

Effects of dietary α -linolenic acid deficiency during pregnancy and lactation on lipid fatty acid composition of liver and serum in the rat

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Summary. The effects of a dietary α -linolenic acid (18 : 3 n-3) deficiency on lipid fatty acid composition of the liver and serum of lactating rats have been studied during three gestations and over three generations. These females were compared to corresponding females which remained sterile.

Two lots of female rats received, respectively, a diet containing lipids either in the form of 1.50 g of sunflower oil per 100 g of diet (deficient diet) or as 1.87 g of soya oil per 100 g of diet (control diet). Both diet contained the same amount of linoleic acid (18 : 2 n-6), *i.e.* 940 mg/100 g of diet, but the sunflower diet supplied 43 times less 18 : 3 n-3 than the soya diet, or 3 mg vs 130 mg/100 g of diet.

Results show that successive gestations appeared to be more efficient means of depleting material n-3 PUFA ⁽¹⁾ stores than successive generations. The 18 : 3 n-3 deficient diet caused a considerable decrease in the level of n-3 polyunsaturated fatty acids (n-3 PUFA) in liver and serum lipids, and particularly of 22 : 6 n-3. This decline was compensated by an increase in the level of n-6 polyunsaturated fatty acids (n-6 PUFA), and particularly by a very high augmentation of 22 : 5 n-6.

The ratio n-6 PUFA/n-3 PUFA in liver phospholipids and in serum lipids was a good index of the adequacy of dietary n-3 PUFA supply.

However, the ratio 22 : 5 n-6/22 : 6 n-3 was a finer index. This ratio appeared to be a reliable index of dietary n-3 PUFA deficiency when it was higher than 1 in serum lipids of a fasting animal.

The proportion of 22 : 5 n-6 as well as the ratios n-6/n-3 and 22 : 5 n-6/22 : 6 n-3, were also increased in the liver phospholipids of lactating females receiving the soya oil diet ; this suggested that a supply of 130 mg/100 g of diet, corresponding to a ratio of n-6/n-3 = 7.2, was not sufficient for these rats during pregnancy and lactation.

A supply of 200 mg of n-3 PUFA/100 g of diet, corresponding to a ratio of n-6/n-3 = 5, is recommended for these animals.

Introduction.

Fatty acids in the n-3 series, of which α -linolenic acid is the main one, are usually found in phospholipids and thus in cellular and subcellular membranes of mammals (Crawford *et al.*, 1969, 1970a, b ; Crawford and Woodford, 1971 ; Gudbjarnason and Halgrímsson, 1976 ; Setchell, 1978).

(1) *Abbreviations* : saturated fatty acids = SFA ; monounsaturated fatty acids = MUFA ; polyunsaturated n-6 fatty acids = n-6 PUFA ; polyunsaturated n-3 fatty acids = n-3 PUFA.

These fatty acids, mostly composed of docosahexaenoic acid (22 : 6 n-3), are very abundant in nervous tissue membranes, particularly in brain (Svennerholm, 1968 ; Alling *et al.*, 1972 ; Galli *et al.*, 1971 ; Eddy and Harman, 1975 ; Cohen and Bernsohn, 1978 ; Bourre *et al.*, 1984) and retina (Anderson and Maude, 1972 ; Benolken *et al.*, 1973 ; Tinoco *et al.*, 1977 ; Aveldano de Caldironi *et al.*, 1981 ; for review, see Tinoco, 1982).

They are also found in serum lipids in the form of 20 : 5 n-3 and 22 : 6 n-3. As a precursor of prostanoids of the n-3 series, 20 : 5 n-3 plays an important role in the regulation of platelet aggregation and prevents the formation of thrombi (Dyerberg *et al.*, 1978 ; Siess *et al.*, 1980 ; Needleman *et al.*, 1979 ; Budowski *et al.*, 1980 ; Sinclair, 1981). Moreover, n-3 PUFA are powerful hypocholesterolemic and hypotriglyceridemic agents (Nordoy, 1965 ; Peifer, 1966 ; Dyerberg *et al.*, 1975 ; Harris *et al.*, 1983 ; Fehily *et al.*, 1983 ; Durand *et al.*, 1985). When the diet is deficient in these n-3 PUFA, n-3 fatty acids are usually replaced by n-6 fatty acids or, more strictly speaking, most of the 22 : 6 n-3 is replaced by 22 : 5 n-6 (Galli *et al.*, 1971, 1974 ; Cohen and Bernsohn, 1978 ; Tinoco *et al.*, 1978 ; Menon and Dhopeswarkar, 1981 ; Nouvelot *et al.*, 1983a,b ; Bourre *et al.*, 1984 ; Youyou *et al.*, 1986).

However, when this occurs, the physiological status is not normal, resulting in an alteration of the electroretinogram (ERG) (Wheeler *et al.*, 1975 ; Neuringer *et al.*, 1984, 1986a, b ; Nouvelot *et al.*, 1985), higher neonatal mortality (Sinclair and Crawford, 1973 ; François *et al.*, 1980 ; Guesnet *et al.*, 1986), decreased learning ability (Galli *et al.*, 1975 ; Lamptey and Walker, 1978), changes in enzyme activities which may alter membrane physiology (Bernsohn and Spitz, 1974 ; Sun and Sun, 1974 ; McMurchie and Raison, 1979 ; Flier *et al.*, 1985), and neurological disorders (Holman *et al.*, 1982).

Now, membrane structures, and particularly those of the nervous system, develop intensely during the foetal period and lactation (Sinclair and Crawford, 1972 ; Crawford *et al.*, 1981 ; Bourre, 1984 ; Menon and Dhopeswarkar, 1982). Moreover, the return to a normal lipid fatty acid composition in the membranes is very slow when a sufficient amount of n-3 PUFA is supplied to previously deprived animals (Youyou *et al.*, 1986).

All these data show that it is important to correctly estimate the n-3 PUFA requirement in females during gestation and lactation.

In a previous paper, we studied the effects of maternal dietary α -linolenic acid deficiency on neonatal survival of the young (Guesnet *et al.*, 1986).

The aim of the present work was to show the effects of dietary α -linolenic acid deficiency on lipid fatty acid composition in the liver and serum of female rats after pregnancy-lactation and to obtain information on the α -linolenic acid requirements of these animals.

Material and methods.

