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# Dairy fat blends high in α-linolenic acid are superior to n-3 fatty-acid-enriched palm oil blends for increasing DHA levels in the brains of young rats

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**Abstract**

Achieving an appropriate docosahexaenoic acid (DHA) status in the neonatal brain is an important goal of neonatal nutrition. We evaluated how different dietary fat matrices improved DHA content in the brains of both male and female rats. Forty rats of each gender were born from dams fed over gestation and lactation with a low α-linolenic acid (ALA) diet (0.4% of fatty acids) and subjected for 6 weeks after weaning to a palm oil blend-based diet (10% by weight) that provided either 1.5% ALA or 1.5% ALA and 0.12% DHA with 0.4% arachidonic acid or to an anhydrous dairy fat blend that provided 1.5% or 2.3% ALA. Fatty acids in the plasma, red blood cells (RBCs) and whole brain were determined by gas chromatography. The 1.5% ALA dairy fat was superior to both the 1.5% ALA palm oil blends for increasing brain DHA (14.4% increase, $P<.05$), and the 2.3% ALA dairy blend exhibited a further increase that could be ascribed to both an ALA increase and n-6/n-3 ratio decrease. Females had significantly higher brain DHA due to a gender-to-diet interaction, with dairy fats attenuating the gender effect. Brain DHA was predicted with a better accuracy by some plasma and RBC fatty acids when used in combination ($R^2$ of 0.6) than when used individually ($R^2=0.47$ for RBC n-3 docosapentaenoic acid at best). In conclusion, dairy fat blends enriched with ALA appear to be an interesting strategy for achieving optimal DHA levels in the brain of postweaning rats. Human applications are worth considering.


*Keywords:* Dairy fat; Palm oil; α-Linolenic acid; Brain; Docosahexaenoic acid; Rat neonates

## 1. Introduction

Docosahexaenoic acid (DHA; 22:6n-3) and arachidonic acid (ARA; 20:4n-6) are highly concentrated in mammalian nervous and visual systems [1]. There is an increased demand for n-3 long-chain polyunsaturated fatty acids (LC-PUFA), particularly DHA, to support optimal visual and cognitive development in infants during fetal life and newborn nursing [2–10]. Linoleic acid (LA; 18:2n-6) and α-linolenic acid (ALA; 18:3n-3) are the precursors of long-chain n-6 and n-3 fatty acids, respectively. Although they can be synthesized from their respective precursor fatty acids [11–14] and although it has been shown in rats that supplementation of mothers with ALA or DHA leads to the same LC-PUFA accretion in maternal, fetal and newborn brains [15], synthesis (especially DHA synthesis) could be insufficient to cover growth needs [16–19]. Therefore, it may be necessary to increase the dietary intake of DHA and/or increase the synthetic capacity for metabolizing ALA to DHA in mothers and newborns.

Infant formulas have been gradually replacing mother's milk for more than 50 years and, at least in Europe and the United States, are usually prepared with vegetable oils. The compositions of these formulas are controlled in terms of fat composition for most fatty acids and especially essential fatty acids (European Economical Community rules) [20]. Furthermore, in a recent attempt to mimic the composition of mother's milk as close as possible [21–23], long chain n-3 and n-6 fatty acids (DHA and ARA) have been added.

Throughout the ages, infant formulas have been prepared with dairy fat, which, to some extent, is less different from breast milk for some components that are not present in vegetable fat formulas (i.e., cholesterol and short-chain FA) [24]. For example, the short- or medium-chain fatty acids in milk fat are more efficiently absorbed and might be beneficial for health [25]. However, the

*Abbreviations:* DHA, docosahexaenoic acid; ARA, arachidonic acid, ALA: alpha-linolenic acid, LA: linoleic acid, EPA: eicosapentaenoic acid, DPA: docosapentaenoic acid, PUFA: polyunsaturated fatty acids, VLC-PUFA: very-long-chain polyunsaturated fatty acids; ELOVL2, elongase of long-chain fatty acids 2; FADS1, fatty acid desaturase 1; FADS2, fatty acid desaturase 2; RBC, red blood cell; FA, fatty acid; GC, gas chromatography; PCA, principal component analysis; OPLS-DA, partial least-squares discriminant analysis; VIP, variable importance index; OPLS, orthogonal partial least-squares.

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use of dairy fat for infant formulas is still a matter of debate in various countries [26,27].

In an attempt to validate the potential replacement of vegetable fats with dairy fat in infant formulas, we used the rat as a nutritional model to compare the effects of blends based on dairy fat instead of palm oil, which provide the same quantities of essential fatty acids, on brain fatty acids. The levels of ALA and LA in these experimental diets followed the recommended and commonly used values in most commercial vegetable fat formulas. For this purpose, sunflower and rapeseed oils were added to maintain the levels of ALA (1.5%) and LA (16%) and the n-6/n-3 ratio within the recommended values [28,29].

Because human milk contains DHA (0.2%–0.4%) and ARA (0.4%–0.8%), it has been proposed that formulas that replace breast-feeding should be supplemented with these long-chain n-3 fatty acids. Therefore, we also compared the previous dairy and palm blends to a classical ALA-enriched (1.5%) palm blend with DHA and ARA levels similar to those used in infant formulas (0.12% and 0.40% of fatty acids).

Finally, we evaluated the potential of a dairy fat blend enriched in ALA (2.3%) by increasing only the rapeseed oil component, such that the increase of ALA could be associated with a reduction in the n-6/n-3 ratio (5 instead of 10), which is now recommended and considered to be beneficial for facilitating an increase in the specific bioconversion to long-chain n-3 and DHA. Each of these modifications agrees with the recommended European Economical Community rules.

In the present work, we compared the effects of different butter-ALA-enriched blends and palm-ALA regular blends (with or without supplementation with long-chain n-3) on the restoration of the fatty acid profiles of brains from ALA-deficient postweaning rats. We also determined the potential of rat plasma and red blood cell (RBC) FAs, which are also reasonably accessible in human newborns, to predict brain DHA levels.

## 2. Materials and methods

### 2.1. Animals and experimental design

The protocol was conducted following the Guidelines for the Care and Use of Experimental Animals and was approved by the local ethics committee. Rats were housed in our animal facility under controlled conditions for light (lights on from 8:00 a.m.–8:00 p.m.), temperature (22°C±1°C) and hygrometry (55%–60%). They received a semisynthetic diet (Table 1) and water *ad libitum*. The restoration of n-3 fatty acids was studied using different blends of lipids in the first generation of postweaning rats (males and females) deficient in n-3 and born from ALA-poor dams. For this purpose, female Wistar rats (6 weeks of age fed with a chow diet) were fed a 5% fat (w/w) semisynthetic diet for 6 weeks before mating and during gestation and lactation with reduced n-3 fatty acid levels (ALA 0.4% of fatty acids) obtained with a palm/soya (97/3) oil blend. They were mated for a period of 7 days (one male+one female per cage). After weaning for 4 weeks, young pups (females and males, $n$=10 each) from ALA-deficient dams received a 10% fat (w/w) diet for 6 weeks. Two groups received diets including rapeseed and sunflower oils to provide identical ALA (1.5% of fatty acids) and LA (13% to 16% of fatty acids) levels, respectively, blended with palm oil (P1) or butter (B1) made from summer milk. Group P2 received a palm diet similar to P1 with supplementation of DHA and ARA (0.12% and 0.4% of FA, respectively). Group B2 received a butter diet similar to B1 that included an increased proportion of rapeseed oil to provide higher ALA levels (2.3% of fatty acids). Butter was provided by Lactalis; sunflower, soya and rapeseed oils were provided by Lesieur; palm oil was provided by Van de Moorteele; DHA (from refined fish oil) was provided as ROPUFA by DSM Nutritional Products; ARA (from fungi) was provided as ARASCO by Martek; and the diet preparations were performed by SAFE (France). Body weights of pups were measured each week. Food intake was monitored during the last 2 weeks of the postweaning period.

Table 1
Dietary fatty acid compositions (g/100 g diet)

| Fatty acids | Diet 10% lip | Diet 10% lip | Diet 10% lip | Diet 10% lip |
|---|---|---|---|---|
| | P1 | P2 | B1 | B2 |
| | Palm blend ALA1.5% | Palm blend ALA1.5% +DHA ARA | Butter blend ALA 1.5% | Butter blend ALA 2.3% |
| 14:0 | 0.08 | 0.08 | 0.75 | 0.7 |
| 16:0 | 3.62 | 3.62 | 1.99 | 1.87 |
| 18:0 | 0.41 | 0.41 | 0.96 | 0.9 |
| 18:1 | 3.84 | 3.84 | 2.66 | 2.94 |
| 18:2n-6 | 1.6 | 1.6 | 1.39 | 1.33 |
| 18:3n-3 | 0.16 | 0.16 | 0.15 | 0.23 |
| 18:2n-6/18:3n-3 | 10.27 | 10.27 | 9.09 | 5.71 |
| SFA | 4.17 | 4.17 | 4.1 | 3.85 |
| SMC[a] | 0 | 0 | 0.61 | 0.57 |
| 22:6n-3 | | 0.012 | | |
| 20:4n-6 | | 0.04 | | |

P1: palm/rapeseed/sunflower (75/10/15); P2: palm/rapeseed/sunflower (75/10/15)+DHA+ARA; B1: butter fat/rapeseed/sunflower (72/10/18); B2: butter fat/rapeseed/-sunflower (67/19/15).
SFA, saturated fatty acids; SMC, short–medium chain.
[a] SMC includes C4:0, C6:0, C8:0, C10:0 and C12:0.

