Effect of Age and α-Linolenic Acid Deficiency on Δ6 Desaturase Activity and Liver Lipids in Rats

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The combined effects of age and of diet deficient in n-3 fatty acids on Δ6 desaturation of linoleic acid and on lipid fatty acid composition were studied in the liver of the rat at 2, 6, 12, 18 and 24 mon of age. The profiles of Δ6 desaturase activity and fatty acid composition were studied in the deficient rats refed, at these different ages, either with 18:3n-3 (mixture of peanut and rapeseed oils) or with 20:5n-3 + 22:6n-3 (fish oil) diets for 2, 4, 8 or 12 wk. Results showed that the liver Δ6 desaturation activity in the control rats remained high at 2 and 6 mon, decreased by 30% from 6 to 12 mon, and then remained stable from 12 to 24 mon. In the deficient rats, this activity remained high during the entire period studied. Thus, the profile of liver Δ6 desaturase activity after puberty was not related to age only; it also depended on the polyunsaturated fatty acid (PUFA) n-6 and n-3 balance in the diet. In the controls, in parallel with the Δ6 desaturase activity, PUFA metabolism could be divided into three periods: a "young" period, and "old age" period, separated by a period of transition between 6 and 12 mon. Recovery from PUFA n-3 deficiency occurred at all ages but in a different manner depending on whether the rats were "young" or "old." Recovery was faster if long-chain n-3 PUFA rather than α-linolenic acid were supplied in the diet.

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Some long-chain polyunsaturated fatty acids (PUFA) of tissue and serum lipids are supplied directly by the diet, but most are derived by desaturation and elongation of their precursors, linoleic and α-linolenic acids. The first step in this process is catalyzed by a microsomal enzyme, Δ6 desaturase. The two precursor fatty acids compete for this enzyme (1,2); 18:3n-3 is desaturated at a higher rate than 18:2n-6. In the presence of the two dietary fatty acids and at equal concentrations, 18:3n-3 effectively inhibits the desaturation of 18:2n-6 (3). Δ6 Desaturase activity is also inhibited by the products of the desaturation-elongation process due to feedback inhibition by 20- and 22-carbon fatty acids (4,5). Studies on rats after puberty have shown that age affects Δ6 desaturase activity (6–8). Some investigators showed that this activity decreased progressively with age (6,7), others that it fluctuated (8). These variations in enzyme activity were reported to be either accompanied by changes in tissue lipid composition (6,7), or not (8). Other authors have studied the fatty acid profile in the liver of rats ranging in age from 1.5 to 40 mon; only the proportion of 20:4n-6 was shown to decrease with age (9). When animals given an α-linolenic acid-deficient diet were refed a balanced PUFA diet, the composition of tissue phospholipids returned to normal within variable times depending on the organ studied. However, these studies were carried out only on young animals, and the n-3 PUFA used in refeeding was derived from vegetable oil (10,11).

The aim of the present work was to study (i) the combined effects of age and dietary n-3 PUFA deficiency on changes in the activity of Δ6 desaturase on 18:2n-6 and changes in the fatty acid composition of liver total lipids in male rats between 2 and 24 mon of age, and (ii) the combined effects of age and of refeeding with α-linolenic acid or its long-chain derivatives on the two parameters.

MATERIALS AND METHODS

Animals and diets. Two generations of female Wistar rats were given a diet containing lipids in the form of African peanut oil (5%, w/w) low in α-linolenic acid; this diet (deficient) supplied about 900 mg of linoleic acid but only 5 mg of α-linolenic acid/100 g of diet. Two weeks before mating, the deficient second-generation females were divided into two groups. The first group continued to receive the deficient diet while the second group was given a diet in which the peanut oil was replaced by a mixture of peanut and rapeseed oils (50:50). This diet (control) supplied the same amount of linoleic acid as the deficient diet but also about 200 mg of α-linolenic acid/100 g of diet (n-6/n-3 = 5). At weaning, the male progeny of these two female groups were given the same diet as their respective dam, and animals were killed when 2, 6, 12, 18 or 24 mon old. At each of these ages some deficient animals were refed with n-3 PUFA, one-half with the control diet described above and the other half with a diet containing a mixture of peanut oil and cod liver oil (92.5–7.2%, respectively); thus, the first half was refed with n-3 PUFA with α-linolenic acid and the other with a mixture of eicosapentaenoic and docosahexaenoic acids (EPA plus DHA). The latter diet supplied about 900 mg of n-6 PUFA (like the other diets) and 100 mg of long-chain n-3 PUFA/100 g of diet. This amount of long-chain n-3 PUFA was chosen because of a preliminary study had shown that it was equivalent to 200 mg of α-linolenic acid, which is sufficient to satisfy the n-3 PUFA requirement (11). Some animals were killed at 2, 4, 8 or 12 wk (T2, T4, T8 and T12) after refeeding with n-3 PUFA. The composition of the diets and the fatty acid composition of the lipids are given in Tables 1 and 2.

