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Original article

Dietary myristic acid at physiologically relevant levels increases the tissue content of C20:5 n-3 and C20:3 n-6 in the rat

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Abstract – This study was designed to investigate the effect of myristic acid on the biosynthesis and metabolism of highly unsaturated fatty acids, when it is supplied in a narrow physiological range in the diet of the rat (0.2–1.2% of total dietary energy). Three experimental diets were designed, containing 22% of total dietary energy as lipids and increasing doses of myristic acid (0.71, 3.00 and 5.57% of total fatty acids). Saturated fat did not exceed 31% of total fat and the C18:3 n-3 amount in each diet was strictly equal (1.6% of total fatty acids). After 7 weeks, the diets had no effect on plasma cholesterol level but greatly modified the liver, plasma and adipose tissue saturated, monounsaturated and polyunsaturated fatty acid profiles. Firstly, daily intakes of myristic acid resulted in a dose-dependent tissue accumulation of myristic acid itself. Palmitic acid was significantly increased in the tissues of the rats fed the higher dose of myristic acid. A dose-response accumulation of tissue C16:1 n-7 as a function of dietary C14:0 was also shown. Secondly, a main finding was that, among n-3 and n-6 polyunsaturated fatty acids, a dose-response accumulation of liver and plasma C20:5 n-3 and C20:3 n-6 (two precursors of eicosanoids) as a function of dietary C14:0 was shown. This result suggests that dietary myristic acid may participate in the regulation of highly unsaturated fatty acid biosynthesis and metabolism.

dietary myristic acid / highly unsaturated FA biosynthesis and metabolism / rat

1. INTRODUCTION

Myristic acid (C14:0) usually accounts for small amounts (less than 1 wt%) of total fatty acids (FA) in animal tissues but is abundant in milk fat (7–12 wt% of total FA) [1] or in copra and palmist oils (15–23 wt% and 15–17 wt%, respectively). Epidemiological and clinical studies have shown that dietary fats containing high levels of saturated fatty acids (usually more than 15% of total energy) induce an increase in plasma total- and low density lipoprotein (LDL)-cholesterol concentrations in humans [2, 3]. Among saturated FA, myristic acid (given at more than 10% of total energy) consistently seems to increase animal and human blood cholesterol concentrations more than other FA [4, 5]. From these studies, it has therefore been suggested that myristic acid may be the most hypercholesterolemic saturated FA, followed by lauric acid (C12:0)

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and palmitic acid (C16:0). The biological mechanisms involved in this increase are (i) a decrease in hepatic LDL receptor activity [5, 6], thereby affecting plasma lipoprotein clearance and (ii) an increase in LDL-cholesterol production rate. In a recent study, Kummrow et al. [7] showed that myristic acid, compared to oleic acid and docosahexaenoic acid (DHA), may increase the secretion of dense apoB-100-lipoprotein particles in rat hepatoma cells.

The estimated amount of myristic acid consumed in European Countries is, however, far less than 10% of total dietary energy. It corresponds to 4–8 g.day^{-1} i.e., 0.5 to 2.0% of total energy, coming essentially from dairy fat [8]. At these physiological levels (0.5 to 2.4% of total dietary energy), recent studies analyzing the effects of increasing doses of dietary myristic acid in hamsters showed a small increase in plasma total cholesterol that only reflected an increase in the level of high density lipoprotein (HDL)-cholesterol and not in the level of LDL-cholesterol [9, 10]. In young men, meals rich in myristic acid also result in a higher HDL-cholesterol concentration within 24 h than meals rich in stearic acid, without affecting the other lipoprotein parameters [11]. Finally, Dabadie et al. [12, 13] showed preliminary results suggesting that in humans, daily intakes of myristic acid from milk (up to 1.8% of total energy) not only resulted in an increase in blood HDL-cholesterol but also in an increase in DHA in the plasma cholesterol ester fraction. From this last result, the hypothesis that dietary myristic acid may have an effect on highly unsaturated FA metabolism of the (n-3) series can be related to recent results obtained by our group, showing that in cultured rat hepatocytes, myristic acid increases Δ6-desaturase activity, in a dose-dependent manner [14]. Besides, it is now well known that an increase in EPA (eicosapentaenoic acid) and DHA could be of interest for human health and prevention of cardiovascular disease.

Therefore, it seems to be important to re-evaluate the metabolic fate and effects of dietary myristic acid when it is supplied in a narrow physiological range, using a diet that meets the Recommended Dietary Allowances adopted by a large number of countries. In the present study, we investigated in rats whether the content of C14:0 in the diet has an effect on the fatty acid profiles of the liver, plasma and adipose tissue. Diets were designed to contain 22% of total dietary energy as lipids (10 g·100 g^{-1} diet) and increasing doses of myristic acid (from 0.7 to 5.6% of FA, i.e., from 0.2 to 1.2% of total dietary energy). Saturated fat did not exceed 31% of total fat and the ratio of linoleic acid (C18:2 n-6)/α-linolenic acid (C18:3 n-3) was between 5 and 7.

2. MATERIALS AND METHODS

2.1. Chemicals

Solvents and chemicals were obtained from Fisher (Elancourt, France) or Sigma (St-Quentin-Fallavier, France). [1-^{14}C]-α-linolenic acid was from Perkin Elmer Life Sciences (Le Blanc Mesnil, France) and [1-^{14}C]-eicosatrienoic acid was from American Radiolabeled Chemicals (Isobio, Fleurus, Belgium). Standard FA for gas chromatography (GC) and high performance liquid chromatography (HPLC) analyses were from Sigma. Fractionated butter fat was given by Lactalis (Retiers, France). Kits for plasma cholesterol and triacylglycerol (TAG) were purchased from DiaSys (Immunochimie, Bouffemont, France).