### 2.2. Dietary fatty acid compositions

The compositions of the diets and the n-3 fatty acid contents of the dietary fats are provided in Table 1. The semisynthetic diets, provided by SAFE (F-89290 Augy), contain lipids (10%), casein (22%), cornstarch (41%), sucrose (20%), cellulose (2%), mineral '205B SAFE' (4%), vitamin '200 SAFE' (1%) and methionine (DL) (0.16%).

### 2.3. Tissue collection

Young pups (10 weeks old) were killed by decapitation between 8:00 a.m. and 11:00 a.m. in a nonfasting state. The blood was collected on heparin (10 IU/ml) cooled on ice, and plasma was separated by 20-min centrifugation (3500 t/min at 4°C) from RBCs that were rinsed with saline solution (NaCl 0.9%). The brain was promptly removed, placed on an ice-cooled watch glass, rinsed with cold saline solution (NaCl 0.9%) and weighed. Tissues were immediately immersed in liquid nitrogen. The plasma, RBC and brains were stored at −80°C for later analysis.

### 2.4. Lipid extraction and fatty acid derivatization into methyl esters

#### 2.4.1. Brain lipid extraction

Half of one brain was weighed and lyophilized. The samples were then pulverized using a motorized potter for homogenization. A dry weight corresponding to 65 mg of fresh tissue was rehydrated to 80% and extracted with 1.5 ml of hexane/isopropanol (3/2, v/v) using a protocol from Schwarz et al. [30]. After centrifugation (9500*g*, 10 min, 4°C), the upper phase (hexane) was recovered and evaporated to dryness under nitrogen. The dry residue was stored under a nitrogen atmosphere at −80°C before fatty acid derivatization.

#### 2.4.2. RBC lipid extraction

RBC lipids were extracted with 1.5 ml of hexane/isopropanol (3/2, v/v) using a protocol from Schwarz et al. [30]. After centrifugation (9500g, 10 min, 4°C), the upper phase (hexane) was recovered and evaporated to dryness under nitrogen. The dry residue was stored under a nitrogen atmosphere at −80°C before derivatization of the component fatty acids into methyl esters.

#### 2.4.3. Derivatization in fatty acid methyl esters

Stock solutions of the reagents used in the method of Lepage and Roy [31] were prepared just prior to each experiment. For each brain and RBC lipid extraction sample, 1.9 ml of stock solution (1.8 ml of methanol and 100 µl of acetyl chloride) was freshly prepared and added to the dry residue. For plasma, 250 µl of plasma and 1.9 ml of the stock solution were combined in screw-capped glass tubes. The tubes were capped and heated at 100°C for 60 min. The tubes were allowed to cool at room temperature. Hexane (1 ml) was added to the brain and RBC samples (distilled water was added elsewhere), and the tubes were vortexed for 30 s. The upper organic phase was collected with a Pasteur pipette. This extraction procedure was repeated to optimize lipid extraction. The combined hexane solutions were dried under a stream of nitrogen, and the dry residue was then redissolved in 80 µl (RBC sample), 100 µl (plasma sample) or 200 µl (brain sample) of hexane, transferred to gas chromatography (GC) vials and capped under nitrogen.

#### 2.4.4. Fatty acid analysis by fast GC

The analysis by fast GC was performed on a 0.5-µl sample injected in split mode with a hydrogen flow rate of 10 ml/min. The column was a capillary column (BP×70, 10 m×0.1 mm ID×0.2 µm film thickness) (SGE International Pty. Ltd., Australia). The temperature program was as follows: initial, 60°C with a 0.5-min hold; ramp, 20°C/min to 200°C, 7°C/min to 225°C with a 1-min hold and then 160°C/min to 250°C with a 1-min hold. The instrumental conditions were as follows: The carrier gas was $H_2$ at a flow rate of 61.4 cm/s and a constant head pressure of 206.8 kPa; flame ionization detector was set at 280°C; the air and nitrogen make-up gas flow rates were 450 ml/min and 45 ml/min, respectively; the injector split ratio was 200:1; the

detector sampling frequency was 50 Hz; the autosampler injections had a volume of 0.5 μl; and the run time for a single sample was 13.23 min with a sample injection-to-injection time of 16 min.

*2.4.5. RNA extraction and gene expression*

Total RNA from the liver was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The RNA concentration was determined spectrophotometrically. For DNA synthesis, reverse transcription was performed with Superscript II (Invitrogen) according to the manufacturer's protocol. Real-time quantitative polymerase chain reaction was performed on cDNA, as described previously, using Stratagene Mx 3005P (Stratagene, Cedar Creek, TX, USA) and the SYBR Green Master mix kit (Eurogentec, Philadelphia, PA, USA). The values were expressed as ratios of RNA levels relative to one control rat (diets with a 1.5% ALA palm blend) using ΔΔ(Ct) in duplicate [32]. The mRNA levels of Δ5 desaturase (NM_053445), Δ6 desaturase (NM_031344) and elongase 2 (NM_001109118) were measured (see primer sequences in supplemental Table 1).

*2.5. Statistical analysis*

All multivariate data analyses and modeling were performed using Simca-P12 software (Umetrics, Umeå, Sweden). Analysis of variance (ANOVA) was executed using Statview (SAS Institute Inc., Cary, NC, USA). Principal component analysis (PCA) was used to study the model overview and trends. In this study, multicriteria assessment [33] for multivariate statistics was used to identify fatty acid biomarkers across groups. To further study the specific effects of gender and diet on the brain, RBCs and plasma fatty acids, an orthogonal projection to latent structures discriminant analysis (OPLS-DA) was used. An orthogonal calculation filters out variations in the *X*-variables that are uncorrelated to the *Y*-variable. This makes the orthogonally treated data more precise and easier to interpret. The S-plot method that combines the contribution/-covariance [p1] and the reliability/correlation [p(corr)1] from the OPLS-DA model was used to select statistically significant fatty acids based on the differences between groups [34]. The loading plot with jack-knifed confidence intervals (99% CIs) displayed the uncertainty of each variable, and the smaller span of the CI gives more credibility to the selected variable. The variables with jack-knifed CIs across zero were excluded [33]. The variable importance coefficient (VIP) value reflecting the influence of each fatty acid on the classification was used as an additional selection criterion (VIP value≥1). The variables meeting all three criteria (i.e., VIP>1, |pCorr(1)|>0.50 and the exclusion of zero from the span of jack-knifed CIs) were selected as potential biomarkers across the different groups.

The changes in brain fatty acids after the dietary challenge were additionally explored with Cytoscape 2.7 [35], which visualizes the complex interactions among fatty acids. The brain fatty acids in the rats were also displayed using a heat map generated by PermutMatrix 1.9.3 (http://www.lirmm.fr/~caraux/PermutMatrix/).

The relationship between RBC and plasma fatty acids and brain DHA and the identification of the brain DHA biomarkers were analyzed by the orthogonal partial least-squares (OPLS) regression method.

Whenever necessary, an ANOVA and multiple regression analysis were performed using Statview (SAS Institute Inc., Cary, NC, USA). A $\alpha$-risk of 0.05 was the significance statistical threshold used for these tests. Statistically relevant results and uncertainty estimates obtained with OPLS were also calculated using the jack-knife method (a resampling method) with 99% CIs [36]. Finally, we also used a multiblock OPLS procedure [37] to determine the influence of diet or gender on the fatty acid profile in the brain, RBC or plasma. A model validation that checks for overfitting is presented in the supplemental material.

## 3. Results

At the end of the postweaning period, the bodyweights did not differ in the male groups (372±35 g, 405±41 g, 379±26 g and 360±60 g for P1, P2, B1 and B2, respectively) or in the female groups (227±17 g, 229±17 g, 246±13 g and 240±40 g for P1, P2, B1 and B2, respectively). In addition, the food intake was similar across dietary treatments, as monitored during the last 2 weeks of the postweaning period (for male groups: 22.6±2.3, 21.3±1, 22.2±1.9 and 22.8±1.3 g/d; for female groups: 17±1, 18±2, 18±1 and 18±1 g/d for P1, P2, B1 and B2, respectively).

