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Original article

The retina is more susceptible than the brain and the liver to the incorporation of *trans* isomers of DHA in rats consuming *trans* isomers of alpha-linolenic acid

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Abstract – *Trans* polyunsaturated fatty acids are formed during heat treatments of vegetable oils from polyunsaturated fatty acids containing *cis* double bonds. After dietary intake, they are distributed in the body and are incorporated into nervous tissues including the retina. Since nervous tissues are known to be rich in n-3 fatty acids such as docosahexaenoic acid (DHA), we studied the ability of the retina and the brain to incorporate *trans* isomers of DHA formed in vivo from the dietary precursor *trans* α-linolenic acid. Wistar rats were fed with *trans* isomers of α-linolenic acid for 21 months. A linear incorporation of *trans* DHA and a decrease in *cis* DHA was observed in the retina, whereas no major changes were observed in the brain. In parallel to the modifications in retinal *cis* and *trans* DHA levels, the retinal functionality evaluated by the electroretinogram showed defects in animals that consumed *trans* α-linolenic acid. These results suggest that the mechanisms leading to the incorporation of *cis* and *trans* fatty acids are quite different in the retina when compared to the brain and the liver, the retina being more susceptible to changes in the dietary lipid contribution.

dietary *trans* polyunsaturated fatty acids / rat / retina / cerebral cortex / electroretinography

1. INTRODUCTION

The central nervous system including the retina mainly contains lipids rich in n-6 and n-3 polyunsaturated fatty acids (PUFA). Since these lipids are mostly phospholipids playing a structural role and not related to energy metabolism, they directly contribute to the functioning of neuronal membranes. Because they are

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mainly esterified with PUFA containing high numbers of double bonds, phospholipids facilitate membrane fusion mechanisms involved in neurotransmission but also membrane fluidity, which represents an important factor for an optimal isomerization of rhodopsin in the retinal photoreceptor outer segments. The most ubiquitous retinal n-3 PUFA is docosahexaenoic acid (DHA: 22:6 n-3) containing 6 double bonds [1, 2]. Its prevalence and constancy in the brain and the retina represents suggestive evidence of the important role it plays within neuronal cells including photoreceptors [3, 4]. This biochemical importance has already been confirmed by many studies showing that dietary n-3 fatty acid deficiency resulting in neuronal DHA depletion gives rise to a large range of defects in terms of cerebral serotoninergic and dopaminergic neurotransmissions [5–7], of retinal electrophysiologic response [8–11] and of visual acuity [12, 13]. These defects are effective even if the decrease in DHA is balanced by docosapentaenoic acid, a 22 carbon- and 5 double bond-PUFA from the n-6 family (DPA n-6; 22:5 n-6). Mechanistic evidences were provided to explain the retinal functional deficits observed in cases of n-3 deficiency and the lower efficiency of DHA replacement by DPA. Litman et al. showed that the rate of the first step of visual transduction (coupling of metarhodopsin II to the retinal G protein) was enhanced in DHA bilayers relative to less unsaturated phospholipids [14]. The same group went further by proving that the reduction of the phospholipid acyl chain unsaturation gives rise to a down-regulation of individual steps in retinal G protein-coupled receptor pathways as illustrated by a reduced rhodopsin activation, a reduced rhodopsin-transducin coupling, a reduced cGMP phosphodiesterase activity, and a slower formation of the metarhodopsin II-G protein complex [15].

Natural PUFA usually have their double bonds under the *cis* configuration. However, heat treatments of PUFA-rich vegetable oils such as deodorization or frying processes induce the isomerization of the *cis* double bond into a *trans* double bond [16–18]. As a consequence, *trans* PUFA such as *trans* isomers of α-linolenic acid (18:3 n-3) can be found in dietary oils [19–21] and various food products [22, 23]. After dietary ingestion, *trans* isomers of α-linolenic acid are converted into longer-chain *trans* PUFA by following the same elongation and desaturation enzymatic pathways as their natural isomer *cis* α-linolenic acid [24, 25] By these mechanisms, *trans* isomers of DHA could be detected in cerebral and retinal lipids of rodents who consumed a diet in which a part of α-linolenic acid was isomerized into *trans* isomers [25–27]. In addition to the formation of *trans* α-linolenic acid, a second consequence of the geometrical isomerization of *cis* α-linolenic acid in vegetable oils is the decrease of the bioavailability of *cis* α-linolenic acid itself. Hence, the dietary consumption of a diet in which a part of α-linolenic acid is isomerized into *trans* isomers may also lead to a smaller formation of longer-chain *cis* PUFA, particularly *cis* DHA. According to the crucial importance of DHA in neuronal membranes, a decrease in its bioavailability might have some functional consequences resembling those observed in chronic n-3 PUFA deficiencies.

In this paper, we report the comparative status of *cis* and *trans* isomers of DHA in hepatic, retinal and cerebral lipids of rats consuming for a very long time a diet in which a part of α-linolenic acid was isomerized into *trans* α-linolenic acid. For the first time, we are able to provide data that proves that the brain is more protected than the retina against the incorporation of *trans* DHA. We also document the consequences of *trans* DHA incorporation into the retina on its functionality evaluated by electroretinography.

