6 for each group).

called "Δ4 desaturation" index, was also higher in females after 5 weeks of ALA supplementation, suggesting that DHA synthesis from *n*−3 DPA was more efficient in females and/or that *n*−3 DPA was more readily displaced from phospholipids by neoformed DHA. Our results support the data from a recent study showing that the DHA content in liver PC and EPG was higher in female rats than in male rats at the age of 15 weeks [15]. Lower *n*−3 DPA content and higher DHA content were also found in erythrocytes of women compared to men [25], supporting our proposal that the DHA/*n*−3 DPA ratio could be used as a metabolic marker for comparing gender-related differences in DHA synthesis.

Although the DHA content was higher in the female plasma, the male and female cerebral cortex DHA contents were similar. These data suggest that, due to the preferential uptake of plasma DHA through the blood–brain barrier, differences in circulating phospholipids between males and females were not large enough to alter the accretion of DHA in their cerebral cortex. Males were thus able to accumulate DHA into their cerebral phospholipids at the same level as that of females, or, from a reverse point of view, the additional production of DHA in the liver of sexually mature females was not particularly "intended" for the brain, but probably for storage and mobilization upon future pregnancy.

Several mechanisms have been proposed to explain gender-related differences in PUFA status. They include the partitioning of ALA between β-oxidation, adipose tissue storage and ALA conversion into DHA [12,26]. In the present study, we focused on the possible differential expression of converting enzymes. We compared their mRNA expression in males and females placed in a nutritional condition of metabolic stimulation. Our data show that the mRNA content of Δ5- and Δ6-desaturase genes (*FADS1* and *FADS2*) was significantly greater in the liver of females compared with males, suggesting an up-regulation of their transcription and/or a reinforcement of their stabilization. Our data also indicated that the Δ5-desaturase protein content in the liver was higher in females. One probable interpretation of our results is that, in the nutritional situation of *n*−3 PUFA replenishment, females have a greater liver capacity for DHA synthesis from ALA due to an accentuated stimulation of the desaturase expression. The higher desaturation indexes in the female liver support our hypothesis that differential regulation of desaturase expression takes account of the higher DHA status in females. Although desaturation activities were not investigated, our findings that the (*n*−3) "Δ4"desaturation index of liver phospholipid classes increased in males and females throughout the 5 weeks of ALA supplementation and that this index reached a higher value in females as soon as the age of 5 weeks give phenotype evidence that sexual dimorphism influenced the increasing capacity of liver to complete DHA synthesis. The (*n*−6) Δ5 and (*n*−6) Δ6 indexes were also significantly higher in 8-week-old females than in males, in EPG and PS for Δ5, and in PC and PS for Δ6. Differential regulation of phospholipid metabolism (at the level of acyltransferases and methyltransferases) may explain that the amplitude of changes in the Δ5 and Δ6 indexes varied within the different phospholipid classes. Moreover, these two indexes were drawn from the *n*−6 fatty acids, not from those of the *n*−3 series. Therefore, it may be supposed that gender concurrently influenced the capacity of the liver to synthesize the Δ5 and Δ6 desaturated derivatives of LA (18:3*n*−6 and AA). As a whole, the data on desaturation indexes and on Δ5- and Δ6-desaturase expression strongly suggest that up-regulation of desaturases in female liver takes account of the higher accretion of polyunsaturated derivatives in membrane phospholipids (i.e., DHA from ALA and, secondarily, 18:3*n*−6 and AA from LA).

We also examined the expression of enzymes in the final step of the PUFA conversion pathway. No difference in ACOX1, multifunctional protein 1 (MFP1) and MFP2 expressions was observed between males and females, suggesting that their transcription was not influenced by gender. Therefore, we suppose that the differences in the "Δ4" desaturation index reflect the Δ6 desaturation step rather than the β-oxidation step (see Fig. 1). The results on FABPs (similar hepatic levels of FABP3 and FABP5 in males and females, and lower mRNA expression of FABP7 in females) do not support the hypothesis that differential fatty acid transport and/or trafficking contributed to gender differences in DHA content.

Few studies have investigated the regulation of Δ5- and Δ6-desaturases by sex hormones [19]. Two studies showed that sex hormone treatment of female rats reduced Δ5 and Δ6 activities measured on *n*−6 PUFA in liver microsomes [27,28]. However, they investigated a short-term estradiol treatment (<2 days), and no study has compared the expression of Δ5- and Δ6-desaturases in the liver and brain of growing males and females. Mammalian Δ5- and Δ6-desaturases introduce a double bond into the carbon chain of fatty acids. They contain a common cytochrome-*b*5-like domain and two membrane-spanning domains, and their expressions in human tissues are quite similar [29]. Their *FADS1* and *FADS2* genes, respectively, are located close to each other on chromosome 11q12–q13.1, but in an opposite 5′-to-3′ orientation [29,30]. Their specific promoters have not yet been located, but their proximity might suggest a coordinately governed transcription [31]. Whether these promoters contain a response element to sex hormone has not been demonstrated. The differential recruitment of liver transcription factors [PPARs, retinoid X receptor (RXR) and sterol-regulatory element-binding protein 1c (SREBP1c)] in the sex-hormone-dependent stimulation of desaturase expression may also be hypothesized. Several studies have shown that PPARα expression in the rodent liver is higher in males than in females, and that this gender-related difference is organ-specific [32,33]. On the contrary, SREBP1c is more highly expressed in females than in males [34]. Since both SREBP1c and PPARα are putative modulators of the transcription of Δ5- and Δ6-desaturase [35], it may be supposed that sex hormones (estrogens and androgens) and their cognate receptors differentially interfere with SREBP1c and/or PPARα signaling in males and females. In the present study, we found that the mRNA expression of liver PPARα was reduced by 1.8-fold in 8-week-old females compared to age-matched males, indicating that a higher mRNA expression of desaturases in female liver compared to male livers occurred concurrently with a lower expression of PPARα. The opposite effects of gender on desaturases and PPARα suggest that complex cross-talks between PPARα signaling and other transcription factors that were not examined herein (SREBP1c, liver X receptor and so on) could underlie the effects of sex hormones. Male and female livers both expressed estrogen receptor (ER) α at the age of 8 weeks (not ERβ), suggesting that the ERα pathway is potentially susceptible to regulate the transcription of liver metabolism genes. However, the mRNA level of ERα was not significantly higher in the female liver, indicating that differential expression of desaturases in males and females was not directly related to difference in their mRNA levels of liver ERα.

In conclusion, the present findings clearly show that the DHA content in plasma and liver phospholipids is higher in female rats than in male rats, both raised on a diet containing a suboptimal amount of ALA. These gender-related differences have no consequences on the cerebral cortex DHA content. Our results on PUFA composition are in agreement with human studies that showed higher circulating DHA concentrations in women plasma. Our data strongly suggest that the up-regulation of Δ5- and Δ6-desaturase expression in the liver of sexually mature females contributes to their greater capacity for synthesizing DHA from dietary ALA. These results are consistent with the regulation of *n*−3 PUFA metabolism by sex hormones and imply that hormonal status and its evolution throughout life should be taken into account when nutritional recommendations are established.

Acknowledgments

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