### 3.1. Model overview and trends

The list of the fatty acids analyzed in plasma, RBCs and brain along with the statistical significance with regard to the dietary effect of the gender is shown in three tables (Tables 2, 3 and 4). Since it is not easy to interpret the data and to restitute the underlying information from such complex set of data, PCA was used as an unsupervised statistical method to study the differences in brain, plasma and RBC fatty acids in male and female rats fed with four different diets. Such method allowed to reduce the tissue fatty acid profiles of the rats shown in the tables to one data point in the plots, with the axis representing the percent of explained variance as principal components, The diet and gender effects can be decomposed along the first and second components, respectively (Fig. 1). The butter-based-diet-fed rats clustered along the right side of the first component, whereas the palm-oil-based-diet-fed rats clustered on the left side. Similarly, females were virtually all located above the shaft, while males were below. The diet effect appeared more substantial (34% of the variance) than the gender effect (20%) in explaining the differences in the fatty acid profiles in various tissues. Hierarchical principal component analysis on the fatty acid profiles in the brain, RBCs and

Table 2
Values (% total fatty acids) of plasma fatty acids among female and male rats fed with P1, P2, B1 and B2 diets

| | Plasma fatty acids | | | | *P* value | | |
|---|---|---|---|---|---|---|---|
| | P1 | P2 | B1 | B2 | Diet effect | Gender effect | Diet effect×gender effect |
| 14:0 | 0.661±0.050$^{c}$ | 0.743±0.063$^{c}$ | 2.104±0.107$^{b}$ | 2.524±0.131$^{a}$ | <.001 | .002 | .0408 |
| 16:0 | 24.898±0.794$^{a}$ | 24.919±0.554$^{a}$ | 22.759±0.489$^{b}$ | 21.007±0.456$^{c}$ | <.001 | <.001 | .0547 |
| 17:0 | 0.142±0.004$^{b}$ | 0.150±0.007$^{b}$ | 0.286±0.013$^{a}$ | 0.307±0.007$^{a}$ | <.001 | <.001 | .0002 |
| 18:0 | 10.391±0.751 | 8.833±0.744 | 10.499±0.746 | 10.146±0.509 | .0001 | <.001 | .0008 |
| 16:1n-9 | 0.403±0.022$^{b}$ | 0.355±0.025$^{c}$ | 0.491±0.025$^{a}$ | 0.441±0.019$^{a,b}$ | <.001 | <.001 | .1285 |
| 16:1n-7 | 2.811±0.343$^{b}$ | 2.829±0.375$^{b}$ | 4.069±0.427$^{a}$ | 3.225±0.247$^{a,b}$ | <.001 | .0005 | .0269 |
| 18:1n-9 | 23.756±0.737$^{c}$ | 27.515±0.956$^{a}$ | 24.337±0.678$^{b}$ | 26.233±0.635$^{a,b}$ | <.001 | <.001 | .0193 |
| 18:1n-7 | 2.363±0.282 | 2.545±0.293 | 2.929±0.416 | 2.315±0.228 | .0246 | <.001 | .0002 |
| 20:1 | 0.470±0.026$^{b}$ | 0.557±0.035$^{a}$ | 0.578±0.032$^{a}$ | 0.621±0.027$^{a}$ | .0006 | <.001 | .1251 |
| 24:1 n-9 | 0.285±0.023$^{a}$ | 0.235±0.029$^{a,b}$ | 0.189±0.017$^{b}$ | 0.237±0.019$^{a,b}$ | .0065 | <.001 | .3796 |
| 18: 2 n-6 | 13.159±0.176$^{b}$ | 13.373±0.265$^{b}$ | 13.603±0.420$^{b}$ | 15.116±0.414$^{a}$ | <.001 | .0104 | .0465 |
| 18: 3 n-6 | 0.243±0.015$^{a}$ | 0.194±0.011$^{b}$ | 0.223±0.015$^{a,b}$ | 0.263±0.024$^{a}$ | .0266 | <.001 | .0128 |
| 20: 3 n-6 | 0.503±0.017$^{c}$ | 0.418±0.018$^{b}$ | 0.618±0.021$^{a}$ | 0.617±0.029$^{a}$ | <.001 | .1544 | .437 |
| 20: 4 n-6 | 15.653±1.017$^{a}$ | 13.191±1.086$^{a,b}$ | 12.539±0.856$^{b}$ | 11.465±0.756$^{b}$ | .0002 | <.001 | .1163 |
| 22: 4 n-6 | 0.193±0.011$^{a}$ | 0.171±0.008$^{a,b}$ | 0.159±0.006$^{b}$ | 0.133±0.007$^{c}$ | <.001 | <.001 | .2972 |
| 22: 5 n-6 | 0.544±0.022$^{a}$ | 0.428±0.018$^{b}$ | 0.444±0.036$^{b}$ | 0.168±0.010$^{c}$ | <.001 | .9233 | .0014 |
| 18: 3 n-3 | 0.409±0.023$^{c}$ | 0.511±0.021$^{b}$ | 0.562±0.036$^{b}$ | 0.977±0.043$^{a}$ | <.001 | .0007 | .1507 |
| 20: 5 n-3 | 0.151±0.011$^{c}$ | 0.151±0.014$^{c}$ | 0.286±0.025$^{b}$ | 0.574±0.050$^{a}$ | <.001 | <.001 | .0005 |
| 22: 5 n-3 | 0.168±0.006$^{c}$ | 0.175±0.007$^{c}$ | 0.208±0.011$^{b}$ | 0.323±0.017$^{a}$ | <.001 | .0002 | .1662 |
| 22: 6 n-3 | 2.005±0.100$^{b}$ | 2.038±0.106$^{a,b}$ | 2.190±0.085$^{a,b}$ | 2.304±0.093$^{a}$ | .0012 | <.001 | .501 |

Results are mean±S.D. of all detected plasma fatty acids ($n$=16 rats per group). Values in the same column that do not share a common superscript$^{a-d}$ are significantly different at $P$<.05 (one-way ANOVA followed by Bonferroni post hoc test).
P1: 1.5%-ALA palm blend diet; P2: 0.12% DHA/0.4% ARA-supplemented 1.5%-ALA palm blend diet; B1: 1.5%-ALA butter blend diet; B2: 2.3%-ALA butter blend diet. $N$=16 rats per group.

Table 3
Values (% total fatty acids) of RBC fatty acids among female and male rats fed with P1, P2, B1 and B2 diets

| | RBC fatty acids | | | | *P* value | | |
|---|---|---|---|---|---|---|---|
| | P1 | P2 | B1 | B2 | Diet effect | Gender effect | Diet effect*gender effect |
| 14:0 | 0.150±0.010$^{c}$ | 0.147±0.009$^{c}$ | 0.363±0.010$^{b}$ | 0.415±0.014$^{a}$ | <.001 | <.001 | .1249 |
| 16:0 | 25.602±0.636$^{a}$ | 25.795±0.566$^{a}$ | 22.906±0.504$^{b}$ | 22.838±0.421$^{b}$ | <.001 | <.001 | <.001 |
| 17:0 | 0.224±0.003$^{c}$ | 0.222±0.007$^{c}$ | 0.373±0.010$^{b}$ | 0.422±0.012$^{a}$ | <.001 | .0214 | .0106 |
| 18:0 | 16.369±0.700 | 15.998±0.693 | 17.610±0.639 | 16.794±0.656 | <.001 | <.001 | .6877 |
| 16:1n-9 | 0.172±0.004$^{c}$ | 0.157±0.006$^{d}$ | 0.213±0.005$^{b}$ | 0.244±0.005$^{a}$ | <.001 | <.001 | .0009 |
| 16:1n-7 | 0.235±0.017$^{b}$ | 0.265±0.024$^{b}$ | 0.425±0.034$^{a}$ | 0.388±0.023$^{a}$ | <.001 | <.001 | .0208 |
| 18:1n-9 | 7.348±0.132$^{c}$ | 7.922±0.168$^{b}$ | 7.908±0.082$^{b}$ | 8.553±0.142$^{a}$ | <.001 | .0056 | .1392 |
| 18:1n-7 | 2.127±0.111 | 2.273±0.126 | 2.455±0.126 | 2.432±0.113 | <.001 | <.001 | .1264 |
| 20:1 | 0.245±0.020 | 0.251±0.005 | 0.237±0.006 | 0.260±0.009 | .2812 | <.001 | .059 |
| 24:1 n-9 | 1.294±0.080$^{a}$ | 1.196±0.068$^{a}$ | 0.862±0.018$^{b}$ | 1.003±0.012$^{b}$ | <.001 | <.001 | <.001 |
| 18: 2 n-6 | 6.403±0.070$^{d}$ | 6.828±0.186$^{c}$ | 7.336±0.139$^{b}$ | 8.366±0.159$^{a}$ | <.001 | .1959 | .2099 |
| 18: 3 n-6 | 0.050±0.002$^{b}$ | 0.049±0.002$^{b}$ | 0.062±0.002$^{a}$ | 0.067±0.002$^{a}$ | <.001 | .1705 | .7454 |
| 20: 3 n-6 | 0.478±0.018$^{b}$ | 0.524±0.019$^{b}$ | 0.744±0.031$^{a}$ | 0.811±0.026$^{a}$ | <.001 | <.001 | .0006 |
| 20: 4 n-6 | 27.851±0.334$^{a}$ | 27.367±0.239$^{a,b}$ | 26.886±0.263$^{b}$ | 25.313±0.254$^{c}$ | <.001 | .0001 | .106 |
| 22: 4 n-6 | 1.876±0.051$^{a}$ | 1.806±0.040$^{a}$ | 1.803±0.029$^{a}$ | 1.328±0.048$^{b}$ | <.001 | .0098 | .0174 |
| 22: 5 n-6 | 1.343±0.041$^{a}$ | 1.115±0.032$^{b}$ | 1.274±0.051$^{a}$ | 0.637±0.029$^{c}$ | <.001 | .0018 | <.001 |
| 18: 3 n-3 | 0.037±0.001$^{c}$ | 0.043±0.002$^{b}$ | 0.044±0.001$^{b}$ | 0.083±0.002$^{a}$ | <.001 | .0003 | .7029 |
| 20: 5 n-3 | 0.109±0.005$^{c}$ | 0.117±0.005$^{c}$ | 0.221±0.007$^{b}$ | 0.489±0.026$^{a}$ | <.001 | <.001 | <.001 |
| 22: 5 n-3 | 0.769±0.014$^{c}$ | 0.793±0.023$^{c}$ | 1.011±0.023$^{b}$ | 1.590±0.040$^{a}$ | <.001 | <.001 | .0016 |
| 22: 6 n-3 | 3.314±0.045$^{c}$ | 3.521±0.068$^{b}$ | 3.543±0.046$^{b}$ | 3.852±0.092$^{a}$ | <.001 | .7586 | .3846 |