2. MATERIALS AND METHODS

2.1. Animals and diets

All experiments were performed in accordance with the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research and with the French legislation (personal agreement number 21CAE086 and animal quarters agreement number A21 231010EA). The animals were housed in animal quarters under controlled temperature (21 ± 1 °C) and light conditions (12-h light/12-h dark cycle). The light intensity measured at various locations of the animal quarters was less than 20 lux. Two groups of weanling male Wistar rats (Janvier's breeding, Le Genest-St-Isle, France) were used. The animals were issued from dams fed with conventional diets deprived of *trans* fatty acids during gestation and lactation. At weaning, the rats were fed ad libitum for 21 months with two standard diets differing only in their fatty acid composition and particularly in *cis* and *trans* isomers of α-linolenic acid: (i) a control diet providing α-linolenic acid in its natural form ("*cis*" group); (ii) a diet in which a part of α-linolenic acid was isomerized into *trans* α-linolenic acid ("*trans*" group). The *trans* isomers of α-linolenic acid were a mixture of 9*cis*,12*cis*,15*trans* 18:3 n-3 (51% of total isomers), 9*trans*,12*cis*,15*cis* 18:3 n-3 (36% of total isomers), 9*trans*,12*cis*,15*trans* 18:3 n-3 (11% of total isomers), 9*cis*,12*trans*,15*trans* 18:3 n-3 (1% of total isomers) and 9*cis*,12*trans*,15*cis* 18:3 n-3 (1% of total isomers). The detailed composition of the diets is presented in Tables I and II.

The diets were prepared from oil mixtures kindly provided by Lesieur (Coudekerque-Branche, France). Briefly, bleached canola oil was used after deodorization for 52.5 h at 205 °C under 3 mbar [28] to provide *trans* α-linolenic acid. Isomerized sunflower oil (270 °C, 18 h) was added in the mixtures in order to balance the *trans* linoleic acid (18:2 n-6) levels. The fatty acid compositions of oil mixes and final diets were compared in order to avoid any fatty acid supply from non lipidic ingredients (particularly casein). No significant differences were observed (data not shown).

Table I. Composition of the diets.

Ingredient	Amount ($g.kg^{-1}$ diet)
Casein	180
Corn starch	460
Sucrose	230
Cellulose	20
Fat	50
Mineral mix[1]	50
Vitamin mix[2]	10

[1] Composition ($g.kg^{-1}$): sucrose, 110.7; $CaCO_3$, 240; K_2HPO_4, 215; $CaHPO_4$, 215; $MgSO_4,7H_2O$, 100; NaCl, 60; MgO, 40; $FeSO_4,7H_2O$, 8; $ZnSO_4,7H_2O$, 7; $MnSO_4,H_2O$, 2; $CuSO_4,5H_2O$, 1; $Na_2SiO_7,3H_2O$, 0.5; $AlK(SO_4)_2,12H_2O$, 0.2; K_2CrO_4, 0.15; NaF, 0.1; $NiSO_4,6H_2O$, 0.1; H_2BO_3, O.1; $CoSO_4,7H_2O$, 0.05; KIO_3, 0.04; $(NH_4)6Mo_7O_{24},4H_2O$, 0.02; LiCl, 0.015; Na_2SeO_3, 0.015; NH_4VO_3, 0.01.

[2] Composition ($g.kg^{-1}$): sucrose, 549.45; retinyl acetate, 1; cholecalciferol, 0.25; DL-a-tocopheryl acetate, 20; phylloquinone, 0.1; thiamin HCl, 1; riboflavin, 1; nicotinic acid, 5; calcium pantothenate, 2.5; pyridoxine HCl, 1; biotin, 1; folic acid, 0.2; cyanobalamin, 2.5; cholin HCl, 200; DL-methionin, 200; p-aminobenzoic acid, 5; inositol, 10.

2.2. Tissue collection and lipid analysis

The animals were sacrificed by decapitation. The liver was removed as well as the brain. A sample of the cerebral cortex was isolated from the frontal area. The eyes were enucleated and the retina was

Table II. Fatty acid composition of the dietary lipids (% of total fatty acid methyl esters).

	Experimental groups	
	"*cis*"	"*trans*"
16:0	4.5	4.4
16:1n-7	0.2	0.1
18:0	3.9	3.3
18:1 n-9	68.5	68.5
trans 18:1	0.7	0.7
18:2n-6	18.9	19.0
trans 18:2	0.2	0.3
18:3n-3	**2.0**	**1.3**
***trans* 18:3**	**0**	**0.7**
Total 18:3	**2.0**	**2.0**
20:0	0.3	0.3
20:1n-9	0.3	0.8
22:0	0.5	0.6

The *trans* isomers of α-linolenic acid were a mixture of 9*cis*,12*cis*,15*trans* 18:3 n-3 (51% of total isomers), 9*trans*,12*cis*,15*cis* 18:3 n-3 (36% of total isomers), 9*trans*,12*cis*,15*trans* 18:3 n-3 (11% of total isomers), 9*cis*,12*trans*,15*trans* 18:3 n-3 (1% of total isomers) and 9*cis*,12*trans*,15*cis* 18:3 n-3 (1% of total isomers).

