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# Comparative effects of well-balanced diets enriched in α-linolenic or linoleic acids on LC-PUFA metabolism in rat tissues

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ABSTRACT

The intake of the essential fatty acid precursor α-linolenic acid (ALA) contributes to ensure adequate n-3 long-chain polyunsaturated fatty acid (LC-PUFA) bioavailability. Conversely, linoleic acid (LA) intake may compromise tissue n-3 PUFA status as its conversion to n-6 LC-PUFA shares a common enzymatic pathway with the n-3 family. This study aimed to measure dietary ALA and LA contribution to LC-PUFA biosynthesis and tissue composition. Rats were fed with control or experimental diets moderately enriched in ALA or LA for 8 weeks. Liver Δ6- and Δ5-desaturases were analyzed and FA composition was determined in tissues (red blood cells, liver, brain and heart). Hepatic Δ6-desaturase activity was activated with both diets, and Δ5-desaturase activity only with the ALA diet. The ALA diet led to higher n-3 LC-PUFA composition, including DHA in brain and heart. The LA diet reduced n-3 content in blood, liver and heart, without impacting n-6 LC-PUFA composition. At levels relevant with human nutrition, increasing dietary ALA and reducing LA intake were both beneficial in increasing n-3 LC-PUFA bioavailability in tissues.



## 1. Introduction

The health benefits of n-3 long chain polyunsaturated fatty acids (LC-PUFA), particularly eicosapentaenoic acid (20:5 n-3, EPA) and docosahexaenoic acid (22:6 n-3, DHA), have been demonstrated in many studies. For example, EPA and DHA are precursors of eicosanoids and docosanoids involved in anti-inflammatory, anti-thrombotic and anti-aggregating signaling, and can promote vasodilatation [1]. More specifically, some studies recently underlined the involvement of the DHA-derived neuroprotectin D1 in cell survival and brain protection against neurodegenerative disorders such as Alzheimer disease [2,3]. EPA and DHA can be obtained from the diet, especially *via* fish and seafood. Since marine food sources are characterized by a limited stock and a declining nutritional quality [4–7], it seems useful to develop alternative sources to meet dietary n-3 PUFA requirements. As EPA and DHA are biosynthesized from α-linolenic acid (18:3 n-3, ALA), consumption of this essential precursor could be a complementary method to ensure sufficient n-3 PUFA bioavailability. However, numerous studies in humans show that dietary supplementation in ALA generally leads to increased circulating amounts of EPA and docosapentaenoic acid (22:5 n-3, DPA), but not DHA [8,9]. This observation is consistent with the limited capacity of ALA conversion into DHA in humans, estimated to be lower than 0.05% [10]. Human studies are limited to blood measurements whereas animal studies are useful for assessing the biodistribution of n-3 PUFA in organs and tissues. DHA content in rodent tissues such as the brain or heart can thus be raised after ALA supplementation [11–13], even if this observation seems to be particularly promoted by moderate ALA supplementation levels [14].

LC-PUFA biosynthesis from ALA involves desaturases, elongases and peroxysomal β-oxidation in the liver (Fig. 1). The conversion of the n-6 linoleic acid (18:2 n-6, LA) to n-6 LC-PUFA shares a common enzymatic pathway and can thus compete with the ALA conversion, particularly for Δ6-desaturation, which is considered a rate-limiting step [15]. Because of known competition between n-6 and n-3 FA in this pathway, n-6 PUFA metabolism must be taken into consideration while studying the conversion from ALA to n-3 LC-PUFA. This concern is epidemiologically relevant because dietary LA intake has sharply risen over the past decades due to the increased consumption of vegetable oils such as soybean oil, resulting in a dietary LA/ALA ratio that now exceeds 10 [16,17]. This imbalance is associated with the development of cardiovascular, metabolic and neuropsychiatric disorders [18,19], and arguments in favor of a reduction in LA intake were recently raised [20,21], but still need more experimental support. Therefore, it is important to get a better understanding of dietary ALA and LA metabolism in order to refine recommended intakes for these FA. In this regard, the liver is the main site of LA and ALA

*Abbreviations:* LC-PUFA, Long chain polyunsaturated fatty acid; ALA, α-linolenic acid; LA, linoleic acid; Fads, Fatty acid desaturase.

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synthesis into long-chain PUFAs and their secretion into the plasma. Organs such as the brain and heart have a limited capacity to synthesize long-chain PUFAs, and therefore obtain them directly from the plasma pool. This would suggest that dietary alterations of LA or ALA, the substrates for liver desaturase and elongase enzymes, might alter the elongation-desaturation capacity of the liver and tissue fatty acid composition.

The present study provided a comprehensive assessment of erythrocyte, liver heart and brain fatty acid status in relation to liver Δ5- and Δ6-desaturase expression and activity, with the aim of examining the dependency of extra-hepatic tissue fatty acid composition on liver enzyme activity. This issue was addressed by comparing the effects of moderate ALA and LA dietary enrichment. Previous studies in rats analyzed the impact of ALA and LA supplementation on lipid tissue composition or hepatic desaturation [12,14,22–24], but the present work provides complementary findings for two reasons. First, we used moderately enriched diets with ALA and LA composition set to values achievable in a human diet for these FA (when expressed in energy %). In the control diet, ALA and LA composition was respectively 0.6% and 2.5% of energy, with a LA/ALA ratio of 4.2/1, which is close to nutritional recommendation determined by the French Agence Nationale de Securite Sanitaire (ANSES) [25,26] The LA-enriched diet contained 0.6% ALA and 4.8% LA and the ALA-enriched diet 2.2% ALA and 2.5% LA (in % of energy), resulting in a LA/ALA ratio of 8/1 and 1.1/1, respectively. Second, we determined the effect of these diets on (1) the fatty acid composition in erythrocytes, liver, heart and brain, and (2) the expression and activity of the hepatic Δ5- and Δ6-desaturases.