Results are mean±S.D. of all detected RBC fatty acids ($n$=16 rats per group). Values in the same column that do not share a common superscript$^{a-d}$ are significantly different at $P$<.05 (one-way ANOVA followed by Bonferroni post hoc test).
P1: 1.5%-ALA palm blend diet; P2: 0.12% DHA/0.4% ARA-supplemented 1.5%-ALA palm blend diet; B1: 1.5%-ALA butter blend diet; B2: 2.3%-ALA butter blend diet. $N$=16 rats per group.

plasma indicated that the brain is less sensitive to the diet and gender effects (supplemental data, Fig. 1).

### 3.2. Gender effects analysis

To further emphasize the differences between the females and the males regardless of diet, a supervised OPLS-DA procedure was used. This is an extension of PCA that allows the testing of whether individuals belong to a predefined class (gender) based on their biological signatures (their fatty acid profiles) and the determination of which individual variables (the individual fatty acids in the profile) drives class assignment. Fig. 2A emphasizes the gender differences in the brain, plasma and RBC. The first component discriminates the intergroup differences (gender), and the second orthogonal component determines the intragroup variation. The female group can be clearly separated from the male group based on the first component, and validation tests indicated that no overfitting occurred (see supplemental data). The discriminant analysis indicated that the difference between males and females depended on specific fatty acid changes that enabled class assignment (e.g., male or female) for most rats (Fig. 2A). Statistically significant fatty acids based on the differences between males and females were selected by crossing the visual results of the S-plot (Fig. 2B), the loading $P$ values of each fatty acid with statistical levels determined by the jack-knifed 99% CIs (Fig. 2C) and the variable importance values determined by the SIMCA software (VIP values>1 with CIs over 95%

Table 4
Brain fatty acids values (% total fatty acids) among female and male rats fed with the P1, P2, B1 and B2 diets

| | Brain fatty acids | | | | *P* value | | |
|---|---|---|---|---|---|---|---|
| | P1 | P2 | B1 | B2 | Diet effect | Gender effect | Diet effect*gender effect |
| 14:0 | 0.118±0.002$^{b}$ | 0.122±0.002$^{a,b}$ | 0.127±0.002$^{a}$ | 0.128±0.003$^{a}$ | <.001 | .1975 | .6109 |
| 16:0 | 18.655±0.150 | 18.577±0.135 | 18.794±0.193 | 18.741±0.140 | .779 | .1002 | .8065 |
| 17:0 | 0.143±0.002$^{b}$ | 0.148±0.003$^{b}$ | 0.168±0.002$^{a}$ | 0.170±0.002$^{a}$ | <.001 | .9796 | .0637 |
| 18:0 | 20.658±0.209 | 20.708±0.082 | 20.997±0.115 | 20.875±0.097 | .1193 | .0197 | <.001 |
| 16:1n-9 | 0.134±0.002$^{b,c}$ | 0.131±0.002$^{c}$ | 0.136±0.002$^{a,b}$ | 0.140±0.002$^{a}$ | .0038 | .0075 | .1928 |
| 16:1n-7 | 0.315±0.004$^{b}$ | 0.332±0.007$^{b}$ | 0.353±0.007$^{a}$ | 0.350±0.007$^{a}$ | <.001 | <.001 | .6052 |
| 18:1n-9 | 16.146±0.130$^{a,b}$ | 16.407±0.153$^{a}$ | 15.896±0.231$^{b}$ | 16.272±0.171$^{a,b}$ | .216 | .0797 | .1745 |
| 18:1n-7 | 3.610±0.050$^{a,b}$ | 3.706±0.033$^{a}$ | 3.709±0.034$^{a}$ | 3.561±0.041$^{b}$ | .0222 | .6667 | .3049 |
| 20:1 | 2.210±0.110$^{a,b}$ | 2.409±0.082$^{a}$ | 2.100±0.074$^{b}$ | 2.111±0.067$^{b}$ | .0249 | .3434 | .0315 |
| 24:1 n-9 | 2.178±0.091$^{a}$ | 2.242±0.061$^{a}$ | 1.921±0.076$^{b}$ | 1.874±0.057$^{b}$ | .0008 | .5679 | .5466 |
| 18: 2 n-6 | 0.492±0.009$^{c}$ | 0.532±0.012$^{b}$ | 0.541±0.011$^{b}$ | 0.585±0.017$^{a}$ | <.001 | <.001 | .4403 |
| 18: 3 n-6 | 0.038±0.002$^{b}$ | 0.040±0.001$^{a,b}$ | 0.042±0.001$^{a}$ | 0.043±0.001$^{a}$ | .0552 | .3583 | .1025 |
| 20: 3 n-6 | 0.351±0.012$^{b}$ | 0.368±0.010$^{a,b}$ | 0.392±0.008$^{a}$ | 0.387±0.008$^{a}$ | .0052 | .0001 | .9562 |
| 20: 4 n-6 | 9.951±0.108 | 9.727±0.062 | 9.898±0.110 | 9.962±0.068 | .1031 | .0989 | .0047 |
| 22: 4 n-6 | 3.506±0.031$^{a}$ | 3.386±0.039$^{b}$ | 3.350±0.022$^{b}$ | 3.235±0.034$^{c}$ | <.001 | .4233 | .363 |
| 22: 5 n-6 | 2.577±0.071$^{a}$ | 2.128±0.064$^{b}$ | 2.222±0.064$^{b}$ | 1.473±0.046$^{c}$ | <.001 | .1579 | <.001 |
| 18 :3n-3 | Tr | Tr | Tr | Tr | | | |
| 20: 5 n-3 | 0.002±0.000 | 0.005±0.000$^{c}$ | 0.006±0.000$^{b}$ | 0.010±0.001$^{a}$ | <.001 | .011 | .1689 |
| 22: 5 n-3 | 0.089±0.003$^{c}$ | 0.092±0.0042$^{c}$ | 0.103±0.002$^{b}$ | 0.134±0.002$^{a}$ | <.001 | .0036 | .7054 |
| 22: 6 n-3 | 11.755±0.221$^{d}$ | 12.896±0.136$^{c}$ | 13.445±0.145$^{b}$ | 14.167±0.125$^{a}$ | <.001 | .0252 | <.001 |

Results are mean±S.D. of all detected brain fatty acids ($N$=16 rats per group). Values in the same column that do not share a common superscript$^{a-d}$ are significantly different at $P$<.05 (one-way ANOVA followed by Bonferroni post hoc test). ALA values are not reported since this fatty acid is present in only trace amount in the brain and cannot be confidently compared among dietary treatments.
P1: 1.5%-ALA palm blend diet; P2: 0.12% DHA/0.4% ARA-supplemented 1.5%-ALA palm blend diet; B1: 1.5%-ALA butter blend diet; B2: 2.3%-ALA butter blend diet. $N$=16 rats per group.
Tr, trace amount.