1. Animals.

The experimental designs used have been described by Guesnet *et al.* (1986) (fig. 1).

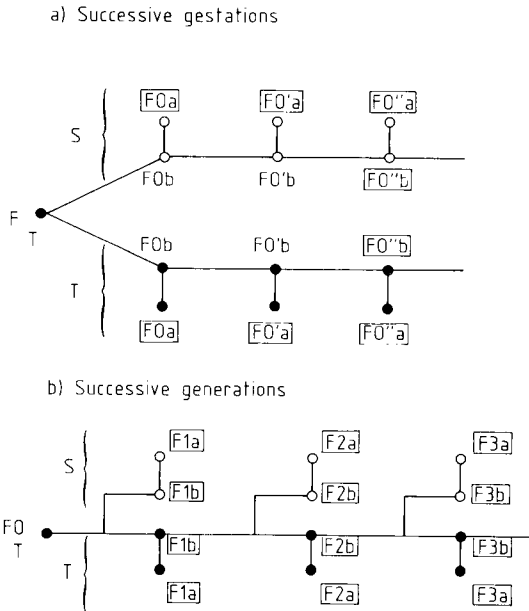


FIG. 1. — *Experimental designs.*

● : sunflower oil diet (T) ; ○ : soya oil diet (S) ; a : sterile females ; b : fertile females.

Design 1. Successive gestations. — From weaning, about 100 female Wistar rats (F0) received a semi-synthetic diet (table 1) in which the lipids were supplied exclusively by 1.5 % sunflower oil poor in α -linolenic acid (0.2 %). At 8 weeks of age, or 2 weeks before mating, they were divided into two groups ; one continued to receive the sunflower diet and the other was given a diet in which the sunflower oil was replaced by 1.87 % of soya oil containing a high level of α -linolenic acid (7.4 %).

Both diets contained 940 mg of linoleic acid/100 g diet, but the sunflower diet had only 3 mg of α -linolenic acid while the soya diet contained 130 mg (table 2).

The F0 rats were mated at 10 weeks and the gravid ones F0b again at 18 weeks at the end of lactation ; those which had a second litter F0'b, nursed it and were then mated a third time at 26 weeks. We thus obtained females which had three successive gestations (F0''b).

TABLE 1
Diet composition (per kg).

	Soya oil (S) diet	Sunflower oil (T) diet
Casein (1)	220	220
DL Methionine (1)	1.6	1.6
Cellulose (1)	20	20
Starch (1)	459.7	463.4
Saccharose (1)	230	230
Oil	18.7	15.0
Vitamin mixture	10	10
Mineral mixture (2)	40	40

(1) United States Biochemical Corp., Cleveland (USA). The vitamin mixture used is the vitamin diet for fortification mixture.

(2) Composition of the mineral mixture/100 g: CaHPO₄, 2 H₂O, 38.0; K₂HPO₄, 24.0; CaCO₃, 18.1; NaCl, 7.0; MgO, 2.0; MgSO₄, 7 H₂O, 9.0; FeSO₄, 7 H₂O, 0.7; ZnSO₄, H₂O, 0.5; MnSO₄, H₂O, 0.5; CuSO₄, 5 H₂O, 0.1; NaF, 0.1; Al₂(SO₄)₃, K₂SO₄, 24 H₂O, 0.02; KI, 0.008; CoCO₃, 0.008; Na₂SeO₃, 5 H₂O, 0.001.

TABLE 2
Fatty acid composition of dietary lipids.

Fatty acids	Diet	
	Soya oil (S)	Sunflower oil (T)
C14 : 0	0.3	0.3
C16 : 0	10.1	6.4
C17 : 0	0.2	—
C18 : 0	5.6	3.9
C20 : 0	0.4	0.3
C22 : 0	0.5	0.7
Σ saturated	17.2	11.6
C16 : 1	—	0.2
C18 : 1	21.4	21.6
C20 : 1	0.3	0.2
Σ monounsaturated	21.7	22.0
C18 : 2 n-6	53.5	66.4
C18 : 3 n-3	7.4	0.2
Fatty acids/100 g of diet :		
C18 : 2 n-6 (mg)	940.0	936.0
C18 : 3 n-3 (mg)	130.0	3.0
n-6/n-3	7.2	333.4

Dietary lipid fatty acid composition was analysed by gas chromatography of fatty acid methyl esters in the following conditions : Packard model 427 chromatograph ; glass capillary column ; stationary phase FFAP ; carrier gas pressure H₂ : 0.6 bar ; temperature : 190 °C ; detection by flame ionization.

One group of 6 females was killed at the end of the third lactation (group F0''b). This lot was compared to lots of 6 sterile females each of the stages studied (F0a : 0 gestation ; F0'a : 1 gestation ; F0''a : 2 gestations).

Design II. Successive generations. — In each generation 40 to 50 females of the sunflower lineage (T) were divided into two groups at 8 weeks of age ; one was kept on the sunflower diet and the other was given the soya diet (S). These rats were then mated at 10 weeks of age.

Groups of 6 females each were killed at the end of lactation along with 6 sterile littermates.

In both designs, the females remained with males during one sexual cycle (4 days) ; the latter were stock-breeding rats receiving a commercial diet. Twenty day later, the gravid females were put into individual cages. The third day post-partum, the litters were equalized to 10 pups each, except the F1 and F3 litters which could not be equalized to more than 7 and 8 pups each, respectively. The litters were weaned when 21 days old. Feed intake measured during gestation and lactation has been shown to be independent of the diet during both periods (Guesnet *et al.*, 1986).

The rats were killed by bleeding. The blood of each lot was pooled and centrifuged after coagulation. The liver, heart, brain and eyes were taken, weighed and pooled ; the serum and organs were freeze-dried and stored at -80°C .

The fatty acid composition of the brain, eyes and heart of the different groups will be published in another article.

2. Analytical methods.

Total lipids were extracted by the method of Folch *et al.* (1957). Phospholipids and neutral lipids were separated using the method of Borgström (1952). Serum fatty acid composition was determined after direct saponification under reflux (Gandemer *et al.*, 1980).

Fatty acid composition was determined by gas-liquid chromatography (Packard 427) of methyl esters on a glass capillary column (D = 0.3 mm ; L = 45 m ; phase = FFAP 0.5 % ; flame ionization detector).

Results.

