Effects of Age and Dietary Essential Fatty Acids on Desaturase Activities and on Fatty Acid Composition of Liver Microsomal Phospholipids of Adult Rats

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The combined effects of age and dietary n-6 and n-3 fatty acids were studied in 3-, 6- and 9-month-old rats. At each age, two groups were fed diets containing 5% (w/w) of vegetable oils rich in either 18:3n-6 (borage group) or 18:3n-6 plus 18:4n-3 (black currant group), for a period increasing with age. A control group was fed the essential fatty acids 18:2n-6 and 18:3n-3 only. For each group, Δ6, Δ5 and Δ9 desaturase activities were measured in liver microsomes, and fatty acid composition was determined in microsomal phospholipids. Desaturase activity varied as a function of age and dietary lipids. Δ6 Desaturation of 18:3n-3 was more sensitive to these factors while Δ6 desaturation of 18:2n-6 and Δ9 desaturation were more dependent on season than the other two. Desaturase activity was influenced more by the black currant than by the borage diet, especially at 6 and 9 months of age. A large proportion of arachidonic acid was maintained in the microsomes independent of the diet. Changes in the fatty acid composition did not strictly reflect the differences in desaturase activities. The effects of the two factors (age and diet) on the activities of the desaturases are complex, suggesting that the enzymes are susceptible to other factors as well.
***Lipids 26,* 127–133 (1991).**

It is well established that in animals unsaturated fatty acids are synthesized by a series of alternating position-specific desaturation and malonyl-CoA-dependent elongation steps. The enzymatic conversion of fatty acids to their corresponding desaturated products has been particularly well studied in rat liver microsomes. Different desaturases introduce double bonds at Δ9, Δ6, Δ5 or Δ4 position in the fatty acid chain (1). Thus, γ-linolenic acid (18:3n-6) and stearidonic acid (18:4n-3) are synthesized by Δ6 desaturation, a rate-limiting step in the metabolism of linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3), which are the main dietary essential fatty acids (EFA). Another enzyme, the Δ5 desaturase, converts dihomo-γ-linolenic acid (20:3n-6) into arachidonic acid (20:4n-6), whereas a Δ9 desaturase is responsible for the transformation of stearic acid, a non-essential saturated fatty acid (18:0), into oleic acid (18:1n-9). Desaturations of fatty acids by liver microsomes are influenced by numerous hormonal and nutritional factors (2–7) and are sensitive to circadian variations (5,8). Losada and Peluffo (9) observed that a cold environment decreased the Δ9 desaturase activity while Δ6 desaturation was insensitive to temperature variations.

Physiological factors, such as age, may induce modifications of desaturase activities. Strouve-Vallet and Pascaud (10) investigated Δ6 desaturation in liver microsomes and brain during the first month of rat growth. Satomi and Matsuda (11) examined the microsomal Δ6 desaturation of linoleic acid in rat liver fetus. Peluffo and Brenner (12) demonstrated that in rat liver Δ6 desaturation of 18:2n-6 and 18:3n-3 decreased while Δ9 desaturation of 18:0 increased at 12 months of age as compared to 3 months. Recently, Bordoni *et al.* (13) showed that a linear decrease of Δ6 desaturase activity occurred with age (1- to 22-month-old rats) and compared the Δ6 desaturation of 18:2n-6 to that of 18:3n-3 (14). However, the standard diets provided to the animals did not allow the influence of dietary essential fatty acids in enriched diets to be studied. Blond *et al.*(15) studied Δ6 and Δ5 desaturations in Zucker rats with age, but only during a short period of growth, *i.e.*, 6, 9 and 12 weeks.

Evening primrose seed oil is a well-known source of γ-linolenic acid. The use of dietary γ-linolenic acid avoids the Δ6 desaturation step. Other species, such as *Boraginaceae*, are also known to contain significant amounts of 18:3n-6 (16). Thus, borage oil also can be used to supply diets with this fatty acid. Black currant seed oil (17), a Ribes seed oil, is proposed currently as a source of both stearidonic acid and 18:3n-6. Hoy *et al.* (18) demonstrated that liver Δ6 and Δ5 desaturase activities were not modified by dietary 18:3n-6 when rats were fed evening primrose oil or safflower oil for 11 weeks. In contrast, the Δ5 desaturase activity was depressed in young animals fed a diet containing 18:3n-6 (19). Choi and Sugano (20), using a diet containing evening primrose oil, showed a decrease of liver Δ6 desaturase activity in 8-month-old rats but not in 3-week-old animals. These previous studies dealing with dietary fatty acids, age and desaturations usually reported results only for certain ages (often for young rats) and more particularly for Δ9 and Δ6 desaturation. A time-course investigation of Δ9, Δ6 and Δ5 desaturation together with a liver microsome fatty acid compositional study was needed.