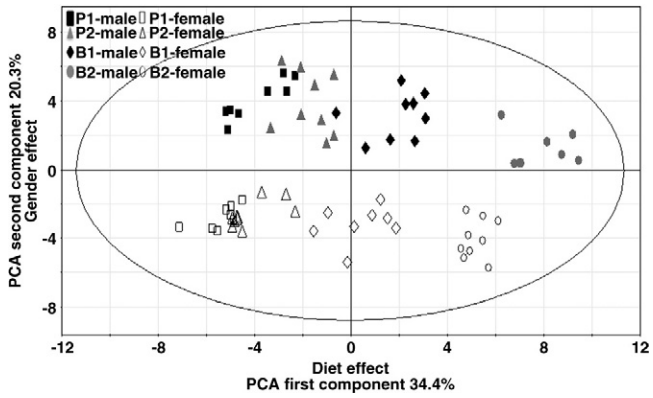


Fig. 1. PCA score plots derived from the fatty acid data in the brain, plasma and RBC of rats. Seven principal components were calculated by cross-validation, explaining 79.7% of the total variance. The first two principal component displayed in the reported PCA plot explained 54.9% of the variance. P1, palm oil blend diet with the ALA level 1.5% of FA; P2, palm oil blend with an ALA level 1.5% of FA and supplementation with DHA (0.12% of total fatty acids) and ARA (0.4% of total fatty acids); B1, butter blended with rapeseed oil to bring ALA content to 1.5% of total fatty acids; B2, butter blended with rapeseed oil to bring ALA content to 2.3% of total fatty acids. $N$=20 rats per group.

after jack-knifing). In Fig. 2B, the relevant variables satisfying our selection criteria are marked in a square and were considered to be the biomarker fatty acids distinguishing males and females. The corresponding univariate statistical $P$ values and the fatty acids content are shown in supplemental Table 2. Compared to male rats, female rats have increased 18:0 (stearic acid, +36.8% in RBCs and 72.4% in plasma), 20:4n-6 (ARA, 57.7% in plasma) and 22:6n-3 (DHA, 35.6% increase in plasma) and, conversely, decreased 16:0 (palmitic acid, 15.2% in RBCs and 13.8% plasma) and delta-9 desaturated fatty acids (16:1n-7, palmitoleic, 51.5% in plasma; 18:1n-9, oleic acid, 16.2% in plasma; and 18:1n-7, *cis*-vaccenic acid, 32.7% in plasma and 62.2% in RBCs). It is interesting to note that no differences were observed for brain DHA between males and females when similar diet were compared (Fig. 2B and 2C).

### 3.3. Analysis of dietary effects

In addition to gender, the dietary component of the fatty acid changes across various tissues was examined by a similar OPLS-DA modeling technique (Fig. 3). Significant dietary group differences were observed in both female and male rats, allowing a 100% confidence on dietary group assignment among the rats (Fisher's $P$ value of $4.8\times10^{-21}$). The distances among the four dietary groups in the two-dimensional plot allowed the evaluation of dietary effects on the fatty acid profiles of various organs. For instance, compared with the 1.5%-ALA palm blend diet (P1), the DHA- and ARA-supplemented 1.5%-ALA palm blend diet (P2) induced the fewest fatty acid changes than the two anhydrous butter diets (B1 and B2), with the 2.3%-ALA butter blend diet (B2) inducing the greatest variation. Furthermore, the fatty acid signatures of the rats that were fed the two kinds of anhydrous butter (B1 and B2) could be distinguished from the rats that had received the palm-oil-based diets with or without DHA and ARA (P2 and P1) by their first principal components. Moreover, a DHA gradient in the brain was observed, with lower values observed in the P1 (11.8% of total fatty acids) and P2 diets (12.9%), higher values seen in the B1 diet (13.4%) and the highest values seen in B2 diet (14.2%) (Table 3).

We then performed pairwise OPLS-DA comparisons to pinpoint the main dietary effects on the fatty acid profiles. In each of the following OPLS-DA plots, the first component represents the dietary effect, and the second orthogonal component represents the gender effect. Validation tests for all the following models were performed and found no overfitting (see Fig. 3 of supplemental data).

The 1.5%-ALA butter-blend-diet-fed (B1) rats could be differentiated from the corresponding 1.5%-ALA palm-blend-diet-fed (P1) rats based on the first component (Fig. 4A) (Fisher's $P$ value=$1.8\times10^{-9}$ and cross validation (CV)-ANOVA $P$ value=$1.6\times10^{-14}$). Interestingly, the distance between males and females in B1 was substantially smaller than in P1, indicating a lower gender effect in B1-diet-fed rats than in P1-diet-fed rats ($P$<.05, statistical levels displayed in the supplemental Fig. 2). The two diets induced differences in the tissue fatty acids in the first component that enabled 100% class assignment (Fig. 4A) (Fisher's $P$ value=$1.8*10^{-9}$ and CV-ANOVA $P$ value=$1.6*10^{-14}$). The S-plot procedure identified eight fatty acids that were highly responsive to the dietary challenge ($P$ values and comparative content summarized in supplemental Table 3) (Fig. 4B). Compared to 1.5%-ALA palm blend diet, the 1.5%-ALA butter blend diet increased ($P$<.0001) the brain DHA by 14.4% ($P$<.0001) with a commensurate 13.8% decrease of n-6 brain docosapentaenoic acid (DPA) ($P$<.0001) and increased the RBCs DPA n-3 by 31.3%. The butter-based diet also increased myristic acid compared to the palm oil blend (218.2% and 140.0% in the plasma and RBCs, respectively).

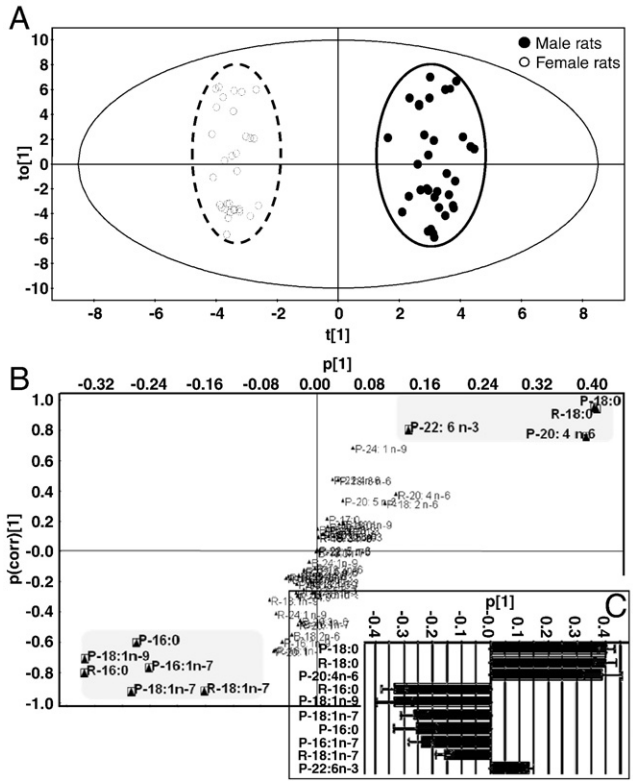


Fig. 2. Strategy for the identification of relevant fatty acids, showing differences between females and males. OPLS-DA model calculated from fast GC fatty acids data; the $R^2Y$ (explained class variance) and $Q^2Y$ (class prediction ability) were 0.95 and 0.946, respectively. (A) Two-dimensional OPLS-DA score plot showing group discriminations according to gender effects; the percentage of variation of the fatty acids (R2X) explained by each component $t$ is indicated. Each rat was correctly assigned as either female or male, according to its fatty acid profile in brain (B), plasma (P) and RBC (R) (100% of correct class assignment, $P$=$2.9\times10^{-19}$). (B) S-plots of the first component. Discriminating fatty acids (variable importance in projection coefficients with significant value >1) are marked in a square. (C) The fatty acids weight coefficients $p$ marked with a square in panel (B) were significant as determined with the jack-knife CI error bars. Lower-left square of the plot: fatty acids increasing in male rats and decreasing in female rats; upper-right square of the plot: fatty acids increasing in female rats and decreasing in male rats.

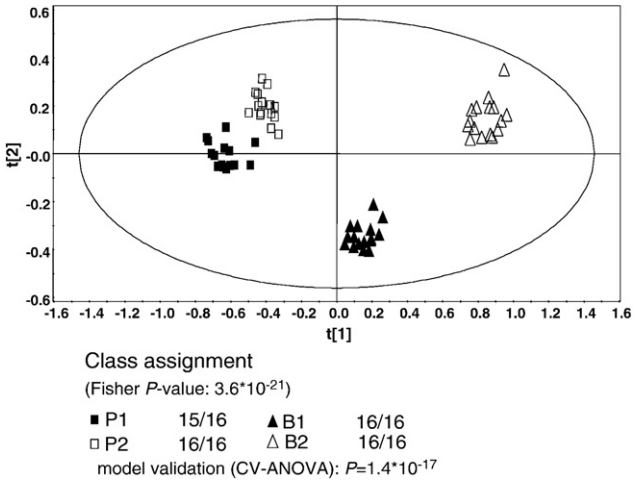


Fig. 3. Characterization of the brain, plasma and RBCs fatty acids changes induced by the dietary challenges. Two-dimensional OPLS-DA score plot showing group discrimination according to the dietary treatments; percentage of variance of the fatty acid variables ($R^2X$) explained by each component *t* is indicated. The group assignment for individual rats was calculated using three significant components to explain 84.5% of the fatty acid variations. The model allowed 100% confidence on dietary group assignment among the rats (Fisher's *P* value of $4.8\times10^{-21}$). P1: 1.5%-ALA palm blend diet; P2: 0.12% DHA/0.4% ARA-supplemented 1.5%-ALA palm blend diet; B1: 1.5%-ALA butter blend diet; B2: 2.3%-ALA butter blend diet. *N*=16 rats per group.