Measurements of Δ6 desaturase activity. Non-fasted animals were killed between 8:00 and 9:00 a.m. to ensure optimal conditions for Δ6 desaturase measurement (12) and to avoid any variation in Δ6 desaturase activity due to circadian rhythm (13). The liver was rapidly excised and weighed. One part was used to measure enzyme activity and the rest for fatty acid analysis.

Specific Δ6 desaturase activity was assayed using the method of Blond and Lemarchal (14) as modified by Bourre *et al.* (15). The liver was homogenized at 4°C in 0.25 M saccharose buffer containing 0.05 M Na_2HPO_4, 2 mM glutathione, pH 7.4 (5 mL of buffer/2 g fresh tissue). The homogenate was centrifuged at 12,000 × *g* for 15 min

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Abbreviations: DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TFA, total fatty acids.

TABLE 1

Diet Composition (g/kg)

Constituents	g/kg
Casein	220.0
DL Methionine	1.6
Mineral mixture[a]	40.0
Vitamin mixture[b]	10.0
Cellulose	20.0
Oil[c]	50.0
Starch	439.0
Saccharose	219.4

[a]Composition (g/100 g):$CaHPO_4 \cdot 2H_2O$, 38.0; K_2HPO_4, 24.0; $CaCO_3$, 18.0; NaCl, 6.9; MgO, 2.0; $MgSO_4 \cdot 7H_2O$, 9.0; $FeSO_4 \cdot 7H_2O$, 0.86; $ZnSO_4 \cdot H_2O$, 0.5; $MnSO_4 \cdot H_2O$, 0.5; $CuSO_4 \cdot 5H_2O$, 0.1; NaF, 0.08; $CrK(SO_4)_2 \cdot H_2O$, 0.05; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.002; KI, 0.004; $CoCO_3$, 0.002; $Na_2SeO_3 \cdot 5H_2O$, 0.002.

[b]Total vitamin supplement, United States Biochemical Corp. (Cleveland, OH).

[c]Control diet consists of a mixture of peanut oil and rapeseed oil (50:50), deficient diet consists of peanut oil, refeeding diets consist of a mixture of peanut oil and rapeseed oil (50:50) or a mixture of peanut oil and cod liver oil (92.5:7.5).

to precipitate cell debris, mitochondria and nuclei. The supernatant contained both microsomes and cytosol. The latter increased the activity of acyl-CoA synthetase activity, thus favoring the formation of acyl-CoA (14) serving as substrates of desaturases. The supernatant was diluted twice with the same buffer before the protein content was determined by the method of Lowry *et al.* (16). Five mg of protein and 100 nmol of [1-^{14}C]18:2n-6 (2 μCi, 20 μL) were added. A final volume of 2 mL was obtained using the same buffer as before containing the following cofactors: Na_2HPO_4 (50 mM) ATP (7.5 mM), $MgCl_2$ (3.8 mM), NADPH (0.2 mM), NADH (0.5 mM) and CoA (0.2 mM). The tubes were incubated for 30 min with shaking. The reaction was stopped by the addition of 1 mL of KOH in 12% ethanol. Thirty μg each of several standard commercial fatty acids (18:2n-6, 18:3n-6, 20:3n-6, 20:4n-6, 20:5n-3, 22:6n-3) were added to facilitate identification of chromatographic fractions. After saponification at 100°C for 30 min, 0.4 mL of 10 N HCl was added, the fatty acids were extracted using hexane, followed by methylation with 1 mL of 14% boron trifluoride for 1 h at 100°C. The fatty acid methyl esters were extracted with hexane, purified by thin-layer chromatography using a mixture of petroleum ether/diethyl ether (80:20, vol/vol), and then were localized by autoradiography. The methyl ester band was scraped from the plate and extracted successively with hexane (2 times 3 mL) and diethyl ether (2 times 3 mL). The methyl esters were finally separated according to their degree of unsaturation by $AgNO_3$ thin-layer chromatography. Plates were impregnated with 10% $AgNO_3$ in acetonitrile for 15 min, then dried at 100°C for 20 min.