In general, for a given diet and for females which had the same physiological status, the factors studied did not change significantly over successive generations or gestations. Only some results on F0'' females, which had 3 successive gestations, showed significant modification.

Thus, our analysis included mostly comparisons (1) between the effects of the two diets on females which had the same physiological status and (2) between females at the end of lactation and the corresponding ones which remained sterile to determine the effect of reproduction.

TABLE 3
Effect of dietary α -linolenic acid deficiency on liver lipid levels of sterile female rats (a) or those at the end of lactation (b).

N ^o of gestations Age at mating (week)	Successive gestations						Successive generations									
	F0		F0'		F0''		F0		F1		F2		F3			
	a	b	a	b	a	b	a	b	a	b	a	b	a	b		
1st	10		18		26		1st	10	1st	10	1st	10	1st	10		
2nd							2nd		2nd		2nd		2nd			
3rd							3rd		3rd		3rd		3rd			
Live weight (g)	S 282 ± 21 T 282 ± 23	276 ± 20 275 ± 22	321 ± 20 327 ± 13	329 ± 29 324 ± 28	282 ± 21 282 ± 23	232 ± 20 228 ± 17	249 ± 19 230 ± 8	246 ± 20 244 ± 17	245 ± 9 255 ± 14	246 ± 20 244 ± 17	246 ± 20 244 ± 17	246 ± 20 244 ± 17	246 ± 20 244 ± 17			
Liver weight (g)	S 6.0 ± 0.4 T 6.3 ± 0.2	7.2 ± 0.4 7.2 ± 0.5	8.2 ± 0.5 9.6 ± 0.6	16.6 ± 2.1 15.9 ± 3.0	6.0 ± 0.4 6.3 ± 0.2	11.7 ± 2.8 11.7 ± 2.8	7.4 ± 0.7 5.8 ± 0.5	6.1 ± 0.8 5.8 ± 0.5	10.5 ± 3.7 13.7 ± 1.7	6.1 ± 0.8 5.8 ± 0.5	6.1 ± 0.8 5.8 ± 0.5	6.1 ± 0.8 5.8 ± 0.5	6.1 ± 0.8 5.8 ± 0.5			
Hepatosomatic index (1)	S 2.1 ± 0.3 T 2.2 ± 0.3	2.6 ± 0.3 2.6 ± 0.4	2.6 ± 0.3 2.9 ± 0.3	5.1 ± 1.1 4.9 ± 1.4	2.1 ± 0.3 2.2 ± 0.3	5.0 ± 1.6 5.1 ± 1.6	3.0 ± 0.5 2.5 ± 0.3	2.7 ± 0.6 2.5 ± 0.4	4.3 ± 1.7 5.5 ± 1.0	2.7 ± 0.6 2.5 ± 0.4	2.7 ± 0.6 2.5 ± 0.4	2.7 ± 0.6 2.5 ± 0.4	2.7 ± 0.6 2.5 ± 0.4			
Total lipids (mg/g)	S 86 T 66	83 83	84 77	236 231	86 66	113 140	71 83	84 82	207 180	71 72	207 180	71 72	178 147			
Neutral lipids (mg/g)	S 51 T 31	46 46	46 42	202 200	51 51	80 106	32 46	46 44	174 143	34 40	146 120	34 40	146 120			
Phospholipids (mg/g)	S 35 T 35	37 37	38 35	34 31	35 35	33 34	39 37	38 38	33 37	33 32	33 32	33 32	32 27			
Total neutral lipids/liver	S 306 T 195	331 331	377 403	3 353 3 180	306 195	936 1 240	237 267	313 290	1 827 1 959	207 232	1 752 1 320	207 232	1 752 1 320			

S = soya oil ; T = sunflower oil ; (1) = liver weight % body weight.
 The means are shown with their standard deviation.

1. Female weight (table 3).

Neither the number of generations, lactation nor α -linolenic acid deficiency had any effect on rat live weight ; this weight was related only to animal age, as shown by the higher weight of group F0".

2. Liver.

2.1. *Fresh weight* (table 3). — Liver weight was independent of diet. On the other hand, it was closely related to physiological status since it was about 2-fold higher in rats at the end of lactation than in the corresponding sterile females (6 to 8 vs 11 to 16 g). The hepatosomatic index (liver weight % body weight), which was about 2.5 in sterile rats, reached about 5.0 in females at the end of lactation.

2.2. *Lipid level* (table 3). — Liver total lipids also seemed to be independent of diet but was always higher in females at the end of lactation than in the sterile ones (mean : 78 mg/g vs 180 mg/g fresh tissue) (\times 2.3). However, this difference was due exclusively to neutral lipids (mean : 42 mg/g fresh tissue in sterile rats vs 145 mg/g in those at the end of lactation) (\times 3.5). Phospholipid level (about 35 mg/g fresh weight) was independent of diet and of physiological status. As compared to the whole liver, the livers of sterile females included 200 to 300 mg of neutral lipids, while those of females at the end of lactation included 1,000 to 2,000 mg or a mean 6-fold more. However, it should be noted that the animals which had three successive lactations (group F0"b) had a total hepatic content of neutral lipids which was greater than 3,000 mg due to the high weight of the organ and its high concentration of that type of lipids.

2.3. Fatty acid composition of liver lipids.

2.3.1. Phospholipids (table 4).

a) *SFA + MUFA*. The SFA of liver phospholipids constituted 35 to 40 % of all the phospholipids ; they were primarily represented by palmitic (about 15 %) and stearic (20 to 25 %) acids. Neither α -linolenic acid deficiency nor lactation had any significant effect on the level of these fatty acids in liver phospholipids.

In our experimental conditions, the proportion of MUFA in liver phospholipids varied between 8 and 20 % ; their main element was oleic acid (C18 : 1 n-9) which alone represented more than 50 % of this type of fatty acid. α -linolenic acid deficiency in sterile animals had no effect on the mean MUFA level in liver phospholipids, but lactation caused a significant increase of these fatty acids ; this increase was slightly less in the sunflower (11 \rightarrow 15 %) than in the soya (11 \rightarrow 17 %) rats.

b) *n-6 PUFA*. n-6 PUFA are the most abundant constituents of liver phospholipids ; 37 to 48 % of all the fatty acids belong to this family. Their total level was always higher in sunflower rats (about 46 %) than in soya ones (about

TABLE 4

Effect of dietary α -linolenic acid deficiency on phospholipid fatty acid composition in the liver of sterile female rats (a) or at the end of lactation (b).