Nutritional experiments were therefore carried out on 3-, 6- and 9-month-old rats. The use of borage and black currant seed oils permitted the study of the effect of a partial substitution of 18:3n-6 and 18:4n-3 for 18:2n-6 and 18:3n3, respectively, on the desaturation capacity of rat liver microsomes as well as on the composition of microsomal phospholipids.

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Abbreviations: Bc, black currant; Bg, borage; C, control; EFA, essential fatty acids; HPLC, high performance liquid chromatography; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

MATERIALS AND METHODS

Animals and diets. Male Wistar rats, supplied by INRA (Jouy-en-Josas, France), were randomized into three groups according to age and housed in stainless steel cages in a well-ventilated room maintained at 22 ± 2°C on a 12 hr/12 hr light/dark cycle. Diets and tap water were provided *ad libitum*. After weaning, the rats were fed the basal diet (control diet) for 7, 16 and 26 weeks. It contained (g/kg): casein, 220; D,L-methionine, 1.6; cellulose, 20; starch, 440; saccharose, 218; mineral mixture, 40; vitamin mixture, 10; and oil mixture (50 % peanut oil + 50 % rapeseed oil), 50 (21). Then, within each group, the animals were again divided into three groups (three rats per group). The first group continued to receive the control diet (group C). The two other groups were given a diet in which the oil mixture was replaced by 5% (w/w) fat including borage oil (group Bg) or 5% (w/w) fat containing black currant seed oil (group Bc) for 2, 5 and 7 weeks, respectively, in the three increasing age groups. For 100 g of diet, the n-6 fatty acids represented 945, 973 and 988 mg for C, Bc and Bg groups, respectively; the n-3 fatty acids represented 188 mg for each diet. The three oil mixtures had a n-6/n-3 fatty acid ratio of about 5.2 and similar contents of n-6 and n-3 fatty acids. The fatty acid compositions of these mixtures are reported in Table 1. The three groups of animals were killed at 3, 6 and 9 months. To minimize seasonal variations in desaturase activities, two groups of three rats were used at 4–6 month intervals throughout the year, for a given age and for a given diet. Means were calculated on the 6 values.

Chemicals. [1-^{14}C]Stearic acid (52 mCi/mmol, 97.5% radiochemical purity) and [1-^{14}C]linoleic acid (58 mCi/mmol, 99% radiochemical purity), were purchased from CEA (Gif sur Yvette, France). [1-^{14}C]α-Linolenic acid and [2-^{14}C]dihomo-γ-linolenic acid (56 mCi/mmol, 96% radiochemical purity) were purchased from the Amersham Radiochemical Centre (Amersham, U.K.). The reported radiopurity of each substrate was that indicated by the manufacturer. Each substrate was diluted in ethanol with unlabelled fatty acid to a specific activity of 10 mCi/mmol. Unlabelled fatty acids, coenzymes and biochemicals were provided by Sigma Chemical Co. (St. Louis, MO) and by NuChek Prep (Elysian, MN). All other chemicals purchased from Sigma and Merck (Darmstadt, Germany) were of analytical grade.

Isolation of microsomes. Rats were killed by exsanguination between 07:00 and 08:00 hr to avoid any circadian variation (22) and to insure conditions of high desaturase activity (8). Livers were excised, rinsed with ice-cold physiological saline solution, blotted, weighed and cut into thin slices. Liver (3.5 g) was homogenized at 4°C in a Potter-Elvejhem homogenizer with 6 volumes of 0.05 M phosphate buffer (pH 7.4) and 0.25 M sucrose solution. Homogenates were centrifuged at 13,000 × *g* for 20 min to sediment cell fragments, mitochondria and nuclei. The microsomal pellet was obtained by centrifugation of the supernatant at 105,000 × *g* for 60 min.

Microsomal pellets were resuspended in 0.4 mL supernatant and 0.8 mL of 0.05 M phosphate buffer (pH 7.4) and 0.25 M sucrose solution for determination of desaturase activities. Microsomal proteins were estimated by the method of Layne (23) with fatty acid-free bovine serum albumin as standard.

