SPANDIDOS PUBLICATIONS

The metabolic syndrome of ω3-depleted rats. I. Liver data

WILLY J. MALAISSE[1], NURDAN BULUR[1], YING ZHANG[1], MIRJAM HACQUEBARD[2], LAURENCE PORTOIS[2], ABDULLAH SENER[1] and YVON A. CARPENTIER[2]

Laboratories of [1]Experimental Hormonology, [2]Experimental Surgery, Université Libre de Bruxelles, Brussels, Belgium

Received March 19, 2009; Accepted April 24, 2009

DOI: 10.3892/ijmm_00000214

Abstract. Second-generation rats depleted in long-chain polyunsaturated ω3 fatty acids were recently proposed as a novel animal model for the metabolic syndrome. In the present study, a dietary deprivation of ω3 acids for 3-7 months was found sufficient to provoke in 6-week-old normal rats the same alteration of the fatty acid content and profile of liver phospholipids and triglycerides as that otherwise prevailing in the second-generation ω3-depleted rats, with emphasis on a severe decrease in their ω3 fatty acid content, alterations in the relative contribution of and ratio between selected long-chain polyunsaturated ω6 fatty acids, saturated and monodesaturated fatty acids and precursors of nervonic acid, and liver steatosis. When the ω3-depleted rats were exposed, after the first 7 months of the present experiments and for 2-4 weeks to a diet supplemented with 5% (w/w) flaxseed oil, most of these hepatic variables returned towards or beyond control values. In both the ω3-depleted rats and control animals, however, the eventual exposure to the flaxseed oil-enriched diet failed to suppress liver steatosis and, on the contrary, provoked a further increase in liver triglyceride content. It is proposed, therefore, that the present approach represents a simple and realistic animal model to study the consequences of ω3-depletion. Moreover, the results suggest that to oppose such consequences, e.g. liver steatosis, it may be necessary to combine the dietary supply of ω3 acids with a suitable control of food intake, in both qualitative and quantitative terms.

Introduction

Recent publications have drawn attention to the fact that second-generation rats depleted in long-chain polyunsaturated ω3 fatty acids present several features of the metabolic syndrome, including visceral obesity, liver steatosis, insulin resistance, hypertension and consequent cardiac hypertrophy (1-6). The major aim of the present experiments was to find out whether a comparable situation prevails in rats exposed to a dietary manipulation more likely to simulate the conditions presently often leading to a depletion of the ω3 fatty acids in Western populations. For such a purpose, normal female 6-week-old rats were exposed for 3 and 7 months to either a control diet or an ω3-depleted diet. Thereafter, the ω3-deficient rats (ω3D rats) were given access for 2 and 4-5 weeks to the same ω3-deficient diet now enriched with 5% (w/w) flaxseed oil. For purpose of comparison, the control rats were also exposed from day 271 to 302 after birth to their control diet enriched with 5% (w/w) of either soybean or flaxseed oil.

The present first report deals mainly with the measurements of the fatty acid content and profile of liver phospholipids and triglycerides in the eight groups of rats mentioned above. The fatty acid content and profile of lipids in other organs (brain, duodenum-jejunum, caecum, colon), as well as the time course for changes in body weight and information on parametrial fat weight, plasma glucose and insulin concentrations will be presented in further reports.

Materials and methods

Forty-eight 6-week-old female normal rats (Iffa Credo, L'Arbresle, France) were housed in groups of 6 rats each, and given access to tap water and either a control diet (AO3; SAFE, Villemoison-sur-Orge, France) or an ω3-depleted diet. The lipid composition of these two diets is given in Table I. The control and ω3-depleted diet contained 5% (w/w) lipids from soya and sunflower, respectively, the C18:3ω3 weight percentage being below the limit of detection in the latter diet. Two groups of 6 control rats each and two groups of ω3D rats each were sacrificed after 3 and 7 months of exposure to their respective diets, i.e. 97±1 and 264±1 days after birth. From day 271 after birth and for the next 4-5 weeks, the control rats were given access to the control diet enriched with 5% (wt/wt) soybean or flaxseed oil (Table I). Also from day 271 after birth, the ω3D rats were given access for either 2 or 4-5 weeks to their ω3-depleted diet enriched with 5% (wt/wt) flaxseed oil (Table I).

In the Tables, the following symbols are used: 3mC and 7mC for the control rats examined 3 and 7 months after the onset of the present experiments, 3mD and 7mD for the ω3D rats also examined 3 and 7 months after the start of the experiments, 7mC/4wS and 7mC/4mF for the control rats

Correspondence to: Professor Willy J. Malaisse, Laboratory of Experimental Hormonology, Université Libre de Bruxelles, 808 Route de Lennik, B-1070 Brussels, Belgium
E-mail: malaisse@ulb.ac.be

Key words: ω3-depleted rats, liver phospholipids, liver triglycerides, fatty acid pattern

Table I. Fatty acid composition of diets (‰ of total fatty acid content).

Oil	Soya (5%)	Sunflower (5%)	Soya (10%)	Soya (5%) Flax (5%)	Sunflower (5%) Flax (5%)
C6:0	0.4	-	-	-	0.2
C8:0	-	0.3	-	-	0.1
C10:0	-	0.4	-	-	0.3
C12:0	1.0	0.6	0.1	0.2	0.4
C14:0	3.0	2.4	2.0	1.9	1.5
C16:0	138.9	64.6	113.7	83.3	54.9
C16:1ω7	6.5	-	-	3.2	0.8
C18:0	25.8	37.6	27.2	26.5	33.3
C18:1ω9	178.3	232.9	201.1	171.3	194.8
C18:2ω6	548.6	650.7	559.9	341.0	394.4
C18:3ω6	-	-	-	-	-
C20:0	2.2	-	3.4	0.8	0.8
C18:3ω3	60.2	-	71.6	351.0	310.3
C20:1ω9	4.7	-	4.5	4.2	1.2
C18:4ω3	0.9	0.5	0.4	0.4	-
C20:2ω6	1.5	1.1	1.2	1.2	1.0
C20:3ω6	0.6	0.6	0.6	0.4	0.4
C22:0	2.0	5.8	2.7	1.4	3.2
C20:4ω6	1.8	-	0.7	1.1	0.4
C22:1ω9	-	0.5	-	1.3	0.5
C20:5ω3	6.9	0.4	3.1	3.0	0.2
C24:0	0.3	1.7	-	1.1	1.4
C22:3ω3	0.4	-	-	-	-
C22:4ω6	-	-	0.2	0.2	-
C22:5ω3	1.1	-	0.8	-	-
C22:6ω3	15.0	-	6.5	6.5	-

exposed during the last 4-5 weeks to either the soybean (S) or flaxseed (F) oil-enriched diets, and 7mD/2wF and 7mD/4wF for the ω3D rats exposed for either 2 or 4-5 weeks to the flaxseed oil-enriched diet.

The rats were eventually euthanized by carbon dioxide inhalation. Blood was punctured from the heart and a piece of liver sampled for measuring the fatty acid content and pattern of hepatic phospholipids and triglycerides. The lipids were extracted (7), separated by thin-layer chromatography (8), and their fatty acid pattern determined by gas-liquid chromatography (9).

All results are presented as means ± SE together with either the number of individual determinations (n) or degree of freedom (df). The statistical significance of differences between mean values was assessed using Student's t-test and confirmed by variance analysis with Bonferroni post-test.

Results

Liver protein content. The liver protein content failed to differ significantly in control and ω3D rats, whether 3 months ($p>0.9$) or 7 months ($p>0.6$) after the onset of the present experiments (Table II). It also failed to be significantly affected when the control animals ($p>0.2$ or more) or ω3D rats ($p>0.5$ or more) were exposed for 2 to 4-5 weeks to the diets enriched with soybean or flaxseed oil.

Liver phospholipids. Total fatty acid content. The total fatty acid content of phospholipids was significantly lower in ω3D rats than in control animals, whether 3 months or 7 months after the onset of the present experiments (Table II). It decreased with increasing age in both the control animals ($p<0.005$) and ω3D rats ($p<0.05$). When the control rats were exposed for 4-5 weeks to the diets enriched with soybean or flaxseed oil, the mean value for the total fatty acid content of liver phospholipids increased. Such an increase achieved statistical significance in the case of the flaxseed oil-rich diet ($p<0.05$), and even in the case of the soybean oil-rich diet ($p<0.005$) after exclusion of one abnormally low value (13.3 mg/g) recorded in the latter group of rats.

When the ω3D rats were exposed to the ω3-enriched diet, the total fatty acid content of liver phospholipids progressively increased ($p<0.005$) and eventually reached a mean value no more significantly different ($p>0.1$) from that recorded at the same age in the control rats.

Long-chain polyunsaturated ω3 fatty acids. The relative weight content of long-chain polyunsaturated ω3 fatty acids was

Protein and total fatty acid content (mg/g wet wt.) of liver phospholipids and triglycerides.