The OPLS-DA model clearly separated the 1.5% butter blend diet (B1) from the 2.5%-ALA butter blend diet (B2) (Fig. 5A) (Fisher's *P* value=$8.6\times10^{-10}$ and CV-ANOVA *P* value=$6.9467\times10^{-11}$). The S-plot (Fig. 5B) shows the extent to which each fatty acid is either over- or underrepresented in the rats fed with the two anhydrous butter diets (B1 and B2). The fatty acids in the bottom-left area were increased, and the ones in the upper-right area were decreased by the anhydrous butter diet with greater ALA levels (B2). Twelve fatty acids appeared to be significantly affected and are summarized in supplementary Table 4. For instance, compared to the 1.5%-ALA butter blend diet (B1), the 2.3%-ALA butter blend diet (B2) increased the brain DHA by 5.4% (*P*<.0001), along with eicosapentaenoic acid (EPA) in plasma (100.7%) and RBC (121.3%) (*P*<.0001), and decreased the n-6 DPA in the brain (33.7%), RBCs (50.0%) and plasma (62.2%) (*P*<.0001).

The distances between the rats fed the palm oil diet augmented with DHA and ARA (P2) and the rats fed the palm oil blend diet without DHA and ARA (P1) were significantly different (Fig. 6A) (Fisher's *P* value=$1.8\times10^{-9}$ and CV-ANOVA ""*P* value=$1.6\times10^{-14}$). In the S-plot, the fatty acids in the bottom-left area of Fig. 6B were decreased, and the ones in the upper-right area were increased by the P2 diet. Four fatty acids appeared to be especially modified by the dietary treatments (Fig. 6B and supplementary Table 5). Namely, the 1.5%-ALA palm blend supplemented with DHA and ARA increased brain DHA by 9.7% and plasma 18:1n-9 by 15.8%. In turn, this was accompanied by a decrease in both RBCs and plasma DPA n-6 (17.4% and 17%, *P*<.0001, respectively) despite the increased uptake of the parent ARA.

To analyze the relationships among the various fatty acids in the brain after our dietary treatments, we performed a pairwise correlation analysis of the brain fatty acids and displayed the correlation matrix as an interactive relationship network (Fig. 7A). This was transformed into a heat map showing the content of each of the fatty acid displayed in the network across the dietary treatments (Fig. 7B). Under our stringent conditions (Pearson correlation coefficient≥0.5), 18 of the 19 fatty acids demonstrated strong pairwise relationships (Fig. 7A). In the brain, 22:6n-3 (DHA) and 24:1n-9 correlated with the greatest number of other fatty acids (seven neighbors each with three in common). Thus, they appeared to be important hubs in the determination of the relative fatty acids balance in the brain. They were also found to be negatively associated (Fig. 7A and 7B). Interestingly, 17:0, a marker of milk fat intake that is increased by present dairy-based diets (Fig. 7B), was also found to be positively associated with all long-chain n-3 PUFAs. In that instance, the DHA content in the brain was significantly increased by both butter diets compared to the palm oil diets. Furthermore, other n-3 PUFAs, such as 22:5n-3 (DPAn-3) and 20:5n-3 (EPA), were positively correlated with DHA and negatively correlated with very-long-chain n-6 fatty acids [primarily DPA (22:5-6) and secondarily DTA (22:4n-6)]. Both 24:1n-9 and 22:5n-6 were decreased in the butter diets compared to the palm diets.

### 3.4. Prediction of brain DHA by the fatty acids in RBC and plasma

Next, we intended to use the fatty acid profiles in RBCs and plasma to predict brain DHA levels under our dietary conditions. Using an OPLS regression, we selected the most predictive fatty acids in RBCs and plasma to explain brain DHA content (VIP coefficient of the OPLS procedure >1 at a 95% CI). These were RBCs DPAn-3, EPA, 20:3n-6, ALA, DPAn-6 and LA, and plasma ALA, EPA, DPAn-3 and DPAn-6. The 10 predictive fatty acid values were then reintroduced into a second

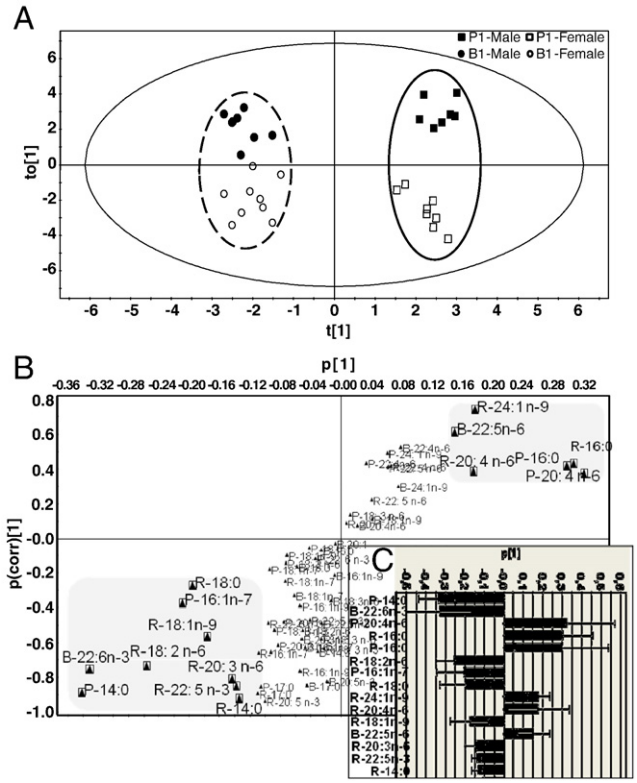


Fig. 4. OPLS-DA model with brain (B), RBCs (R) and plasma (P) fatty acid data in the palm and butter based-diets providing identical ALA intakes (P1 and B1 diets, respectively). (A) OPLS-DA model from tissues fatty acids; the $R^2Y$ (explained class variance) and $Q^2Y$ (class prediction ability) were 0.97 and 0.95, respectively. OPLS-DA score plot shows group discrimination according to the dietary treatments (100% class assignment, Fisher's *P* value=$1.8\times10^{-9}$). The group assignment for individual rats was calculated using one significant component to explain 94.6% of fatty acid variations. (B) S-plot, fatty acid variations with significant variable importance in projection coefficients >1 are marked within a square. (C) The fatty acids weight coefficients *p* marked with a square in panel (B) were significant as determined with the jack-knife CI error bars. Lower-left square of the plot: fatty acids increasing with the B1 diet (1.5%-ALA butter blend) and decreasing in the P1 diet (1.5%-ALA palm blend); upper-right square of the plot: fatty acids increasing with the P1 diet (1.5%-ALA palm blend) and decreasing with the B1 diet (1.5%-ALA butter blend).

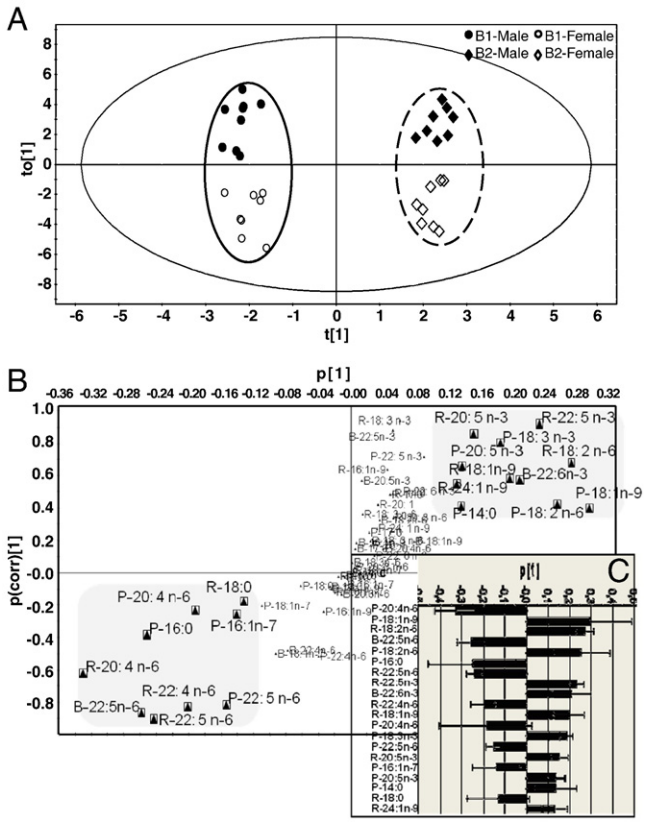


Fig. 5. OPLS-DA model with brain (B), RBCs (R) and plasma (P) fatty acid data between the rats fed the anhydrous butter-based diets with different ALA levels (1.5% and 2.3% of total fatty acid) (B1 and B2 diets, respectively). (A) OPLS-DA score plot showing group discrimination according to the dietary treatments (100% class assignment, Fisher's $P$ value$=8.6\times10^{-10}$); the $R^2Y$ (explained class variance) and $Q^2Y$ (class prediction ability) were 0.98 and 0.94, respectively. The group assignment for individual rats was calculated using one significant component to explain 98.4% of fatty acid variations. (B) S-plot, fatty acid variations with significant variable importance in projection coefficients >1 are marked with a square. (C) The fatty acids weight coefficients $p$ marked within a square in panel (B) were significant as determined with the jack-knife CI error bars. Lower-left square of the plot: fatty acids increasing in rats fed with the B2-based diet (2.3%-ALA butter blend) and decreasing with the B1-based diet (1.5%-ALA butter blend); upper-right square of the plot: fatty acids increasing in rats fed with the B1-based diet (1.5%-ALA butter blend) and decreasing with the B2-based diet (2.3%-ALA butter blend).