Lots	Successive gestations						Successive generations												
	F0		F0'		F0''		F0			F1			F2			F3			
	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b	
C16:0	S	14.0	14.1	14.5	15.0	14.0	13.5	16.2	13.9	15.6	14.8	15.0							
	T	14.5	14.2	13.6	16.7	14.5	15.1	16.9	14.0	15.9	12.9	15.7							
C18:0	S	21.4	24.0	16.3	20.2	21.4	24.3	21.9	21.0	17.9	23.1	18.8							
	T	26.2	23.1	21.8	20.0	26.2	23.2	22.8	19.3	21.3	25.7	21.7							
Σ SFA	S	35.7	38.5	31.3	35.9	35.7	38.2	38.6	36.0	34.1	38.6	34.7							
	T	41.6	38.0	36.2	37.7	41.6	39.0	40.4	32.9	37.6	39.8	36.0							
C18:1 n-9	S	4.8	5.3	6.0	10.0	4.8	5.2	10.0	8.9	12.7	5.0	11.0							
	T	4.6	5.8	7.1	10.0	4.6	4.7	10.3	6.7	10.0	4.5	10.5							
Σ MUFA	S	8.8	9.2	11.2	15.4	8.8	9.1	14.3	15.0	19.2	8.9	17.4							
	T	8.7	10.6	14.7	14.9	8.7	10.1	14.3	11.0	14.8	8.4	15.3							
C18:2 n-6	S	7.8	6.3	6.8	5.8	7.8	6.4	6.2	5.9	7.1	7.1	6.7							
	T	6.6	5.9	5.0	5.5	6.6	5.5	5.2	6.5	6.7	6.9	6.9							
C20:4 n-6	S	29.4	29.4	29.7	28.6	29.4	29.9	26.4	26.0	24.4	29.0	26.3							
	T	33.0	31.7	29.7	31.7	33.0	31.6	31.2	32.3	32.5	32.2	29.7							
C22:4 n-6	S	0.4	0.4	0.3	0.5	0.4	0.4	0.5	1.0	0.4	0.6	0.7							
	T	0.8	0.9	0.9	0.7	0.8	0.8	0.9	0.8	0.5	1.0	0.7							
C22:5 n-6	S	1.3	1.4	1.3	2.7	1.3	1.6	2.4	2.5	3.3	2.0	2.7							
	T	4.2	7.5	7.0	5.5	4.2	6.1	4.4	5.9	4.8	6.7	4.9							
Σ n-6 PUFA	S	39.8	38.2	40.0	38.8	39.8	39.1	36.6	36.6	37.0	39.5	37.8							
	T	45.7	46.8	43.8	44.8	45.7	45.9	42.9	45.5	45.6	47.8	43.7							
C20:5 n-3	S	0.3	0.2	0.6	0.6	0.3	0.3	0.7	0.9	0.9	0.3	1.1							
	T	—	0.3	0.7	0.2	—	0.3	—	—	0.2	0.2	0.2							
C22:5 n-3	S	0.7	0.7	0.9	0.9	0.7	0.8	1.0	0.9	1.0	0.8	1.0							
	T	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2							
C22:6 n-3	S	14.6	13.0	13.4	7.5	14.6	12.2	7.6	9.1	7.8	11.5	7.1							
	T	3.7	3.9	3.4	1.9	3.7	4.2	2.6	3.5	2.8	3.6	2.6							
Σ n-3 PUFA	S	15.7	13.9	15.3	9.4	15.7	13.4	9.7	11.5	9.7	13.0	9.4							
	T	3.9	4.4	4.5	2.4	3.9	5.0	2.9	3.7	3.2	4.1	3.0							
Σ (n-6) + (n-3)	S	55.5	52.1	55.3	48.2	55.5	52.5	46.3	48.1	46.7	52.5	47.2							
	T	49.6	51.2	48.3	47.2	49.6	50.9	45.8	49.2	48.8	51.9	46.7							
n-6:n-3	S	2.5	2.7	2.6	4.1	2.5	2.9	3.8	3.2	3.8	3.0	4.0							
	T	11.7	10.6	9.7	18.7	11.7	9.2	14.8	12.3	14.2	11.6	14.6							

S = soya oil ; T = sunflower oil.
The percentages of fatty acids not shown are : C14 : 0 : 0.7-1.5 % ; C16 : 1 n-7 : 0.4-1.0 % ; C16 : 1 n-9 : 6.9-8.4 % ; C18 : 1 n-7 : 2.4-3.8 %.

38 %). This overall superiority in these rats was due mostly to the constantly high, relative level of 22 : 5 n-6 ; the rest was imputed to 20 : 4 n-6 and 22 : 4 n-6. On the other hand, 18 : 2 n-6 was no longer abundant in sunflower rats, showing that it supplied longer-chain derivatives of its series when n-3 PUFA was deficient.

The level of 22 : 5 n-6 was usually higher in soya females at the end of lactation than in the corresponding sterile ones ; the reverse was true in sunflower animals.

c) *n-3 PUFA*. The level of n-3 PUFA in liver phospholipids varied considerably and according to diet and physiological status. In sterile females, this level was 13 to 16 % with the soya diet and 4 to 5 % with the sunflower diet ; in females at the end of lactation, it was 9 to 10 % with the soya diet and 3 % with the sunflower regime. These variations in level were due to the three n-3 PUFA elements of liver phospholipids, i.e. 20 : 5 n-3, 22 : 5 n-3 and 22 : 6 n-3, but mostly to the latter (90 %) which is a basic component of membrane structure.

d) *n-6 + n-3 and n-6/n-3*. In spite of some fluctuations (FOa, FO" a), it cannot be affirmed that dietary α -linolenic acid deficiency had an effect on overall PUFA level in liver phospholipid in either sterile females or those at the end of lactation. In general, n-6 PUFA, and particularly 22 : 5 n-6, compensated for the lack of 22 : 6 n-3. However, lactation caused a decrease in this level in both series of rats (52 \rightarrow 47 %) ; this decrease was compensated by an increase in the MUFA level (see above).

In sterile females, the n-6/n-3 ratio was about 3 in soya group phospholipids and about 11 in sunflower group phospholipids ; in rats at the end of lactation, this ratio was about 4 for the first group and 15 for the second.