Rats	Protein (mg/g)	Phospholipids (mg/g)	Triglycerides (mg/g)	Triglycerides/Phospholipids
3mC	241±4 (6)	27.9±0.9 (5)	2.62±0.22 (6)	0.090±0.011 (5)
3mD	241±5 (6)	20.5±0.6 (5)^{e}	5.04±0.53 (6)^{e}	0.248±0.026 (5)^{f}
7mC	232±2 (5)	22.1±0.8 (5)	3.11±0.31 (5)	0.138±0.011 (5)
7mD	234±4 (6)	18.8±0.4 (6)^{e}	4.75±0.38 (6)^{d}	0.247±0.024 (6)^{e}
7mC/4wS	240±6 (6)	24.0±2.1 (6)	5.21±0.73 (6)	0.215±0.021 (6)
7mC/4wF	228±9 (6)	25.1±0.8 (6)	4.80±0.71 (6)	0.190±0.026 (4)
7mD/2wF	239±6 (6)	19.5±1.0 (6)	6.11±0.97 (6)	0.298±0.038 (6)
7mD/4wF	231±9 (6)	23.8±0.3 (6)^{e}	7.78±1.57 (6)	0.297±0.057 (6)

Values are means ± SE; nos. in parentheses indicate the no. of individual determinations for each value. The statistical indices ($^{a}p<0.05$; $^{b}p<0.025$; $^{c}p<0.02$; $^{d}p<0.01$; $^{e}p<0.005$; $^{f}p<0.001$) refer to the differences with the preceding line. The symbols for each group of rats are defined in Materials and methods.

Table III. Weight percentage of long-chain polyunsaturated ω3 fatty acids in liver phospholipids.

Rat	C18:3ω3	C20:5ω3	C22:5ω3	C22:6ω3
3mC	0.2±0.1 (6)	1.3±0.2 (6)	1.3±0.1 (6)	17.9±0.7 (6)
3mD	0.0±0.0 (6)^{c}	0.0±0.0 (6)^{f}	0.0±0.0 (6)^{f}	4.3±0.3 (6)^{f}
7mC	0.2±0.0 (5)	1.2±0.3 (5)	1.2±0.0 (5)	16.3±0.8 (5)
7mD	0.0±0.0 (6)^{f}	0.0±0.0 (6)^{e}	0.1±0.0 (6)^{f}	3.5±0.2 (6)^{f}
7mC/4wS	0.2±0.0 (6)	0.6±0.1 (6)	1.1±0.1 (6)	15.2±0.7 (6)
7mC/4wF	0.9±0.1 (6)^{f}	3.9±0.6 (6)^{f}	2.3±0.1 (6)^{f}	13.9±0.7 (6)
7mD/2wF	0.7±0.1 (6)	3.0±0.8 (6)	1.8±0.1 (6)	15.3±0.6 (6)
7mD/4wF	0.7±0.1 (6)	3.4±0.5 (6)	1.8±0.2 (6)	15.0±1.2 (6)

Same presentation as in Table II.

much lower in the liver phospholipids of ω3D rats as compared to control animals of the same age (Table III).

When the control animals were exposed for 4-5 weeks to the flaxseed oil-rich diet, the relative weight content of all long-chain polyunsaturated ω3 fatty acids, except C22:6ω3, became significantly higher than that found in either the control animals examined after 7 months exposure to the control diet or the control animals given access to the soybean oil-rich diet for the last 4-5 weeks before sacrifice. Except in the case of C20:5ω3 ($p<0.02$), the weight percentage of long-chain polyunsaturated ω3 fatty acids in liver phospholipids failed to differ significantly in the latter two groups of animals (Table III).

When the ω3D rats were given access to the ω3-enriched diet, the relative weight content of C18:3ω3, C20:5ω3 and C22:5ω3 reached, within 2 weeks, steady-state values significantly higher ($p<0.001$) than those recorded in the control rats during the first 7 months of the present experiments. Only the relative weight of C22:6ω3 in liver phospholipids remained slightly lower ($p<0.05$) in the ω3D rats given access for 2-5 weeks to the ω3-enriched diet (15.1±0.6%; n=12) than in the control rats examined during the first 7 months of the present experiments (17.1±0.6%; n=11).

The C20:5ω3/C18:3ω3 ratio was significantly lower ($p<0.005$) in the control animals fed an oil-enriched diet (3.61±0.27; n=12) than in the control animals examined during the first 7 months of the present experiments (5.68±0.63; n=10). Relative to the results found in the control animals exposed for 7 months to the control diet, the C22:6ω3/C20:5ω3 ratio was twice higher ($p<0.025$) after feeding these animals the soybean oil-enriched diet, but about four times lower ($p<0.01$) when the control rats were given access to the flaxseed oil-enriched diet. The C22:5ω3/C22:6ω3 ratio was virtually identical in the control rats before and after exposure to the soybean oil-enriched diet, whilst being doubled ($p<0.001$) after exposure of the control rats to the flaxseed oil-enriched diet.

The C20:5ω3/C18:3ω3 ratio in liver phospholipids failed to differ significantly ($p>0.1$) in the control animals examined during the first 7 months of the present experiments (5.68±0.63; n=10) and in the ω3D rats given access to the ω3-enriched diet (4.44±0.52; n=12). The C22:6ω3/C20:5ω3 ratio was lower ($p<0.005$), however, in the latter rats

Table IV. Ratio between selected long-chain polyunsaturated ω3 fatty acids in liver phospholipids.

Rats	C20:5ω3/C18:3ω3	C22:6ω3/C20:5ω3	C22:5ω3/C22:6ω3 (10^{-3})
3mC	4.97±0.69 (5)	15.79±2.40 (6)	74.6±6.7 (6)
3mD	N.C.	N.C.	0.0±0.0 (6)[f]
7mC	6.39±1.05 (5)	15.76±3.41 (5)	75.0±4.5 (5)
7mD	N.C.	N.C.	30.9±5.9 (5)[f]
7mC/4wS	3.01±0.32 (6)	30.38±3.96 (6)	74.6±6.6 (6)
7mC/4wF	4.20±0.26 (6)[e]	4.10±0.75 (6)[f]	165.1±14.7 (6)[f]
7mD/2wF	4.26±1.00 (6)	8.54±2.87 (6)	119.9±7.4 (6)
7mD/4WF	4.62±0.42 (6)	5.33±1.38 (6)	128.3±24.5 (6)

Same presentation as in Table II. N.C.: not calculable.

Table V. Absolute values (mg/g) for C20:5ω3 and C22:6ω3 weight content of liver phospholipids.

Rat	C20:5ω3	C22:6ω3
7mC	0.28±0.06 (5)	3.61±0.24 (5)
7mC/4wF	0.97±0.14 (6)	3.49±0.23 (6)
- increment	+ 0.69±0.17 (*9*)	- 0.12±0.34 (*9*)
7mD	0.00±0.00 (6)	0.66±0.04 (6)
7mD/4wF	0.81±0.12 (6)	3.56±0.30 (6)
- increment	+ 0.81±0.12 (*10*)	+ 2.90±0.30 (*10*)

Values are means ± SE; nos. in parentheses indicate the no. of individual determinations (lower case) or degree of freedom (italics).

(6.94±1.59; n=12) than in the control animals (15.77±1.92; n=11). Inversely, the C22:5ω3/C22:6ω3 ratio in liver phospholipids was higher ($p<0.005$) in the ω3D rats given access to the ω3-enriched diet (124.1±12.3‰; n=12) than in the control animals (74.8±4.0‰; n=11). The latter value was itself higher ($p<0.001$) than that recorded in the ω3D rats also examined during the first 7 months of the present experiments (Table IV).

Table V provides the absolute values for the C20:5ω3 and C22:6ω3 content of liver phospholipids in the control animals and ω3D rats examined shortly before and 4-5 weeks after exposure to the flaxseed oil-enriched diets. In both the control animals and ω3D rats, the liver phospholipid content in C20:5ω3 was increased ($p<0.005$ or less) as a result of exposure to the ω3-enriched diet. Such an increase failed to differ significantly ($p>0.5$) in the control animals and ω3D rats. The liver phospholipid content in C22:6ω3 failed, however, to change significantly ($p>0.7$) in the control rats during exposure to the flaxseed oil-enriched diet. In the ω3D rats, such a content increased by 2.90±0.30 mg/g (df=10; $p<0.001$), the latter value being significantly different ($p<0.001$) from both the change in the C22:6ω3 content of liver phospholipids in the control animals also exposed for 4-5 weeks to the ω3-enriched diet and the increase in the C20:5ω3 content of liver phospholipids in the same ω3D rats.

Long-chain polyunsaturated ω6 fatty acids. The weight percentage of C18:2ω6 in liver phospholipids was lower in ω3D rats than in control animals (Table VI). This coincided with lower weight percentage for both C20:2ω6 and C20:3ω6 in the ω3D rats than in the control animals. Relative to the mean corresponding values found at the same age in the latter animals, the values recorded in the ω3D rats indeed averaged 82.8±6.3% (df=21; $p<0.02$) in the case of C20:2ω6 and 75.4±5.8% (df=21; $p<0.001$) in the case of C20:3ω6, these two percentages failing to differ significantly ($p>0.3$) from one another. The weight percentages of C20:4ω6 and C22:4ω6 in liver phospholipids were much higher, however, in ω3D rats than in control animals. Even the weight percentage of C18:3ω6 was higher ($p<0.05$) in the ω3D rats than in the control animals, averaging in the former rats 159.1±19.8% (n=12) of the mean corresponding values found at the same age in the control animals (100.0±15.7%; n=11).

