Effects of α-linolenic acid deficiency and age on oxidative phosphorylation and fatty acid composition of rat liver mitochondria

Ofelia Angulo-Monroy, Philippe Guesnet, and Georges A. Durand

I.N.R.A., Laboratoire de Nutrition et Sécurité Alimentaire, 78352 Jouy-en-Josas Cédex, France

Two weeks before mating, female rats from n-3 *PUFA deficient lineage were divided into two groups; the first group continued to get the* n-3 *PUFA deficient diet (peanut oil) and the second one received a control diet (peanut and rapeseed oil mixture). Total phospholipid fatty acid composition, respiration, and succinic dehydrogenase (SDH) activity were studied in liver mitochondria from 14-, 30-, and 90-day-old male rats delivered by these two experimental groups. Moreover, the principal phospholipid classes (PC, PE, CL) content and F_1F_0 ATPase activity were studied in liver submitochondrial (inner) membrane from 30-day-old rats. The results showed that dietary* n-3 *PUFA deficiency did not modify cholesterol and phospholipid levels in total mitochondrial lipids whatever the animal age. However, 22:6* n-3 *(DHA) level in phospholipids was considerably reduced by this deficiency; this reduction was compensated by an increase in 22:5* n-6 *and 20:4* n-6 *so that the total polyunsaturated fatty acid sum (*n-6 + n-3*) was not modified. It did not alter basal and stimulated succinic dehydrogenase specific activity, state 3, state 4, neither respiratory control ratio. However, the rate of oxidative phosphorylation pathway was doubled between 14- and 30-day-old animals independently of diet. The relative proportions of the major phospholipid classes in submitochondrial membrane were not altered. Also, there was no significant effect on F_1F_0 ATPase activity in submitochondrial membrane. Finally, the considerable reduction of DHA level and the parallel increase of the* n-6/n-3 *ratio in mitochondrial membrane phospholipids did not influence mitochondrial physiological activity, at least with regard to experimental conditions used and to the parameters studied.*

Keywords: α-linolenic acid deficiency; oxidative phosphorylation; mitochondria; growth

Introduction

Polyunsaturated fatty acids (PUFA) are, with cholesterol, the main basic components of the lipid matrix of biological membranes. Their relative proportions determine to a great extent the biophysical and physiological properties of these membranes.[1-4] Moreover, many studies have demonstrated that membrane lipid composition could be modified rapidly and profoundly by dietary fats (for review, see reference 5).

N-3 PUFA, of which α-linolenic acid (18:3 *n*-3) is the precursor, are usually found in all cellular and subcellular membranes of mammals mainly in the form of docosahexaenoic acid or DHA (22:6 *n*-3) (for review, see reference 6). In brain and retina, the abundance of DHA in phospholipids suggests that *n*-3 PUFA may play a specific role in the physiology of nervous tissue membranes. Some studies on retinal and brain function in *n*-3 PUFA deficient rat and rhesus monkey support the evidence of the essentiality of these fatty acids.[7,8] However, no study has been reported on the effects of α-linolenic acid deficiency upon the physiology of membranes other than nervous membranes. Some of them contain appreciable proportions of 22:6 *n*-3 (i.e., heart and liver mitochondria, where the level of DHA can reach up to 15% of total fatty acids in rats fed a diet with a dietary *n*-6/*n*-3 ratio of about 5 to 10.[9,10]) Subsequently, in these membranes, one PUFA

Address reprint requests to Dr. Georges Durand, Director of Research, Laboratoire de Nutrition et Sécurité Alimentaire, I.N.R.A., 78352 JOUY-en-JOSAS CEDEX, France.
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Table 1 Diet composition (g/kg)

	Deficient diet	Control diet
Casein	220	220
DL methionine	1.6	1.6
Cellulose	20	20
Starch	438.4	438.4
Saccharose	220	220
Vitamin mixture[a]	10	10
Mineral mixture[b]	40	40
Peanut oil[c]	50	25
Rapeseed oil[c]	—	25