TABLE 2

Fatty Acid Composition of Dietary Lipids[a]

	Diets		
	Peanut oil	Peanut oil (50%) Rapeseed oil (50%)	Peanut oil (92.5%) Cod liver oil (7.5%)
Fatty acids (%)			
14:0	0.4	0.5	0.9
16:0	11.9	10.1	10.9
18:0	2.9	2.3	3.3
20:0	1.2	0.8	1.3
22:0	1.9	1.1	2.3
24:0	0.9	0.6	1.0
Total SFA	19.2	15.4	19.7
16:1n-7	0.6	0.7	1.6
18:1n-7	3.1	6.0	2.9
18:1n-9	56.1	53.3	52.3
20:1n-9	0.9	0.8	1.7
22:1n-11	—	—	0.8
Total MUFA	60.7	60.8	59.3
18:2n-6	19.9	19.8	18.5
20:4n-6	—	—	0.2
Total n-6 PUFA	19.9	19.8	18.7
18:3n-3	0.1	3.7	0.3
20:5n-3	—	—	0.8
22:5n-3	—	—	0.3
22:6n-3	—	—	0.9
Total n-3 PUFA	0.1	3.7	2.3
(n-6) + (n-3)	20.0	23.5	21.0
n-6/n-3	199.0	5.3	8.1
Total n-6 PUFA (mg/100/g of diet)	935.0	931.0	879.0
Total n-3 PUFA (mg/100/g of diet)	5.0	173.9	108.0

[a]Abbreviations: SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

The mobile phase was a mixture of petroleum ether/diethyl ether (50:50, vol/vol). The fractions were localized by autoradiography, and the radioactive distribution was determined using a plate reader (Automatic TLC Linear Analyzer Berthold, La Garenne Colombes, France). Results were expressed in pmol of 18:3n-6 formed/mg protein/min incubation.

Fatty acid composition of liver total lipids. Liver lipids were extracted from frozen dried liver with a chloroform/methanol mixture (2:1, vol/vol) using the method of Folch *et al.* (17) as modified by Pollet *et al.* (18). The lipid extracts were then transmethylated (19). After extraction with hexane, the methyl esters were analyzed by gas chromatography, using a Carlo Erba (Rueil Malmaison, France) chromatograph with an automatic on-column injector, a flame-ionization detector and capillary-type Carbowax C.P. Wax 52 C.B. column. Data were processed using a Stang microcomputer (Pavillon-sous Bois, France) and Nelson software (Cupertino, CA).

Analysis of results. This study required a large number of animals: 144 male rats were killed at various ages. Nevertheless, only a small number of animals could be studied at each time point (3 or 2 rats). Liver total lipid fatty acid composition was analyzed for only two rats per time period; thus common statistical procedures could not be applied. High mortality in the groups of rats 24 mon of age or older did not permit measurements at 12 wk of refeeding.

RESULTS

Effect of age and dietary α-linolenic acid deficiency. In control rats, the specific Δ6 desaturase activity (Fig. 1) remained constant at 2 and 6 mon; it decreased by about 30% between 6 and 12 mon and then did not change between 12 and 24 mon. Enzyme activity in α-linolenic acid-deficient animals was comparable to that in controls at 2 and 6 mon, but instead of decreasing thereafter it remained high during the entire period studied; thus, from 12 to 24 mon, enzyme activity was 50% higher than in controls.

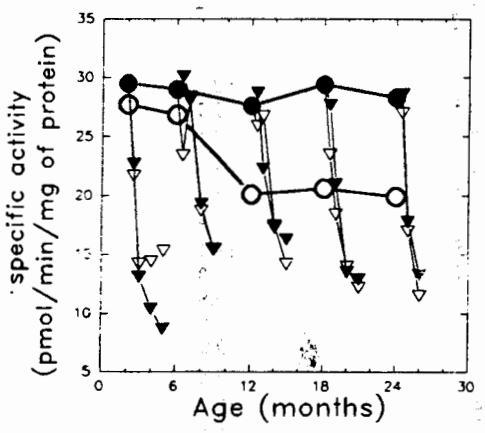

FIG. 1. Effect of age and α-linolenic acid deficiency on Δ6 desaturase activity in the rat. Effect of refeeding n-3 polyunsaturated fatty acids (PUFA) according to age and nature of n-3 PUFA (α-linolenic acid or long-chain n-3 PUFA). Assays of Δ6 desaturase were carried out as described in Materials and Methods; ○, control rats; ●, deficient rats; ▽, 18:3n-3 refed rats; ▼, 20:5n-3 + 22:6n-3 refed rats. Values are the mean of three or two rats/diet.