removed. Samples were stored at −20 °C in a chloroform/methanol mixture (2:1, v:v) until analysis. Total lipids from the liver, the retina and the cerebral cortex were extracted according to the Folch procedure [29]. Total phospholipids were separated from neutral lipids by the method of Juanéda and Rocquelin [30] and were transesterified with sodium methylate according to Christie et al. [31]. The fatty acid methyl esters (FAME) were analysed on a Hewlett-Packard (Palo Alto, CA, USA) 5890 series II gas chromatograph equipped with a split/splitless injector, a flame ionization detector, and a BPX 70-silica capillary column (120 m × 0.5 mm i.d. film thickness 0.25 μm; SGE, Melbourne, Australia). The injector and the detector were maintained at 250 °C and 280 °C, respectively. Hydrogen was used as a carrier gas (inlet pressure 300 kPa). The oven temperature was fixed at 60 °C for 1 min, then increased from 60 to 175 °C at a rate of 20 °C.min^{-1} and left at this temperature until the end of the analysis. FAME were identified by comparison with commercial or synthetic standards and quantified using the DIAMIR software (JMBS Ins., Portage, MI, USA).

2.3. ERG measurement

The electroretinogram (ERG) was recorded in vivo before the beginning of dietary treatments and after 6, 12, 18 and 21 months of diet. The following ERG measurement procedures were adapted from those described by Doly et al. [32]. Prior to ERG recordings, the rats were dark-adapted for at least 3 h. All further procedures were carried out under dim red light (λ > 650 nm) and at a constant temperature of 25 °C. The animals were anaesthetized with an intramuscular injection of ketamine (120 mg.kg^{-1} body weight) and xylazine (6 mg.kg^{-1} body weight) in a saline solution. Their pupils were dilated with 0.5% tropicamide (Ciba Vision Ophthalmics, Blagnac, France). An irrigating solution (BSS, Alcon Laboratories, Rueil Malmaison, France) was used to prevent corneal desiccation. After 10 min, the corneal electrode was put in place. The ERG was recorded via the corneal electrode (thin silver wire with a 3 mm-ring end) and a reference electrode was placed on the rat's tongue. The retina was stimulated by a photostimulator (model PS33 PLUS, Grass Telefactor, Astro-Med Inc., West Warwick, RI, USA) delivering light flashes (white light, 6500 mcds/m^2) to the eye through fiber optics and a white sphere that mimics a Ganzfeld dome. One flash was delivered every minute and the average of ten individual ERG was considered as one measurement. The ERG

response was amplified using a low-pass filter setting of 1 Hz and a high-pass filter of 1000 Hz. After amplification, the signal was digitized and processed. The amplitudes were determined for each recording and were measured from the baseline (a-wave) or from the peak of the a-wave (b-wave).

2.4. Statistical analysis

The results are expressed as means ± standard deviation (SD). Statistical analyses were performed using the Statistical Analysis System (SAS Institute, Cary, NC, USA). The ANOVA procedure and the Newman-Keuls test were used between the two groups. *p* values lower than 0.05 were considered as significant.

3. RESULTS

3.1. Time-course of incorporation of *cis* and *trans* isomers of DHA in hepatic, retinal and cerebral phospholipids

No incorporation of *trans* DHA was observed in phospholipids from the brain, the liver and the retina of rats of the "*cis*" group even after 21 months of diet. By contrast, feeding animals with *trans* α-linolenic acid ("*trans*" group) led to the incorporation of *trans* DHA in hepatic, cerebral and retinal phospholipids in various proportions. The *trans* isomer of DHA that was found in tissues was the 19*trans* isomer.

As shown in Figure 1, the levels of *trans* DHA in retinal phospholipids were higher when compared to those of cerebral and hepatic phospholipids. At the beginning of the experiment and before any dietary treatment, no isomers of *trans* DHA were detected in the retina, in the brain and in the liver. By feeding the animals with *trans* isomers of α-linolenic acid, *trans* isomers of DHA were incorporated into the retina, the brain and the liver, which allowed their detection after 6 months of