2.2. Diets

The diets were prepared at the Unité de Production d'Aliments Expérimentaux (UPAE, INRA, Jouy-en-Josas, France). They were isocaloric and varied only in the type of fat used, in order to contain different amounts of myristic acid. The basal composition of the diets was as follows (g·100 g^{-1}): 42.6 corn starch, 19.8 casein, 21.3 sucrose, 0.9 cellulose, 3.6 mineral mix,

0.9 vitamin mix, 0.9 agar-agar and 10.0 lipid. To meet essential fatty acid requirements in each diet, 5 g lipid·100 g^{-1} diet were from an oil mixture containing 70% olive oil, 25% rapeseed oil and 5% corn oil. The diets differed only in the natural fat giving the other 5 g lipid·100 g^{-1} diet. The natural fats used were fractionated butter fat (BU), lard (LA) and a mixture (50% each) of fractionated butter fat and lard (BL). Whatever the diet, fat provided 22% of total dietary energy. Table I shows the FA composition of the three diets, analyzed by GC. The LA, BL and BU diets contained 0.71% of myristic acid (0.16% of total dietary energy), 3.00% (0.66%) and 5.57% (1.22%), respectively (Tab. I). The cholesterol in the diets was the following (g·100 g^{-1}): 0.11 for LA, 0.46 for BL and 0.66 for BU.

Table I. Fatty acid composition (wt%) of the three experimental diets.

Fatty acid	LA	BL	BU
C10:0	0.04	0.68	1.34
C12:0	0.05	1.03	2.12
C14:0	0.71	3.00	5.57
C16:0	16.94	16.85	16.70
C18:0	8.10	6.42	4.46
C20:0	0.36	0.39	0.26
C22:0	0.09	0.09	0.11
SFA	26.29	28.46	30.56
C14:1 n-5	0.02	0.41	0.86
C16:1 n-9	0.20	0.25	0.25
C16:1 n-7	1.52	1.61	1.66
C18:1 n-9	54.85	54.34	54.10
C18:1 n-7	2.65	2.15	1.77
C20:1 n-9	0.66	0.50	0.33
MUFA	59.90	59.26	58.97
C18:2 n-6	11.94	10.35	8.43
C18:3 n-6	0.03	0.09	0.16
C20:3 n-6	0.04	0.05	0.06
C20:4 n-6	0.09	0.09	0.10
n-6 PUFA	12.10	10.58	8.75
C18:3 n-3	1.63	1.60	1.59
C20:5 n-3	0.04	0.05	0.07
C22:5 n-3	0.04	0.05	0.06
n-3 PUFA	1.71	1.70	1.72
18:2/18:3 ratio	7.3	6.5	5.3

2.3. Animals

Animal protocols were in compliance with European Union Guidelines for animal care and use. Eighteen Sprague-Dawley male rats (60 g body weight) were obtained from the R. Janvier breeding center (Le-Genest-St-Isle, France) and maintained on rat chow (nutriment A04 purchased from UAR, Epinay-sur-Orge, France) with free access to water for one week before the study. Then, the rats were randomly assigned to three groups ($n = 6$ per group) and fed with the three diets described above (LA, BL and BU) for 7 weeks. At the end of this period, the rats were fasted overnight. On the following morning, they were anaesthetized with an intraperitoneal injection of penthobarbital (7.5 mg·100 g^{-1} body weight). Blood samples were drawn into heparinated tubes by cardiac puncture. The liver and epididymal fat were removed, weighed, snap-frozen in liquid nitrogen and stored at –80 °C. The plasma was separated from blood cells by centrifugation (2500 *g*, 10 min, 4 °C). Plasma total cholesterol and TAG were assayed with commercial enzymatic kits, according to the manufacturer's instructions, using a 96-well microtiter plate reader (EL 340, Bio-Tek, Bioblock, Strasbourg, France).

2.4. Lipid extraction and FA analysis

Lipids from the liver were extracted from 3 g-samples homogenized in a mixture of dimetoxymethane/methanol (4:1 v/v) and incubated for 30 min before extraction [15]. After evaporation of the solvents, the lipids were dissolved in 25 mL chloroform. The lipids from epididymal adipose tissue were extracted from 100 mg tissue, homogenized

in a mixture of dimetoxymethane/methanol and dissolved in 1 mL of chloroform. The lipids from plasma were extracted from 1 mL-samples with a mixture of hexane/isopropanol (3:2 v/v), after acidification with 1 mL HCl 3 mol·L^{-1} [16], and dissolved in 1 mL of chloroform. Lipid species (from liver and plasma) were separated by thin-layer chromatography using silicagel H plates and a mixture of hexane:diethylether:acetic acid (85:15:1 v/v/v). Phospholipids (PL), non esterified fatty acids (NEFA), triacylglycerol (TAG) and cholesterol esters (CE) were scraped off the plate and extracted with 2 mL of diethyl ether (for TAG, NEFA and CE) or 2 mL of methanol for PL, as described previously [16]. Total lipid extracts from the liver, plasma and adipose tissue, and lipid species from the liver and plasma were saponified for 30 min at 70 °C by 1 mL of 0.5 mol·L^{-1} NaOH in methanol and then methylated with 1 mL BF_3 (14% in methanol) for 15 min at 70 °C. Fatty acid methyl esters were extracted twice with pentane and analyzed by GC [15] using an Agilent Technologies 6890N (Bios Analytique, Toulouse, France) with a split injector (40:1) at 250 °C and a bonded silica capillary column (30 m × 0.25 mm internal diameter; BPX 70; SGE, Villeneuve-St-Georges, France) with a polar stationary phase of 70% cyanopropylpolysilphenylene-siloxane (0.25 µm film thickness). Helium was used as the carrier gas (average velocity 24 cm·s^{-1}). The column temperature program started at 150 °C, ramped at 2 °C·min^{-1} to 220 °C, and held at 220 °C for 10 min. The flame ionization detector temperature was 250 °C. Identification of methyl ester peaks was based on retention times obtained for methyl esters prepared from FA standards.