2.3.2. Neutral lipids (table 5).

a) *SFA and MUFA*. The SFA constituted 30 to 40 % of the total fatty acids of liver neutral lipids, or slightly less than in the phospholipids. These fatty acids were represented mostly by palmitic acid (85 to 90 %).

The SFA level was not modified by 18 : 3 n-3 deficiency in sterile animals but increased slightly and independently of diet at the end of lactation (33 \rightarrow 38 %).

The MUFA were the most abundant in liver neutral lipids, forming 47 to 60 % of all the fatty acids in this type of lipids. As in phospholipids, the dominant element was oleic acid (75 to 90 % of total MUFA).

As in phospholipids, the MUFA level was unaffected by dietary α -linolenic acid deficiency in sterile females but was always higher at the end of lactation (mean : 50 – 58 %).

b) *n-6 PUFA*. This group of fatty acids was found in limited levels in liver neutral lipids (2.4 to 21.2 %).

In sterile rats, the n-6 PUFA level was not significantly influenced by diet, but it was still slightly increased in sunflower animals.

In rats at the end of lactation, diet had no influence but the effect of lactation was considerable because the mean levels of these PUFA dropped from

TABLE 5

Effect of dietary α -linolenic acid deficiency on neutral lipid fatty acid composition in the liver of sterile female rats (a) or at the end of lactation (b).

Lots	Successive gestations				Successive generations											
	F0	F0'	F0''	F0'''	F0		F1		F2		F3		F4		F5	
	a	a	a	b	a	b	a	b	a	b	a	b	a	b	a	b
C16:0	S	27.3	19.2	28.3	31.9	27.3	28.0	31.9	28.8	32.5	28.5	30.9	28.5	30.9	28.5	30.9
	T	28.0	28.9	29.6	36.1	28.0	28.7	32.6	29.3	32.7	26.9	31.6	26.9	31.6	26.9	31.6
Σ SFA	S	31.5	32.8	33.5	36.9	31.5	31.9	37.3	33.9	38.9	33.2	36.0	33.2	36.0	33.2	36.0
	T	32.3	32.4	34.4	37.1	32.3	32.4	37.8	32.7	38.6	31.4	37.1	31.4	37.1	31.4	37.1
C18:1 n-9	S	38.8	38.1	36.3	46.5	38.8	40.0	46.2	34.0	43.5	37.3	48.0	37.3	48.0	37.3	48.0
	T	39.1	39.0	36.3	46.7	39.1	40.0	46.9	38.3	45.4	37.8	45.9	37.8	45.9	37.8	45.9
Σ MUFA	S	49.5	50.0	50.7	59.1	49.5	51.1	57.2	48.6	56.6	47.1	59.5	47.1	59.5	47.1	59.5
	T	50.8	50.4	50.9	58.1	50.8	52.0	58.4	49.8	57.8	46.7	58.0	46.7	58.0	46.7	58.0
C18:2 n-6	S	12.3	10.6	8.2	2.7	12.3	9.9	2.6	10.1	2.2	12.7	2.6	12.7	2.6	12.7	2.6
	T	10.5	10.0	8.6	1.8	10.5	8.9	2.3	11.3	2.6	14.3	2.7	14.3	2.7	14.3	2.7
C20:4 n-6	S	2.9	3.1	2.3	0.7	2.9	2.3	0.8	2.8	0.6	3.7	0.6	3.7	0.6	3.7	0.6
	T	3.1	4.1	1.6	0.4	3.1	2.8	0.7	3.6	0.4	3.8	0.8	3.8	0.8	3.8	0.8
C22:4 n-6 + C22:5 n-6	S	0.4	0.5	0.8	—	0.4	0.8	0.2	0.7	0.1	0.6	—	0.6	—	0.6	—
	T	0.8	1.0	0.7	—	0.8	1.1	0.2	0.8	—	1.3	0.3	1.3	0.3	1.3	0.3
Σ n-6 PUFA	S	16.5	15.1	11.9	3.8	16.5	13.6	4.0	14.9	3.1	17.7	3.5	17.7	3.5	17.7	3.5
	T	16.5	16.8	13.3	2.4	16.5	15.4	3.6	17.2	3.3	21.2	3.9	21.2	3.9	21.2	3.9
C18:3 n-3	S	0.7	0.5	0.7	—	0.7	0.8	0.3	0.4	0.3	0.5	0.2	0.5	0.2	0.5	0.2
	T	0.1	—	0.1	—	0.1	0.2	—	0.1	0.2	—	—	—	—	—	—
C20:5 n-3	S	0.3	0.3	0.9	—	0.3	0.7	0.6	0.3	0.5	0.3	0.3	0.3	0.3	0.3	0.3
	T	0.1	—	0.8	—	0.1	0.2	—	0.1	0.1	0.2	0.1	0.2	0.1	0.2	0.1
C22:5 n-3 + C22:6 n-3	S	0.9	1.0	1.3	0.2	0.9	1.1	0.6	1.1	0.5	0.8	0.4	0.8	0.4	0.8	0.4
	T	1.0	0.2	0.9	—	1.0	0.8	0.2	0.2	0.1	0.5	0.4	0.5	0.4	0.5	0.4
Σ n-3 PUFA	S	2.1	1.9	3.4	0.2	2.1	3.2	1.5	2.4	1.4	1.7	0.9	1.7	0.9	1.7	0.9
	T	1.2	0.5	1.8	—	1.2	1.2	0.2	0.5	0.4	0.7	0.3	0.7	0.3	0.7	0.3
Σ (n-6) + (n-3)	S	18.6	17.0	15.3	4.0	18.6	16.8	5.5	17.3	4.5	19.4	4.4	19.4	4.4	19.4	4.4
	T	17.7	17.3	15.1	2.4	17.7	16.6	3.8	17.7	3.7	21.9	4.2	21.9	4.2	21.9	4.2
n-6/n-3	S	7.8	7.9	3.5	19.0	7.8	4.2	2.7	6.2	2.2	4.4	3.9	4.4	3.9	4.4	3.9
	T	13.7	33.6	7.4	—	13.7	12.8	18.0	34.4	8.2	30.3	13.0	30.3	13.0	30.3	13.0

S = Soya oil; T = sunflower oil.