[a] Total vitamin supplement, United States Biochemical Corp., Cleveland, OH.
[b] Composition g/100 g: $CaHPO_4,2H_2O$, 38.0; K_2HPO_4, 24.0; $CaCO_3$, 18.0; NaCl, 6.9; MgO, 2.0; $MgSO_4,7H_2O$, 9.0; $FeSO_4,7H_2O$, 0.86; $ZnSO_4,H_2O$, 0.5; $MnSO_4,H_2O$, 0.5; $CuSO_4,5H_2O$, 0.1; NaF, 0.08; $CrK(SO_4)_2,H_2O$, 0.05; $(NH_4)_6Mo_7O_{24},4H_2O$, 0.002; KI, 0.004; $CoCO_3$, 0.002; $Na_2SeO_3,5\ H_2O$, 0.002.
[c] Gift from the Company "Lesieur Alimentaire" (92100 Boulogne Billancourt, France).

Table 2 Fatty acid composition of dietary lipids

	Diet	
Fatty acids %	Deficient diet[a]	Control diet[b]
Σ Saturated	20.1	14.7
Σ Monounsaturated	59.0	60.1
18:2 *n*-6	20.8	21.0
18:3 *n*-3	0.1	4.2
Fatty acids/100 g of diet:		
18:2 *n*-6 (mg)	1 008	1 035
18:3 *n*-3 (mg)	5	204
n-6/*n*-3	202	5.1

Note: Lipid fatty acid composition was analyzed by gas chromatography of fatty acid methyl esters in the following conditions: Packard model 427 chromatograph; glass capillary column coated with Carbowax 20M (Chrompack, France); carrier gas pressure H_2:0.8 bar; temperature: 190° C; detection by flame ionization.
[a] Deficient: peanut-oil diet.
[b] Control: rapeseed-peanut oil diet.

out of two acylated at the β-position of phospholipids is DHA.

The interest in studying the physiological function of *n*-3 PUFA in the liver mitochondria is manifest because the level of 22:6 *n*-3 in mitochondrial phospholipids was decreased rapidly during the first three days of total PUFA deficiency in the rat,[10] and, although the influence of total essential fatty acid deficient diets upon transport and oxidative phosphorylation has received considerable attention since 1960,[11] few studies have provided useful information on the respective importance of arachidonic acid (20:4 *n*-6) and DHA upon this metabolic pathway.[12,13] Some studies have shown that an alteration on the *n*-6/*n*-3 PUFA ratio in liver mitochondrial phospholipids produced a change on F_1F_0 ATPase activity.[14,15]

To assess the role of *n*-3 PUFA (particularly DHA) on the hepatic mitochondrial oxidative phosphorylation, this study was undertaken on α-linolenic acid deficient growing rats by measuring succinic dehydrogenase activity and mitochondrial respiration, as well as F_1F_0 ATPase activity in relation to phospholipid class proportions in submitochondrial (inner) membrane.

Methods and materials

Animals and diets

Thirty Wistar female rats from a *n*-3 PUFA deficient lineage (second generation) were used as previously described.[16] They received a semisynthetic diet (*Table 1*) in which lipids were supplied by 5% peanut oil, deficient in α-linolenic acid (*Table 2*).

Two weeks before mating at 10 weeks of age, two groups were constituted: the first group ("deficient") continued to get the α-linolenic deficient diet and the second one received a control diet containing 5% of an equal mixture of rapeseed and peanut oils. Both diets supplied 1,000 mg of linoleic acid/100 g diet; however, the control diet contained 200 mg of α-linolenic acid against 5 mg for the deficient one. After delivery, females were caged individually and the litters were equalized to 10 pups each. Then, the young male pups were sacrificed during suckling (14 days), after weaning (30 days), and at adult age (90 days).

Respiration and succinic dehydrogenase activity measurements in relation to total phospholipid fatty acid composition were evaluated in total mitochondria from rats at three different ages (14, 30, and 90 days). F_1F_0 ATPase activity and phospholipid classes content were measured in submitochondrial membrane from 30-day-old rats only.