## 2. Material and methods

### 2.1. Chemicals

Chemicals were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France) and solvents used in lipid analysis were from Fisher Scientific (Elancourt, France).

### 2.2. Animals and diets

All protocols complied with the European Union Guideline for animal care and use (2003/35/CEE). Eighteen Sprague-Dawley rats (60 g weight) were purchased from Janvier breeding center (Le Genest Saint Isle, France). They had free access to water and food. They were fed with rodent chow (Special Diet Services, Witham, UK) for one week until their random allotment into three groups (6 rats per group). Each group was then fed during 8 weeks with the experimental diets (control, ALA or LA). The diets were isoenergetic and isolipidic, and contained 10% of fat (21% of energy), 198 g of casein, 416 g of starch, 213 g of sucrose, 36 g of mineral mix, 9 g of cellulose, 9 g of vitamin mix and 9 g of Agar–Agar per kg.

The diets were made from a combination of commercial vegetable oils. Olive, rapeseed, corn and flaxseed oil composition was 33.9:30:7.7:0 in the control, 37.3:22.7:5.5:17 in the ALA diet and 27.3:28:27.8:0 in the LA diet (in % of fat). Fatty acids from triglycerides (12:0, 14:0, 16:0 and 18:0) (TCI Europe, Zwijndrecht, Belgium) were added to the oil blend to obtain isolipidic and isoenergetic diets. The dietary FA compositions are given in Table 1. The supplementation of the ALA and LA diets with essential precursors was physiologically relevant (respectively +1.6% and +2.2% of energy) and was compensated with palmitic acid from tripalmitin in the control diet. The diets were prepared at the Unité de Production d'Aliments Expérimentaux (INRA, Jouy en Josas, France).

After an overnight fast, rats were finally anesthetized with two successive intraperitoneal injections of thiopental (37.5 mg/kg each) (Nesdonal, Merial, Lyon, France) and blood samples were collected by cardiac punctures. The liver, brain and heart were removed, weighted, snap-frozen in liquid nitrogen and stored at −80 °C.

### 2.3. Lipid extraction and FA analysis

Lipids from frozen tissues were extracted twice separately with 4 ml and 2 ml dimethoxymethane:methanol (4:1 v/v) after homogenization with an Ultra-Turrax®. After acidification with 1 ml HCl 3 M, plasma and red blood cell lipids (from 500 μl sample) were extracted twice separately with 4 ml and 2 ml hexane:isopropanol (3:2 v/v).

Total lipids were saponified at 70 °C for 30 min with NaOH (0.5 M) in methanol and methylated at 70 °C for 15 min with $BF_3$ (14% in methanol). Fatty acid methyl esters were extracted twice

**Table 1**
FA composition (in % of total fatty acid) of the experimental diets.

| In % of FA | Control | ALA | LA |
|---|---|---|---|
| **12:0** | 2.6 | 2.6 | 2.6 |
| **14:0** | 5.5 | 6.0 | 6.0 |
| **16:0** | **26.5** | 16.2 | 16.0 |
| **18:0** | 4.1 | 4.1 | 3.9 |
| **20:0** | 0.3 | 0.3 | 0.3 |
| **ΣSFA** | 39.0 | 29.2 | 28.8 |
| **16:1 n-7** | ND | 0.4 | ND |
| **18:1 n-7** | 1.8 | 1.8 | 1.7 |
| **18:1 n-9** | 44.6 | 46.2 | 44.2 |
| **ΣMUFA** | 46.4 | 48.3 | 45.9 |
| **18:2 n-6** | 11.6 | 12.0 | **22.4** |
| **18:3 n-3** | 3.0 | **10.3** | 3.0 |
| **Σ PUFA** | 14.6 | 22.3 | 25.4 |
| **LA/ALA** | 3.9 | 1.2 | 7.5 |
| **In % of energy** | **Control** | **ALA** | **LA** |
| **18:2 n-6** | 2.5 | 2.6 | 4.8 |
| **18:3 n-3** | 0.6 | 2.2 | 0.6 |

ND: not detectable.

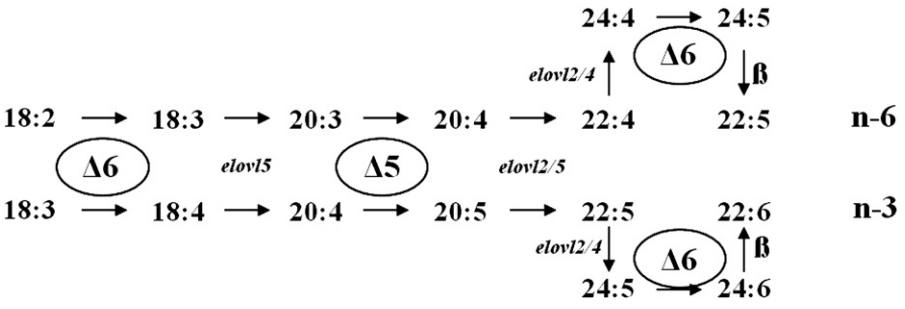


**Fig. 1.** n-6 and n-3 LC-PUFA biosynthesis from essential precursors in mammals involves common enzymes to catalyze the desaturation, elongation and partial β-oxidation steps. Δx: Δx-desaturase, elovl: elongation of very long chain, β: partial β-oxidation.