The percentages of C14:0, C18:0, C16:0, C16:1 n-9, C16:1 n-7 and C18:1 n-7 did not vary very significantly; C14 = 1%; C18 = 2.3%; C16:1 n-9 = 0.6%; C16:1 n-7 = 6-10%; C18:1 n-7 = 2.5-4%; C20:3 n-9 = 0.2-0.3%.

16 to 3.5 %. The mean levels of all the fatty acids of this series varied : 18 : 2 n-6 dropped from 10.5 to 2.4 % ; 20 : 4 n-6 from 2.9 to 0.6 % ; and the sum of 22 : 4 n-6 + 22 : 5 n-6 from 0.8 % to practically zero.

c) *n-3 PUFA*. The proportion of n-3 PUFA in neutral lipids was influenced by dietary deficiency of 18 : 3 n-3 in both groups of rats.

While the 18 : 3 n-3 level was about 2.5 % in sterile soya females, it remained at about 1 % in the corresponding sunflower rats. At the end of lactation, this level decreased to 1-1.5 % in the soya females and to less than 0.5 % in the sunflower ones.

d) *n-6 + n-3 and n-6/n-3*. In sterile rats, dietary deficiency did not significantly change the level of total PUFA, considering the low percentage of n-3 PUFA in relation to n-6 PUFA and the slight progression of the latter in sunflower females.

In rats at the end of lactation, total PUFA were considerably lower in soya rats (17 → 4.6 %) and in sunflower rats (18 → 3.5 %) ; this total was not related to 18 : 3 n-3 deficiency.

Given the fact that the proportion of n-3 PUFA in neutral lipids was often very low, it was difficult to obtain a precise analysis of these fatty acids ; the result was relative large errors in their estimation and thus wide variability of the n-6/n-3 ratio. The ratio was always higher in sunflower females than in soya ones with the same physiological status ; moreover, it was usually higher in sterile rats than in those at the end of lactation due to preferential mobilization of n-3 PUFA.

3. Fatty acid composition of total serum lipids (table 6).

a) *SFA and MUFA*. Neither diet nor lactation has any effect on the SFA level in serum lipids (about 33 %). These fatty acids were represented mostly by palmitic and stearic acids ; contrary to that observed in liver phospholipids, palmitic acid was more abundant.

Total serum lipids included 20 to 30 % of MUFA, the main one being oleic acid. In sterile females, diet had no effect on these fatty acids.

Lactation caused a slight increase in the MUFA level in soya females but none in sunflower animals, so that at the end of lactation, there were significant deviations between the two groups (28 to 29 % in soya rats vs 22 to 24 % in sunflower rats).

b) *n-6 PUFA*. In sterile females, diet had no effect on serum lipid levels in this type of fatty acids. However, α -linolenic acid deficiency caused n-6 PUFA level in serum lipids of sunflower lactating rats to increase (39 → 42 %), while this level in soya females decreased (39 → 35 %) ; this resulted in a marked mean deviation between the two groups at the end of lactation.

As in liver phospholipids, arachidonic acid was by far the most abundant n-6 PUFA and caused most of the variation in total n-6 PUFA level. The level of 22 : 5 n-6 was always higher in sunflower rats (2 %) while it varied between 0.5 and 1 % in soya rats.

c) *n-3 PUFA*. The level of n-3 PUFA in serum lipids was 2 to 3-fold less in

TABLE 6

Effect of dietary α -linolenic acid deficiency on serum lipid fatty acids in sterile female rats (a) or at the end of lactation (b).

Lots	Successive gestations						Successive generations								
	F0		F0'		F0''		F0		F1		F2		F3		
	a	b	a	b	a	b	a	b	a	b	a	b	a	b	
C16: 0	S	18.9	16.7	17.5	17.7	18.9	19.1	15.1	16.4	16.0	18.5				
	T	18.2	18.0	17.7	17.7	18.2	18.0	16.6	15.5	15.6	17.3				
C18: 0	S	13.0	13.6	12.9	14.3	13.0	9.8	11.2	14.9	15.4	11.0	12.1			
	T	13.1	13.6	14.1	14.3	13.1	15.4	10.5	16.7	15.3	13.5	11.1			
Σ SFA	S	33.9	32.3	33.2	32.5	33.9	32.5	31.9	32.6	34.5	32.0	34.0			
	T	33.0	33.3	34.0	34.0	33.0	33.2	33.6	34.7	32.0	34.5	31.2			
C18: 1 n-9	S	18.1	15.1	17.0	21.3	18.1	20.4	24.2	18.3	22.0	18.6	22.4			
	T	18.9	18.5	17.7	18.1	18.9	18.8	17.6	18.9	16.2	18.8	13.9			
Σ MUFA	S	23.7	20.7	23.9	28.2	23.7	26.6	28.6	24.5	29.0	24.7	28.6			
	T	26.6	24.6	25.5	24.2	26.6	25.1	23.3	25.8	22.4	25.0	22.5			
C18: 2 n-6	S	8.8	9.6	8.0	6.8	8.8	7.7	9.0	9.5	6.3	9.2	7.8			
	T	9.1	7.9	7.2	6.0	9.1	7.3	8.3	7.9	7.4	9.6	10.7			
C20: 4 n-6	S	27.2	31.3	28.3	26.4	27.2	28.0	26.0	26.8	24.7	25.6	22.6			
	T	25.6	28.5	28.0	30.9	27.9	27.9	30.7	27.2	32.9	27.4	29.4			
C22: 4 n-6	S	0.3	0.4	0.3	0.3	0.3	0.2	0.3	0.6	0.5	0.4	0.5			
	T	0.8	0.6	0.7	0.6	0.8	0.5	0.5	0.5	0.5	0.5	0.4			
C22: 5 n-6	S	0.5	0.6	0.4	0.8	0.5	0.2	0.6	0.6	0.8	0.8	0.9			
	T	1.8	2.4	2.1	2.2	1.8	2.2	1.3	1.7	2.0	1.7	1.6			
Σ n-6 PUFA	S	37.6	43.0	38.4	35.5	37.6	36.8	37.0	38.8	33.9	38.8	33.8			
	T	38.9	40.2	39.3	40.7	38.9	39.7	41.3	36.5	44.1	38.8	44.2			
C18: 3 n-3	S	0.3	0.2	0.3	0.1	0.3	—	0.4	0.3	0.1	0.3	0.2			
	T	—	—	—	—	—	0.1	—	—	—	—	—			
C20: 5 n-3	S	0.6	0.4	0.6	0.5	0.6	0.4	0.3	0.4	0.5	0.4	0.8			
	T	0.2	0.2	0.2	0.3	0.2	0.2	0.3	0.2	0.1	0.4	0.3			
C22: 5 n-3	S	0.6	0.4	0.5	0.4	0.6	0.5	0.3	0.6	0.5	0.8	0.5			
	T	0.2	0.2	0.1	0.1	0.2	0.3	0.3	0.2	0.3	0.2	0.4			
C22: 6 n-3	S	3.3	3.0	3.1	1.8	3.3	3.2	1.5	2.8	1.5	2.7	2.0			
	T	1.0	0.9	0.9	0.7	1.0	1.4	1.2	1.5	1.1	1.1	1.1			
Σ n-3 PUFA	S	4.8	4.0	4.5	2.8	4.8	4.1	2.5	4.1	2.6	4.2	3.5			
	T	1.4	1.3	1.2	1.1	1.4	2.0	1.8	1.9	1.5	1.6	1.8			
Σ (n-6) + (n-3)	S	42.4	47.0	42.9	38.3	42.4	40.9	39.5	42.9	36.5	43.0	37.3			
	T	40.3	41.5	40.5	41.8	40.3	41.7	43.1	40.4	45.6	40.4	46.0			
n-6/n-3	S	7.8	10.7	8.5	12.7	7.8	9.0	14.8	9.5	13.0	9.2	9.7			
	T	27.8	30.9	32.7	37.0	27.8	19.8	22.9	20.3	29.4	24.2	24.5			