Mitochondrial preparation

To maintain a maximum respiratory control ratio of mitochondria, 30- and 90-day-old animals were fasted overnight and then refed for three hours (7:00 a.m. to 10:00 a.m.) before the sacrifice. Then, the liver was excised rapidly, washed, and cut up in a cold homogenization medium 10 mM HEPES/KOH buffer, pH 7.4 containing succinate and ADP as described by Reinhart et al.[17] This technique allowed the rapid preparation of a relatively pure mitochondrial fraction with a high acceptor control ratio of respiration. Briefly, 5 g (14 days old) or 10 g (30 and 90 days old) of liver were homogenized respectively with 5 ml or 10 ml of cold homogenization medium using a Teflon potter homogenizer (Thomas C., size C.) using three up and down strokes at 1,000 rpm. Then, the homogenate was layered on to a step percoll gradient (19, 31, 42, and 52%, vol/vol) (Pharmacia, St. Quentin en Yvelines, France) and centrifuged at 20,000 rpm (31,400 × *g*) for 1 min in a Beckman JA-20 rotor. The mitochondrial fraction was collected at the 42%/52% interface and immediately used for the respiratory succinic dehydrogenase activities or submitochondrial membrane preparation used for the measurement of F_1F_0 ATPase activity.

Protein was assayed by the method of Bradford[18] in presence of sodium deoxycholate (0.4 g/100 ml). Isolated mitochondria was monitored for purity by electron microscopy and showed few endoplasmic reticulum in the preparation.

Total mitochondrial lipid analysis

Mitochondrial total lipids were extracted by the Folch et al. procedure[19] in the presence of butylhydroxytoluene (BHT) at 0.02% (wt/vol) (20 mg/100 ml). Cholesterol was assessed enzymatically after Wolff's method.[20] Total phospholipids amounts were determined by measuring the total phosphorus as described by Bartlett et al.[21] Protein, the element of reference, was determined by the method of Lowry et al.[22] Fatty acid composition of total mitochondrial phospholipids was determined by separation of fatty acid methyl esters (FAME) by gas-liquid chromatography (Packard 427, capillary column Chrompack coated with Carbowax 20 M).

Respiration measurements and succinic dehydrogenase activity in isolated mitochondria

Oxygen consumption (respiration) by isolated mitochondria was determined polarographically at 37° C in the absence (state 4) or in the presence (state 3) of ADP with a clark-oxygen electrode as described by Estabrook.[23] 1 mg of mitochondrial protein was added to the oxygen buffer (4.5 ml) consisting of 50 mM KCl, 100 mM sucrose, 10 mM KH_2PO_4, 2 mM $MgCl_2$, 1 mM EDTA, and 15 mM Tris/HCl, pH 7.4. Sodium succinate (50 μmol) was used as oxidizable substrate in the measurement of mitochondrial respiration. The first state 3 (ADP non-limiting)/state 4 (ADP limiting) respiration cycle was used in all measurements after adding 2.5 μmol ADP. The respiratory control ratio was obtained by the state 3/state 4 ratio.

Succinic dehydrogenase activity (SDA) was measured as described by Bachman et al.[24] in absence (basal) or in presence (stimulated) of ADP (20 mM) and NADH (15 mM).

Submitochondrial membrane preparation

Fresh rat liver mitochondria prepared from total liver as described earlier was homogenized with pyrophosphate buffer (0.1 M) and exposed to sonic oscillation in an ultrasonic homogenizer (Labsonic 2 000, 50 W) at 0° C for 2 min using Kagawa's technique.[25] The homogenized mixture was centrifuged at 26,000 × *g* (15,000 rpm in a Beckman JA 20 rotor) for 15 min; the resulting supernatant was centrifuged at 100,000 × *g* (45,000 rpm in a Beckman 50 Ti rotor) for 1 hr. The precipitate was washed with 0.25 M sucrose to recuperate the membrane preparation.

Submitochondrial phospholipid class analysis

After total lipid extraction as previously described, the phospholipid classes from submitochondrial membrane were separated by one-dimensional thin layer chromatography using commercial purified standards (Sigma) as controls. Samples were applied to silica gel H plates (0.5 mm) and developed in a solvent system of chloroform:methanol:acetic acid:water (25:15:4:2 by vol). After developed plates were dried, areas corresponding to phospholipid classes were scraped off. Only the major phospholipid classes (phosphatidylcholine = PC; phosphatidylethanolamine = PE; cardiolipin = CL) were quantified by Bartlett's method.[21]

Table 3 α-Linolenic acid deficiency in the rat: effects on body and liver weight, and on mitochondrial protein content of liver during growth