TABLE 1

Fatty Acid Composition (% moles) of Dietary Lipids

Fatty acids	Control[a]	Black currant[b]	Borage[c]
14:0	0.2	0.8	0.5
16:0	7.7	26.1	18.1
16:1n-7	0.3	—	0.2
18:0	2.9	5.7	4.5
18:1n-9	57.3	39.8	49.6
18:1n-7	2.3	1.1	—
18:2n-6	20.1	16.3	17.3
18:3n-6	—	4.4	3.6
18:3n-3	4.0	3.2	4.0
18:4n-3	—	0.8	—
20:0	1.1	0.6	0.5
20:1n-9	1.5	0.3	1.3
22:0	1.8	0.9	0.4
24:0	0.8	—	—
SFA/PUFA[d]	0.2	0.8	0.5
Σ n-6	20.1	20.7	20.9
Σ n-3	4.0	4.0	4.0
n-6/n-3	5.0	5.2	5.2

[a]Control: (rapeseed + peanut) oils.
[b]Black currant: (black currant seed + peanut + palm) oils.
[c]Borage: (borage + rapeseed + hydrogenated palm) oils.
[d]SFA, saturated fatty acids; PUFA, polyunsaturated fatty acids.

Enzyme assays in vitro. Five mg of microsomal proteins were incubated in an open flask. Two quantities of each substrate in ethanolic solution were used: a nonsaturating level, 50, 60, 30 and 40 nmol and a saturating level, 100, 120, 80 and 120 nmol for 18:0, 18:2n-6, 20:3n-6 and 18:3n-3, respectively. These levels and the effect of microsomal protein concentration and incubation time were established in preliminary experiments (unpublished data). Incubations were performed at 37°C for 15 min in a shaking water bath with a total volume of 2.1 mL incubation medium containing 72 mM phosphate buffer, pH 7.4, 4.8 mM $MgCl_2$, 0.5 mM coenzyme A, 3.6 mM ATP and 1.2 mM NADPH. Incubations were stopped by adding 15 mL chloroform/methanol (1:1, v/v). Lipids were saponified and methylated as reported by Slover and Lanza (24). The distribution of radioactivity between substrate (stearic, linoleic, dihomo-γ-linolenic or α-linolenic acid) and desaturation product (oleic, γ-linolenic, arachidonic or stearidonic acid) was determined by the reversed phase high performance liquid chromatography (HPLC) method described by Narce *et al.* (25), using a Waters Chromatograph and a Lichrocart column (Superspher RP 18, 250 mm × 4 mm i.d. Waters, Milford, MA). After separation by HPLC, each fraction corresponding to a fatty acid methyl ester was collected at the detector outlet (differential refractometer) and its radioactivity was directly measured in the solvent by liquid scintillation counting. The conditions established by the authors permitted a good separation of the different unsaturated fatty acid methyl esters. Moreover, the desaturation product was eluted before its substrate,

avoiding possible radioactive contamination. Enzyme activity was calculated from the desaturation product/substrate ratio and was expressed as nmoles of substrate transformed in 15 min with 5 mg of total microsomal proteins.

Fatty acid analysis. Lipids from aliquots of liver microsomes were extracted according to Delsal (26). Phospholipids were obtained by liquid column chromatography following the procedure described by Hirsch and Ahrens (27). After addition of heptadecanoic acid, fatty acids were methylated in the presence of methanolic BF_3 and extracted as previously described (24). The fatty acid methyl esters were analyzed by capillary gas-liquid chromatography on a model 417 Becker-Packard apparatus (Packard Instrument, Rungis, France) equipped with a laboratory-made 50 m × 0.3 mm i.d. glass capillary column coated with Carbowax 20 M. Gas chromatographic peaks were identified on the basis of their retention time relative to methyl heptadecanoate. Peak area was measured using a Delsi model Enica 21 computing integrator (Delsi Instruments, Suresnes, France).

Statistical analysis. All data are presented as the mean ± SE for n=6 animals. After analysis of variance using a Duncan's multiple range test, means were compared according to the least significant difference ($p<0.05$).

RESULTS

Table 2 shows that, at each age, the composition of dietary lipids had no significant effect on body and liver weights and on microsomal protein content of liver microsomes. In each dietary group, body weight increased between 3 and 6 months, but in the control group it also increased between 6 and 9 months.

Tables 3 and 4 report the desaturation activity, first expressed as quantities in Table 3 (nanomoles of desaturation product) and second, as conversion percentages (Table 4). The Δ9 desaturase showed the highest activity independent of age and diet. This activity clearly increased between 3 and 6 months but then decreased at 9 months to approximately the three month-level. The diet had no significant influence. At a 50-nmol substrate concentration, the conversion percentage was about 1.5 times higher than at a substrate concentration of 100 nmol.