2.5. Liver total lipids and total cholesterol

A 5 mL sample from liver lipids was taken to dryness and weighed to determine liver total lipid content, expressed as mg·g^{-1} liver. Another sample of liver lipids was used to determine total cholesterol content according to the Liebermann reaction. Briefly, a 2 mL aliquot of the chloroform solution was mixed with 4 mL of acetic anhydride and 5 drops of concentrated sulfuric acid, and incubated for 30 min. Absorbance was measured at 680 nm. A calibration curve was done with standard cholesterol solutions in chloroform.

2.6. Desaturase assays

Fresh liver samples (3 g) were homogenized in 12 mL of 50 mmol·L^{-1} phosphate buffer (pH 7.4) containing 0.25 mol·L^{-1} sucrose. The samples were centrifuged twice at 10 000 *g* for 30 min. The resulting post-mitochondrial supernatant was diluted 3 times and used for a Δ6-, and Δ5-desaturase assay as described previously [15]. Enzymatic activity was determined using a 1-mL assay mixture containing 100 µL of supernatant (600 to 800 µg protein), 150 mmol·L^{-1} phosphate buffer (pH 7.16), 6 mmol·L^{-1} $MgCl_2$, 7.2 mmol·L^{-1} ATP, 0.54 mmol·L^{-1} CoA and 0.8 mmol·L^{-1} NADH. The reaction was started with the addition of 60 nmol of [1-^{14}C]-α-linolenic acid or [1-^{14}C]-eicosatrienoic acid (740 MBq·mmol^{-1}) for Δ6- or Δ5-desaturase, respectively. The incubation was carried out in a shaking water bath at 37 °C for 20 min. The reactions were stopped by adding 1 mL of 2 mol·L^{-1} KOH in ethanol. Control assays were performed by adding KOH in ethanol before adding the substrate. Each assay mixture was then heated for 30 min at 70 °C. The FA were liberated by acidification, extracted with diethylether and dried. Fatty acids were converted to fatty acid naphthacyl esters [17] and separated on HPLC (Alliance 2695 integrated system, Waters, St-Quentin-en-Yvelines, France). The peaks corresponding to radiolabeled FA (substrate and product of each desaturase assay) were collected and subjected to liquid scintillation counting (Packard Tri-Carb 1600 TR, Meriden, CT). From the amount of radioactivity found in the product vs. the radioactivity recovered in the

Table II. Physiological parameters of rats fed the three experimental diets for 7 weeks.

		LA	BL	BU
Weights	Body (g)	468.4 ± 35.7	465.4 ± 33.5	450.6 ± 13.5
	Liver (g)	12.80 ± 2.01	12.58 ± 0.58	12.47 ± 0.81
Liver	Lipids ($mg \cdot g^{-1}$)	80.54 ± 8.00	79.30 ± 12.13	84.44 ± 14.82
	FA content ($mg \cdot g^{-1}$)	40.40 ± 5.17	37.32 ± 6.85	41.16 ± 8.79
	Cholesterol ($mg \cdot g^{-1}$)	7.32 ± 1.13	7.99 ± 2.78	8.13 ± 1.93
Plasma	Cholesterol ($mg \cdot mL^{-1}$)	0.78 ± 0.14	0.72 ± 0.08	0.73 ± 0.13
	TAG ($mg \cdot mL^{-1}$)	1.60 ± 0.47	1.31 ± 0.49	1.77 ± 0.48
	FA content ($mg \cdot mL^{-1}$)	1.49 ± 0.34	1.50 ± 0.31	1.45 ± 0.46
Adipose tissue	FA content ($mg \cdot g^{-1}$)	809.8 ± 233.9	744.2 ± 77.0	708.6 ± 65.9

Data are mean ± SD ($n = 6$). No significant difference was shown in these parameters between the three groups.

substrate, the enzyme activity could be determined and expressed as pmol substrate converted to product per min per mg of protein. Protein in the supernatant used for the desaturase assays was determined by a modified Lowry procedure [18].

2.7. Results expression and statistical analysis

The values reported are means ($n = 6$) ± SD. *P*-values were calculated using the Student test for two-group comparisons. The differences were considered significant at a *P*-value of less than 0.05.

3. RESULTS

3.1. Physiological status

Rats were fed the LA, BL and BU diets for 7 weeks. At the end of this period, body weights, liver weights, and concentrations of liver lipids, plasma TAG, hepatic and plasma total cholesterol were analyzed in 6 rats for each group. The amounts of total FA in the liver, plasma and adipose tissue are also presented. Table II shows that there was no significant difference in these parameters among the three groups.

3.2. Saturated fatty acid profiles of the tissues

The LA, BL and BU diets were experimentally designed to contain increasing amounts of myristic acid (0.71%, 3.00% and 5.57%, respectively) (Tab. I). After 7 weeks of these three diets, the composition of total FA was analyzed in the liver (Tab. III), plasma (Tab. IV) and adipose tissue (Tab. V) of six rats for each diet. The results showed that myristic acid accounted for about 0.9% of total FA in the liver, 1.3% in the plasma and 3.5% in adipose tissue of the rats fed the BU diet. All these values were significantly higher than the C14:0 percentages measured in the tissues of the rats fed the LA diet. For each tissue, the results obtained in the BL group were just between those found in the BU and the LA groups. These results show a dose-response enrichment of tissue myristic acid as a function of dietary myristic acid (Fig. 1A). A similar result was obtained in every lipid species (data not shown).

The LA, BL and BU diets contained almost the same percentage of palmitic acid (16.7 to 16.9% of total FA, Tab. I). After 7 weeks of each diet, the results showed that in the liver (Tab. III) and adipose tissue (Tab. V) of the rats fed the BU diet, the

Table III. Fatty acid composition (wt%) of liver total lipids of the rats fed the three experimental diets.