Age	Diet	Body weight (g)	Liver weight (g)	Protein/liver (mg/g)
14 days	Control[a]	31.6 ± 3.0	1.0 ± 0.3	5.2 ± 1.9
	Deficient[b]	28.4 ± 5.8	1.0 ± 0.4	5.1 ± 1.4
30 days	Control[a]	82.3 ± 11.7	2.9 ± 0.7	12.8 ± 4.2
	Deficient[b]	82.6 ± 22.4	2.8 ± 0.7	11.9 ± 3.6
90 days	Control[a]	313.8 ± 10.5	5.4 ± 0.2	22.4 ± 3.2
	Deficient[b]	316.8 ± 21.5	5.4 ± 0.2	21.7 ± 2.2

Note: Values are the mean ± SD. For body and liver weight, the number of values is 35, 15, and 10 at 14, 30, and 90 days of age, respectively. For mitochondrial protein content of liver, the number of determinations is 7, 5, and 5, respectively.
[a] Control: rapeseed-peanut oil diet.
[b] Deficient: peanut-oil diet.

F_1F_0 ATPase activity

F_1F_0 ATPase activity of rat liver submitochondrial membrane was determined by following the hydrolysis of exogenous ATP to ADP + Pi using the method of Kagawa.[25]

Data analysis

Data were analyzed by using Student's *t* test.

Results

Body and liver weights and mitochondrial protein content

Regardless of diet, body and liver weights were respectively about 30 g and 1 g at 14 days, 82 g, and 2.8 g at 30 days, and 315 g and 5.4 g at 90 days of age (*Table 3*).

As previously observed,[26] there were no significant effects of α-linolenic acid deficiency on either body weight or liver weight in the animals.

Similarly, total liver mitochondrial protein content was not modified by the deficiency. We only observed

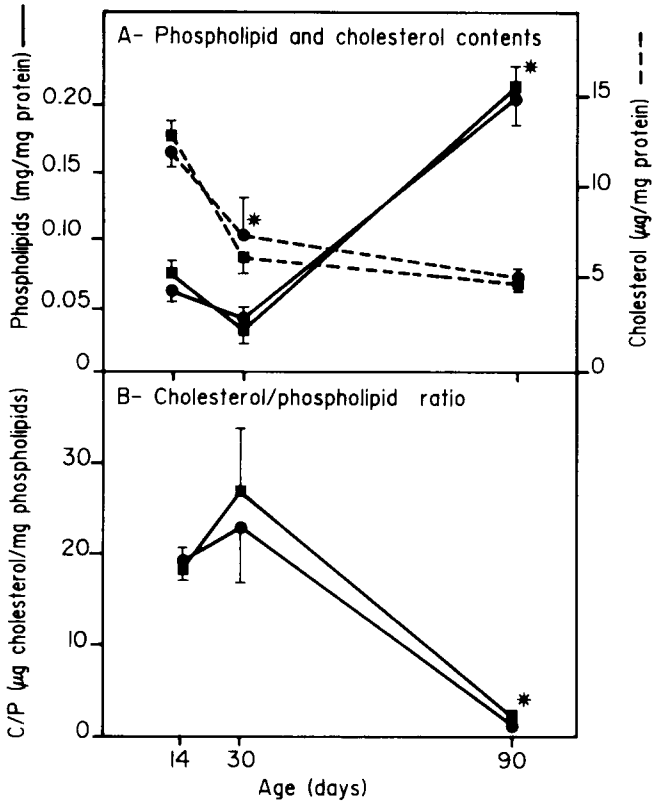

Figure 1 α-Linolenic acid deficiency during growth in the rat. Effects on lipid composition of mitochondria. Part A: phospholipid and cholesterol contents; Part B: cholesterol/phospholipid ratio. ● control rats; ■ deficient rats. Values are expressed as means ± SD for 7 (14 days) and 5 (30 and 90 days) determinations; * denotes significantly different ($P < 0.05$) from the precedent age, but not from the following.

an increase of protein content during growth; so, between 14 and 90 days of age, this content was increased 4 fold (about 22 mg protein/g liver vs 5 mg protein/g liver.