The two other desaturases were less active than the Δ9 desaturase. The Δ5 desaturation of 20:3n-6 was more active than the Δ6 desaturation of 18:2n-6 or 18:3n-3 in any of the experimental situations. As for Δ9 desaturation, Δ5 desaturation progressed at a higher rate in the presence of a lower concentration of substrate (30 nmol). The activity increased from 3 to 6 months, at which time it reached a plateau. The influence of diet appeared only at 6 and 9 months when black currant and borage oils had been ingested over a longer time period. In 3-month-old animals, the desaturation of n-3 fatty acid was much higher than that of n-6 fatty acid for all dietary groups. The difference disappeared by 6 and 9 months. The conversion percentage was the same at the two concentrations of the two substrates in contrast to results obtained with the Δ9 and Δ5 desaturases. The effect of season (Table 5) was investigated in the 6-month-old rats. The Δ9 desaturation activity markedly increased in winter in all dietary groups. Δ5 Desaturation activity was also higher in winter but only at high concentration of substrate and only for the C and Bc groups. At the same saturating concentration of substrate (120 nmol), the Δ6 desaturation of the n-3 fatty acid was not affected by the season, whereas the desaturation of the n-6 fatty acid was greatly increased in winter.

Table 6 presents the percentages of the major fatty acids of liver microsomal phospholipids at the different ages and for the different diets. Palmitic and stearic acids were the main saturated fatty acids. Their cumu-

TABLE 2

Body Weight, Liver Weight and Protein Content of Liver Microsomes of Rats. Effects of Age and Dietary Lipids[a]

Age (months)	Dietary group	Body weight (g)	Liver weight (g)	Liver weight/ body weight (%)	Microsomal proteins (mg/g of liver)
3	C[b]	327 ± 27^{x}	11.5 ± 0.6^{w}	3.51 ± 0.11^{y}	19.12 ± 3.46^{z}
	Bc[c]	330 ± 33^{x}	12.2 ± 1.5^{wx}	3.70 ± 0.13^{z}	16.40 ± 2.14^{yz}
	Bg[d]	331 ± 17^{x}	12.7 ± 0.9^{wxy}	3.85 ± 0.08^{z}	17.19 ± 2.42^{yz}
6	C	411 ± 27^{y}	12.7 ± 1.2^{wxy}	3.08 ± 0.15^{vwx}	15.35 ± 2.17^{y}
	Bc	448 ± 20^{yz}	13.9 ± 1.8^{yz}	3.12 ± 0.11^{wx}	16.22 ± 1.96^{yz}
	Bg	440 ± 52^{y}	14.2 ± 0.9^{yz}	3.22 ± 0.15^{x}	16.82 ± 3.37^{yz}
9	C	479 ± 40^{z}	14.2 ± 1.5^{z}	2.97 ± 0.13^{vw}	17.08 ± 2.79^{yz}
	Bc	442 ± 38^{yz}	13.4 ± 0.8^{xyz}	3.01 ± 0.17^{vw}	18.95 ± 1.07^{z}
	Bg	460 ± 26^{yz}	13.4 ± 1.8^{xyz}	2.92 ± 0.19^{v}	15.97 ± 2.33^{y}

[a]Results are means ± SE for n=6 animals in each group. After analysis of variance (Duncan's multiple range test), means were compared in each column (three groups and three diets) according to the least significant difference, and classified according to increasing order. Means assigned different manuscript letters were significantly different ($p<0.05$).

[b]Control diet.

[c]Black currant seed oil diet.

[d]Borage oil diet.

lated percentages generally decreased with age in the three groups. The influence of diet at each age was not evident. The decrease in saturated fatty acid content was generally compensated (especially in the control group) by an increase of the monounsaturated fatty acids 16:1n-7 and 18:1n-9, the products of Δ9 desaturation. The percentage of linoleic acid did not really change with age and diet. The arachidonic acid content did not increase with age in the C group. With the experimental diets containing γ-linolenic acid, an increase in arachidonic acid was observed, but only at 9 months (corresponding to ingestion of 18:3n-6 for a longer time). This caused a similar variation in the total n-6 fatty acid content. The ratio 20:4n-6/18:2n-6, which reflects the overall conversion of 18:2n-6 to 20:4n-6, did not generally change between 3 and 9 months in each dietary group. However, the ratio was higher in the black currant and borage dietary groups (especially the latter). Docosahexaenoic acid (22:6n-3) was the major n-3 fatty acid in the microsomal phospholipids. The percentage of 22:6n-3 tended to increase with age for each group. At 9 months, the total n-3 fatty acid content was higher than at 3 months, especially in the two groups on the experimental diets, Bc and Bg. The presence of stearidonic acid in the black currant oil did not induce any specific changes.

DISCUSSION

The purpose of this study was to see whether dietary manipulation could overcome a possible age-related decrease in PUFA content of tissue lipids due to a decrease in desaturation activity. A decrease in Δ6 desaturation is of special concern as Δ6 desaturation is the major regulating reaction step in EFA metabolism.