Exposure of the control rats to the soybean oil-enriched diet failed, as a rule, to affect the weight percentage of long-chain polyunsaturated ω6 fatty acids in liver phospholipids. The C20:2ω6 and C20:4ω6 relative weight content of such phospholipids was somewhat higher ($p<0.01$ or less), however, in these rats than the mean corresponding value found in the control rats during the first 7 months of the present experiments. When compared to the control rats fed for 4-5 weeks the soybean oil-enriched diet, the control rats exposed for the same period to the flaxseed oil-enriched diet displayed a higher relative weight content of C18:2ω6 and lower relative weight contents of C20:2ω6, C20:4ω6 and C22:4ω6. Except in the case of C20:2ω6, such differences represent a mirror image of those otherwise provoked by exposure of the rats to an ω3-deprived diet. The results recorded in the control rats after exposure to the flaxseed oil-enriched diet also differed from those recorded in the control rats before such an exposure by higher weight percentages of C18:2ω6 ($p<0.05$) and C20:3ω6 ($p<0.005$) and lower weight percentages of C20:2ω6 ($p<0.01$), C20:4ω6 ($p<0.001$) and C22:4ω6 ($p<0.025$).

In the ω3D rats given access for 2-5 weeks to the ω3-enriched diet, the weight percentage of C18:2ω6 (11.4±0.4%;

Weight percentage of long-chain polyunsaturated ω6 fatty acids in liver phospholipids.

Rat	C18:2ω6	C18:3ω6	C20:2ω6	C20:3ω6	C20:4ω6	C22:4ω6
3mC	12.8±0.6 (6)	0.1±0.0 (6)	0.4±0.0 (6)	0.6±0.0 (6)	27.0±0.7 (6)	0.1±0.0 (6)
3mD	9.3±0.2 (6)[f]	0.2±0.0 (6)	0.3±0.0 (6)	0.4±0.0 (6)[f]	38.4±0.3 (6)[f]	1.0±0.1 (6)[f]
7mC	13.8±0.9 (5)	0.2±0.0 (5)	0.3±0.0 (5)	0.6±0.0 (5)	27.1±0.8 (5)	0.2±0.0 (5)
7mD	10.2±0.7 (6)[c]	0.3±0.0 (6)[c]	0.3±0.0 (6)[c]	0.5±0.0 (6)[a]	39.1±0.4 (6)[f]	1.0±0.0 (6)[f]
7mC/4wS	13.7±0.3 (6)	0.1±0.0 (6)	0.5±0.0 (6)	0.7±0.1 (6)	29.6±0.6 (6)	0.2±0.0 (6)
7mC/4wF	15.0±0.4 (6)[a]	0.1±0.0 (6)	0.3±0.0 (6)[f]	0.8±0.0 (6)	23.6±0.5 (6)[f]	0.1±0.0 (6)[f]
7mD/2wF	11.1±0.2 (6)	0.2±0.0 (6)	0.2±0.0 (6)	0.7±0.1 (6)	27.0±1.2 (6)	0.1±0.0 (6)
7mD/4wF	11.7±0.8 (6)	0.2±0.0 (6)	0.2±0.0 (6)	0.7±0.0 (6)	26.1±0.4 (6)	0.1±0.0 (6)

Same presentation as in Table II.

Table VII. Ratio between selected long-chain polyunsaturated ω6 fatty acids in liver phospholipids.

Rats	C20:2ω6/C18:2ω6 (10^{-3})	C18:3ω6/C18:2ω6 (10^{-3})	C20:3ω6/C18:3ω6	C20:4ω6/C20:3ω6	C22:4ω6/C20:4ω6 (10^{-3})
3mC	29.2±2.0 (6)	9.7±1.0 (5)	5.1±0.6 (5)	44.3±3.3 (5)	6.5±0.2 (5)
3mD	35.9±2.6 (6)[a]	21.3±3.9 (5)[c]	2.4±0.3 (5)e	91.7±7.8 (6)[f]	25.8±1.7 (6)[f]
7mC	24.2±1.3 (5)	11.4±1.7 (5)	4.3±0.5 (5)	44.4±3.9 (5)	7.2±0.4 (5)
7mD	25.2±1.7 (6)	26.7±1.9 (6)[f]	1.9±0.1 (6)[f]	76.2±2.1 (6)[f]	25.6±1.3 (6)[f]
7mC/4wS	35.4±2.5 (6)	10.9±1.3 (6)	4.7±0.7 (6)	47.2±4.7 (6)	6.4±0.3 (6)
7mC/4wF	18.1±1.1 (6)[f]	9.2±0.3 (6)	6.0±0.3 (6)	30.9±1.3 (6)[d]	4.4 ±0.2 (6)[f]
7mD/2wF	16.9±1.1 (6)	13.8±1.2 (6)	4.8±0.6 (6)	41.4±6.0 (6)	5.2±0.3 (6)
7mD/4wF	14.2±1.2 (6)	13.2±0.6 (6)	4.7±0.2 (6)	37.3±2.5 (6)	5.0±0.4 (6)

Same presentation as in Table II.

n=12) was higher ($p<0.01$) than that recorded in the ω3D rats during the first 7 months of the present experiments (9.7±0.4%; n=12), but remained nevertheless lower ($p<0.02$) than that found over the same period of 7 months in the control animals (13.3±0.5%; n=11). Likewise, the weight percentage of C20:2ω6 remained lower ($p<0.001$) in the ω3D rats exposed to the ω3-enriched diet (0.18±0.01%; n=12) than in the control animals (0.35±0.02%; n=11). The weight percentages of C18:3ω3, C20:3ω6, C20:4ω6 and C22:4ω6 changed rapidly and markedly, however, in response to the exposure of the ω3D rats to the ω3-enriched diet, reaching values comparable to those recorded in the control animals.

As judged from the ratio between selected long-chain polyunsaturated ω6 fatty acids in liver phospholipids, the activity of Δ6-desaturase (C18:3ω6/C18:2ω6) and Δ5-desaturase (C20:4ω6/C20:3ω6) as well as elongase (C20:2ω6/C18:2ω6 and C22:4ω6/C20:4ω6), appeared, as a rule, significantly higher in ω3D rats than in control animals (Table VII). Only the C20:3ω6/C18:3ω6 ratio was significantly lower in ω3D rats than in control animals. Except for a significant increase ($p<0.01$) in the phospholipid C20:2ω6/C18:2ω6 ratio, the exposure of the control rats to the soybean oil-enriched diet failed to affect significantly the ratio between selected long-chain polyunsaturated ω6 fatty acids. Compared to the values recorded in these control rats exposed to the soybean oil-enriched diet, those recorded in the control rats exposed to the flaxseed oil-enriched diet demonstrated significant decreases in the C20:2ω6/C18:2ω6, C20:4ω6/C20:3ω6 and C22:4ω6/C20:4ω6 ratios, all these differences representing a mirror image of those otherwise caused by exposure of the rats to the ω3-deprived diet.

When the ω3D rats were given access to the ω3-enriched diet, the phospholipid C20:2ω6/C18:2ω6 ratio became lower ($p<0.001$) than that recorded in the control animals. The C18:3ω6/C18:2ω6 ratio also decreased, whilst remaining slightly higher ($p<0.02$) than that recorded in the control animals. The C20:3ω6/C18:3ω6 and C20:4ω6/C20:3ω6 ratios resumed values comparable ($p>0.2$ or more) to those found in control rats. The C22:4ω6/C20:4ω6 ratio event became significantly lower ($p<0.001$) than that measured in the control animals.

A last finding duly merits to be underlined. The results so far presented do not refer to the C22:5ω6 content of liver phospholipids because it had not been measured in our usual procedure. Nevertheless, it should not be ignored that such a content was close to the limit of detection in control rats, whilst being far-from-negligible in ω3D rats. For instance, after 3 and 7 months of dietary ω3 deprivation, it averaged, respectively, 1.82±2.8 and 1.34±0.14 mg/g wet weight (n=6 in both cases). These two mean values, which did not differ

Table VIII. Weight percentage of saturated and monodesaturated fatty acids in liver phospholipids.