Isolated mitochondria

Lipid composition. The effect of α-linolenic acid deficiency on lipid composition is reported in *Figure 1*. No appreciable difference in mitochondrial lipid content was noted between the two groups of animals. Total phospholipid content increased about 130% during growth between 14 days and 90 days of age. On the contrary, cholesterol content decreased twofold between 14 days and 90 days of age. Consequently, these modifications caused a slight but not significant increase in the total cholesterol/phospholipids ratio between 14 and 30 days of age, and a 10-fold decrease at 90 days of age (1.6 vs 18–24 µg/mg).

The mitochondrial phospholipid fatty acid composition (*Table 4*) showed that total saturated fatty acids (SFA) constituted 35–37% of all fatty acids whatever the age and the group studied; the main fatty acids were 16:0 and 18:0 (16–20%). As to monounsaturated fatty acids (MUFA), their levels varied between 12% and 15% and the main fatty acid was oleic acid (6–8%). The α-linolenic acid deficiency had no effect on the mean SFA and MUFA level in mitochondrial phospholipids whatever the age.

Among polyunsaturated fatty acids (PUFA), those of the *n*-6 family were the most abundant. They constituted around 75% of total PUFA in the control group versus 95% in the deficient one, due mainly to the increased membrane concentration of 22:5 *n*-6 and 20:4 *n*-6; thus, 22:5 *n*-6 membrane concentration increased from 4- to 9-fold in deficient rats.

As expected, *n*-3 PUFA mitochondrial membrane content was reduced dramatically by the deficient diet. Their level was 10–13% in the control rats, and 2–3% in the deficient ones. These variations were mostly due to 22:6 *n*-3 which is the major fatty acid of this family (83–89%). Nevertheless, as *n*-6 PUFA compensated for the lack of 22:6 *n*-3, the *n*-6 + *n*-3 PUFA level was maintained at 50% of total fatty acids and the 22:5 *n*-6/22:6 *n*-3 ratio was 0.1% in control animals and 2.4 to 3.5 in deficient animals.

Succinic dehydrogenase activity and respiration. The α-linolenic acid deficiency appeared to have no effect on basal and stimulated (SDH) activity (*Figure 2A*). Basal activity increased from 10 to 17–20 µmol/min per mg protein between 14-to-30-day-old rats, then reached a plateau until 90 days of age. For stimulated activity, the same pattern was noted except for 14 days of age, in which the enzyme activity in control rats showed a markedly but nonsignificantly higher activity than that of deficient rats (98 µmol/mg protein per min ± 30 vs 68 µmol/mg protein per min ± 15).

Neither the oxygen consumption nor the respiratory control ratio were altered by the deficient status (*Figure 2B*). An activated-oxygen-consumption (state 3) increase (×2) was noted between 14- and 30-day-old animals independently of dietary group; this higher oxygen consumption was maintained at 90 days of age. The same increase was observed between 14 and 30 days of age for the basal oxygen consumption rate (state 4) but it was followed by a nonsignificant decrease at 90 days of age.

These modifications in mitochondrial respiration were reflected by an increase (×2) of respiratory control ratio between 14- and 90-day-old rats (*Figure 2C*).

Submitochondrial membrane

Phospholipid classes distribution (PC, PE, CL). The proportion of phospholipid classes from liver submitochondrial membrane of rats fed the deficient diet was not significantly different from that of rats fed the control diet (*Table 5*).

F_1F_0 ATPase activity. The results showed that a dietary deficiency in 18:3 *n*-3 did not influence the liver-submitochondrial F_1F_0 ATPase activity (*Table 6*).

Table 4 α-Linolenic acid deficiency in the rat. Effects on liver mitochondrial total phospholipid fatty acid composition