Fatty acid	LA	BL	BU
C12:0	0.02 ± 0.01[a]	0.04 ± 0.01[b]	0.06 ± 0.01[c]
C14:0	0.47 ± 0.09[a]	0.63 ± 0.18[a]	0.91 ± 0.20[b]
C16:0	19.82 ± 1.19[a]	20.06 ± 1.11[a]	21.93 ± 1.16[b]
C18:0	11.23 ± 1.54[a]	11.19 ± 2.29[a]	9.53 ± 1.65[a]
C20:0	0.06 ± 0.01[a]	0.05 ± 0.01[a]	0.21 ± 0.04[b]
SFA	31.60 ± 1.00[a]	31.96 ± 1.62[a]	32.64 ± 0.99[a]
C14:1 n-5	0.03 ± 0.01[a]	0.08 ± 0.02[b]	0.14 ± 0.04[c]
C16:1 n-9	0.43 ± 0.06[a]	0.40 ± 0.12[a]	0.45 ± 0.08[a]
C16:1 n-7	2.28 ± 0.44[a]	2.73 ± 1.01[a,b]	3.56 ± 0.99[b]
C18:1 n-9	32.07 ± 3.56[a]	31.84 ± 3.95[a]	33.01 ± 4.38[a]
C18:1 n-7	4.16 ± 0.36[a]	4.20 ± 0.65[a]	4.15 ± 0.25[a]
C20:1 n-9	0.25 ± 0.04[a]	0.22 ± 0.03[a,b]	0.19 ± 0.04[b]
MUFA	39.23 ± 3.90[a]	39.47 ± 5.30[a]	41.50 ± 5.26[a]
C18:2 n-6	8.50 ± 0.42[a]	7.94 ± 0.77[a,b]	7.01 ± 0.92[b]
C20:2 n-6	0.10 ± 0.01[a]	0.08 ± 0.01[b]	0.06 ± 0.02[b]
C20:3 n-6	0.40 ± 0.02[a]	0.57 ± 0.08[b]	0.64 ± 0.21[b]
C20:4 n-6	14.38 ± 2.23[a]	14.24 ± 2.66[a]	12.33 ± 2.70[a]
C22:4 n-6	0.24 ± 0.01[a]	0.20 ± 0.05[a,b]	0.16 ± 0.03[b]
C22:5 n-6	0.24 ± 0.04[a]	0.23 ± 0.07[a]	0.22 ± 0.06[a]
n-6 PUFA	23.87 ± 2.47[a]	23.26 ± 2.85[a]	20.42 ± 3.48[a]
C18:3 n-3	0.30 ± 0.02[a]	0.27 ± 0.06[a]	0.26 ± 0.07[a]
C20:5 n-3	0.11 ± 0.03[a]	0.18 ± 0.04[a,b]	0.21 ± 0.04[b]
C22:5 n-3	0.32 ± 0.03[a]	0.32 ± 0.05[a]	0.36 ± 0.09[a]
C22:6 n-3	4.57 ± 0.83[a]	4.53 ± 1.08[a]	4.61 ± 1.24[a]
n-3 PUFA	5.30 ± 0.86[a]	5.31 ± 1.07[a]	5.44 ± 1.29[a]

Data are mean ± SD ($n = 6$).

[a,b,c] Significant differences ($P < 0.05$) between the diets are indicated by differing letters.

C16:0 percentages were significantly higher than the values obtained with the two other diets (Fig. 1B). This effect was also apparent in the plasma, but the differences were not significant (Tab. IV). This result shows that the higher dose of dietary myristic acid had an effect on the tissue level of palmitic acid (Fig. 1B).

3.3. Monounsaturated fatty acid profiles of the tissues

Although the three diets contained a very similar % of palmitoleic acid (1.5–1.6%), significant differences appeared in the C16:1 n-7 found in the tissues. In the rats fed the BU diet, palmitoleic acid accounted for 3.6% of total FA in the liver (Tab. III), 3.1% in the plasma (Tab. IV) and 5.6% in adipose tissue (Tab. V). All these values were significantly higher than the C16:1 n-7 amounts found in the rats fed the LA diet. For each tissue, the C16:1 n-7 percentages measured in the BL group were just between those obtained in the BU and LA groups. This result suggests a dose-response accumulation of tissue palmitoleic acid as a function of dietary myristic acid (Fig. 1C).

Table IV. Fatty acid composition (wt%) of plasma total lipids of the rats fed the three experimental diets.