S = Soya oil ; T = sunflower oil.

The percentages of fatty acids not shown are the following : C14 : 0 = 0.5-1.0 % ; C16 : 1 n-9 = 0.3-0.5 % ; C16 : 1 n-7 = 2.6-3.8 % ; C18 : 1 n-7 = 1.9-3.1 % ; 20 : 3 n-9 = 0.4-0.6 %.

sterile sunflower rats than in their soya littermates (2.5 to 5 % vs 1 to 2 %). Lactation lowered this level in the latter from about 4.2 to 2.8 %, while it did not affect that level in sunflower rats (about 1.6 %).

22 : 6 n-3 was again the most abundant n-3 PUFA ; its level remained near 3 % in sterile soya females. In rats with and α -linolenic acid deficiency, the level of this fatty acid was about 1 %. In sunflower rats, 18 : 3 n-3 practically disappeared from serum lipids, while 20 : 5 n-3 and 22 : 5 n-3 always represented less than 0.5 % of total fatty acids.

The ratio 22 : 5 n-6/22 : 6 n-3 was always higher than 1 in sunflower rats and less than 0.5 in soya ones. In the latter, it was always higher in lactating rats than in sterile ones.

d) *n-6 + n-3 and n-6/n-3*. The total PUFA varied between 40 and 47 %, except in soya rats at the end of lactation, where it was always less than 40 % due to the low level of n-6 PUFA and n-3 PUFA in serum lipids.

The ratio n-6/n-3 varied between 8 and 10 in soya animals and between 20 and 30 in sunflower ones. Whatever the diet, it was always higher at the end of lactation than in the sterile littermates, but for different reasons : because of the decrease in n-3 PUFA with the soya diet and the increase of n-6 PUFA with the sunflower diet.

Discussion.

1. *Dietary α -linolenic acid deficiency and liver lipids.*

Our data show that dietary α -linolenic acid deficiency has no marked effect on liver size, including the hepatic hypertrophy of lactating rats that has been shown to be due to deep changes in lipid metabolism (Williamson, 1980). The hypertrophy we observed in this study at the end of lactation was accompanied by an increase in liver lipids, proportional to the increase in liver weight in the case of phospholipids, but much more than proportional ($\times 3.5$) in the case of neutral lipids. This may be related to dietary intake which increased 3-fold during lactation.

On the other hand, the α -linolenic acid deficiency in sterile rats caused large changes in the fatty acid composition of liver lipids :

1.1. *In phospholipids* it caused the n-3 PUFA level to decrease, primarily due to the drop in 22 : 6 n-3. This decrease was generally compensated by an augmentation in n-6 PUFA so that the sum of n-6 + n-3 PUFA remained the same (about 52 % of total fatty acids). This compensation required only a rather small increase in all the n-6 PUFA (+ 20 %), but this increase was considerable if we look at 22 : 5 n-6 ($\times 2$ or 3). This confirms that 22 : 5 n-6 is a true index of n-3 PUFA deficiency (Galli *et al.*, 1974 ; Tinoco *et al.*, 1979 ; Bourre *et al.*, 1984 ; Youyou *et al.*, 1986).

The increase in n-6 PUFA and decrease in n-3 PUFA resulted in an augmentation of the ratio n-6/n-3 which rose from about 3 in soya rats to about 13 in sunflower ones. According to Budowski and Crawford (1985), this values in soya rats would be normal but, as Galli *et al.* (1974) proposed, the ratio 22 : 5 n-6/22 : 6 n-3 can also be used as an index of n-3 PUFA deficiency, at least when the proportion of these two fatty acids is high enough to be precisely analysed.

In our experimental conditions, this ratio is about 0.1 to 0.4 in the controls and between 1 and 2 in deficient rats. Lactation also has its own effect on the respective levels of different types of PUFA in liver phospholipids ; in fact, although it does not significantly change the overall n-6 PUFA level, it increased the 22 : 5 n-6 but decreased the 22 : 6 n-3 in soya rats. The lactation caused a two-fold increase in the 22 : 5 n-6/22 : 6 n-3 ratio, indicating that the α -linolenic acid supply of the soya diet was not sufficient for lactating females, either in absolute value (mg 18 : 3 n-3/100 g feed) and/or in relative value (n-6/n-3). This could explain why neonatal loss was still high (about 5 %) in the progeny of soya females (Guesnet *et al.*, 1986).

On the contrary, in sunflower females, the ratio 22 : 5 n-6/22 : 6 n-3 was not significantly changed by lactation (mean : 1.76 vs 2.04) ; this ratio seemed to reach a limit, beyond which the dam's physiology might be seriously altered.