	Age					
	14 days (n = 7)		30 days (n = 5)		90 days (n = 5)	
	Diets					
Fatty acids	Control[a]	Deficient[b]	Control[a]	Deficient[b]	Control[a]	Deficient[b]
16:0	19.7 ± 1.0	17.3 ± 1.7	16.1 ± 1.4	15.9 ± 1.0	16.4 ± 0.8	16.1 ± 1.2
18:0	15.7 ± 1.3	16.8 ± 0.8	18.0 ± 1.4	18.2 ± 1.3	19.0 ± 0.5	20.4 ± 0.5
ϵ SFA	37.2 ± 2.2	35.3 ± 2.1	34.9 ± 2.3	35.1 ± 2.2	36.2 ± 1.4	37.4 ± 1.7
18:1 *n*-9	6.5 ± 1.0	7.5 ± 1.1	8.3 ± 2.7	8.4 ± 1.1	6.0 ± 1.3	6.6 ± 1.1
18:1 *n*-7	3.7 ± 0.5	3.1 ± 0.4	3.6 ± 0.6	3.5 ± 0.3	4.4 ± 0.7	4.0 ± 0.4
ϵ MUFA	11.9 ± 1.6	13.0 ± 1.6	14.4 ± 2.5	14.7 ± 0.8	12.9 ± 1.0	13.3 ± 1.2
18:2 *n*-6	12.6 ± 1.5	13.5 ± 1.2	13.3 ± 2.0	11.9 ± 2.0	12.0 ± 1.2	9.3 ± 0.6[c]
20:3 *n*-6	0.8 ± 0.1	0.8 ± 0.2	0.8 ± 0.1	0.7 ± 0.1	1.5 ± 0.1	1.4 ± 0.2
20:4 *n*-6	21.9 ± 1.6	25.5 ± 1.8[c]	24.0 ± 2.7	28.4 ± 1.6[c]	25.6 ± 2.1	29.0 ± 1.1[c]
22:5 *n*-6	1.1 ± 0.3	7.0 ± 1.1[d]	1.2 ± 1.1	5.0 ± 1.1[c]	0.6 ± 0.0	5.5 ± 0.6[d]
ϵ *n*-6 PUFA	37.4 ± 1.9	48.5 ± 3.2[c]	40.3 ± 3.2	47.6 ± 3.0[d]	40.9 ± 1.7	46.4 ± 1.6[d]
20:5 *n*-3	0.2 ± 0.1	—	0.4 ± 0.1	—	0.4 ± 0.1	0.2 ± 0.1
22:5 *n*-3	1.1 ± 0.1	—	1.0 ± 0.2	0.4 ± 0.2[c]	0.6 ± 0.1	0.4 ± 0.3
22:6 *n*-3	11.9 ± 1.8	2.0 ± 0.3[d]	8.6 ± 2.1	1.9 ± 0.8[d]	8.5 ± 0.8	2.3 ± 0.3[c]
ϵ *n*-3 PUFA	13.4 ± 1.8	2.0 ± 0.3[d]	10.3 ± 2.4	2.3 ± 1.4[d]	9.7 ± 0.7	2.9 ± 0.1[d]
n-6 + *n*-3	50.8 ± 2.7	50.5 ± 3.0	50.6 ± 2.5	49.8 ± 2.7	50.6 ± 2.7	49.3 ± 1.5
n-6/*n*-3	2.8 ± 0.4	24.2 ± 4.6[d]	3.9 ± 2.0	20.6 ± 7.9[d]	4.2 ± 0.1	16.0 ± 1.3[d]
22:5 *n*-6/22:6 *n*-3	0.1 ± 0.0	3.5 ± 0.7[c]	0.1 ± 0.2	2.6 ± 1.2[d]	0.1 ± 0.0	2.4 ± 0.3[d]

Note: Values are means ± SD; n = number of determinations.
Abbreviations: SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.
[a] Control: rapeseed-peanut oil diet.
[b] Deficient: peanut-oil diet.
[c] $P < 0.01$.
[d] $P < 0.001$.

Table 5 α-Linolenic acid deficiency in 30-day-old rats: effect on proportion of phospholipid classes (phosphatidylcholine = PC; phosphatidylethanolamine = PE; cardiolipin = CL) in submitochondrial membranes

Phospholipid classes (μg/mg protein)	Control[a] n = 5	Deficient[b] n = 5
PC	91.6 ± 32.0	134.6 ± 44.2
PE	62.0 ± 22.3	83.7 ± 28.0
CL	31.4 ± 10.1	47.5 ± 18.5

Note: Values are means ± SD; n = number of determinations.
[a] Control: rapeseed-peanut oil diet.
[b] Deficient: peanut oil diet.

Table 6 α-Linolenic acid deficiency in 30-day-old rat: effects on F_1F_0 ATPase activity of submitochondrial membrane

	Control[a] n = 5	Deficient[b] n = 5
F_1F_0 ATPase specific activity (nmol Pi/mg protein/min)	1642 ± 147	1644 ± 160

Values are means ± SD; n = number of determinations.
[a] Control: rapeseed-peanut oil diet.
[b] Deficient: peanut-oil diet.