Rats	C14:0	C16:0	C16:1ω7	C18:0	C18:1ω9
3mC	0.1±0.0 (6)	13.5±0.4 (6)	0.2±0.0 (6)	21.0±0.4 (6)	1.9±0.2 (6)
3mD	0.3±0.1 (6)	15.4±0.2 (6)[e]	0.5±0.1 (6)[e]	25.9±0.4 (6)[f]	2.4±0.1 (6)[a]
7mC	0.2±0.0 (5)	13.7±0.2 (5)	0.3±0.0 (5)	21.0±0.4 (5)	2.0±0.2 (5)
7mD	0.3±0.0 (6)[f]	14.4±0.5 (6)	0.6±0.0 (6)[e]	25.5±0.6 (6)[f]	2.8±0.1 (6)[e]
7mC/4wS	0.2±0.0 (6)	13.4±0.4 (6)	0.2±0.0 (6)	20.5±0.4 (6)	2.1±0.1 (6)
7mC/4wF	0.2±0.0 (6)	12.6±0.3 (6)	0.2±0.0 (6)	22.3±0.3 (6)[d]	2.3±0.2 (6)
7mD/2wF	0.1±0.0 (6)	12.0±0.4 (6)	0.4±0.1 (6)	23.6±0.4 (6)	2.3±0.0 (6)
7mD/4wF	0.2±0.0 (6)	12.5±0.4 (6)	0.4±0.1 (6)	23.2±0.4 (6)	2.1±0.1 (6)

Rats	C20:0	C20:1ω9	C22:0	C22:1ω9	C24:0
3mC	0.1±0.0 (6)	0.1±0.0 (6)	0.3±0.0 (6)	0.2±0.1 (6)	0.8±0.0 (6)
3mD	0.1±0.0 (6)	0.0±0.0 (6)	0.4±0.0 (6)	0.2±0.1 (6)	1.1±0.0 (6)[f]
7mC	0.1±0.0 (5)	0.1±0.0 (5)	0.3±0.0 (5)	0.2±0.1 (5)	0.9±0.1 (5)
7mD	0.1±0.0 (6)	0.1±0.0 (6)	0.4±0.0 (6)[f]	0.1±0.0 (6)	1.1±0.0 (6)
7mC/4wS	0.1±0.0 (6)	0.1±0.0 (6)	0.3±0.0 (6)	0.4 ±0.0 (6)	0.9±0.0 (6)
7mC/4wF	0.1±0.0 (6)	0.1±0.0 (6)	0.3±0.0 (6)[e]	0.2±0.0 (6)[d]	0.9±0.0 (6)
7mD/2wF	0.1±0.0 (6)	0.0±0.0 (6)	0.3±0.0 (1)	0.2±0.0 (6)	0.9±0.0 (6)
7mD/4wF	0.1±0.0 (6)	0.0±0.0 (6)	0.4±0.0 (6)	0.3±0.1 (6)	1.0±0.0 (6)

Same presentation as in Table II.

significantly ($p>0.1$) from one another, yielded a relative weight content of C22:5ω6 in the liver phospholipids of ω3D rats averaging 8.0±0.5% (n=12). The C22:5ω6/C22:4ω6 ratio averaged, in the same rats, 8.98±0.76 (n=12).

Saturated and monodesaturated fatty acids. No C12:0 was detected in the liver phospholipids of any animal. With the sole exception of C20:0, C20:1ω9 and C22:1ω9, all other saturated and monodesaturated fatty acids (C14:0, C16:0, C16:1ω7, C18:0, C18:1ω9, C22:0 and C24:0) displayed higher mean values for their relative weight content in ω3D rats than in control animals (Table VIII). Such an increase was most obvious and achieved statistical significance at both the 3rd and 7th month of the present experiments in the case of C16:1ω7, C18:0 and C18:1ω9.

When the control rats were exposed for 4 weeks to the soybean oil-enriched diet, the weight percentage of C14:0 ($p<0.06$), C20:0 ($p<0.005$) and C22:0 ($p<0.001$) in liver phospholipids became higher than that recorded in the control animals at the end of the 7 first months in the present experiments, whilst the weight percentage of C16:1ω7 became lower ($p<0.005$) in the former rats than in the latter animals. As a rule, there was little to distinguish between the control animals given access to either the soybean or flaxseed oil-enriched diet, except for a higher C18:0 ($p<0.01$) and lower C22:0 ($p<0.005$) relative content in the liver phospholipids of the latter animals, as compared to former ones.

In the 12 ω3D rats given access to the ω3-enriched diet, the weight percentage of C14:0 ($p<0.025$), C16:0 ($p<0.001$), C16:1ω7 ($p<0.07$), C18:0 ($p<0.001$), C18:1ω9 ($p<0.005$) and C24:0 ($p<0.005$) were all lower than in the 12 ω3D rats examined during the first 7 months of the present experiments.

The C16:1ω7/C16:0 ratio was higher in ω3D rats than in control animals, whilst there was little to distinguish between ω3D and control rats in the case of the C18:1ω9/C18:0 ratio (Table IX). The (C18:0 + C18:1ω9)/(C16:0 + C16:1ω7) ratio of liver phospholipids was higher ($p<0.005$) in the ω3D rats (1.84±0.04; n=12) than in the control animals (1.65±0.04; n=11). When the control rats were given access to either the soybean or flaxseed oil-enriched diet, the C16:1ω7/C16:0 ratio became lower ($p<0.05$) than that recorded in the control rats during the first 7 months of the present experiments (20.0±1.6; n=11), whilst the C18:1ω9/C18:0 ratio remained unchanged. The (C18:0 + C18:1ω9/(C16:0 + C16:1ω7) ratio also failed to be affected when the control animals were exposed to the soybean oil-enriched diet. In the control animals exposed to the flaxseed oil-enriched diet, however, such a ratio became unexpectedly higher ($p<0.005$) than that recorded in the control animals during the 7 first months of the experiments.

The activity of Δ9-desaturase as judged from the C16:1ω7/C16:0 or C18:1ω9/C18:0 was unexpectedly not significantly different in ω3D rats exposed to either the ω3-depleted or ω3-enriched diet. In the latter animals, the activity of elongase, as judged from the (C18:0/C18:1ω9)/(C16:0/C16:1ω7) ratio was even further increased in the ω3D rats given access to the ω3-enriched diet, the values recorded in these rats (2.03±0.06; n=12) being higher ($p<0.025$) than that recorded in the ω3D rats when exposed to the ω3-depleted diet (1.84±0.04; n=12).

Ratio between selected saturated and monodesaturated fatty acids in liver phospholipids.

Rats	C16:1ω7/C16:0 (10^{-3})	C18:1ω9/C18:0 (10^{-3})	(C18:0 + C18:1ω9)/(C16:0 + C16:1ω7)
3mC	17.0±1.1 (6)	89.6±9.6 (6)	1.67±0.07 (6)
3mD	31.6±3.7 (6)[e]	91.4±3.8 (6)	1.78±0.04 (6)
7mC	23.5±2.5 (5)	95.6±8.9 (5)	1.63±0.03 (5)
7mD	36.2±2.1 (6)[e]	108.0±6.1 (6)	1.90±0.06 (6)
7mC/4wS	12.3±1.3 (6)	101.3±7.0 (6)	1.68±0.09 (6)
7mC/4wF	14.5±1.1 (6)	104.3±8.1 (6)	1.93±0.08 (6)
7mD/2wF	31.0±6.1 (6)	96.5±1.8 (6)	2.09±0.08 (6)
7mD/4wF	34.2±4.4 (6)	89.5±6.7 (6)	1.96±0.10 (6)

Same presentation as in Table II.

Monodesaturated ω9 fatty acids. At variance with the findings made for most saturated and monodesaturated fatty acids and despite the higher weight percentage of C18:1ω9 in the liver phospholipids of ω3D rats, that of further precursors of nervonic acid (C20:1ω9 and C22:1ω9) only represented in the ω3D rats examined 3 and 7 months after the onset of the present experiments respectively 53.4±15.5% (n=12) and 58.3±6.0% (n=12) of the mean corresponding values recorded at the same age in control animals. Over this period, the weight percentage of C20:1ω9 and C22:1ω9 in liver phospholipids of ω3D rats thus averaged 55.8±8.2% (n=24; p<0.02) of the mean corresponding values found for the same fatty acid(s) at the same age(s) in control animals (100.0±14.7%; n=22).

When the control animals were given access for 4-5 weeks to the soybean oil-enriched diet, the weight percentage of C20:1ω9 and C22:1ω9 was increased (p<0.05) to 159.3±12.0% (n=12) of the mean corresponding values recorded in the control animals exposed for 7 months to the control diet (100.0±26.7%; n=10) and, as such, represented 168.6±13.6% (n=12; p<0.002) of the mean corresponding values found in control animals exposed for 4-5 weeks to the flaxseed oil-enriched diet (100.0±12.0%; n=12). In other words, the weight percentage of C20:1ω9 and C22:1ω9 in liver phospholipids was virtually identical (p>0.8 or more) in control animals just before and 4-5 weeks after exposure to the flaxseed oil-enriched diet.

In the ω3D rats, the alteration in the relative content of nervonic acid precursors in liver phospholipids was apparently opposed, however, when these rats were given access to the ω3-enriched diet. The C22:1ω9/C18:1ω9 ratio indeed increased (p<0.02) from a mean value of 5.7±1.1% (n=12) in the ω3D rats examined during the first 7 months of the present experiments to 11.0±1.5% (n=12) in the ω3D rats given access to the flaxseed oil-enriched diet, the latter value being virtually identical (p>0.8) to that recorded in the control animals (11.5±2.9%; n=11) also examined during the first 7 months of the present experiments. The latter values also failed to differ significantly (p>0.6) from that recorded in the control rats exposed for 4-5 weeks to the flaxseed oil-enriched diet (9.4±1.2%; n=6), which itself only represented half (p<0.005) of the C22:1ω9/C18:1ω9 ratio found in the liver phospholipids of control animals exposed for 4-5 weeks to the soybean oil-enriched diet (19.9±2.3%; n=6).