The experimental approach was to bypass the Δ6 desaturation reaction by adding to the diet γ-linolenic acid (n-6 series) or stearidonic acid (n-3 series). Δ9 Desaturase activity was also studied because it broadly reflects lipogenic activity. In studies with guinea pigs (28), humans (29) and rats (30), it was shown that administration of γ-linolenic acid results in the biosynthesis of arachidonic acid by shunting the Δ6 desaturase step. An increase of n-6 PUFAs has been observed in various organs after the addition to the diet of black currant seed oil or borage oil, both containing γ-linolenic acid.

In our experiments rats were fed low quantities of 18:3n-6 (206.8 mg and 169.1 mg per 100 g of Bc and Bg diets, respectively). Black currant and borage oils were mixed with other oils to maintain the same total levels of n-6 and n-3 fatty acids as in the control group. Experimental diets were given at different time points as a function of age, the duration of feeding being increased from 3 to 9 months of age. The protocol was justified by the aim of this study, which was to compensate for, and not to prevent a possible deficiency in desaturation capacity. Because the turnover rate of tissue lipids supposedly decreases with age, the duration of the substituted experimental diet was increased with age.

Results of *in vitro* desaturation by liver microsomes (Tables 3 and 4) showed that the Δ9 desaturase

TABLE 3

Desaturase Activity in Rat Liver Microsomes. Effects of Age and Dietary Lipids[a]

Dietary group	Age (months)	Δ n-3 18:3 → 18:4		Δ6 n-6 18:2 → 18:3		Δ5 n-6 20:3 → 20:4		Δ9 18:0 → 18:1n-9	
		40	120	60	120	30	80	50	100
		nmoles							
C[b]	3	3.3 ± 0.4^{z}	9.7 ± 0.9^{yz}	3.5 ± 0.5^{xy}	5.3 ± 1.3^{wx}	6.3 ± 0.7^{xy}	8.3 ± 1.1^{x}	21.3 ± 6.2^{yz}	27.9 ± 12.0^{xy}
	6	3.5 ± 0.9^{z}	9.0 ± 1.2^{xy}	5.2 ± 1.2^{z}	8.0 ± 2.6^{z}	9.0 ± 1.0^{z}	12.7 ± 2.1^{y}	30.5 ± 5.4^{z}	43.6 ± 11.5^{z}
	9	3.0 ± 0.5^{yz}	8.2 ± 1.0^{wx}	4.7 ± 0.9^{yz}	6.1 ± 1.1^{xy}	8.5 ± 0.8^{yz}	12.6 ± 2.4^{y}	14.6 ± 5.0^{y}	16.6 ± 7.7^{w}
Bc[c]	3	3.4 ± 0.5^{z}	10.6 ± 1.4^{z}	3.1 ± 0.4^{xy}	3.8 ± 0.5^{vw}	5.8 ± 0.7^{x}	8.5 ± 1.4^{x}	25.0 ± 4.9^{yz}	31.2 ± 8.8^{y}
	6	2.3 ± 0.2^{y}	7.9 ± 0.7^{w}	2.3 ± 0.2^{x}	7.1 ± 2.6^{yz}	5.9 ± 0.9^{x}	16.7 ± 3.5^{z}	30.3 ± 5.0^{z}	42.9 ± 13.8^{z}
	9	2.8 ± 0.8^{yz}	8.2 ± 0.6^{wx}	2.3 ± 0.2^{x}	7.1 ± 2.6^{yz}	9.2 ± 1.6^{z}	14.8 ± 3.5^{yz}	22.1 ± 5.8^{yz}	35.0 ± 10.6^{yz}
Bg[d]	3	2.8 ± 0.5^{yz}	9.2 ± 1.3^{y}	2.4 ± 0.2^{x}	3.5 ± 0.5^{v}	5.2 ± 0.3^{x}	7.4 ± 1.2^{x}	20.6 ± 7.3^{yz}	25.9 ± 10.5^{wxy}
	6	3.1 ± 0.8^{yz}	8.2 ± 1.0^{wx}	4.6 ± 0.7^{yz}	7.2 ± 1.8^{yz}	5.6 ± 0.7^{x}	13.3 ± 1.3^{y}	30.6 ± 3.8^{z}	45.2 ± 13.1^{z}
	9	2.3 ± 0.3^{y}	9.2 ± 1.1^{y}	4.6 ± 1.1^{yz}	8.3 ± 2.1^{z}	10.2 ± 2.1^{z}	16.5 ± 3.9^{z}	15.8 ± 9.9^{y}	20.2 ± 13.8^{wx}

[a]Desaturase activities are expressed as nmoles of substrate converted per 15 min per 5 mg microsomal protein. Results are means ± SE for n=6 animals in each group. After analysis of variance (Duncan's multiple range test), means were compared in each column (three groups and three diets) according to the least significant difference, and classified according to increasing order. Means assigned different manuscript letters were significantly different ($p<0.05$).
[b]Control diet.
[c]Black currant seed oil diet.
[d]Borage oil diet.