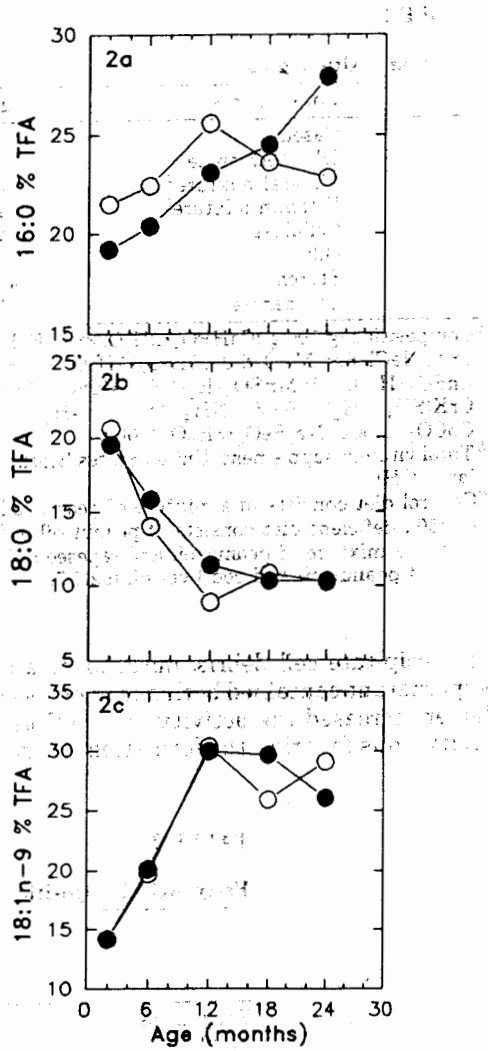

FIG. 2. Effect of age and α-linolenic acid deficiency on levels of the main saturated fatty acids and monounsaturated fatty acids of liver total lipids in the rat. Only values for the major fatty acids are reported. Experimental details are given in Materials and Methods. Data were derived from the percent of total fatty acids (TFA); ○, control rats; ●, deficient rats. Values are the mean of two rats/diet.

Fatty acid composition was also affected by the diet. Palmitic (16:0) and stearic (18:0) acids were the major saturated fatty acids (SFA). In controls, the proportion of palmitic acid was unchanged (21 to 23%) between 2 and 24 mon (Fig. 2a), but stearic acid levels decreased by half between 2 and 12 mon and then remained constant up to 24 mon (Fig. 2b). The 18:0 profile affected the proportion of total SFA which decreased from 44 to 36% between 2 and 12 mon and then remained stable until 24 mon (Table 3). Generally, an n-3 PUFA deficient diet had very little influence on the proportions of liver lipid SFA and monounsaturated fatty acids (MUFA). However, in the deficient rats, 18:0 decreased with age, as in the controls, and 16:0 increased regularly so that, as the decrease of one compensated the increase of the other, the level of total SFA remained almost constant from 2 to 24 mon (between 36 and 40% of total fatty acids). The MUFA time course was similar in control and deficient animals: their proportions doubled between 2 and 12 mon (from 20 to 40%) at

TABLE 3

Effect of Age and of n-3 PUFA Deficiency on Fatty Acid Composition of Liver Total Lipids[a]