1.2 *In neutral lipids* α -linolenic acid deficiency produced modifications similar to those in phospholipids, with an increase in n-6 PUFA compensating a decrease in n-3 PUFA. However, PUFA in this type of lipids, no matter which series they belonged to, occurred in such low proportions that the ratio n-6/n-3 was not a precise index, and the ratio 22 : 5 n-6/22 : 6 n-3 could not be used.

Still the most notable fact was the decrease in the proportion of PUFA in liver neutral lipids of lactating females, due to the uptake of these fatty acids by the mammary gland and to the increase of lipid neosynthesis in the liver.

The decrease of n-6 and especially n-3 PUFA levels was very significant at the end of the third consecutive lactation (group F0"). Successive gestations thus appeared to be a more efficient means of depleting maternal n-3 PUFA stores than successive generations.

2. *Dietary α -linolenic acid deficiency and serum lipids.*

As expected, dietary 18 : 3 n-3 deficiency caused a decrease in the n-3 PUFA level in serum lipids that was marked by the clearance of 18 : 3 n-3 and a considerable decrease in 22 : 6 n-3, which was the most abundant fatty acid of its series. When the diet was deficient in 18 : 3 n-3, this level remained at about 1 %, as previously reported by Durand *et al.* (1985).

But we did not expect that lactation would intensify the decrease in serum lipid n-3 PUFA only in the soya dams ; it was as if the soya females could use their serum stores of this type of fatty acid to enrich their milk, while the sunflower females, having reached a minimal physiological threshold, in this respect, could not. A similar process is observed in the case of liver lipids.

Another remarkable fact was the change in serum lipid n-6 PUFA due to

lactation ; the level of these fatty acids, about equal in all the sterile females, tended to decrease in soya dams but increase in sunflower ones. While the decrease in n-6 PUFA in soya females can be easily explained by mammary gland uptake, it is difficult to understand their increase in sunflower rats. Mammary gland uptake of n-3 PUFA might be selective, resulting in an overload of n-6 PUFA.

In the serum lipids we analysed, the ratio n-6/n-3 ranged between 20 and 40. However, these rats were killed after fasting so the lipids contained no chylomicrons which could reflect the composition of dietary lipids ; moreover, VLDL composition was not analysed separately. The fatty acid composition in the present study only furnishes an inexact picture of the lipids from which the mammary gland usually derives its fatty acids, i.e. the chylomicrons and the VLDL (Williamson, 1980). This ratio n-6/n-3 might be higher in these fractions than in total serum lipids after fasting and would be more similar to the dietary value.

In any case, when the ratio $22 : 5$ n-6/22 : 6 n-3 is higher than 1 in a fasting animal, it appears to be a reliable index of dietary n-3 PUFA deficiency.

Conclusion.

Although in the last few years, and especially since the work of Holman *et al.* (1982), most authors recognize that n-3 PUFA are essential for mammals, few have proposed recommendations concerning the requirement of this type of fatty acids. In a recent review, Tinoco (1982) declared that « the dietary requirement, if any, for n-3 fatty acids in mammals and birds is unknown ». However, Holman *et al.* (1982) estimated this requirement in children as 0.54 % of the caloric supply.

In female rats in reproduction, the results of a previous work (Guesnet *et al.*, 1986) and of the present study seem to indicate that a supply of dietary n-3 PUFA equal to 130 mg/100 g diet and corresponding to a ratio of n-6/n-3 = 7.2, is suboptimal.

From this, it appears that the ratio n-6/n-3 in dietary lipids should be equal to or lower than 5 ; this corresponds to a minimal n-3 PUFA supply of 200 mg/100 g diet, if the n-6 PUFA requirement is 1 000 mg/100 g diet.

This analysis agrees with that of Budowski and Crawford (1985) based on a study of the value of the ratio n-6/n-3 in phospholipids of different animal species and in human milk of different countries.

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Résumé. *Effets d'une déficience alimentaire en acide α -linoléinique pendant la gestation et la lactation sur la composition en acides gras des lipides du foie et du sérum chez le rat.*

Les effets d'un régime alimentaire pauvre en acide α -linoléinique (18 : 3 n-3) sur la composition en acides gras des lipides du foie et du sérum sont étudiés chez des rattes allaitantes au cours de trois gestations et de trois générations. Ces femelles sont comparées à des femelles correspondantes restées stériles.

Pour cela, deux lots de rattes reçoivent respectivement un régime alimentaire dans lequel les lipides sont incorporés soit sous forme d'huile de tournesol, à raison de 1,50 % (régime déficient), soit sous forme d'huile de soja à raison de 1,87 % (régime témoin). Les

deux régimes apportent la même quantité d'acide linoléique (18 : 2 n-6) à savoir 940 mg/100 g d'aliment, mais le régime d'huile de tournesol apporte 43 fois moins de 18 : 3 n-3 que le régime huile de soja, soit 3 mg vs 130 mg/100 g d'aliment.

Les résultats montrent que la succession des gestations semble être un moyen plus efficace que la succession des générations pour conduire à un épuisement des réserves maternelles en acides gras polyinsaturés n-3 (AGPI n-3).

Dans les phospholipides hépatiques et les lipides sériques, le régime déficient en 18 : 3 n-3 provoque une diminution considérable de la teneur des AGPI n-3, en particulier celle du 22 : 6 n-3. Cette diminution est compensée par une augmentation de la teneur des acides gras polyinsaturés n-6 (AGPI n-6), en particulier celle du 22 : 5 n-6. Le rapport AGPI n-6/AGPI n-3 est un bon index de l'adéquation de l'apport alimentaire en AGPI n-3, mais le rapport 22 : 5 n-6/22 : 6 n-3 est un index plus sensible.

La proportion de 22 : 5 n-6 par rapport à l'ensemble des acides gras, ainsi que les rapports n-6/n-3 et 22 : 5 n-6/22 : 6 n-3, sont également augmentés dans les phospholipides hépatiques des femelles allaitantes recevant le régime huile de soja : ceci suggère qu'un apport de 130 mg/100 g d'aliment, correspondant à un rapport n-6/n-3 = 7,2, est insuffisant pour la ratte pendant la période de gestation-lactation.

Un apport de 200 mg d'AGPI n-3/100 g d'aliment, correspondant à une valeur du rapport n-6/n-3 = 5, est préconisé pour cet animal.

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