Fatty acid	LA	BL	BU
C12:0	0.13 ± 0.04[a]	0.21 ± 0.08[a,b]	0.25 ± 0.01[b]
C14:0	0.68 ± 0.09[a]	0.92 ± 0.10[b]	1.29 ± 0.11[c]
C16:0	19.26 ± 1.10[a]	18.96 ± 1.38[a]	20.09 ± 0.68[a]
C18:0	6.41 ± 0.87[a]	6.30 ± 1.03[a]	5.84 ± 1.37[a]
C20:0	0.12 ± 0.01[a]	0.19 ± 0.03[b]	0.29 ± 0.05[c]
SFA	26.62 ± 0.63[a]	26.57 ± 1.90[a]	27.76 ± 1.83[a]
C14:1 n-5	0.13 ± 0.10[a]	0.15 ± 0.03[a]	0.36 ± 0.02[b]
C16:1 n-9	0.46 ± 0.02[a]	0.50 ± 0.03[b]	0.56 ± 0.02[c]
C16:1 n-7	2.18 ± 0.22[a]	2.60 ± 0.55[a,b]	3.15 ± 0.51[b]
C18:1 n-9	33.29 ± 1.91[a]	33.14 ± 1.63[a]	33.92 ± 2.83[a]
C18:1 n-7	3.41 ± 0.21[a]	3.40 ± 0.28[a]	3.78 ± 0.60[a]
C20:1 n-9	0.26 ± 0.05[a]	0.22 ± 0.04[a]	0.25 ± 0.04[a]
MUFA	39.73 ± 1.89[a]	40.01 ± 2.29[a]	42.01 ± 3.16[a]
C18:2 n-6	10.67 ± 0.38[a]	9.87 ± 0.74[b]	8.91 ± 0.62[c]
C20:2 n-6	0.49 ± 0.09[a]	0.58 ± 0.08[a,b]	0.67 ± 0.08[b]
C20:3 n-6	0.33 ± 0.02[a]	0.42 ± 0.08[b]	0.49 ± 0.03[b]
C20:4 n-6	17.34 ± 1.42[a]	17.89 ± 2.78[a]	15.36 ± 2.46[a]
C22:4 n-6	0.39 ± 0.11[a]	0.27 ± 0.09[a,b]	0.22 ± 0.05[b]
C22:5 n-6	0.31 ± 0.04[a]	0.28 ± 0.04[a,b]	0.24 ± 0.03[b]
n-6 PUFA	29.53 ± 1.22[a]	29.31 ± 2.26[a]	25.90 ± 2.52[b]
C18:3 n-3	0.52 ± 0.04[a]	0.50 ± 0.08[a]	0.54 ± 0.07[a]
C20:5 n-3	0.26 ± 0.04[a]	0.40 ± 0.10[b]	0.43 ± 0.12[b]
C22:5 n-3	0.98 ± 0.75[a]	0.64 ± 0.45[a]	1.12 ± 0.83[a]
C22:6 n-3	2.35 ± 0.26[a]	2.57 ± 0.40[a]	2.23 ± 0.26[a]
n-3 PUFA	4.12 ± 0.88[a]	4.11 ± 0.78[a]	4.33 ± 0.79[a]

Data are mean ± SD ($n = 6$).

[a,b,c] Significant differences ($P < 0.05$) between the diets are indicated by differing letters.

A similar result was obtained in every lipid species (data not shown).

The LA, BL and BU diets contained almost the same percentage of oleic acid (54.1 to 54.9% of total FA, Tab. I), whatever the tissue, no difference was shown in the C18:1 n-9 % nor in total monounsaturated FA percentages in the liver (40–42% of total FA) in the rats fed the LA, BL and BU diets (Tab. III).

3.4. n-3 polyunsaturated fatty acid profiles of the tissues

The three diets were designed to contain very similar amounts of C18:3 n-3 (1.5–1.6%). After 7 weeks, α-linolenic acid accounted for similar % in the liver (0.26–0.30%) and in the plasma (0.50–0.54%) of the rats fed the LA, BL and BU diets.

Only trace amounts of highly unsaturated FA of the (n-3) series were present in the LA, BL and BU diets (Tab. I). Highly unsaturated FA from the n-3 series were detected in the liver (Tab. III) and plasma (Tab. IV). In the biosynthesis pathway starting from C18:3 n-3 and going to DHA, a single but major significant difference between the rats fed the BU and LA diets was found in the C20:5 n-3%. Eicosapentaenoic acid (EPA) was significantly higher in the liver (0.21% vs. 0.11%) and in the

Table V. Fatty acid composition (wt%) of adipose tissue total lipids of the rats fed the three experimental diets.

Fatty acid	LA	BL	BU
C12:0	0.15 ± 0.06[a]	0.48 ± 0.03[b]	0.85 ± 0.06[c]
C14:0	1.14 ± 0.08[a]	2.26 ± 0.11[b]	3.49 ± 0.20[c]
C16:0	19.92 ± 0.93[a]	20.14 ± 0.32[a]	21.28 ± 0.76[b]
C18:0	3.66 ± 0.24[a]	2.92 ± 0.24[b]	2.39 ± 0.12[c]
SFA	24.86 ± 0.93[a]	25.79 ± 0.32[b]	28.01 ± 0.90[c]
C14:1 n-5	0.07 ± 0.01[a]	0.27 ± 0.03[b]	0.50 ± 0.04[c]
C16:1 n-9	0.56 ± 0.02[a]	0.56 ± 0.09[a]	0.55 ± 0.12[a]
C16:1 n-7	4.19 ± 0.55[a]	4.84 ± 0.96[a,b]	5.57 ± 0.66[b]
C18:1 n-9	54.18 ± 0.93[a]	53.89 ± 1.49[a,b]	52.19 ± 1.39[b]
C18:1 n-7	5.35 ± 0.34[a]	5.55 ± 0.59[a]	5.60 ± 0.50[a]
MUFA	64.35 ± 0.50[a]	65.10 ± 0.53[a]	64.42 ± 1.01[a]
C18:2 n-6	9.84 ± 0.55[a]	8.26 ± 0.26[b]	6.78 ± 0.25[c]
C18:3 n-3	0.94 ± 0.04[a]	0.85 ± 0.07[b]	0.79 ± 0.04[b]

Data are mean ± SD ($n = 6$).
[a,b,c] Significant differences ($P < 0.05$) between the diets are indicated by differing letters.

plasma (0.43% vs. 0.26%). The values obtained with the rats fed the BL diet were intermediate (0.18% in the liver and 0.40% in the plasma). This result suggests a dose-response accumulation of tissue EPA as a function of dietary myristic acid (Fig. 1D). The increased amounts of C20:5 n-3 in the BU group were confirmed by analyzing the FA composition of the lipid species: compared to the LA group, a 2.5-fold increase of EPA was measured in the liver PL and in the plasma CE fractions (Tab. VI).

Concerning the other (n-3) polyunsaturated fatty acids (PUFA), no significant difference was observed between the three groups in the C22:5 n-3, C22:6 n-3 and in the total n-3 PUFA percentages.

3.5. n-6 polyunsaturated fatty acid profiles of the tissues

The study was especially focused on the (n-3) PUFA and the level of C18:3 n-3 was equilibrated between diets. It was therefore more difficult, when the diets were designed, to obtain exactly the same % of linoleic acid in each diet (Tab. I). The BL diet contained about 10% of C18:2 n-6, the BU diet a little bit less (8.4%) and the LA diet a little bit more (11.9%). After 7 weeks, the % of linoleic acid found in the liver (Tab. III), plasma (Tab. IV) and adipose tissue (Tab. V) of the rats fed the LA, BL and BU diets were related to the decreasing percentages of dietary C18:2 n-6.