	Diet									
	C (2)[b]	D (2)	C (6)	D (6)	C (12)	D (12)	C (18)	D (18)	C (24)	D (24)
Fatty acids (%)										
Total SFA	43.7	39.8	38.1	38.2	36.0	35.9	36.2	36.7	34.3	40.1
Total MUFA	20.5	20.7	28.4	28.8	41.4	39.1	36.4	39.3	38.7	36.1
n-6 ≥ 20 C	18.8	29.0	17.2	23.5	11.4	15.8	11.0	14.2	11.1	14.4
Total n-6 PUFA	29.9	38.6	26.3	28.8	22.6	24.1	22.8	23.3	22.5	22.7
n-3 ≥ 20 C	5.8	1.1	6.8	1.1	3.9	0.8	4.2	0.7	4.0	0.8
Total n-3 PUFA	6.2	1.1	7.1	1.1	4.1	0.8	4.7	0.7	4.5	0.8
n-6 + n-3 ≥ 20 C	24.6	30.1	24.0	24.6	15.3	16.6	15.2	14.9	15.1	15.2
Total (n-6) + (n-3)	36.1	39.7	33.4	33.0	26.7	24.8	27.4	24.0	27.0	23.5
n-6/n-3	4.8	35.1	3.7	29.0	5.5	32.1	4.9	33.0	5.1	28.4
22:5n-6/22:6n-3	0.04	3.70	0.04	3.40	0.05	2.40	0.03	1.60	0.03	1.30
20:4n-6/18:2n-6	1.81	2.82	2.03	2.72	1.08	1.79	1.00	1.58	1.00	1.79

[a]The values are the mean of two or three rats/diet. C, control rats; D, deficient rats; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
[b]Number in parentheses is age in months.

the same time as oleic acid increased from 14 to 28% (Fig. 2c); between 12 and 24 mon there was no change.

As expected, the n-6 PUFA profiles were different in control and deficient animals: in the former the proportion of total n-6 PUFA decreased from 30 to 23% between 2 and 12 mon and then stabilized. This overall relatively moderate decrease was due entirely to arachidonic acid (20:4n-6) which decreased from 18 to 11% between 2 and 12 mon (Fig. 3b), while the proportion of linoleic acid was constant (about 10%) (Fig. 3a). In deficient animals, the proportion of 18:2n-6 (which did not vary with age) was systematically lower than in controls. On the other hand, 20:4n-6 and especially 22:5n-6 levels were higher (Fig. 3c). However, the level of 22:5n-6, higher from 2 to 6 mon, diminished considerably between 6 and 12 mon. After 12 mon, the higher values of 20:4n-6 and 22:5n-6 just compensated for the lower value of 18:2n-6, so that total n-6 PUFA became similar in control and deficient rats after 12 mon (about 23%). The level of 20:3n-6 did not vary with either age or diet. DHA (22:6n-3) was the main n-3 PUFA in controls. It represented 5–6% of n-3 PUFA up to 6 mon, dropped between 6 and 12 mon to about 3.4% and then remained at that level until 24 mon (Fig. 3d). The total n-3 PUFA profile was comparable. In deficient rats, n-3 PUFA were represented only by 22:6n-3, and it proportion remained at about 1% during the whole period studied. Total n-6 + n-3 PUFA in controls remained at the same level from 2 to 6 mon, decreased by about 20% between 6 and 12 mon and then stabilized; in deficient animals, the same pattern was seen but the plateau reached at 12 mon was lower due to a lower proportion of n-3 PUFA which was no longer compensated for by a high n-6 PUFA level (Fig. 3e). If only long-chain PUFA (≥20 C) are considered instead of total PUFA, there was overcompensation for the n-3 PUFA deficiency by n-6 PUFA at 2 mon but not at other time points. In controls, the n-6/n-3 ratio remained about 5 throughout the period studied; it was 6 to 7 times higher in deficient rats (Fig. 3f). The 22:5n-6/22:6n-3 ratio, which may be considered an index of dietary n-3 PUFA deficiency (20), did not change in controls. It was high in deficient rats but decreased progressively with age. In controls the 20:4n-6/18:2n-6 ratio, an index of total Δ6 and Δ5 desaturase activities (21), remained high and stable from 2 to 6 mon, dropped by one-half between 6 and 12 mon and then remained stable until 24 mon. The profile of the deficient rats was comparable but the ratio was higher.