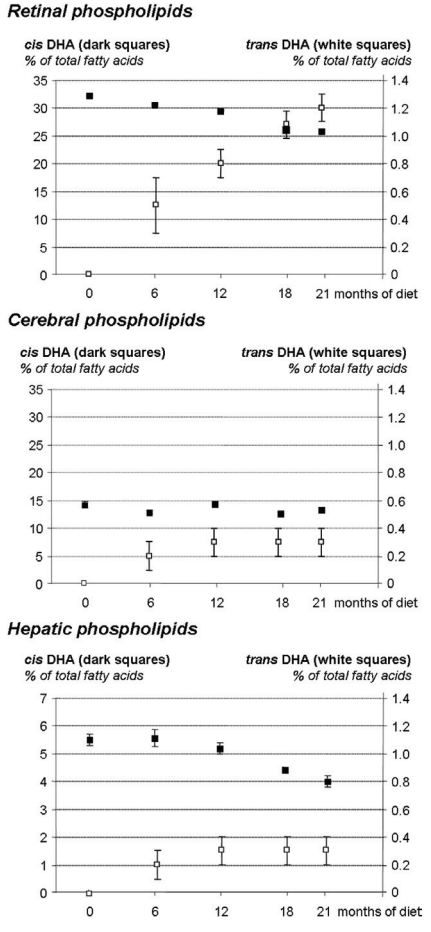

Figure 1. Time-course of incorporation of *cis* and *trans* isomers of DHA in retinal, cerebral and hepatic phospholipids of rats fed for 21 months with *cis* and *trans* isomers of 18:3 n-3 ($n = 6$). A linear incorporation of *trans* DHA and a decrease of *cis* DHA were observed in the retina and the liver whereas the cerebral phospholipids showed minor fluctuations in *cis* DHA. A low incorporation of *trans* DHA was observed in cerebral and hepatic phospholipids.

diet. The incorporation of *trans* DHA was higher in retinal phospholipids since *trans* DHA represented 0.5% of total fatty acids whereas it was only of 0.2% in the cerebral cortex and in the liver. In the retina, a time-dependent incorporation was observed and the levels of *trans* DHA represented 0.8% of total fatty acids after 12 months then 1.1% and 1.2% after 18 and 21 months of diet, respectively. The time-course of incorporation was quite different in the cerebral cortex and in the liver since the amounts of *trans* isomers of DHA were increased to 0.3% of total fatty acids after 12 months of diet and were maintained at this level until the end of the experiment.

Concomitantly to the increase of *trans* DHA in retinal phospholipids, *cis* DHA levels were decreased from 32.2% before the beginning of dietary treatment to 30.6%, 29.6%, 26.2% and 25.9% of total fatty acids after 6, 12, 18 and 21 months of diet, respectively. The same tendency was observed in the liver since the amounts of *cis* DHA were decreased from 5.5% (before nutritional intervention) to 4.0% of total fatty acids after 21 months of diet. In opposition to the retina and the liver, the levels of cerebral *cis* DHA remained constant during the whole experiment (around 14% of total fatty acids), with only some minor fluctuations.

3.2. PUFA composition of cerebral and retinal phospholipids after 21 months of diet

The detailed PUFA composition of retinal, cerebral and hepatic phospholipids after 21 months of diet is given in Table III.

As previously evoked in Figure 1, the amounts of *cis* DHA were significantly decreased in the retina of rats that consumed *trans* α-linolenic acid for 21 months when compared to the controls. Especially in this tissue, the amounts of all n-6 PUFA (except 18:2 n-6 because less represented) were increased in the animals belonging to the "*trans*" group. This was particularly true for DPA n-6 (22:5 n-6) whose level reached 2.4% of total fatty acids whereas it was only of 1.1% in the retina of animals from the "*cis*" group. Because of the concomitant decrease of the *cis* DHA level and the increase in n-6 PUFA in the "*trans*" group the retinal n-6/n-3 ratio was raised from 0.4 to 0.6 but without significantly influencing the retinal total saturated and monounsaturated fatty acid content. *Trans* isomers of PUFA were completely absent in the retina of animals belonging to the "*cis*" group. However, *trans* isomers of 18:3 n-3, 20:5 n-3 and DHA were detected in the retina of animals from the group that did consume *trans* α-linolenic acid. In this tissue, *trans* isomers of 18:3 n-3 and 20:5 n-3 were only present as traces whereas *trans* DHA levels reached 1.2% of total fatty acids. The variations in hepatic phospholipid composition were quite similar to those observed in the retina. In animals belonging to the "*trans*" group the amounts of *cis* DHA were also reduced when compared to the controls (4.0% versus 5.7% of total fatty acids in the "*cis*" group). However in this tissue, only DPA n-6 levels were increased within n-6 PUFA from 1.3% to 1.8% of total fatty acids. As observed in the retina, this was enough together with the decrease in *cis* DHA to raise the n-6/n-3 ratio from 5.5 to 7.2 but without significantly changing the total saturated and monounsaturated fatty acid content. Only, the *trans* DHA incorporation was very different in the liver when compared to the retina. In the animals consuming *trans* isomers of α-linolenic acid, *trans* DHA represented only 0.3% of total fatty acids in the liver whereas it represented 1.2% in retinal phospholipids. The cerebral cortex composition was quite different from the other tissues since dietary supplementation in *trans* α-linolenic did not induce variations in *cis* DHA levels and n-6 PUFA, except in DPA n-6 but without changing the n-6/n-3 ratio and the total

saturated and monounsaturated fatty acid level. As observed in the liver, the incorporation of *trans* DHA was very limited in animals from the "*trans*" group since its level was of 0.3% of total fatty acids.

3.3. Consequences on the retinal function evaluated by electroretinography

The repercussions of *trans* DHA incorporation on the retina were studied by measuring its electrical response. A representative set of ERG traces is presented in Figure 2a.