Trace amounts of (n-6) highly unsaturated fatty acids were present in the LA, BL and BU diets. Highly unsaturated FA of the n-6 series were detected in the liver (Tab. III) and plasma (Tab. IV) of the rats. Several significant differences between the three groups appeared in the n-6 PUFA composition. First, C20:3 n-6 (eicosatrienoic acid) was significantly higher in both the liver and plasma of the rats fed the BU diet (0.64% and 0.49%) compared to that fed the LA diet (0.40% and 0.33%). The

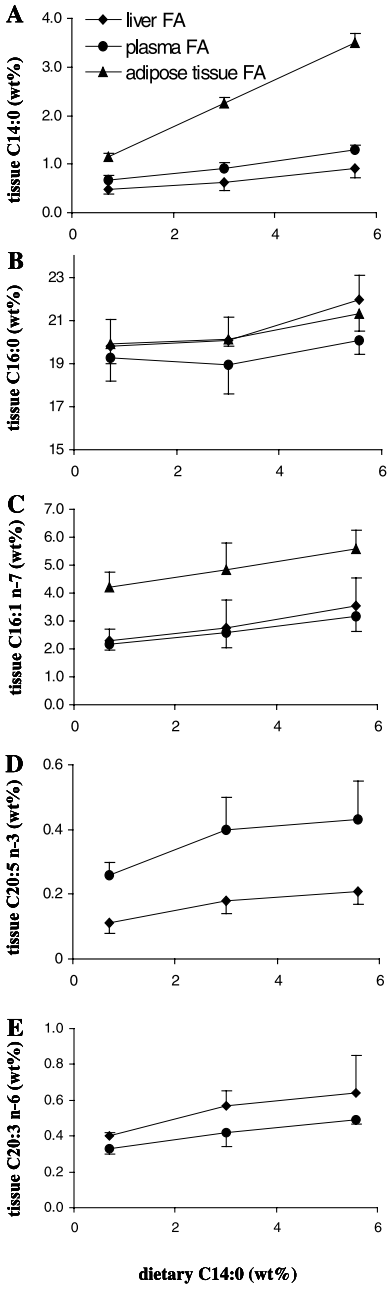

Figure 1. Relationship between the dietary level (wt%) of myristic acid and the tissue level (wt%) of myristic acid itself (**A**), palmitic acid (**B**), palmitoleic acid (**C**), EPA (**D**) and eicosatrienoic acid (**E**) (mean ± SD, $n = 6$).

C20:3 n-6 percentages in the BL group (0.57% in the liver, 0.42 % in the plasma) were between that obtained in the BU and LA groups (Fig. 1E). Compared to the rats fed the LA diet, the increased values of C20:3 n-6 in the tissues of the rats fed the BU diet were confirmed by analyzing the FA composition of lipid species, showing a 1.6-fold increase in the liver and plasma PL fraction (Tab. VI).

Concerning the other (n-6) PUFA, there was no significant difference in arachidonic acid in the liver (Tab. III) and the plasma (Tab. IV) between the three groups, although the mean values obtained in the rats fed the BU diet (12% in the liver and 15% in the plasma) were lower than that measured in the two other groups (14% in the liver and 16–17% in the plasma) because of the lower supply of the C18:2 n-6 precursor in the BU diet. Finally, no significant difference was observed in the total n-6 PUFA percentages in the liver (20–23% of total FA) between the three groups.

3.6. Desaturase activities

Some of the differences found in the PUFA profiles between diets are difficult to explain by a direct effect of the diet (especially the accumulation of C20:5 n-3 and C20:3 n-6 in the rats fed the BU diet). This result led us to hypothesize that desaturase activities could be involved in these modifications. Figures 2A and 2B show that there was, however, no effect of the diets on Δ6- and Δ5-desaturase activities measured in the liver of the rats, after an overnight fasting, using C18:3 n-3 and C20:3 n-6 as substrates, respectively.

4. DISCUSSION

This study was aimed at investigating the effect of myristic acid, at physiologically relevant levels in the diet, on the tissue FA compositions of the rat. Isocaloric diets containing increasing amounts of myristic

Table VI. (n-3) and (n-6) fatty acid composition (wt%) of liver and plasma phospholipids (PL) and cholesterol esters (CE) of the rats fed the three experimental diets.