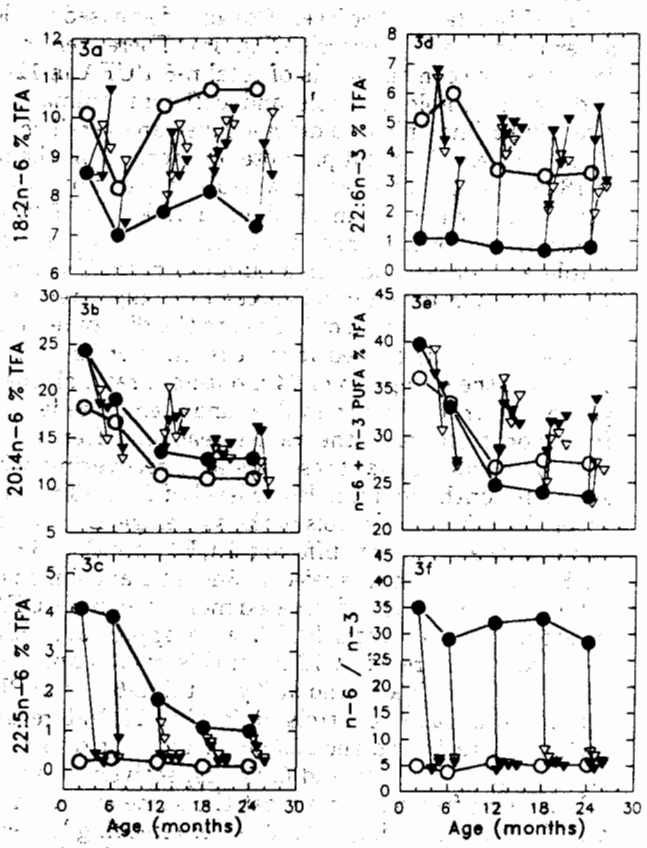

FIG. 3. Effect of age and α-linolenic acid deficiency on the levels of the main polyunsaturated fatty acids (PUFA) of liver total lipids in the rat: Effect of refeeding n-3 PUFA according to age and nature of n-3 PUFA (α-linolenic acid or long-chain n-3 PUFA). Experimental details are given in Materials and Methods. Data were derived from the percent of total fatty acids (TFA); ○, control rats; ●, deficient rats; ▽, 18:3n-3 refed rats; ▼, 20:5n-3 + 22:6n-3 refed rats. Values are the mean of two rats/diet.

Effect of PUFA n-3 refeeding in deficient rats. Generally, the addition of n-3 PUFA to the diet, whether in the form of 18:3n-3 or of 20:5n-3 + 22:6n-3, caused the Δ6 desaturase activity to drop by one-half to a level lower than that in controls of the same age (Fig. 1). The inhibition appeared much sooner in 2-month-old animals, independent of the nature of the PUFA n-3 in the diet. In older animals there was a latency period of about 2 wk before the inhibition became evident, especially when the rats received long-chain n-3 PUFA. Maximal inhibition was obtained only after 8 wk of refeeding.

The addition of 18:3n-3 to the diet of deficient rats modified the fatty acid composition of liver total lipid with a decrease in n-6 PUFA level between 2 and 6 mon. At these two ages, the values obtained at T8 for 2-month-old rats and at T4 for 6-month-old ones were very close to those of controls. This decrease was accompanied by a new balance between the 18:2n-6 on one hand and the 20:4n-6 and the 22:5n-6 fatty acids on the other. At the end of the first two wk of refeeding (T2), the level of 18:2n-6 increased and that of 22:5n-6 decreased; thus, there was a return to normal values (Figs. 3a,3c). The level of 20:4n-6 decreased to a level lower than that of controls (Fig. 3b). There were no differences between the levels of total n-6 PUFA in 12-, 18- and 24-month-old controls and deficient animals, and refeeding α-linolenic acid did not cause an overall change. However, refeeding did promote an increase in the level of 18:2n-6, which reached a plateau at the end of 4 wk but did not return to normal values. The levels of 20:4n-6 returned to normal only at 24 mon; it was practically stable at 18 mon and increased at 12 mon. The level of 22:5n-6 returned to normal values, but only after 8 wk of refeeding. The addition of 18:3n-3 to the diet of n-3 PUFA deficient animals caused an increase in liver 22:6n-3 (Fig. 3d). With the exception of 24-month-old rats, all the age groups returned to control values and even exceeded them. In 6-month-old rats, the values reached after 4 wk of refeeding showed that these rats were recovering, whereas in 24-month-old animals the level of 22:6n-3 remained lower than that in controls of the same age. Due to variations in the levels of different PUFA, total (n-6 + n-3) PUFA decreased in rats refed 18:3n-3 at 2 and 6 mon (Fig. 3e) because n-6 PUFA decreased more than n-3 PUFA increased. However, total PUFA were appreciably higher when rats were refed at 12 mon; this overcompensation disappeared with age, and at 2 yr total (n-6 + n-3) PUFA was the same as in controls. Refeeding 18:3n-3 returned the n-6/n-3 ratio and the 22:5n-6/22:6n-3 ratio to normal values within 4 wk (5 and 0.05, respectively) (Fig. 3f).