TABLE 4

Desaturase Activity in Rat Liver Microsomes Expressed as Conversion Percentages. Effects of Age and Dietary Lipids[a]

Desaturation		Δ6 n-3 18:3 → 18:4		Δ6 n-6 18:2 → 18:3		Δ5 n-6 20:3 → 20:4		Δ9 18:0 → 18:1n-9	
Dietary group	Age (months)	40	120	60	120	30	80	50	100
		nmoles of substrates							
C[b]	3	8.3	8.1	5.8	4.4	21.0	10.4	42.6	27.9
	6	8.8	7.5	4.3	6.7	30.0	15.9	61.0	43.6
	9	7.5	6.8	3.9	5.1	28.3	15.8	29.2	16.6
Bc[c]	3	8.5	8.8	5.2	3.2	19.3	10.6	50.0	31.2
	6	5.8	6.6	3.8	5.9	19.7	20.8	60.6	42.9
	9	7.0	6.8	3.8	5.9	30.7	18.5	44.2	35.0
Bg[d]	3	7.0	7.7	4.0	2.9	17.3	9.3	41.2	25.9
	6	7.8	6.8	7.7	6.0	18.7	16.6	61.2	45.2
	9	5.8	7.7	7.7	6.9	34.0	20.6	31.6	20.2

[a]Conversion percentages were calculated from the desaturation product/substrate ratio.
[b]Control diet.
[c]Black currant seed oil diet.
[d]Borage oil diet.

was more active than the Δ6 and Δ5 desaturases. This high activity of Δ9 desaturation, especially at 6 months, may be related to active lipogenesis in the liver. Growth is most active between 3 and 6 months and the need for fatty acids for tissue synthesis is elevated. Saturated fatty acids provided by the dietary oils were relatively low, enhancing lipogenic reactions which give rise to saturated fatty acids that undergo desaturation by Δ9 desaturase.

In comparison, the activities of Δ5 and, more particularly, of Δ6 desaturases were clearly lower than that of the Δ9 desaturase. The Δ5 and Δ6 desaturations do not have the same physiological significance as the Δ9 desaturation, since they are required in the biosynthesis of PUFAs from dietary linoleic and linolenic acids. The low activity of the Δ6 desaturase means that it constitutes a limiting step in the biosynthesis of the essential PUFAs.

The difference observed regarding the activity of the enzymes with different substrates is in agreement with observations reported previously by others. Brenner and Peluffo (31) demonstrated a more rapid desaturation of α-linolenic acid than of linoleic acid by the Δ6 desaturase. This was confirmed by Marcel *et al.* (32) and by Sprecher (33). Data regarding the Δ5 desaturation rate are sparse and sometimes contradictory. Bernert and Sprecher (34) observed a rate lower than the Δ6 desaturation rate, but others (31,32) found it higher.

The activities of Δ6 and Δ5 desaturases toward n-6 fatty acids increased from 3 to 6–9 months. This corresponds to an active period of growth during which PUFA demand is high for membrane phospholipid synthesis in the newly formed tissues. Desaturation activities adapt themselves to this need probably by the synthesis of new enzyme under hormonal control. The Δ6 desaturation of the n-3 fatty acid was more active than that of the n-6 fatty acid only at 3 months. Nervous tissue is very rich in n-3 PUFAs and at three months of age the brain is still rapidly developing (35), increasing the demand for this type of PUFAs.

Our results regarding the influence of age on the desaturation rates (Tables 3 and 4) can be compared to those reported by Peluffo and Brenner (12) and Bordoni *et al.* (13). These authors studied the Δ6 desaturation in rats fed a diet containing two levels of proteins (12) or a commercial standard diet (13). They observed that Δ6 desaturation activity was lower at 12 or 14 months than at 3 months. This is not contradictory to our results since we observed the maximum at 6 and 9 months; the activity is then likely to decrease. The changes in Δ6 desaturation with age may be similar to those we observed with Δ9 desaturation, but at a slower rate. Results of studies at a more advanced age, *i.e.*, at 12, 18 and 24 months, may answer this question.

The presence of γ-linolenic acid in the diet did not induce any change in the Δ6 desaturation of linoleic acid. In contrast, Δ5 desaturation was slightly more active, especially at 9 months. These low effects are likely due to the low level of γ-linolenic acid in the experimental diets (Bc and Bg). When ingested for a longer time (rats of 9 months of age), the stimulating effect on Δ5 desaturation was more significant. The presence of stearidonic acid (18:4n-3) in black currant seed oil did not affect the Δ6 desaturation of α-linolenic acid, probably because the amount of this n-3 fatty acid in the diet was very low. Choi and Sugano (20) observed an inhibitory effect of dietary γ-linolenic acid on the Δ6 desaturation of linoleic acid. In these experiments, a higher amount of dietary evening primrose oil (9.3 % of γ-linolenic acid) was provided (10% weight of oil in the diet) than in our study.