Liver triglycerides. Total fatty acid content. Whether after 3 or 7 months of exposure to the ω3-depleted diet, the total fatty acid content of liver triglycerides was significantly higher than that recorded at the same age in the control animals (Table II). Likewise, already 3 months after exposure to the ω3-depleted diet, the glycerol content of triglycerides was already significantly higher (p<0.05) in ω3D rats (6.42±0.50 μmol/g; n=6) than in control animals (4.16±0.25 μmol/g; n=6). At that time, the relative magnitude of the increase in the triglyceride liver content was not significantly different (p>0.1) whether judged from their fatty acid or glycerol content, with an overall mean value of 75.1±13.4% (df=20; p<0.001).

When the control rats were exposed for 4-5 weeks to the soybean oil-enriched diet, the total fatty acid content of liver triglycerides, as well as the ratio between such a content and the total fatty acid content of liver phospholipids, became higher (p<0.05 or less) than that found in the control rats before such an exposure. In respect to these two variables, there was no significant difference between the control animals eventually exposed to either the soybean oil-enriched diet or flaxseed oil-enriched diet.

Likewise, the total fatty acid content of liver triglycerides was higher (p<0.05) in the 12 ω3D rats exposed for 2-5 weeks before sacrifice to the ω3-enriched diet (6.94±0.92 mg/g) than in the 12 ω3D rats exposed for 3-7 months to the ω3-depleted diet (4.89±0.31 mg/g).

In the ω3D rats, the triglyceride/phospholipid ratio for the total fatty acid content of these liver lipids failed, however, to differ significantly (p>0.15) after exposure to the ω3-enriched diet (0.297±0.032; n=12) and before such an exposure (0.248±0.018; n=11), both latter mean values being much higher (p<0.001) than that recorded in the control animals during the first 7 months of the present experiments (0.112±0.011; n=10). In the ω3D rats exposed for 4-5 weeks to the flaxseed oil-enriched diet, the mean values for the total fatty acid content of liver triglycerides and for the triglyceride/phospholipid ratio for total fatty acid content were higher than those recorded in the control animals also exposed for

Table X. Weight percentage of long-chain polyunsaturated ω3 fatty acids in liver triglycerides.

Rats	C18:3ω3	C20:5ω3	C22:5ω3	C22:6ω3
3mC	2.7±0.2 (6)	1.2±0.3 (6)	1.6±0.1 (6)	4.7±0.3 (6)
3mD	0.0±0.0 (6)[f]	0.0±0.0 (6)[e]	0.0±0.0 (6)[f]	0.1±0.1 (6)[f]
7mC	2.3±0.2 (5)	1.5±0.4 (5)	1.5±0.2 (5)	4.2±0.7 (5)
7mD	0.0±0.0 (6)[f]	0.0±0.0 (6)[e]	0.0±0.0 (6)[f]	0.0±0.0 (6)[f]
7mC/4wS	2.9±0.1 (6)	0.9±0.2 (6)	1.6±0.2 (6)	4.5±0.6 (6)
7mC/4wF	13.6±1.0 (6)[f]	3.5±0.3 (6)[f]	3.2±0.4 (6)[d]	3.7±0.4 (6)
7mD/2wF	10.6±1.3 (6)	1.6±0.2 (6)	1.3±0.1 (6)	1.5±0.1 (6)
7mD/4wF	12.6±0.7 (6)	1.6±0.0 (6)	1.3±0.1 (6)	1.5±0.2 (6)

Same presentation as in Table II.

Table XI. Weight percentage of long-chain polyunsaturated ω6 fatty acids in liver triglycerides.

Rats	C18:2ω6	C18:3ω6	C20:2ω6	C20:3ω6	C20:4ω6	C22:4ω6
3mC	41.1±1.0 (6)	0.1±0.1 (6)	0.0±0.0 (6)	0.0±0.0 (6)	4.9±0.3 (6)	0.4±0.2 (6)
3mD	31.2±1.3 (6)[f]	0.7±0.1 (6)[d]	0.1±0.1 (6)	0.0±0.0 (6)	3.3±0.2 (6)[e]	0.6±0.0 (6)
7mC	37.2±1.8 (5)	0.4±0.1 (5)	0.2±0.1 (5)	0.5±0.2 (5)	4.2±0.5 (5)	0.8±0.1 (5)
7mD	28.1±2.5 (6)[c]	0.8±0.1 (6)[b]	0.2±0.1 (6)	0.0±0.0 (6)[a]	4.3±0.5 (6)	0.7±0.1 (6)
7mC/4wS	44.8±0.5 (6)	0.8±0.1 (6)	0.4±0.1 (6)	0.5±0.1 (6)	5.0±0.4 (6)	0.7±0.1 (6)
7mC/4wF	34.5±0.9 (6)[f]	0.3±0.1 (6)[d]	0.1±0.1 (6)[a]	0.3±0.1 (6)	2.8±0.1 (6)[f]	0.1±0.1 (6)[e]
7mD/2wF	30.1±2.6 (6)	0.5±0.1 (6)	0.1±0.0 (6)	0.1±0.1 (6)	2.8±0.3 (6)	0.1±0.1 (6)
7mD/4wF	29.6±0.8 (6)	0.5±0.0 (6)	0.0±0.0 (6)	0.0±0.0 (6)	2.1±0.2 (6)	0.1±0.1 (6)

Same presentation as in Table II.

4-5 weeks to the flaxseed oil-enriched diet, the former mean values averaging 159.2±21.2% (n=12; p<0.02) of the corresponding mean values recorded in the control animals (100.0±9.6%; n=12).

Long-chain polyunsaturated ω3 fatty acids. Whilst sizeable amounts of all long-chain polyunsaturated ω3 fatty acids (C18:3ω3, C20:5ω3, C22:5ω3, C22:6ω3) were found in the triglycerides of control animals, such was only once the case (C22:6ω3 relative weight content: 0.3%) out of a total of 48 measurements made in ω3D rats (Table X).

The relative weight content of long-chain polyunsaturated ω3 fatty acids in liver triglycerides were comparable in the control rats before and after exposure to the soybean oil-enriched diet. Except in the case of C22:6ω3, higher values were recorded in the control rats exposed to the flaxseed oil-enriched diet.

When the ω3D rats were given access to the ω3-enriched diet, sizeable amounts of all four long-chain polyunsaturated ω3 fatty acids were invariably found in liver triglycerides. The weight percentage of C18:3ω3 was much higher (p<0.001) in these rats (11.6±0.8%; n=12) than in the control animals examined during the first 7 months of the present experiments (2.5±0.1%; n=4). The weight percentages of C20:5ω3 and C22:5ω3, however, were not vastly different in these two groups of rats, whilst that of C22:6ω3 remained lower (p<0.001) in the ω3D rats exposed for 2-5 weeks to the ω3-enriched diet (1.5±0.1%; n=12) than in the control animals examined 3-7 months after the onset of the present experiments (4.5±0.3%; n=11).

The C20:5ω3/C18:3ω3, C22:6ω3/C20:5ω3 and C22:5ω3/C22:6ω3 ratios failed to differ significantly in the control rats examined during the first 7 months of the present experiments and the control animals eventually exposed to the soybean oil-enriched diet, with respective mean values of 0.52±0.07 (n=11) and 0.32±0.08 (n=6) for the C20:5ω3/C18:3ω3 ratio, 3.52±0.46 (n=10) and 4.20±0.25 (n=5) for the C22:6ω3/C20:5ω3 ratio, and 0.36±0.01 (n=11) and 0.36±0.02 (n=6) for the C22:5ω3/C22:6ω3. The C20:5ω3/C18:3ω3 ratio also failed to differ significantly (p>0.4) in the control animals fed either the soybean or flaxseed oil-enriched diet. In the latter animals, however, the C22:6ω3/C20:5ω3 ratio was much lower (1.09±0.12; n=6; p<0.001) and the C22:5ω3/C22:6ω3 twice higher (0.85±0.06; n=6; p<0.001) than in the former animals.

The C20:5ω3/C18:3ω3 ratio in liver triglycerides was much lower (p<0.001) in the ω3D rats exposed for 2 to 4-5 weeks to the ω3-enriched diet (0.14±0.01; n=12) than in the control

. Ratio between selected long-chain polyunsaturated ω6 fatty acids in liver triglycerides.