Refeeding fish oil had almost the same effect on liver n-6 PUFA as refeeding 18:3n-3. After refeeding long-chain n-3 PUFA, 18:3n-3 was not detected in liver lipids; 20:5n-3 and 22:5n-3 levels were lower than or equal to 0.5% (results not shown). Refeeding with 100 mg of 20:5n-3 + 22:6n-3/100 g of diet resulted in an increase in 22:6n-3 comparable to a supply of 200 mg of 18:3n-3/100 g of diet in 2-, 6- and 12-month-old rats. But in rats of 18 and 24 mon of age, the fish oil diet raised the level of 22:6n-3 more quickly than the rapeseed oil diet. After 2 wk of refeeding, the level was much higher than that seen in control rats of the same age. After this increase the values stabilized. In 12- and 18-month-old rats, the values remained higher than normal but in 24-month animals they returned to control values as observed after 8 wk of refeeding.

DISCUSSION

Effect of age. We found a decrease of 18:0 between 2 and 12 mon and an increase of 18:1n-9 during the same period, corroborating the results of Ulmann *et al.* (8) who found a slight decrease in the sum of 16:0 + 18:0 together with an increase of 16:1n-7 + 18:1n-9 in the liver microsomal phospholipids of rats 3-, 6- and 9-months old. In regard to the PUFA composition of liver total lipids, our study showed that the postpuberty period can be divided into three parts: (i) a period of stability between 2 and 6 mon; (ii) a transition period between 6 and 12 mon during which levels of 20:4n-6, 22:5n-6 and 22:6n-3 decrease; and (iii) another period of stability from 12 to 24 mon. Thus, the PUFA level was lower in old than in young rats. However, our results do not show whether the transition period extends over the whole 6 to 12 mon interval or whether it is shorter. Murawski *et al.* (9) also observed a decrease in the level of total liver lipid 20:4n-6 in rats receiving a standard diet and with ages ranging from 1.5 to 40 mon. But these authors found no variation in the proportions of other PUFA. In contrast to these results, Bordoni *et al.* (6) found that total liver lipid 20:4n-6 levels were not different in rats 4- and 22-months-old.

Our study shows that in animals receiving a balanced PUFA diet the variations in PUFA composition correspond to variations in specific Δ6 desaturase activity which, like total liver lipids, was similar at 2 and 6 mon, decreased between 6 and 12 mon and was stable thereafter. The 20:4n-6/18:2n-6 ratio also fell during this period. However, the 20:3n-6 level did not change. Thus, it seems that only Δ6 desaturase should be involved in the decrease of the 20:4n-6/18:2n-6 ratio; otherwise, we likely would have observed an increase in 20:3n-6 level if Δ5 desaturase was also involved (22). Peluffo and Brenner (23) showed that Δ6 desaturase activity tends to decrease with age in rats receiving a standard diet; but their experiment only concerned rats aged 3 mon and 1 yr and, therefore, any intermediate fluctuations would have been missed. Recently, Ulmann *et al.* (8) studying rats aged 3, 6 and 9 mon receiving a control diet similar to the ones in our study also showed that Δ6 desaturase activity decreased from 6 to 9 mon. However, other authors have not observed the same trend of Δ6 desaturase activity with age; Bordoni *et al.* (6) using liver microsomes of rats at 13 d and 1, 4, 14 and 22 mon which had received a standard diet noted that enzyme activity decreased progressively from 4 to 22 mon. In a similar study on rats aged 1, 6, 10 and 25 mon, Hrelia *et al.* (7) also found a linear decrease in Δ6 desaturase activity with age; in mice, Bourre *et al.* (15) noted that liver Δ6 desaturase activity did not change between 3 wks and 4 mon but progressively decreased between 4 and 17 mon. The discrepancy between the results of our study and those of Ulmann *et al.* (8) on one hand, and those of Bordoni *et al.* (6) and Hrelia *et al.* (7) on the other, could be due to different diet compositions since this, as we have shown, determines the profile of enzyme activity during postpubertal development.