PLS to generate for each rat a single composite predictive latent variable (see Methods). We then compared the regression coefficient (against brain DHA) obtained with the composite variable made with the 10 individual fatty acids to that obtained with 22:5n-3 in RBCs, which was the best brain DHA predictor in our study. The nonlinear prediction index $R^2$ is indicated regardless of whether when 22:5n-3 (Fig. 8A) or the composite latent variable (Fig. 8B) was used. We improved the prediction index from 0.4777 obtained with 22:5n-3 to 0.60 obtained with the composite latent variable.

### 3.5. Gene expression analysis

Finally, to explain the impact of the various diets on the fatty acid profiles, we measured the gene expression of fatty-acid-desaturation/elongation enzymes in the liver, such as the delta 6 desaturase (FADS2), the delta 5 desaturase (FADS1) and the very-long-fatty-acid elongase (ELOVL2) (Table 5). As an overview of the dietary effect on genes expression, the B1 diet induced a relative up-regulation of the three PUFA-synthesizing genes; and B2, a down-regulation. The palm oil blend-based diets were intermediary. As a result, a dietary effect can be observed. To examine the concerted action of the desaturase/elongase gene expression system on the polyunsaturated fatty acid products, we then related the combined genes expression results to the PUFA content in tissues. For this, we performed a PLS analysis, examining whether the desaturation/elongation activity (combining FADS1, FADS2 and ELVOL2 as predicting $X$-variables) could be related to the sum of n-6 or n-3 fatty acids products in plasma, RBCs or brain (dependent $Y$-variables, sum of18:3n-6, 20:3n-6, 20:4n-6, 22:4n-6 and 22:5n-6 in either tissue, or the corresponding sum of 20:3n-3, 20:4n-3, 20:5n-3, 22:5n-3 and 22:6n-3). Although the model can be validated by the permutation test ($R^2Y$ and $Q^2Y$ values decreasing when permuting the $Y$-variables from 0.103 and 0.056 to as low a 0.0023 and −0.048 for $R^2Y$ and $Q^2Y$, respectively), the PLS model was of poor predicting value, with a PUFA variance explained from the desaturation/elongation gene system ($R^2Y$) of 10.3% and a predicted variance explained ($Q^2Y$) of 5.6%, which is considered insufficient. A cross-validation ANOVA test also indicated that from the $Y$-variables (sum of PUFA products), only the n-3 PUFA in RBCs can be related to some extent to the desaturase/elongase activity (21.4% of the explained variance, 16% of the predicted variance, $P$=.021 after cross-validation ANOVA). A multiple regression model was also calculated as a PLS results confirmation, with FADS1, FADS2 and ELVOL2 as independent variables and the sum of n-6 and n-3 fatty acids products in plasma, RBCs or brain as dependent variables. No statistical correlation with the three independent gene expression

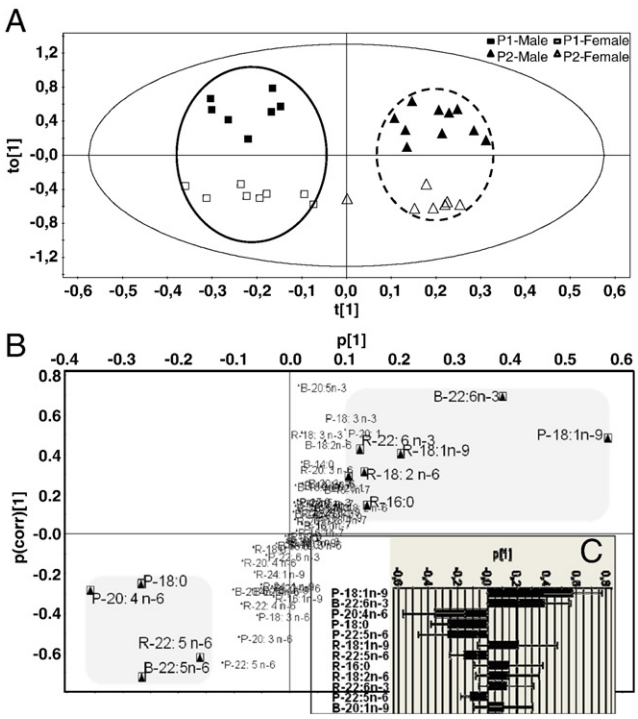


Fig. 6. OPLS-DA model with brain (B), RBCs (R) and plasma (P) fatty acids data of rats fed with the palm-oil-based diets with or without DHA and ARA supplementation (P2 and P1 diets, respectively). (A) OPLS-DA score plot showing group discrimination according to the dietary treatments (100% class assignment, Fisher's $P$ value$=1.8\times10^{-9}$); the $R^2Y$ (explained class variance) and $Q^2Y$ (class prediction ability) were 0.88 and 0.70, respectively. The group assignment for individual rats was calculated using one significant component to explain 86.5% of fatty acid variations. (B) S-plot, fatty acid variations with significant variable importance in projection coefficients >1 are marked with a square. (C) The fatty acids weight coefficients $p$ marked with a square in panel (B) were significant as determined with the jack-knife CI error bars. Lower-left square of the plot: fatty acids increasing in rats fed with the P1 diet (1.5%-ALA palm blend) and decreasing with the P2 diet (DHA/ARA-supplemented 1.5%-ALA palm blend); upper-right square of the plot: fatty acids increasing in rats fed with the P2 diet (DHA/ARA-supplemented 1.5%-ALA palm blend) and decreasing with the P1 diet (1.5%-ALA palm blend).

Table 5
Key polyunsaturated fatty acid gene expression data and statistical significance according to the dietary treatment and gender

| Genes | Diet | | | | *P* values | | |
|---|---|---|---|---|---|---|---|
| | P1 diet | P2 diet | B1 diet | B2 diet | Diet effect | Gender effect | Diet and gender interaction |
| ELOVL2 | $1.01\pm0.11^{a,b}$ | $0.931\pm0.091^{a,b}$ | $1.191\pm0.12^{a}$ | $0.90\pm0.06^{b}$ | .045 | .004 | .048 |
| FADS1 | $1.12\pm0.13^{a,b}$ | $0.93\pm0.09^{b}$ | $1.28\pm0.13^{a}$ | $0.75\pm0.17^{b}$ | .002 | .0003 | .6294 |
| FADS2 | $1.03\pm0.06^{a,b}$ | $1.10\pm0.15^{a}$ | $1.31\pm0.11^{a}$ | $0.68\pm0.128^{b}$ | .003 | .009 | .039 |

Results expressed as mean±S.E.M., in arbitrary unit. $N=12$ rats (6 females, 6 males) per dietary group. Values in the same column that do not share a common superscript$^{a-d}$ are significantly different at $P<.05$ (one-way ANOVA followed by Bonferroni post hoc test).

variables was found, except again for the sum of n-3 long-chain fatty acids in plasma ($R^2$ value of 0.367, adjusted $R^2$ of 0.314, $P=.0008$).

## 4. Discussion

This study investigates the ability of dairy-fat-based diets to modulate the tissue fatty acid profiles in young rats (with an emphasis on brain DHA) by a comparison to corresponding vegetable saturated-fat-based diets. ALA deficiency over both gestation and lactation in the dams was achieved by feeding a palm-oil-blend-based diet (0.4% ALA), and the rat pups were then switched at weaning to either a palm-oil-blend diet (P1) or a butter diet (B1) supplemented with sunflower and rapeseed oils to maintain 16% LA and 1.5% ALA. Two other diets were tested: 1.5%-ALA palm diet supplemented with 0.12% DHA and 0.4% ARA (P2) and a 2.3%-ALA butter diet (B2). The latter was obtained by selectively increasing the rapeseed oil in the blend, which induced a further decrease in the n-6/n-3 ratio from 10 to 5.

The main finding of our study is that an anhydrous dairy-fat-based diet with 1.5% ALA is more efficient than a palm oil blend with as much ALA and 0.12% added DHA and 0.4% ARA for increasing brain DHA levels in postweaning rats. In addition, both anhydrous dairy fats optimized brain DHA levels more than pure vegetable fat blends. The further increase in brain DHA levels observed with the 2.5%-ALA enriched-butter diet compared to the 1.5%-ALA butter diet could be attributed to both the increased level of dietary ALA and the concomitant decrease in the n-6/n-3 ratio induced by the selective enrichment of the blend with rapeseed. Together, these observations clearly demonstrated that brain DHA levels can be substantially improved by dairy-fat-based diets.