Before nutritional treatment, the ERG response of rats was normal with a negative a-wave and a positive b-wave. Oscillatory potentials (OP) were also generated by rods (high frequency, in the raising part of the b-wave) and cones (low frequency, on the top of the b-wave). After 6 months of diet, the ERG responses of animals from "*cis*" and "*trans*" groups were quite similar, particularly in terms of b-wave amplitude. However, the OP were reduced in both groups and particularly those generated by cones. From 12 months of diet, the b-wave amplitude was significantly reduced in animals from the "*trans*" group ($p < 0.05$) (Figs. 2a and 2b). The difference in the b-wave amplitude between the "*cis*" and "*trans*" groups was larger and more significant when increasing the diet duration. Moreover, some differences in the a-wave amplitude were detected after 18 months of diet and were confirmed after 21 months of diet (data not shown).

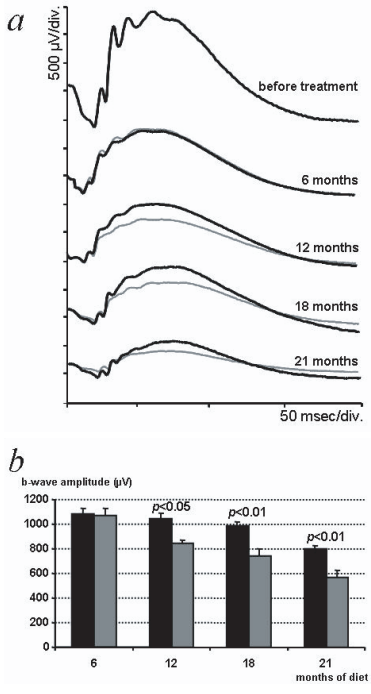

Figure 2. (a) Typical electroretinographic responses of rats fed for 21 months with *cis* isomers of 18:3 n-3 (black curves, n= 6) and *trans* isomers of 18:3 n-3 (grey curves, n = 6). The ERG was defective in animals fed with *trans* isomers of 18:3 n-3 by an alteration of the b-wave from 12 months of diet and of the a-wave from 18 months of diet. **(b)** b-wave amplitude of the ERG in rats fed for 21 months with *cis* isomers of 18:3 n-3 (black bars, n = 6) and *trans* isomers of 18:3 n-3 (grey bars, n = 6) showing a significant decrease in rats fed with *trans* isomers of 18:3 n-3 from 12 months of diet.

4. DISCUSSION

In this paper, we demonstrate that the isomerization of dietary α-linolenic acid leads to a significant incorporation of *trans* DHA in the retina but not in the brain and the liver. First, our results confirmed earlier findings showing that *trans* isomers of 18:3 n-3 can be converted into *trans* isomers of EPA and *trans* isomers of DHA and can be incorporated into neuronal tissues [25–27, 33]. The incorporation of *trans* DHA was noticeable in the retina since it could represent 1.2% of total fatty acids (4.4% of total DHA). Due to its very low level in the brain and in the retina, the amount of *trans* 18:3 n-3 loaded by these tissues directly from the

Table III. PUFA composition of phospholipids from the retina, the cerebral cortex and the liver of rats fed for 21 months with 2.0% of *cis* 18:3 n-3 ("*cis*" group) or 1.3% of *cis* 18:3 n-3 + 0.7% of *trans* 18:3 n-3 ("*trans*" group).

	Retinal phospholipids (n = 6)		Cerebral phospholipids (n = 6)		Hepatic phospholipids (n = 6)	
	"*cis*"	"*trans*"	"*cis*"	"*trans*"	"*cis*"	"*trans*"
18:2 n-6	0.5 ± 0.04	0.4 ± 0.06	0.2 ± 0.02	0.2 ± 0.02	5.5 ± 0.33	5.2 ± 0.18
20:4 n-6	9.7 ± 0.43*	10.9 ± 0.26*	10.4 ± 0.26	10.7 ± 0.12	24.0 ± 0.41	23.6 ± 0.97
22:4 n-6	2.1 ± 0.07*	3.1 ± 0.16*	3.2 ± 0.11	3.2 ± 0.06	0.3 ± 0.04	0.3 ± 0.05
22:5 n-6	**1.1 ± 0.16***	**2.4 ± 0.13***	**1.1 ± 0.08***	**1.5 ± 0.11***	**1.3 ± 0.02***	**1.8 ± 0.16***
18:3 n-3	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
20:5 n-3	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
22:6 n-3	**32.4 ± 1.49***	**25.9 ± 0.20***	**13.0 ± 0.22**	**13.1 ± 0.23**	**5.7 ± 0.50***	**4.0 ± 0.21***
trans 18:3 n-3	nd	< 0.1	nd	< 0.1	nd	< 0.1
trans 20:5 n-3	nd	< 0.1	nd	< 0.1	nd	< 0.1
trans 22:6 n-3	nd	1.2 ± 0.05	nd	0.3 ± 0.01	nd	0.3 ± 0.01
n-6/n-3	**0.4 ± 0.01***	**0.6 ± 0.01***	**1.1 ± 0.03**	**1.2 ± 0.04**	**5.5 ± 0.40***	**7.2 ± 0.24***
sat + mono	53.6 ± 2.08	55.7 ± 0.62	69.0 ± 1.31	68.1 ± 0.56	61.6 ± 1.49	63.8 ± 1.92