Rats	C20:2ω6/C18:2ω6 (10^{-3})	C18:3ω6:C18:2ω6 (10^{-3})	C20:4ω6/C18:2ω6	C22:4ω6/C20:4ω6
3mC	N.A.	20.7 (1)	0.117±0.006 (6)	0.170±0.031 (3)
3mD	9.55±0.54 (2)	21.0±2.8 (6)	0.106±0.004 (6)	0.170±0.009 (6)
7mC	12.56±0.45 (2)	13.8±2.0 (4)	0.113±0.011 (5)	0.202±0.052 (5)
7mD	9.30±0.79 (4)	27.2±1.3 (6)[f]	0.153±0.008 (6)[c]	0.166±0.013 (6)
7mC/4wS	9.63±1.27 (5)	16.6±1.6 (6)	0.112±0.010 (6)	0.163±0.012 (5)
7mC/4wF	7.35±1.10 (2)	13.5±0.4 (6)	0.082±0.002 (6)[c]	0.116 (1)[c]
7mD/2wF	6.03±0.05 (2)	15.8±1.3 (6)	0.095±0.008 (6)	0.079±0.005 (2)
7mD/4wF	8.18 (1)	16.4±0.6 (6)	0.071±0.007 (6)	0.125 (1)

Same presentation as in Table II. N.A., not applicable.

rats examined 139 and 264 days after birth (0.52±0.07; n=11). The C22:6ω3/C20:5ω3 ratio was also lower ($p<0.001$) in the ω3D rats exposed to the ω3-enriched diet for 2 to 4-5 weeks before sacrifice (0.99±0.08; n=12) than in the control animals examined during the first 7 months of the present experiments (3.52±0.46; n=10). Inversely, however, the C22:5ω3/C22:6ω3 ratio was much higher ($p<0.001$) in the ω3D rats given access to the ω3-enriched diet (0.91±0.08; n=12) than in the control animals exposed to the standard diet (0.36±0.01; n=11). It should be underlined, that none of these ratios differed significantly in the ω3D rats exposed for 2 weeks *versus* 4-5 weeks to the ω3-enriched diet, and in the control animals examined 139 *versus* 264 days after birth. Except in the case of the C20:5ω3/C18:3ω3 ratio, which yielded a higher value ($p<0.001$) in the control animals than in the ω3D rats both exposed to the flaxseed oil-enriched diet, the other two ratios between long-chain polyunsaturated ω3 fatty acids, i.e. the C22:6ω3/C20:5ω3 and C22:5ω3/C22:6ω3 ratios, failed to differ significantly ($p>0.4$ or more) in these two groups of rats.

Long-chain polyunsaturated ω6 fatty acids. The relative weight content of C18:2ω6 in liver triglycerides was lower in ω3D rats than in control animals (Table XI). Such was also the case for C20:3ω6 after 7 months dietary ω3 deprivation and for C20:4ω6 after 3 months of dietary ω3 deprivation. Comparable results were recorded in control and ω3D rats in the case of C20:2ω6 and C22:4ω6. The relative weight content of C18:3ω6, however, was higher in ω3D rats than in control animals, such a difference being most pronounced after 3 months of dietary ω3 deprivation.

Whenever different from zero, the C20:2ω6/C18:2ω6 ratio yielded mean values not significantly different in control and ω3D rats (Table XII). After 7 months of ω3 deprivation, the C18:3ω6/C18:2ω6 ratio was twice higher in ω3D rats than in control animals. Even after only 3 months of dietary ω3 deprivation, such a difference was highly significant ($p<0.001$) when all results, including null values, were taken into consideration.

When the control animals were given access to the soybean oil-enriched diet, the C18:2ω6 ($p<0.005$) and C20:2ω6 ($p<0.02$) relative content of liver triglycerides became significantly higher than the mean values recorded in the control animals during the first 7 months of the present experiments. The measurements made in the control animals exposed to the flaxseed oil-enriched diet were lower, for all long-chain polyunsaturated ω6 fatty acids, than the readings recorded in the control animals fed the soybean oil-enriched diet, such a difference only failing to achieve statistical significance ($p<0.09$) in the case of C20:3ω6. The four ratios between selected long-chain polyunsaturated ω6 fatty acids listed in Table XII yielded comparable values in the control animals examined before and after exposure to the soybean oil-enriched diet. Exposure of the control animals to the flaxseed oil-enriched diet lowered the C20:4ω6/C18:2ω6 and C22:4ω6/C20:4ω6 ratios ($p<0.02$) relative to the values found in the control animals fed the soybean oil-enriched diet.

As already mentioned, the sole consistent differences between control and ω3D rats, in terms of the weight percentage of long-chain polyunsaturated ω6 fatty acids in liver triglycerides, consisted in a lower amount of C18:2ω6, contrasting with a higher amount of C18:3ω6 in the ω3D rats than in the control animals. When the former rats were given access of the ω3-enriched diet, the relative weight content of C18:2ω6 in liver triglycerides remained lower than in control animals. However, the relative weight content of C18:3ω6, C20:2ω6, C20:4ω6 and C22:4ω6 became all lower than those found in the ω3D rats after 7 months of exposure to the ω3-deficient diet, such a decrease being highly significant ($p<0.001$) in all cases except C20:2ω6 ($p<0.08$).

Whenever calculable, the C20:2ω6/C18:2ω6 ratio in liver triglycerides was lower ($p<0.025$) in ω3D rats examined after exposure to the flaxseed oil-enriched diet (6.74±0.71; n=3) than in the ω3D rats investigated before such an exposure (9.38±0.52; n=6). The elevated C18:3ω6/C18:2ω6 ratio found after 7 months of ω3 deprivation rapidly returned to a normal value when the ω3D rats were given access to the ω3-enriched diet. Likewise, the elevated C20:4ω6/C18:2ω6 ratio found in the ω3D rats after 7 months of ω3 deprivation rapidly decreased from 0.153±0.008 to 0.095±0.008 and 0.071±0.007 (n=6 in all cases) when the ω3D rats were exposed for 2 and 4-5 weeks, respectively, to the ω3-enriched diet. During the first 7 months of the present experiments, the C22:4ω6/C20:4ω6 ratio in liver triglycerides failed to differ significantly in the

Table XIII. Weight percentage of saturated and monodesaturated fatty acids in liver triglycerides.

Rats	C14:0	C16:0	C16:1ω7	C18:0	C18:1ω9
3mC	0.3±0.2 (6)	21.4±1.0 (6)	1.9±0.4 (6)	2.1±0.2 (6)	17.7±0.9 (6)
3mD	1.4±0.2 (6)[e]	27.9±0.5 (6)[f]	3.5±0.4 (6)[c]	2.6±0.1 (6)[a]	28.6±1.1 (6)[f]
7mC	0.2±0.1 (5)	24.2±2.0 (5)	2.2±0.4 (5)	2.8±0.2 (5)	17.3±0.9 (5)
7mD	1.6±0.1 (6)[f]	32.1±2.9 (6)[a]	3.6±0.2 (5)[a]	3.0±0.3 (6)	25.6±1.0 (6)[f]
7mC/4wS	0.7±0.2 (6)	19.1±0.6 (6)	1.1±0.3 (6)	1.9±0.2 (6)	15.2±0.8 (6)
7mC/4wF	0.5±0.0 (6)	17.6±0.6 (6)	1.1±0.2 (6)	2.6±0.2 (6)[a]	15.8±1.0 (6)
7mD/2wF	0.7±0.2 (6)	21.8±1.7 (6)	2.9±0.7 (6)	2.6±0.3 (6)	22.8±0.9 (6)
7mD/4wF	0.9±0.1 (6)	22.1±0.8 (6)	3.1±0.5 (6)	2.1±0.2 (6)	22.3±0.3 (6)

Same presentation as in Table II.

Table XIV. Ratio between selected saturated and monodesaturated fatty acids in liver triglycerides.

Rats	C16:1ω7/C16:0 (%)	C18:1ω9/C18:0	(C18:0 + C18:1ω9)/(C16:0 + C16)
3mC	8.73±1.89 (6)	8.80±0.65 (6)	0.848±0.066 (6)
3mD	12.68±1.44 (6)	11.16±0.92 (6)	0.993±0.023 (6)
7mC	8.62±1.25 (5)	6.26±0.51 (5)	0.780±0.049 (5)
7mD	11.26±0.59 (6)	8.88±0.97 (6)	0.817±0.090 (6)
7mC/4wS	5.54±1.75 (6)	8.37±0.67 (6)	0.850±0.041 (6)
7mC/4wF	5.97±1.21 (6)	6.03±0.38 (6)[c]	0.999±0.084 (6)
7mD/2wF	12.71±2.02 (6)	9.44±1.22 (6)	1.054±0.063 (6)
7mD/4wF	13.65±1.68 (6)	11.32±1.40 (6)	0.983±0.056 (6)

Same presentation as in Table II.

control animals and ω3D rats, with an overall mean value of 0.177±0.014 (n=20). Even if one ignores the fact that such a ratio yielded null values in 9 out of the 12 ω3D rats given access to the ω3-enriched diet, the mean value recorded in the other 3 rats (0.094 ± 0.015) remained significantly lower ($p<0.02$) than that recorded in the ω3D rats examined after 7 months exposure to the ω3-deficient diet (0.166±0.013; n=6).

Saturated and monodesaturated fatty acids. Detectable amounts of C12:0 in liver triglycerides were only found in five 264-day-old control rats, yielding a mean weight percentage of 0.4±0.1% (n=5).