Our studies confirmed the seasonal variations of desaturation previously observed by Peluffo and Brenner (12). The desaturations were generally more active

TABLE 5

Desaturase Activity of Liver Microsomes of 6-month-old Rats. Effect of the Season[a]

Desaturase		Δ6 n-3		Δ6 n-6		Δ5 n-6		Δ9	
Dietary group	Season	40	120	60	120	30	80	50	100
		nmoles							
C[b]	Summer	2.9 ± 0.9^{y}	9.1 ± 1.2^{z}	4.2 ± 0.4^{x}	5.6 ± 0.7^{w}	9.2 ± 1.1^{z}	11.1 ± 0.8^{x}	26.2 ± 4.4^{y}	33.6 ± 5.6^{z}
	Winter	4.1 ± 0.3^{z}	8.9 ± 1.4^{z}	6.3 ± 0.2^{z}	10.4 ± 0.3^{z}	8.6 ± 0.8^{z}	14.2 ± 1.8^{y}	34.7 ± 0.1^{z}	53.6 ± 0.7^{y}
Bc[c]	Summer	2.4 ± 0.2^{y}	7.7 ± 0.8^{y}	2.5 ± 0.2^{y}	4.6 ± 0.1^{v}	6.8 ± 0.4^{yz}	14.7 ± 0.9^{y}	25.9 ± 0.7^{y}	30.9 ± 3.1^{z}
	Winter	2.2 ± 0.2^{y}	8.1 ± 0.6^{z}	2.1 ± 0.1^{w}	9.7 ± 0.4^{y}	5.1 ± 0.1^{y}	18.7 ± 4.3^{z}	34.6 ± 2.4^{z}	54.9 ± 5.5^{y}
Bg[d]	Summer	2.9 ± 0.7^{y}	8.4 ± 0.2^{z}	3.9 ± 0.2^{x}	5.6 ± 0.2^{w}	6.1 ± 0.2^{y}	13.6 ± 1.3^{y}	27.4 ± 2.5^{y}	34.1 ± 6.9^{z}
	Winter	2.5 ± 0.9^{y}	8.0 ± 1.6^{z}	5.2 ± 0.4^{y}	8.9 ± 0.3^{x}	5.1 ± 0.6^{y}	13.1 ± 1.5^{y}	33.7 ± 0.6^{z}	56.4 ± 2.8^{y}

[a]Results are expressed as in Table 3 for n=3 animals. After analysis of variance (Duncan's multiple range test), means were compared in each column (three diets and two seasons) according to the least significant difference, and classified according to increasing order. Means assigned different manuscript letters were significantly different ($P < 0.05$).
[b]Control diet.
[c]Black currant seed oil diet.
[d]Borage oil diet.

TABLE 6

Major Fatty Acids (% moles) of Liver Microsomal Phospholipids. Effects of Age and Dietary Lipids[a]