[1] Results are mean ± SD; nd, not detected; sat, total saturated fatty acids; mono, total monounsaturated fatty acids; * significantly different between "*cis*" and "*trans*" groups ($p < 0.05$). The *trans* isomer of DHA found in tissues was the 19*trans* isomer.

blood may be low, making it very improbable that *trans* DHA is biosynthesized in situ. Concerning *cis* fatty acids, even if some retinal and cerebral cells such as retinal pigment epithelium and brain astrocytes have the ability to produce DHA from 18:3 n-3 [34, 35], the liver is considered to be the primary site at which DHA is formed from dietary 18:3 n-3 for distribution to target organs via the circulation. Since the synthesis of *trans* PUFA follows the same enzymatic pathways as their natural isomers, we can suppose that *trans* DHA found in the cerebral cortex and in the retina is mostly formed in the liver and then incorporated into these tissues without omitting a lower part synthesized in situ from 22:5 n-3 [36]. However, since the hepatic levels of *trans* DHA did not exceed 0.3% of total fatty acids, we can suppose that the *trans* DHA formed in the liver is rapidly exported to peripheral tissues as demonstrated for *cis* DHA [37].

Considering this hypothesis and since the phospholipid compositions throughout the experiment showed that the time-course of incorporation of *trans* DHA was quite different in the retina when compared to the cerebral cortex, one explanation to these results can be the differential incorporation mechanisms of *trans* PUFA in these tissues. Studies have shown that DHA is found in high proportion in retinal and cerebral microvessels [38, 39], suggesting that the microvascular endothelium and particularly endothelial cells play an important role in the regulation of the PUFA supply to the retina and the brain. Although retinal endothelial and brain-derived endothelial cells are biochemically and structurally very similar, it was shown that they are not identical in their functioning. As an example, it is well known that, unlike the retinal microcirculation, the cerebral microcirculation is much less affected in cases of diabetes [40]. Related

to this, some differences were demonstrated in glucose uptake between retinal and brain-derived endothelial cells [41, 42]. A recent study from Grammas and Riden [43] proved that, compared to brain-derived endothelial cells, retinal endothelial cells have a lower stress related expression of tight-junction associated protein ZO-1 (zonula occludens-1), resulting in an increased permeability. The results obtained here represent one more evidence that the retina is more susceptible than the brain to nutrient uptake and especially to *trans* fatty acids. In the retina, the incorporation of *trans* DHA was linear from 0 to 21 months of diet, suggesting no major selectivity of retinal endothelial cells against *trans* PUFA. Unlike retinal phospholipids, the levels of *trans* DHA in phospholipids from the cerebral cortex rapidly reached their maximal value and did not exceed 0.3% of total fatty acids. However, further work is required to define the biochemical and molecular bases of this differential permeability.

A selective susceptibility of the retina when compared to the brain was also observed for other fatty acids since their compositions varied very differently. In the retina, the levels of the majority of n-6 and n-3 fatty acids were modified whereas only a slight variation in the levels of DPA n-6 was observed in the cerebral cortex. In the retina, lower dietary *cis* 18:3 n-3 led to a 20% decrease of the levels of DHA balanced by a 120% increase of DPA n-6. Other n-6 fatty acids, as 20:4 n-6 and 22:4 n-6 were also found in higher amounts in the retina of animals consuming *trans* α-linolenic acid. As illustrated by the n-6/n-3 ratio, the characteristic pattern of the retina of these animals is typical of a "deficiency-like" status, as previously described in many studies [8, 10]. Thus, we prove here that the very-long term consumption of a diet in which a part of dietary α-linolenic acid is isomerized can have specific consequences on retinal lipids resembling those observed in chronic deficiencies.

The consequences of chronic n-3 PUFA deficiencies on retinal function were already studied [8, 10]. In rats deprived from n-3 PUFA from 2 generations, the main finding concerning the retinal function was the decreased ERG a-wave and b-wave amplitudes. We already demonstrated that the isomerization of dietary α-linolenic acid decreased the ERG b-wave amplitude in rats [26, 44]. Considering the typical pattern of retinal fatty acids (as described above), the defects observed here in the retinal function may be mainly the consequence of the decreased dietary intake of *cis* α-linolenic acid and subsequently the lack of *cis* DHA. However, the *trans* α-linolenic acid contribution leading to the presence of *trans* DHA in the retina has to be taken into account regarding one study showing that the level of rhodopsin activation is reduced in the presence of *trans* fatty acid-rich phospholipids [45]. In our diets, the value represented by 0.7% of *trans* 18:3 n-3 and subsequently the decrease by 0.7% of dietary *cis* 18:3 n-3 due to its isomerization is a good approach of what can be found in the human diet in terms of the percentage of total fatty acids [46] and is very far from a chronic deficiency. This appears to be enough to induce a severe loss in ERG wave amplitudes close to what was observed in total n-3 PUFA deprivation. Although extrapolation from animal studies to human physiology is hazardous, more attention should be paid to the presence of *trans* fatty acids in the human diet.