Effect of dietary n-3 PUFA deficiency. The previous studies on dietary n-3 PUFA deficiency have shown that the deficiency causes an increase in 20:4n-6 and 22:4n-6 levels, and especially in 22:5n-6 levels, to compensate for the decrease in 18:2n-6, 22:5n-3 and especially 22:6n-3 (24–28). Other studies have shown that n-6 PUFA can

completely compensate for n-3 PUFA (22, 29–31) and that total PUFA (n-6 + n-3) remains constant. These studies on n-3 PUFA deficiency were done on 15-day-old and 60-day-old rats. Our results showed that this was not the case in animals from 12 mon of age, and that compensation was not adequate so that the sum of PUFA was lower in n-3 PUFA-deficient animals than in controls. However, if only fatty acids with at least 20 carbon atoms are considered, n-3 PUFA were exactly compensated for by n-6 PUFA. In other words, in the old, deficient animals, increases in 20:4n-6 and 22:5n-6 did not compensate for the decrease in 18:2n-6 but only for the decrease in 22:5n-3, and especially 22:6n-3. Moreover, in n-3 PUFA-deficient rats, results are remarkably consistent in regard to the lower level of 18:2n-6 and the higher level of 20:4n-6 and 22:5n-6 on one hand, and the higher level of Δ6 desaturase activity on the other, as compared to controls. In the deficient animals, specific enzyme activity did not change with age as it did in controls. Thus, it is evident that changes in Δ6 desaturase activity were not related solely to age but were also a function of diet. It is known that the desaturase activities, and especially the Δ6 desaturase activity, also depend on non-lipid dietary factors. These activities moreover depend on hormonal factors, the effects of which being obvious in some pathological conditions, such as diabetes. At last, if the influence of desaturases on the lipid composition of tissue lipids, and in particular that of microsomal phospholipids, is generally assumed, it should be observed that this composition also results from many other metabolic factors (for a review, see Ref. 32).

Effect of n-3 PUFA refeeding. Our results showed that, independent of the nature of dietary n-3 PUFA, the modalities of recovery in young animals (2- and 6-months-old) were clearly different from those in old animals (12-, 18- and 24-months-old). Refeeding tended to decrease the level of total liver lipid PUFA in the young, while this level was markedly increased in old animals so that it reached the level in young controls. Moreover, a supply of long-chain n-3 PUFA permitted a more rapid and more complete recovery than a supply of 18:3n-3, the difference being more evident in older animals. In this study, EPA plus DHA supplementation did not increase the level of 20:5n-3 to the detriment of 20:4n-6, as occurred when a larger amount of these fatty acids was supplied in the diet (33,34). It is likely that, because of the moderate supply of EPA plus DHA (100 mg/100 g of diet), 20:5n-3 was completely transformed into 22:6n-3 to cover the requirements for phospholipid 22:6n-3. The persistently low activity of Δ6 desaturase in refed rats compared to that in controls of the same age could be due to the fact that 22:5n-6 progressively released from phospholipids decreased Δ6 desaturase activity by feedback inhibition. This fatty acid may then be eliminated either by mitochondrial and/or peroxisomal oxidation, or by retroconversion into 20:4n-6. If in old rats the retroconversion is the usual pathway, this would explain why the high 20:4n-6 levels found in n-3 PUFA-deficient rats was maintained or even increased when the animals were refed with n-3 PUFA. This high 20:4n-6 level could in itself contribute to maintaining Δ6 desaturase activity at the low level seen after n-3 PUFA refeeding. In any case, Δ 6 desaturase activity at a given age may depend not only on the relative proportions of n-6 and n-3 PUFA in the current diet but also on the relative proportions in the diet eaten over a longer period of time.

In conclusion, this study shows for the first time that during adult rat life (puberty to old age), PUFA metabolism can be divided into three periods: two of stability ("young" and "old" ages), separated by a transition period. The latter occurs between 6 and 12 mon and its duration remains to be determined. In rats given a balanced PUFA diet, the transition period is characterized by a decrease in liver long-chain PUFA, in particular 22:6n-3 and 20:4n-6. Our results show that the profile of Δ6 desaturase activity as a function of age cannot be dissociated from the n-6 and n-3 PUFA ratio in dietary lipids. Thus, an n-3 PUFA-deficient diet maintains specific Δ6 desaturase activity at a high, constant level throughout adult life, from puberty to old age. Finally, it is possible to recover from multi-generational n-3 PUFA deficiency at all ages but the pattern of recovery is different, depending on whether the animal is in the "young" or the "old age" period. During the "old age" period, recovery is quicker if the diet directly supplies long-chain n-3 PUFA.

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