		Fatty acids							
Dietary group	Age (months)	16:0 + 18:0	16:1n-7 + 18:1n-9	18:2n-6	20:4n-6	22:6n-3	total n-6[e]	total n-3[f]	20:4n-6/ 18:2n-6
C[b]	3	38.5 ± 1.8^{yz}	13.5 ± 2.0^{wx}	7.2 ± 0.6^{xyz}	23.7 ± 3.0^{u}	8.9 ± 1.4^{wxyz}	35.1 ± 3.7^{w}	10.7 ± 2.3^{xy}	3.4 ± 0.4^{x}
	6	39.6 ± 1.5^{z}	14.8 ± 2.2^{xy}	8.0 ± 0.6^{z}	24.8 ± 2.6^{uv}	7.9 ± 1.3^{vw}	35.7 ± 2.6^{wx}	9.6 ± 1.6^{wx}	3.1 ± 0.5^{x}
	9	33.2 ± 1.2^{wx}	17.0 ± 2.8^{z}	7.7 ± 0.5^{yz}	25.7 ± 3.0^{uvw}	9.7 ± 1.1^{xyz}	36.4 ± 1.9^{wx}	12.0 ± 1.0^{zy}	3.3 ± 0.5^{x}
Bc[c]	3	39.8 ± 1.2^{z}	12.5 ± 1.5^{w}	6.1 ± 0.8^{w}	26.8 ± 1.7^{vw}	8.5 ± 0.3^{wx}	36.9 ± 1.6^{wx}	9.8 ± 0.2^{wx}	4.4 ± 0.8^{y}
	6	37.7 ± 2.8^{y}	15.9 ± 1.0^{yz}	6.5 ± 0.3^{w}	26.9 ± 3.1^{vw}	7.1 ± 1.8^{v}	38.8 ± 5.3^{xy}	8.5 ± 1.8^{w}	4.1 ± 0.3^{y}
	9	31.6 ± 2.2^{w}	13.3 ± 1.7^{w}	7.3 ± 0.5^{xy}	31.8 ± 1.7^{yz}	10.0 ± 0.6^{yz}	42.0 ± 1.7^{yz}	12.3 ± 0.6^{z}	4.4 ± 0.3^{y}
Bg[d]	3	38.5 ± 0.8^{yz}	11.0 ± 0.7^{v}	5.3 ± 0.9^{v}	27.9 ± 1.7^{wx}	8.9 ± 0.9^{wxy}	37.1 ± 2.8^{wx}	10.2 ± 0.8^{x}	5.3 ± 0.6^{z}
	6	32.6 ± 0.8^{wx}	13.6 ± 0.3^{wx}	7.4 ± 0.3^{yz}	30.1 ± 0.8^{xy}	9.5 ± 0.2^{xyz}	41.7 ± 1.0^{yz}	10.7 ± 0.2^{xy}	4.1 ± 0.3^{y}
	9	32.3 ± 2.3^{wx}	12.2 ± 0.7^{vw}	6.6 ± 0.3^{wx}	33.5 ± 3.1^{z}	10.1 ± 0.7^{z}	43.6 ± 2.4^{z}	12.1 ± 0.6^{yz}	5.0 ± 0.3^{z}

[a]Results are expressed as molar percentages of total fatty acids. Results are means ± SE for n=6 animals in each group. After analysis of variance (Duncan's multiple range test), means were compared in each column (three groups and three diets) according to the least significant difference, and classified according to increasing order. Means assigned different manuscript letters were significantly different ($p < 0.05$).
[b]Control diet.
[c]Black currant seed oil diet.
[d]Borage oil diet.
[e]Total n-6: include unreported fatty acids: 18:3, 20:2, 20:3, 22:4 and 22:5n-6.
[f]Total n-3: include unreported fatty acids: 18:3, 20:5 and 22:5n-3.

in winter (January) than in summer (June). Surprisingly, the Δ6 desaturation of α-linolenic acid was not affected in contrast to the Δ6 desaturation of linoleic acid. The variations in desaturation activity are likely to follow variations in hormonal secretions. The physiological significance of these modifications is difficult to understand since the rats were submitted to identical environmental conditions throughout the year. The results underline the importance of taking into account seasonal variations when studying the desaturation rates, especially in the course of aging. In our experiments with 3- and 9-month-old rats, two groups of three rats were tested at 4- to 6-month intervals to minimize seasonal variations. Modifications in Δ6 and Δ5 desaturation rates are likely to influence the rate of biosynthesis of essential PUFAs and to modify their pattern in membrane phospholipids. To test this hypothesis, we analyzed the fatty acid composition of microsomal phospholipids at the three different ages and with the three different diets. This choice of membrane appears appropriate for this study because the desaturases are thought to reside in the phospholipid bilayers of the endoplasmic reticulum. The question is often raised whether changes in desaturase activity would change the fatty acid composition of the phospholipids or, conversely, whether modification of phospholipid fatty acids would induce changes in the desaturation rate. Eventually, correlation between the two phenomena could be established. In this work, the fatty acid composition of microsomal phospholipids (Table 6) did not necessarily correlate with changes in desaturase activity. For example, the proportion of monounsaturated fatty acids was not modified between 6 and 9 months, whereas the Δ9 desaturation rate sharply decreased during this period (Tables 3 and 4). Similarly, the proportion of arachidonic acid did not change from 3 to 9 months, whereas the Δ6 and Δ5 desaturation rates increased from 3 to 6 months. However, the type of diet seemed to influence the phospholipid fatty acid composition. The presence of γ-linolenic acid in dietary black currant or borage oil tended to increase the Δ5 desaturation rate and, consequently, the arachidonic acid content of microsomal phospholipids. This property is important since the activity of the rate-limiting Δ6 desaturation step may decrease with age.

ACKNOWLEDGMENTS

This work was supported in part by the Ministère de La Recherche et de l'Enseignement Supérieur (MRES, Aliment 2000), France. We thank J. Gresti for technical assistance in performing chromatographic separations and Drs. M. Suschetet, P.O. Astorg and F. Sauvageot for assisting in the statistical evaluation of our data.

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[Received December 26, 1989, and in revised form September 27, 1990; Revision accepted October 31, 1990]