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REFERENCES

[1] Connor WE, Neuringer M, Reisbick S. Essential fatty acids: the importance of n-3 fatty acids in the retina and brain. Nutr Rev 1992, 50: 21–29.

[2] Fliesler SJ, Anderson RE. Chemistry and metabolism of lipids in the vertebrate retina. Prog Lipid Res 1983, 22: 79–131.

[3] Crawford MA, Sinclair AJ. Nutritional influences in the evolution of the mammalian brain. In: CIBA Foundation Symposium on Lipids, malnutrition and the developing brain (Ed), Amsterdam, 1972, p 267–287.

[4] Sinclair AJ. Long chain polyunsaturated fatty acids in mammalian brain. Proc Nutr Soc 1975, 34: 287–291.

[5] Aid S, Vancassel S, Poumes-Ballihaut C, Chalon S, Guesnet P, Lavialle M. Effect of a diet-induced n-3 PUFA depletion on cholinergic parameters in the rat hippocampus. J Lipid Res 2003, 44: 1545–1551.

[6] Kodas E, Galineau L, Bodard S, Vancassel S, Guilloteau D, Besnard JC, Chalon S. Serotoninergic neurotransmission is affected by n-3 polyunsaturated fatty acids in the rat. J Neurochem 2004, 89: 695–702.

[7] Zimmer L, Vancassel S, Cantagrel S, Breton P, Delamanche S, Guilloteau D, Durand G, Chalon S. The dopamine mesocorticolimbic pathway is affected by deficiency in n-3 polyunsaturated fatty acids. Am J Clin Nutr 2002, 75: 662–667.

[8] Bourre JM, Francois M, Youyou A, Dumont O, Piciotti M, Pascal G, Durand G. The effects of dietary alpha-linolenic acid on the composition of nerve membranes, enzymatic activity, amplitude of electrophysiological parameters, resistance to poisons and performance of learning tasks in rats. J Nutr 1989, 119: 1880–1892.

[9] Watanabe I, Kato M, Aonuma H, Hashimoto A, Naito Y, Moriuchi A, Okuyama H. Effect of dietary alpha-linolenate/linoleate balance on the lipid composition and electroretinographic responses in rats. Adv Biosci 1987, 62: 563–570.

[10] Weisinger HS, Vingrys AJ, Sinclair AJ. Effect of dietary n-3 deficiency on the electroretinogram in the guinea pig. Ann Nutr Metab 1996, 40: 91–98.

[11] Weisinger HS, Vingrys AJ, Sinclair AJ. The effect of docosahexaenoic acid on the electroretinogram of the guinea pig. Lipids 1996, 31: 65–70.

[12] Neuringer M, Connor WE, Lin DS, Barstad L, Luck S. Biochemical and functional effects of prenatal and postnatal omega-3 fatty acid deficiency on retina and brain in rhesus monkey. Proc Natl Acad Sci USA 1986, 83: 4021–4025.

[13] Uauy R, Hoffman DR. Essential fatty acid requirements for normal eye and brain development. Semin Perinatal 1991, 15: 449–455.

[14] Litman BJ, Niu SL, Polozova A, Mitchell DC. The role of docosahexaenoic acid containing phospholipids in modulating G protein-coupled signaling pathways: visual transduction. J Mol Neurosci 2001, 16: 237–242.

[15] Niu SL, Mitchell DC, Lim SY, Wen ZM, Kim HY, Salem N Jr, Litman BJ. Reduced G protein-coupled signaling efficiency in retinal rod outer segments in response to n-3 fatty acid deficiency. J Biol Chem 2004, 279: 31098–31104.

[16] Ackman RG, Hooper SN. Linoleic acid artefacts from deodorization of oils. J Am Oil Chem Soc 1974, 51: 42–49.

[17] Grandgirard A, Sebedio JL, Fleury J. Geometrical isomerization of linoleic acid during heat treatment of vegetable oils. J Am Oil Chem Soc 1984, 61: 1563–1568.

[18] Sebedio JL, Grandgirard A, Prevost J. Linoleic acid isomers in heat-treated sunflower oils. J Am Oil Chem Soc 1988, 61: 362–366.

[19] O'Keefe SF, Wiley V, Gaskins S. Geometrical isomers of essential fatty acids in liquid infant formulas. Food Res Intern 1994, 27: 7–13.

[20] Sebedio JL, Grandgirard A, Septier C, Prevost J. État d'altération de quelques huiles de friture prélevées en restauration. Rev Fr Corps Gras 1987, 34: 15–18.

[21] Wolff RL. Trans-polyunsaturated fatty acids in French edible rapeseed and soybean oil. J Am Oil Chem Soc 1992, 69: 106–110.

[22] Chardigny JM, Wolff RL, Mager E, Bayard CC, Sebedio JL, Martine L, Ratnayake WMN. Fatty acid composition of French infant formulas with emphasis on the content and detailed profile of trans fatty acids. J Am Oil Chem Soc 1996, 73: 1595–1601.

[23] Ratnayake WMN, Hollywood R, O'Grady E. Fatty acids in Canadian margarines. Can Inst Sci Technol 1991, 24: 81–86.