		Fatty acid	LA	BL	BU
C 18:3 n-3	Liver	PL	ND	ND	ND
		CE	0.67 ± 0.25[a]	0.52 ± 0.08[a]	0.59 ± 0.09[a]
	Plasma	PL	0.52 ± 0.06[a]	0.32 ± 0.14[b]	0.31 ± 0.14[b]
		CE	ND	ND	ND
C 20:5 n-3	Liver	PL	0.11 ± 0.04[a]	0.29 ± 0.10[b]	0.31 ± 0.14[b]
		CE	ND	ND	ND
	Plasma	PL	ND	ND	ND
		CE	0.31 ± 0.05[a]	0.48 ± 0.13[b]	0.73± 0.12[c]
C 22:5 n-3	Liver	PL	0.63 ± 0.04[a]	0.59 ± 0.08[a]	0.78 ± 0.14[a]
		CE	ND	ND	ND
	Plasma	PL	0.41 ± 0.01[a]	0.43 ± 0.06[a]	0.59 ± 0.25[a]
		CE	ND	ND	ND
C 22:6 n-3	Liver	PL	10.35 ± 1.05[a]	9.41 ± 0.85[a]	10.61 ± 1.23[a]
		CE	0.28 ± 0.16[a]	0.29 ± 0.09[a]	0.28 ± 0.07[a]
	Plasma	PL	5.45 ± 0.46[a]	5.17 ± 0.51[a]	5.67 ± 0.30[a]
		CE	1.43 ± 0.19[a]	1.27 ± 0.24[a]	1.56 ± 0.22[a]
C 18:2 n-6	Liver	PL	6.22 ± 0.95[a]	6.60 ± 0.90[a]	6.37 ± 0.94[a]
		CE	6.57 ± 0.66[a]	5.68 ± 0.56[b]	4.69 ± 0.30[c]
	Plasma	PL	6.66 ± 0.13[a]	7.36 ± 1.20[a,b]	7.81 ± 1.02[b]
		CE	9.09 ± 0.52[a]	9.58 ± 1.05[a]	9.27 ± 1.18[a]
C 20:3 n-6	Liver	PL	0.68 ± 0.07[a]	0.92 ± 0.25[a,b]	1.07 ± 0.22[b]
		CE	ND	ND	ND
	Plasma	PL	0.77 ± 0.12[a]	1.00 ± 0.31[a,b]	1.25± 0.25[b]
		CE	0.30 ± 0.02[a]	0.44 ± 0.07[b]	0.49 ± 0.05[b]
C 20:4 n-6	Liver	PL	31.95 ± 5.17[a]	29.29 ± 0.64[a]	27.90 ± 2.19[a]
		CE	3.77 ± 0.43[a]	3.17 ± 1.54[a,b]	2.62 ± 0.71[b]
	Plasma	PL	30.33 ± 0.65[a]	28.33 ± 1.41[b]	26.50 ± 0.97[c]
		CE	53.84 ± 3.09[a]	44.08 ± 6.70[b]	44.73 ± 6.93[b]

Data are mean ± SD ($n = 6$).
[a,b,c] Significant differences ($P < 0.05$) between the diets are indicated by differing letters. ND = not detected.

acid (from 0.71 to 5.57% of total FA) were designed (Tab. I) and given to rats for 7 weeks.

Although our goal was not to focus on plasma lipoprotein and cholesterol metabolism, several physiological lipidic parameters were measured (Tab. II). The results showed that under our experimental conditions, no effect on plasma cholesterol concentrations or on hepatic cholesterol level

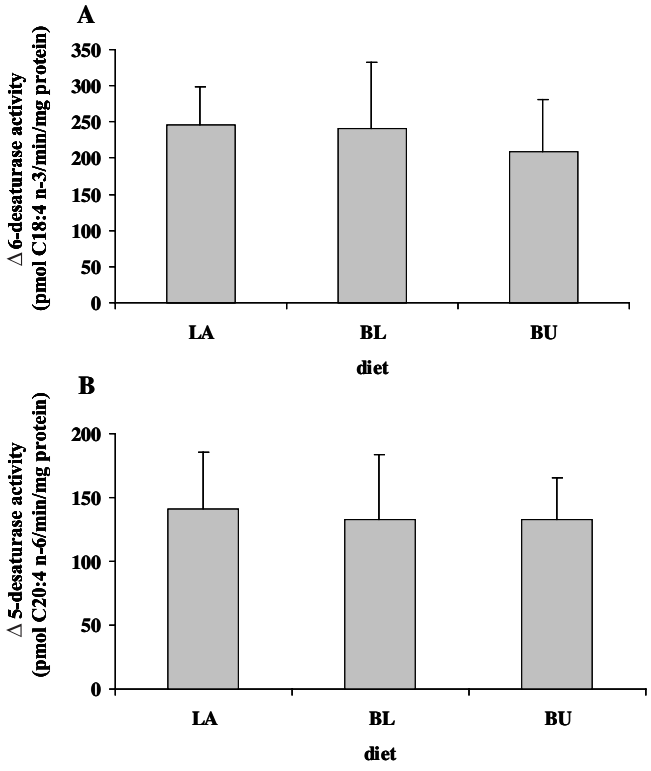

Figure 2. The effect of the 3 diets on desaturase activities (**A**) Δ6-desaturase, (**B**) Δ5-desaturase, (mean ± SD, $n = 6$). No significant difference was shown between the three groups.

was shown in response to increasing amounts of myristic acid in the physiological range we chose. The rat is not the best model for the study of human plasma lipoprotein and cholesterol metabolism, because of its low cholesteryl ester transfer protein (CETP) activity and high HDL-cholesterol/total cholesterol ratio [19]. However, this result shows that myristic acid at physiologically relevant levels, in a diet containing 30% fat as saturated fatty acids, has no effect on plasma total cholesterol concentration in rats, compared to diets very low in myristic acid (LA diet). Moreover, we observed that the level of dietary cholesterol had no effect on the lipid values in the plasma and liver. This result was in accordance with a recent study showing that, in the hamster, an increase in the level of plasma HDL-cholesterol resulting in a slight and not significant increase in plasma total cholesterol concentration, was related to the amount of C14:0 (0.5 to 2.4%) in the diet [9, 10].

By studying the tissue FA compositions of the rats (Tabs. III-V), the results showed that variations in the myristic acid content of the diets had an effect on the tissue myristic acid level and more widely on saturated FA composition. Our results show that the tissue % of myristic acid are highly correlated to the dietary % of myristic acid (Fig. 1A). In agreement with our study, Wolk et al. [8] showed that C15:0 but also C14:0 content in adipose tissue is a valid biomarker for dairy fat intake in men. Whatever the diet, our results show that the amount of myristic acid was higher in the adipose tissue than in the plasma, and higher in the plasma than in the liver, showing that the reliability between dietary and tissue myristic acid depends on the tissue

analyzed and probably on the ability of this tissue to metabolize myristic acid that comes from the diet. The lower level of myristic acid in the liver compared to plasma and adipose tissue suggests that it has been rapidly metabolized and especially highly oxidized, as previously shown in cultured rat hepatocytes [16, 20]. Compared to the two other diets, however, this increased β-oxidation rate producing higher levels of acetyl residues in the rats fed the BU diet did not result in an increased biosynthesis of cholesterol in the liver (Tab. II).