The n-6 to n-3 ratio has been recognized as an important factor driving the bioconversion of ALA into DHA because of the competition between the parent n-3 and n-6 fatty acids for the desaturation and elongation pathways. This could explain the increase in brain DHA levels observed in the two dairy-fat diets. The other possibility would be that the various diets caused selective desaturase and elongase gene activation. We were unable to find such relationship that could explain to a large extent the fatty acid variations in the tissues that we examined. Thus, mechanisms operating post-traductionally on the desaturation pathways are more important for governing very-long-chain PUFA (VLC-PUFA) tissue accretion that direct effects on the desaturase and elongase gene expression system, as observed by others [38]. In that respect, the dairy fats differed from the plant oils in their content of short- and medium-chain fatty acids. ALA is a one of the best β-oxidation substrates [39], whose activity can prevent its

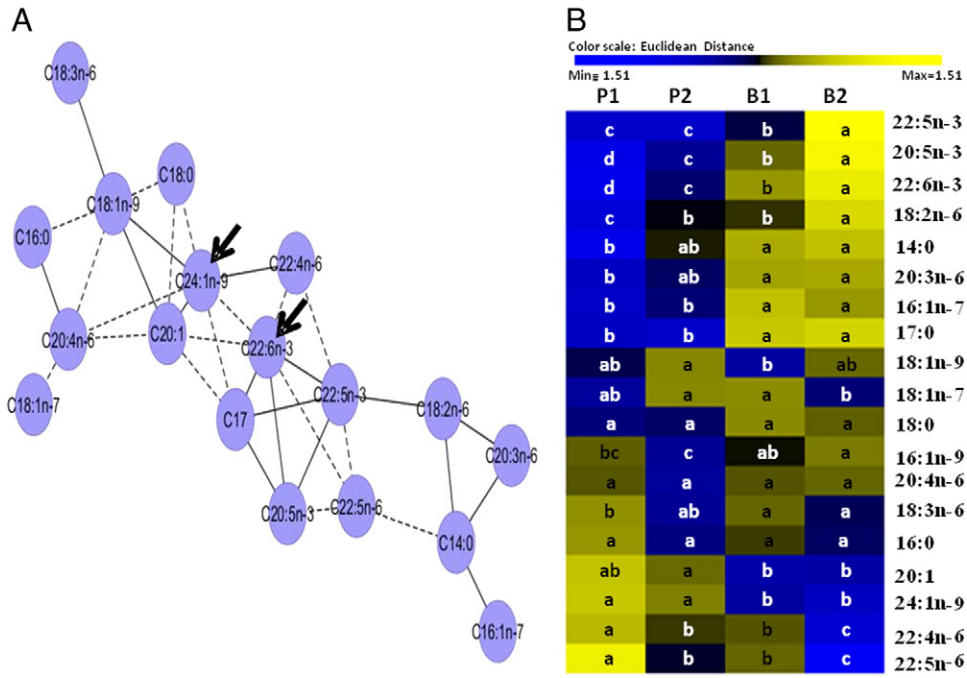


Fig. 7. Fatty acid correlation network in the brains of both male and female pups fed with the various diets. (A) Correlation plots indicating pairwise correlations among brain fatty acids of both male and female pups that have a Pearson correlation coefficient square value over or equal to 0.5 ($\rho>0.7$). Only the strongest relationships remained after this stringent filtering (18 of the 19 fatty acids can be visualized). Nodes represent the annotated fatty acids, the edges with solid lines represent positive coefficients, and the edges with dashed lines represent negative coefficients. Arrows show the fatty acids displaying the greatest number of correlations with $\rho>0.7$ (hubs). (B) Heat map showing the selected fatty acid content displayed in the interaction network according to the dietary challenges (yellow boxes: increase, blue boxes: decrease, black boxes: no relative change) with rows describing the fatty acids and columns describing individual rats; *P* values comparing fatty acid content across the dietary challenges are indicated (a, b, c, d: a fatty acid not sharing the same letter is significantly different; $P<.05$).

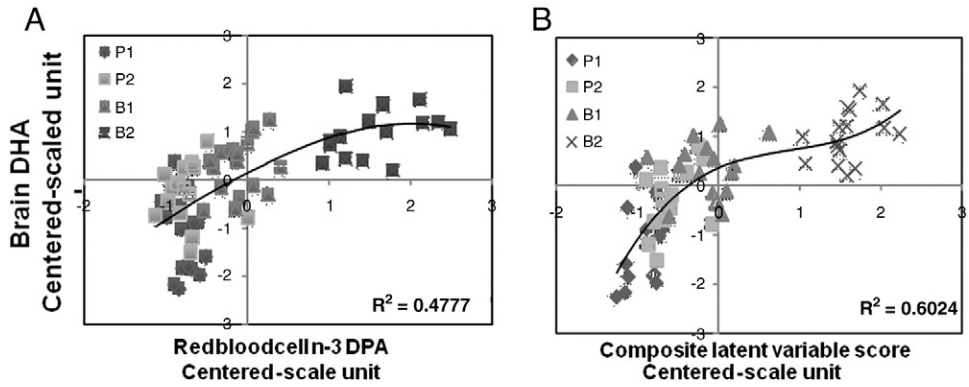


Fig. 8. (A) Relationship between RBCs lipid DPA n-3 and brain lipid DHA. The content of brain lipid DHA was significantly and positively correlated with the content of DPAn-3 in RBC lipids. (B) Relationship between a selection (best OPLS index, variable importance in projection coefficient values >1) of RBC and plasma fatty acids (RBC DPAn-3, EPA, C20:3n-6, ALA, DPAn-6 and LA, and plasma ALA, EPA, DPAn-3 and DPAn-6) and brain lipid DHA. The individual values of the selected fatty acids were reduced to one composite latent variable per individual prior to calculation (see Methods section). The nonlinear prediction index $R^2$ is indicated, whether when DPA panel (A) or the composite latent variable in panel (B) is used.

conversion into VLC-PUFA. Short-chain fatty acids, such as those found in dairy fats, are also highly oxidized after absorption [40,41] and may thereby spare ALA from oxidation and favor ALA partitioning towards the desaturation and elongation pathways. The possible sparing of ALA from β-oxidation by short-chain fatty acids is a plausible explanation for our observation that better bioconversion of ALA into DHA is obtained with the dairy fat blend B1 compared to the corresponding palm oil blend P1. Both have as much ALA (1.5%), with the same n-6/n-3 ratio. However, this needs to be precisely addressed and deserves careful examination.

In our dietary conditions, the brain fatty acid profiles appeared to be more protected against environmental influences (such as nutritional changes) than RBCs or plasma. This also holds true for gender influences. This appears to be an illustration of tighter control of membrane lipid fatty acids to preserve optimal brain function. Nonetheless, as observed by others [42], changes in tissue fatty acid levels due to both dietary and gender influences occurred even in the brain. In fact, when excluding the influence of diet, our model predicted that brain DHA is not influenced by gender per se. Only a gender/diet interaction can reveal the differences between males and females that drive brain DHA variations. Likewise, we found the butter-based diets attenuated the gender influence to a greater extent than the palm-oil-based diets. The brain DHA levels were lower in males than in females in palm-oil-based diet P1 (11.0% vs. 12.6%) but comparable in males and females in the counterpart diet P2 that provided both preformed DHA and ARA (12.91% vs. 12.88%). An explanation would be that the gender effect on ALA conversion is negligible from the moment that preformed DHA is given to males. This observation also strongly supports that a specific gender effect should be considered in the design of infant formula because of a possible gender/diet interaction effect.

A great deal of concern in infant nutrition focuses on specific biomarker fatty acids that can be easily accessed and used as surrogates for brain DHA status [43]. For this purpose, RBCs n-3 DPA was found to be the most reliable indicator in our study. Some have found that RBC DHA is best for predicting brain DHA [44,45], whereas others obtained poor prediction with that fatty acid, similar to our study [46]. Since no single fatty acid varies independently of the others, we investigated the interdependence of brain DHA and the other fatty acids measured in the plasma and RBCs. Our goal was to reveal a fatty acid signature in peripheral tissues that could predict the DHA content in neuronal tissues better than any single fatty acid. Using a multivariate selection procedure (VIP coefficients of OPLS), we selected 10 fatty acids in the plasma and RBCs that, together, can serve as markers of DHA accretion in the brain. The composite variable calculated from these 10 fatty acids improved prediction compared to RBC n-3 DPA, which we had found to be the most strongly associated with brain DHA. We also compared the predictive performance of our composite index to the unsaturated fatty acid index of Stark [47] and found that our composite index was still better ($R^2$=0.54 calculated from RBC fatty acids and 0.41 calculated from plasma fatty acids, whereas our result was 0.60). Due to the contradictory results found across the literature in finding a reliable and universal biomarker of brain DHA, the strategy of using a composite biomarker could be an interesting alternative but, at this stage, still requires validation in other models.

The interaction network displaying the interactions among the brain fatty acids further underlines the role of DHA as an important player for the general determination of membrane fatty acid composition. The central positions of DHA and nervonic acid (24:1n-9) in the interaction network suggest that any conditions that change their content in brain membranes would affect the overall fatty acid profile and, thus, neuronal function.

In conclusion, our study shows that a dairy fat blend providing as little as 1.5% ALA is superior to the plant oil blend even when the recommended DHA and ARA levels are exogenously provided. A further increase in DHA can be even observed when increasing by 50% the levels of ALA in the dairy matrix. The dietary conditions can reveal a gender effect with regard to brain PUFA, especially brain DHA. In our context, a composite variable made by aggregation of selected blood fatty acids can predict brain DHA levels with a reasonably good confidence and constitute an interesting strategy to be validated in other dietary contexts and for the human situation.

We believe that our findings are worth considering in a human perspective.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jnutbio.2011.10.011.

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