[24] Chardigny JM, Sebedio JL, Grandgirard A, Martine L, Berdeaux O, Vatele JM. Identification of novel *trans* isomers of 20:5n-3 in liver lipids of rats fed a heated oil. Lipids 1996, 31: 165–168.

[25] Grandgirard A, Piconneaux A, Sebedio JL, O'Keefe SF, Semon E, Le Quere JL. Occurrence of geometrical isomers of eicosapentaenoic and docosahexaenoic acids in liver lipids of rats fed heated linseed oil. Lipids 1989, 24: 799–804.

[26] Chardigny JM, Bonhomme B, Sebedio JL, Juaneda P, Vatele JM, Doly M, Grandgirard A. Effects of dietary *trans* polyunsaturated fatty acids on the rat electroretinogram. Nutr Res 1998, 18: 1711–1721.

[27] Chardigny JM, Bron A, Sebedio JL, Juaneda P, Grandgirard A. Kinetics of incorporation of n-3 *trans* fatty acids in the retina. Nutr Res 1994, 14: 909–917.

[28] Henon G, Kemeny Z, Recseg K, Zwobada F, Kovari K. Deodorization of vegetable oils. Part 1: Modelling the geometrical isomerization of polyunsaturated fatty acids. J Am Oil Chem Soc 1999, 76: 73–81.

[29] Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 1957, 226: 497–509.

[30] Juaneda P, Rocquelin G. Rapid and convenient separation of phospholipids and non phosphorus lipids from rat heart using silica cartridges. Lipids 1985, 20: 40–41.

[31] Christie WW, Sebedio JL, Juaneda P. A practical guide to the analysis of conjugated linoleic acid. Inform 2001, 12: 147–152.

[32] Doly M, Droy-Lefaix MT, Braquet P. Retinal electrophysiology and retinotoxicity. In: Droy-Le Faix MT, Ferradini C, Christen Y (Eds), Advances in *Ginkgo biloba* Extract Research: *Ginkgo biloba* Extract (EGb 761) as a Free-Radical Scavenger, Paris, 1993, p 61–71.

[33] Grandgirard A, Bourre JM, Julliard F, Homayoun P, Dumont O, Piciotti M, Sebedio JL. Incorporation of *trans* long-chain n-3 polyunsaturated fatty acids in rat brain structures and retina. Lipids 1994, 29: 251–258.

[34] Moore SA, Yoder E, Murphy S, Dutton GR, Spector AA. Astrocytes, not neurons, produce docosahexaenoic acid (22:6 omega-3) and arachidonic acid (20:4 omega-6). J Neurochem 1991, 56: 518–524.

[35] Wang N, Anderson RE. Synthesis of docosahexaenoic acid by retina and retinal pigment epithelium. Biochemistry 1993, 32: 13703–13709.

[36] Pawlosky RJ, Ward G, Salem N Jr. Essential fatty acid uptake and metabolism in the developing rodent brain. Lipids. 1996, 31: S103–S107.

[37] Scott BL, Bazan NG. Membrane docosahexaenoate is supplied to the developing brain and retina by the liver. Proc Natl Acad Sci USA 1989, 86: 2903–2907.

[38] Benistant C, Dehouck MP, Fruchart JC, Cecchelli R, Lagarde M. Fatty acid composition of brain capillary endothelial cells: effect of the coculture with astrocytes. J Lipid Res 1995, 36: 2311–2319.

[39] Lecomte M, Paget C, Ruggiero D, Wiernsperger N, Lagarde M. Docosahexaenoic acid is a major n-3 polyunsaturated fatty acid in bovine retinal microvessels. J Neurochem 1996, 66: 2160–2167.

[40] Kern TS, Engerman RL. Capillary lesions develop in retina rather than cerebral cortex in diabetes and experimental galactosemia. Arch Ophthalmol 1996, 114: 306–310.

[41] Rajah TT, Olson AL, Grammas P. Differential glucose uptake in retina- and brain-derived endothelial cells. Microvasc Res 2001, 62: 236–242.

[42] Tang J, Zhu XW, Lust WD, Kern TS. Retina accumulates more glucose than does the embryologically similar cerebral cortex in diabetic rats. Diabetologia 2000, 43: 1417–1423.

[43] Grammas P, Riden M. Retinal endothelial cells are more susceptible to oxidative stress and increased permeability than brain-derived endothelial cells. Microvasc Res 2003, 65: 18–23.

[44] Acar N, Chardigny JM, Bonhomme B, Almanza S, Doly M, Sebedio JL. Long-term intake of *trans* (n-3) polyunsaturated fatty acids reduces the b-wave amplitude of electroretinograms in rats. J Nutr 2002, 132: 3151–3154.

[45] Niu SL, Mitchell DC, Litman BJ. Trans fatty acid derived phospholipids show increased membrane cholesterol and reduced receptor activation as compared to their cis analogs. Biochemistry. 2005, 44: 4458–4465.

[46] Wolff RL. Ubiquité et caractéristiques des isomères *trans* de l'acide linolénique. OCL 1995, 2: 391–400.