Analyzing the tissue level of palmitic acid gives additional information on the metabolic utilization of dietary myristic acid. The significantly higher % of palmitic acid in the tissues of the rats fed the BU diet (Tabs. III and V), compared with the two other diets, did not correlate with the dietary % of palmitic acid, which was similar in the LA, BL and BU diets. Therefore, this result suggests that, as previously shown in cultured rat hepatocytes [16, 21], the elevated C16:0 values found in the rats fed the BU diet reflect a rapid elongation of myristic acid (initially provided by the diet) to palmitic acid. It is interesting to note that the tissue % of C16:0 is, however, not correlated to the dietary C14:0% (Fig. 1B), since the BL diet, which contained 3.0% of C14:0, led to similar C16:0 content as the LA diet (0.7% of C14:0). Therefore, there may be a threshold level of myristic acid daily intake (between 3.0 and 5.6% of total dietary FA) that may exceed the β-oxidation capacity of the organism, thereby directing the additional myristic acid to a conversion to C16:0.

Among monounsaturated FA, palmitoleic acid was higher in all the tissues of the rats fed the BU diet, compared with the two other groups (Tabs. III-V). This effect can probably be explained by the metabolic fate of part of dietary myristic acid that is first elongated to palmitic acid and then Δ9-desaturated to C16:1 n-7, as previously suggested in cultured rat hepatocytes [16]. Our results suggest that high levels of dietary myristic acid can contribute to increase the tissue level of palmitoleic acid (Fig. 1C).

In animal cells, the availability of n-6 and n-3 very-long-chain PUFA depends on the diet providing the essential precursors (i.e., C18:2 n-6 and C18:3 n-3) and derivatives, and on the activity of the enzymes involved in highly unsaturated FA biosynthesis. In order to be able to compare the three groups, the three diets were designed to contain almost only the n-6 and n-3 precursors and not the derivatives, in a ratio that meets human essential FA requirements (between 5 and 7) and the priority was to equilibrate the C18:3 n-3 amount between the diets (1.5–1.6%). After 7 weeks, in the n-3 PUFA, a main finding was that although the three diets contained the same amounts of C18:3 n-3, EPA was significantly higher in the liver and plasma of the rats fed the BU diet compared to those fed the LA diet (Tabs. III and IV). In addition, considering the intermediate level of C14:0 in the BL diet, Fig. 1D suggests that there is a dose-response accumulation of tissue EPA as a function of dietary myristic acid. In a human study, Dabadie et al. [12] showed that myristic acid intakes of 1.2% and 1.8% total energy were associated with a significant increase in cholesteryl ester DHA and a smaller but still significant increase in EPA. Although these results showing an increase in n-3 PUFA as a function of dietary myristic acid are in agreement with our study, we did not obtain the same effect on DHA in rats as in humans.

The n-6 PUFA profiles are not as easy as n-3 PUFA profiles to analyze because the C18:2 n-6 precursor was not exactly equilibrated between the diets. These slightly different levels could introduce alternative and/or complementary effects to those obtained by increasing the level of C14:0. Since dietary linoleic acid was lower in the BU diet (8.4%) compared to the LA diet (11.9%), one would have expected that, if not all, at least some of the PUFA of the n-6 series would have been lowered in the BU group, after 7 weeks of these diets. This

hypothesis was verified (Tabs. III and IV) for C20:4 n-6 (although no significant differences appeared), C22:4 n-6, and C22:5 n-6 in the plasma (Tab. IV). On the contrary, C20:3 n-6 was significantly higher in both the liver and plasma of the rats fed the BU diet (especially in the PL fraction) compared to the rats fed the LA diet. It is unlikely that dietary trace amounts of C20:3 n-6 and its precursor C18:3 n-6 in the BU diet may have contributed to all this effect, since they represented only 0.06 and 0.16% of total FA in the BU diet (Tab. I). Finally, it is unlikely that the lower content of C18:2n-6 in the BL and BU diets (compared to the LA diet) could influence the long chain derivative biosynthesis since C20:3n-6 increased to the same extent as C20:5n-3.

A dose-response accumulation of tissue C20:3 n-6 as a function of dietary C14:0 is suggested when the three diets were analyzed (Fig. 1E). Eicosatrienoic acid (C20:3 n-6) is a precursor of eicosanoids of the 1-series and also a substrate of Δ5-desaturase leading to the biosynthesis of arachidonic acid.

A higher tissue level of C20:5 n-3 and C20:3 n-6 may reflect increased biosynthesis from the essential precursors or decreased conversions to longer highly unsaturated FA. However, no significant difference between the three groups appeared in the arachidonic acid and DHA levels. Therefore, the PUFA profiles suggest that at least Δ6-desaturase activity in the n-3 and n-6 pathways and also Δ5-desaturase activity in the n-3 pathway may have been increased in the BU group. In a previous study carried out on cultured rat hepatocytes, we showed that myristic acid indeed increases Δ6-desaturase activity, in a dose-dependent manner [14]. In the present dietary study, however, no difference between groups was shown in the liver Δ6- and Δ5-desaturase activities (Figs. 2A and 2B). We assume that measuring these activities after an overnight fasting may have hidden potential differences between diets since it is now known that the expression of these two enzymes is induced after fasting [22]. However, desaturase assays are routinely designed after fasting [15] because high variability in desaturase activities comes from the nutritional status of the animals. While dietary PUFA are known to suppress the expression and activity of Δ6- and Δ5-desaturases [23, 24], the effect of individual saturated FA has been less explored.

Altogether, these results show that among saturated FA, daily intake of myristic acid at physiological levels has a great impact on the tissue FA composition and especially on the level of C20:5 n-3 and C20:3 n-6. This could be of importance, since these PUFA are two of the three known precursors of eicosanoids.

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