Discussion

PUFA lipid composition of mitochondria

In this study, α-linolenic acid deficiency caused a drop in the amount of DHA (22:6 *n*-3) compensated by an increased level of 22:5 *n*-6 (+ 400% to 900%), as well as by that of arachidonic acid (20:4 *n*-6) (+ 15%). The extent of the modification of the *n*-6 and *n*-3 PUFA composition of deficient mitochondrial phospholipids presented in this paper are in agreement with previous results on liver phospholipids, brain cells, brain mitochondria, and microsomes in the rat[27,28]; several studies have shown that the 22:5 *n*-6/22:6 *n*-3 ratio in phospholipid membranes when higher than 1[27,29] may be a reliable index of *n*-3 PUFA deficiency. In this study, this ratio is included between 2.4 and 3.5. The total percent of PUFA (*n*-6 + *n*-3) is not altered and represents about 50% of total fatty acids.

During growth, we did not observe appreciable changes in PUFA pattern of liver mitochondrial phospholipids whatever the dietary group considered. Meanwhile, between 14 and 30 days of age, we observed a slight increase of arachidonic acid content which was compensated by a decrease of 22:6 *n*-3 content (control group) and of 18:2 *n*-6 and 22:5 *n*-6 contents (deficient group). During the perinatal life, some studies have pointed out changes of PUFA composition of all mitochondrial liver phospholipids,[30,31]

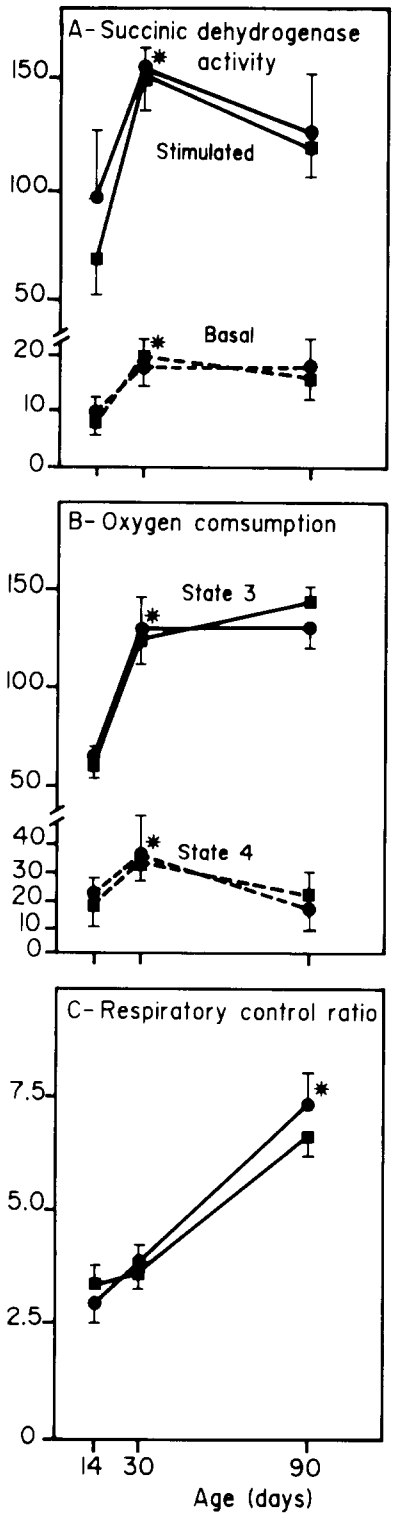

Figure 2 α-Linolenic acid deficiency during growth in the rat. Effects on succinic dehydrogenase activity (part A), oxygen consumption (part B), and on respiratory control ratio (part C). Succinic dehydrogenase activity is expressed as μmol reduced 2,4 dichloroleucoindophenol/mg protein per min. The oxygen consumption is expressed as μmol O_2/mg protein per min. ● control rats; ■ deficient rats. Values are expressed as means ± SD for 7 (14 days) and 5 (30 and 90 days) determinations; * denotes significantly different ($P < 0.05$) from the precedent age, but not from the following.