The mean values for the weight percentages of C14:0, C16:0, C16:1ω7, C18:0 and C18:1ω9 in liver triglycerides were always higher in ω3D rats than in control animals, whether after 3 or 7 months of exposure to their respective diets (Table XIII). Such a difference only failed to achieve statistical significance in one out of ten instances, i.e. in the case of C18:0 in the rats examined 7 months after the onset of the present experiments.

The weight percentage of C14:0 was higher ($p<0.05$) in the control rats after than before exposure to the soybean oil-enriched diet. The four other fatty acids listed in Table XIII yielded lower mean values, however, after than just before exposure to the same diet; they averaged after exposure to this diet 70.5±5.0% (n=24; $p<0.001$) of the mean corresponding values (100.0±5.3%; n=20) recorded in the control animals at the end of the first 7 months of the present experiments. Except for a somewhat higher weight percentage ($p<0.05$) of C18:0 in the control rats exposed to the flaxseed oil-enriched diet, no other significant difference was found for the relative contribution to liver triglycerides of the five fatty acids listed in Table XIII.

When the ω3D rats were offered access to the ω3-enriched diet, the mean weight percentages of C14:0, C16:0, C16:1ω7, C18:0 and C18:2ω6 in liver triglycerides became lower than those recorded in the ω3D rats after 7 months of exposure to the ω3-deprived diet. Such a decrease failed to achieve statistical significance in the case of the poorly abundant C16:1ω7 ($p>0.3$) and C18:0 ($p<0.07$) fatty acids, whilst being highly significant in the case of C14:0 ($p<0.001$), C16:0 ($p<0.001$) and C18:1ω9 ($p<0.001$).

Except for the presence of a small amount of C20:1ω9 (0.2%) in one 264-day-old ω3D rat, no C20;0, C20:1ω9, C22:0, C22:1ω9 and C24:0 was detected in the liver triglycerides of control or ω3D rats up to the age of 264 days. Likewise, among these five saturated and monodesaturated

s, only C20:1ω9 was detected in one control animal o the soybean oil-enriched diet (0.2%) and in ω3D rats given access to the ω3-enriched diet, with a mean relative weight content of 0.3±0.2% (n=3).

The mean values for both the C16:1ω7/C16:0 and C18:1ω9/C18:0 ratios in liver triglycerides were higher in ω3D rats than in control animals, whether after 3 or 7 months exposure to their respective diet (Table XIV). Such ratios averaged, in the ω3D rats, 136.1±6.3% (n=24; $p<0.001$) of the mean corresponding values found in control animals of the same age (100.0±6.8%; n=22). When the ω3D rats were given access to the ω3-enriched diet, the same ratios failed to return towards control values and, on the contrary, displayed a progressive increase. They indeed averaged, relative to the corresponding mean values found in the ω3D rats after 7 months of exposure to the ω3-deprived diet (100.0±5.8%), 109.7±10.8 and 124.3±10.4% (n=12 in all cases) after 2 and 4-5 weeks of exposure to the ω3-enriched diet, respectively. The progressive increase in these percentages as a function of the length of exposure to the ω3-enriched diet yielded a correlation coefficient of 0.4366 (df=34; $p<0.01$).

The activity of elongase, as judged from the (C18:0 + C18:1ω9)/(C16:0 + C16:1ω7) ratio failed to differ significantly ($p>0.2$) in the control animals (0.817±0.042; n=11) and ω3D rats (0.905±0.052; n=12), and in the latter rats, failed to change significantly ($p>0.009$) during exposure to the ω3-enriched diet. Likewise, it failed to differ significantly in the control animals before and after exposure to the soybean oil-enriched diet ($p>0.6$) and in the control animals fed either the latter diet or the flaxseed oil-enriched diet ($p>0.1$).

Discussion

Igarashi *et al* (10) recently documented the depletion of liver phospholipid and triacylglycerol in C18:3ω3, C20:5ω3, C22:5ω3 and C22:6ω3 prevailing in male rats exposed for 15 weeks after weaning to a diet containing as sole long-chain polyunsaturated ω3 fatty acid 0.25 μmol/g C18:3ω3 (as distinct from 7.8 μmol/g in the control diet). The investigations conducted by these authors differed, however, in several respects from the present study. First, in these investigations, male rats were exposed to the ω3-depleted diet from weaning onwards, whilst the present study aimed at exploring the consequence of a dietary ω3 deprivation initiated in female rats already 6-week-old. Second, in the prior investigations, the rats were exposed to diets containing 10% fat in which C12:0 and C14:0 accounted for 50.7±2.2% of total fatty acid content, as distinct from the diets offered during the first seven months of the present experiments containing only 5% fat in which C12:0 and C14:0 represented only 0.35±0.05% of total fatty acid content. Such differences may well account for the abnormally high liver triacylglycerol content (64.2±5.0 μmol/g liver; n=10) found in the control rats examined by Igarashi *et al* (10), as compared ($p<0.001$) to 10.2±0.7 μmol/g (n=11) in the control rats of the present study. Last, the latter situation may well account for the fact that no further significant increase of liver total triacylglycerol content was observed by Igarashi *et al* (10) in their ω3-depleted rats, whilst the occurrence of liver steatosis in our ω3-depleted rats represents an essential feature of the metabolic syndrome in these animals.

The present results document that a dietary deprivation of ω3 fatty acids for no more than 3 months is sufficient to cause a severe depletion of these fatty acids in both liver phospholipids and triglycerides. This coincided in both lipid fractions with a number of other changes in fatty acid content and pattern.

In the case of liver phospholipids, attention is drawn to the decrease in their total fatty acid content, and perturbations in the content of long-chain polyunsaturated ω6 fatty acids, saturated and monodesaturated fatty acids and precursors of nervonic acid. The relative weight contents of C18:2ω6, C20:2ω6 and C20:3ω6 were indeed lower and those of C18:3ω6, C20:4ω6, C22:4ω6 and C22:5ω6 higher in the ω3D rats than in control animals. The activity of Δ6-desaturase (C18:3ω6/C18:2ω6 ratio) and Δ5-desaturase (C20:4ω6/C20:3ω6 ratio) and Δ9-desaturase (C16:1ω7/C16:0 ratio) appeared also increased in the ω3D rats. Likewise, the weight contents of C16:1ω7, C18:0 and C18:1ω9 were increased in the ω3D rats. Nevertheless, the weight percentage of C20:1ω9 and C22:1ω9 in liver phospholipids only represented in the ω3D rats about half of the corresponding control values. The postulated increase in Δ5-desaturase and Δ6-desaturase liver activity is in agreement with the increased mRNA levels and increased microsomal activities of these enzymes observed by Igarashi *et al* (11) in the liver of rats exposed to an ω3-deficient diet for 15 weeks after weaning. These authors also reported increased mRNA and activity levels of elongase 2 and 5 in the same ω3-depleted rats. These findings coincide with the increased C22:4ω6/C20:4ω6 ratio here found in the liver phospholipids of ω3-depleted rats.

Despite a normal protein content, the total fatty acid and glycerol content of triglycerides were increased in the ω3D rats. The relative weight content of C18:2ω6 in liver triglycerides was again decreased and the C18:3ω6/C18:2ω6 ratio increased, as already noted in liver phospholipids. Likewise, as also observed in phospholipids, the relative weight content of most saturated (C14:0, C16:0) and monodesaturated (C16:1ω7, C18:1ω9) fatty acids, as well as the C16:1ω7/C16:0 and C18:1ω9/C18:0 ratios in liver triglycerides, were all increased in the ω3D rats.

All these features duplicate those found in second generation ω3D rats (3,12,13). They suggest, therefore, that the present experimental device is appropriate to assess the metabolic consequences of a depletion in ω3 fatty acids, with emphasis on the development of liver steatosis.

A more nuanced situation prevailed when the ω3D rats were exposed to an ω3-enriched diet.

On one hand, when the ω3D rats were exposed to the flaxseed oil-enriched diet, several hepatic variables moved towards or even beyond control values. In the ω3D rats, the increase in the liver phospholipid content provoked by the exposure to this ω3-rich diet was much lower for C20:5ω3 than C22:6ω3. Such a preferential enrichment of liver phospholipids in C22:6ω3 relative to C20:5ω3 was recently also observed in ω3D rats (2nd generation) injected intravenously 60 min before sacrifice with a novel medium-chain triglyceride:fish oil emulsion containing equal amounts of

C20:5ω3 and C22:6ω3 (14). When control rats are injected with the same emulsion, the liver phospholipid content of C20:5ω3 is also significantly increased, whilst such is no more the case for the C22:6ω3 content of liver phospholipids (3). Likewise, in the present study, the C22:6ω3 content of liver phospholipids failed to change significantly in the control animals during exposure to the flaxseed oil-enriched diet, in sharp contrast to the finding made, under the same experimental conditions, in ω3D rats. These converging observations are compatible with the view that the stepwise conversion of C20:5ω3 to C22:6ω3 is more efficient in ω3D rats than in control animals. The C22:5ω3/C22:6ω3 ratio also became higher than that found in the control animals. This coincided with restoration of a normal total fatty acid content of liver phospholipids, a rise of the C18:2ω6 relative weight content, and normalization of the C18:3ω6, C20:6ω6, C20:4ω6 and C22:4ω6 phospholipid content, as well as most ratios between selected long-chain polyunsaturated ω6 fatty acids. Likewise, the weight percentage of most saturated and mono-desaturated fatty acids, including precursors or nervonic acid, in liver phospholipids moved back towards control values. In the liver triglycerides, the weight percentage of long-chain polyunsaturated ω3 fatty acids also reached normal or higher than normal values, except once again in the case of C22:6ω3. Nevertheless, significant differences between control animals and ω3D rats exposed to the flaxseed oil-enriched diet were encountered in the case of several ratios between selected ω3 fatty acids in liver triglycerides. In these triglycerides, like in phospholipids, the relative weight content of C14:0, C16:0 and C18:1ω9 moved towards normal values when the ω3D rats were exposed to the ω3-enriched diet.