the proportion of phospholipid classes remaining unmodified.[32] These authors showed a consistent decrease in the relative amounts of 20:4 *n*-6 and 22:6 *n*-3 between 1-day-old and 4-day-old rat, but no subsequent changes until adult age. Yet, Wolff[10] showed the same decline in 22:6 *n*-3 content and increase of 20:4 *n*-6 content in mitochondrial phospholipids of rat liver from weaned to 30-day-old rats. These changes seem to be a general feature with regard to similar variations in erythrocyte and plasma phospholipids.[33]

The α-linolenic acid deficiency did not modify total phospholipid and cholesterol contents of liver mitochondrial lipids in the growing rat nor the content of phospholipid classes of submitochondrial membranes. We only noted an age-related change in either total phospholipid and cholesterol content which led to a marked decrease in the cholesterol/phospholipid ratio between 30 and 90 days of age. The significance and the mechanism for these alterations are unknown. We only know that the increase in total cholesterol and cholesterol/phospholipid ratio is related to an alteration of mitochondrial membrane associated functions such as transport activities.[34,35]

Physiological activity of mitochondria

In mammals, functional effects of *n*-3 PUFA on nervous tissue physiology are well documented and have been discussed recently.[7] First, α-linolenic acid deficiency altered retinal function: it produced a perturbation in the electroretinogram both in the rat and the infant rhesus monkey, and impaired the visual acuity in the latter one.[8,36-40] Second, it diminished performance of behavior and learning tasks in the rat,[41-43] even in a case of moderate alterations in *n*-3 deprivation.[42] In neural membrane, some enzymatic activities were affected specifically: 5′-nucleotidase,[38,44] Na+,K+-ATPase, and 2′,3-cyclic nucleotide 3′-phosphodiesterase.[38] In our study, in spite of a dramatic reduction in DHA level in the mitochondrial membrane, no effect was noted on succinic dehydrogenase activity, respiration, nor F_1F_0 ATPase activity by the *n*-3 PUFA deficient status. These results are consistent with those of Williams et al.[12] who showed that methyl docosahexanoate supplementation in PUFA deficient rats had no effects on the restoration of normal oscillation states of mitochondria. In fact, it has been shown that the *n*-6/*n*-3 PUFA ratio in mitochondrial phospholipids did not alter the specific activity of succinate cytochrome c reductase but modified that of F_1F_0 ATPase in liver mitochondria.[52] It is well known that respiration and oxidative phosphorylation are highly dependent on the total PUFA content of mitochondrial phospholipids.[45] A total PUFA deficiency caused the uncoupling of oxidative phosphorylation and increased ATPase activity in rat liver mitochondria,[13,46] in spite of some controversies.[15,47,48] Moreover, the enrichment of the diet with linoleic acid decreased the ADP/O ratio but increased oxygen consumption rate of rat liver mitochondria[49]; in the heart,

this enrichment decreased oligomycin-sensitive F_1-ATPase activity.[14,50]

The *n*-6/*n*-3 PUFA ratio in mitochondrial phospholipids was claimed to be the main factor controlling the phase transition temperatures of some enzymes of the respiratory pathway of the inner mitochondrial membrane[51,52]: in our study the 4-to-8-fold increase in the *n*-6/*n*-3 PUFA ratio had no effect on liver mitochondrial function. However, the *n*-6/*n*-3 PUFA ratio modifications are mainly due to the decrease in 22:6 *n*-3 compensated by the increase in 22:5 *n*-6 and not by an appreciable changement in 20:4 *n*-6.[51-53] Finally, total PUFA content is maintained.

Many studies have emphasized that cardiolipin content and its 18:2 *n*-6 level specifically regulate enzyme activities of the respiratory pathway as F_1F_0 ATPase[54,55] and cytochrome C oxidase.[55-57] Our results showed that F_1F_0 ATPase activity in submitochondrial membrane was unchanged by the dietary *n*-3 PUFA deficiency. Similarly, the cardiolipin content was maintained which emphasizes the lack of effect of dietary α-linolenic acid deficiency on liver mitochondrial oxidative phosphorylation.

In conclusion, our results showed that the reciprocal replacement of 22:6 *n*-3 by *n*-6 PUFA, and especially by 22:5 *n*-6, in liver mitochondrial phospholipids of the *n*-3 PUFA deficient growing rat did not alter respiration and oxidative phosphorylation.

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