On the other hand, however, under the same experimental conditions, the (C18:0/C18:1ω9)/(C16:0 + C16:1ω7) ratio in liver phospholipids, which was higher in ω3D rats than in control animals, was further increased in the ω3D rats exposed to the flaxseed oil-enriched diet. More importantly, the total fatty acid content of liver triglycerides, which was also abnormally high in the ω3D rats, was also further increased when the latter rats were given access to the ω3-enriched diet. Thus, at variance with the situation found in second-generation ω3-depleted rats examined 60 min after the intravenous injection of a medium-chain triglyceride:fish oil emulsion (3,12), the dietary supply of ω3 fatty acids to the ω3D rats failed, in the present experiments, to correct liver steatosis and, on the contrary, caused a further increase in liver triacylglycerol content.

Two factors may well account for the latter unexpected finding.

First, it may be attributable, in part at least, to the fact that the total triglyceride content of the flaxseed oil-enriched diet was twice higher than that of the ω3-deprived diet offered to the ω3D rats during the first 7 months of the present experiments. This proposal is consistent with the finding that, in the control rats, exposure to either the soybean or flaxseed oil-enriched diets, which both also contained an amount of triglycerides twice higher than the control diet offered to the control rats during the first 7 months of the present experiments, also increased the liver triglyceride content well beyond the modest rise that could, otherwise, be expected, from the progressive ageing of these animals. In the case of the soybean oil-enriched diet, such an increase could not be attributed to any enrichment of either liver phospholipids or triglycerides in long-chain polyunsaturated ω3 fatty acids.

Second, the ω3D rats, when exposed to the flaxseed oil-enriched diet, display a rapid and dramatic increase in both body weight and parametrial adipose tissue weight, such an increase not being observed in the control rats exposed to either the soybean or flaxseed oil-enriched diet (15). Hence, an increase in food intake, as conceivably attributable to the supply of ω3 fatty acids to the ω3D rats (16-18), may also participate in the aggravation of liver steatosis in these rats.

Considering these two converging factors, it may, quite rightly, be objected that the inclusion in the present study of a further group of ω3D rats eventually exposed to either the control diet, i.e. that containing only 5% (wt/wt) of soybean oil, or to a diet only containing 5% of flaxseed oil, would have been desirable.

In conclusion, the present experiments documents that a dietary deprivation of ω3 fatty acids for a few months is sufficient to duplicate the changes in the fatty acid content and pattern of liver phospholipids and triglycerides otherwise found in second generation ω3-depleted rats. The present procedure may thus be recommended as a more simple and realistic approach to explore the metabolic and functional consequences of such a depletion. Our findings draw attention, however, to the idea that in order to correct both the depletion in ω3 fatty acids and its undesirable consequences, such as liver steatosis, it may be necessary to combine the dietary supply of long-chain polyunsaturated ω3 fatty acids with a suitable control of food intake, in both qualitative and quantitative terms.

Acknowledgments

This study was supported by a grant from the Belgian Foundation for Scientific Medical Research (3.4574.07), and by Convention 5459 (Project WALNUT-20) from the Région Wallonne (Namur, Belgium). We are grateful to A. Chwalik, A. Dufour and E. Hupkens for technical assistance and C. Demesmaeker for secretarial help.

References

1. Armitage JA, Pearce AD, Sinclair AJ, Vingrys AJ, Weisinger RS and Weisinger HS: Increased blood pressure later in life may be associated with perinatal n-3 fatty acid deficiency. Lipids 38: 459-464, 2003.
2. Cancelas J, Prieto PG, Villanueva-Peñacarrillo ML, Zhang Y, Portois L, Sener A, Carpentier YA, Valverde I and Malaisse WJ: Glucose intolerance associated to insulin resistance and increased insulin secretion in rats depleted in long-chain polyunsaturated ω3 fatty acids. Horm Metab Res 39: 823-825, 2007.
3. Carpentier YA, Peltier S, Portois L, Sebedio JL, Leverve X and Malaisse WJ: Rapid reduction of liver steatosis in ω3-depleted rats injected with a novel lipid emulsion. Horm Metab Res 40: 875-879, 2008.
4. Oguzhan B, Sancho V, Acitores A, Villanueva-Peñacarrillo ML, Portois L, Chardigny J-M, Sener A, Carpentier YA and Malaisse WJ: Alteration of adipocyte metabolism in ω3 fatty acid-depleted rats. Horm Metab Res 38: 789-798, 2006.
5. Oguzhan B, Zhang Y, Louchami K, Courtois P, Portois L, Chardigny J-M, Malaisse WJ, Carpentier YA and Sener A: Pancreatic islet function in ω3 fatty acid-depleted rats. Glucose metabolism and nutrient-stimulated insulin release. Endocrine 29: 457-466, 2006.

S, Malaisse WJ, Portois L, Demaison L, Novel-Chate V, gny J-M, Sebebio JL, Carpentier YA and Leverve XM: Acute *in vivo* administration of a fish oil-containing emulsion improves post-ischemic cardiac function in n-3-depleted rats. Int J Mol Med 18: 741-749, 2006.
7. Folch J, Lees M and Sloane-Stanley GH: A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226: 497-509, 1957.
8. Dahlan W, Richelle M, Kulapongse S, Rössle C, Deckelbaum RJ and Carpentier YA: Effects of essential fatty acid contents of lipid emulsions on erythrocyte polyunsaturated fatty acid composition in patients on long-term parenteral nutrition. Clin Nutr 11: 262-268, 1992.
9. Lepage G and Roy CC: Direct transesterification of all classes of lipids in a one step reaction. J Lipid Res 27: 114-120, 1986.
10. Igarashi M, DeMar JC, Ma K, Chang L, Bell JM and Rapoport SI: Upregulated liver conversion of α-linoleic acid to docosahexaenoic acid in rats on a 15 week n-3 PUFA-deficient diet. J Lipid Res 48: 152-164, 2007.
11. Igarashi M, Ma K, Chang L, Bell JM and Rapoport SI: Dietary n-3 PUFA deprivation for 15 weeks upregulates elongase and desaturase expression in rat liver but not brain. J Lipid Res 48: 2463-2470, 2007.
12. Carpentier YA, Peltier S, Portois L, Sener A and Malaisse WJ: Rapid lipid enrichment in ω3 fatty acids: Liver data. Int J Mol Med 21: 367-373, 2008.
13. Louchami K, Zhang Y, Oguzhan B, Delporte C, Portois L, Carpentier YA, Genten F, Danguy A, Malaisse WJ and Sener A: Rapid changes in liver lipid composition and pancreatic islet K^+ handling and secretory behaviour provoked by the intravenous administration of a medium-chain triglyceride:fish oil emulsion to long-chain polyunsaturated ω3 fatty acid-depleted rats. Int J Mol Med 18: 1047-1055, 2006.
14. Peltier S, Portois L, Malaisse WJ and Carpentier YA: Preferential enrichment of liver phospholipids in docosahexaenoate relative to eicosapentaenoate in ω3-depleted rats injected with a medium chain triglyceride:fish oil emulsion. Prostaglandins Leukot Essent Fatty Acids 78: 27-32, 2007.
15. Sener A, Zhang Y, Bulur N, Louchami K, Malaisse WJ and Carpentier YA: The metabolic syndrome of ω3-deficient rats. II. Body weight, adipose tissue mass and glycemic homeostasis. Int J Mol Med 24: 125-129, 2009.
16. Goncalves CG, Ramos EJ, Romanova IV, Suzuki S, Chen C and Meguid MM: Omega-3 fatty acids improve appetite in cancer anorexia, but tumor resecting restores it. Surgery 139: 202-208, 2006.
17. Ramos EJ, Romanova IV, Suzuki S, Chen C, Ugrumov MV, Sato T, Goncalves CG and Meguid MM: Effects of omega-3 fatty acids on orexigenic and anorexigenic modulators at the onset of anorexia. Brain Res 1046: 157-164, 2005.
18. Dunlap S and Heinrichs SC: Neuronal depletion of omega-3 fatty acids induces flax seed dietary self-selection in the rat. Brain Res 1250: 113-119, 2009.