

The metabolic syndrome of ω 3-depleted rats. IV.

Intestinal phospholipid ω 3 fatty acids

MIRJAM HACQUEBARD, LAURENCE PORTOIS, WILLY J. MALAISSE and YVON A. CARPENTIER

Laboratory of Experimental Surgery, Université Libre de Bruxelles, 808 Route de Lennik, B-1070 Brussels, Belgium

Received August 19, 2009; Accepted September 21, 2009

DOI: 10.3892/ijmm_00000304

Abstract. A dietary deprivation in long-chain polyunsaturated ω 3 fatty acids initiated in 7-week old normal rats provokes within 3 to 7 months the appearance of several features of the metabolic syndrome. Likewise, within 2 to 4-5 weeks exposure to a flaxseed oil-enriched diet, these anomalies are rapidly corrected. The present study deals with the ω 3 fatty acid content of intestinal phospholipids under the same experimental conditions. For the sake of comparison, the control rats were given access during the last 4-5 weeks to either a soybean or flaxseed oil-enriched diet. In control rats, the relative weight content of ω 3 fatty acids as well as their product/precursor ratio differed in distinct segments of the intestinal tract (duodenum, jejunum, caecum, colon). Within 3 months of ω 3-deprivation, the intestinal content of C18:3 ω 3, C20:5 ω 3 and C22:5 ω 3 reached values below the limit of detection, whilst the C22:6 ω 3 content progressively decreased down to 10-20% of control values. Within 2 weeks of exposure to the ω 3-rich diet, the C18:3 ω 3, C20:5 ω 3 and C22:5 ω 3 content of intestinal phospholipids were higher than control values, whilst that of C22:6 ω 3 progressively returned to a normal level during the 2 to 4-5 weeks exposure to the flaxseed oil-enriched diet. The results collected in the intestinal cells, which are the first cells exposed to each given diet, reinforce the view that the present animal model is quite suitable to assess the metabolic consequences of both ω 3 fatty acid deprivation and replenishment.

Introduction

In the first 3 reports in this series, it was documented that a dietary deprivation of long-chain polyunsaturated ω 3 fatty acids initiated in 7-week-old normal rats was sufficient within 3 to 7 months to reproduce several features of the metabolic syndrome, including liver steatosis, visceral obesity and insulin

resistance, as otherwise found in second-generation rats depleted in these ω 3 fatty acids (ω 3D rats) (1-3). The time course for the repletion of ω 3 fatty acids in liver and brain was also investigated when the ω 3D rats were given access for 2 to 4-5 weeks to a flaxseed oil-enriched diet. Moreover, for the sake of comparison, the various phospholipid variables were measured in the control rats given access for the last 4-5 weeks of the present experiments to either a soybean or flaxseed oil-enriched diet.

Since the cells of the gastrointestinal tract are the first exposed to either an ω 3-depleted or ω 3-enriched diet, attention was also paid, in the same animals as those examined in our prior studies, to the changes in the fatty acid content and profile of phospholipids at 4 levels of such a tract, namely in the duodenum, jejunum, caecum and colon. These intestinal measurements provide several pieces of information. First, they allow to compare, in control animals, the phospholipid fatty acid pattern in different segments of the gastrointestinal tract. Second, they document the time course for changes in intestinal phospholipid fatty acid patterns, when normal rats are deprived of a dietary supply of ω 3 fatty acids. Third, they also document the time course for the reversal of these changes in ω 3D rats exposed to an ω 3-enriched diet.

The present report presents the data collected in the 8 groups of rats under consideration and concerning the weight content of distinct long-chain polyunsaturated ω 3 fatty acids in the duodenum, jejunum, caecum and colon phospholipids.

Materials and methods

The 8 groups of 5-6 female rats each were the same as those indicated in our first report in this series (1). Briefly, 4 of these groups included control rats exposed for 3 or 7 months to a diet containing 5% (wt/wt) soybean oil and then given access for 4-5 weeks to the same diet enriched with either another 5% of soybean oil or 5% of flaxseed oil. The other 4 groups consisted of rats exposed for 3 or 7 months to a diet containing 5% sunflower oil and then given access for 2 or 4-5 weeks to the same diet enriched with 5% flaxseed oil. The fatty acid composition of these diets and the modalities of sacrifice and tissue sampling were also described in our prior publication (1). The small and large bowel segments were removed from mesenteric and vascular connections and sequentially removed from the peritoneum. Segments used for intestinal mucosae fatty acid analysis were the duodenum (5 cm distal to the pylorus), jejunum (20 cm distal from duodenum), caecum and

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: ω 3-depleted rats, intestinal phospholipids, ω 3 fatty acids

colon (segment between caecum and rectum). The lumen of each intestinal segment was thoroughly flushed with ice-cold 9 g NaCl/l to clear intestinal contents. Intestinal segments were then split lengthwise and the mucosa was gently scraped. Mucosal samples were immediately immersed in liquid nitrogen and stored at -80°C for fatty acid analyses. The lipids were extracted (4), separated by thin-layer chromatography (5), and their fatty acid pattern determined by gas-liquid chromatography (6).

The present experiments were conducted in accordance with the principles of the Animal Experimentation Ethics Committee of Brussels Free University Medical School and approved by this Committee.

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 ω 3-depleted rats (ω 3D) also examined 3 and 7 months after the start of the experiments, 7mC/4wS and 7mC/4wF for the control rats eventually exposed for 4-5 weeks to either the soybean (S) or flaxseed (F) oil-enriched diets, and 7mD/2wF and 7mD/4wF for the ω 3D rats eventually exposed for 2 or 4-5 weeks to the flaxseed oil-enriched diet.

All results are presented as mean values (\pm SEM) 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

Relative weight content of long-chain polyunsaturated ω 3 fatty acids. In the control rats, the most abundant long-chain polyunsaturated ω 3 fatty acids was C22:6 ω 3 in all segments of the intestinal tract (Table I). Relative to the total amount of phospholipid fatty acids, it represented $46.91 \pm 1.97\%$ (n=14) in the duodenum-jejunum, $45.17 \pm 2.01\%$ (n=11) in the caecum and $42.44 \pm 3.35\%$ (n=11) in the colon, these 3 mean values not being significantly different from one another ($p > 0.2$ or more). Relative to the mean amount of C22:6 ω 3, that of other ω 3 fatty acids differed, on occasion, significantly in distinct segments of the intestinal tract (Fig. 1). For instance, that of C18:3 ω 3 averaged $11.3 \pm 0.7\%$ (n=14, *versus* $100.0 \pm 3.6\%$) in the duodenum-jejunum and $10.2 \pm 0.7\%$ (n=11, *versus* $100.0 \pm 4.2\%$) in the caecum, as distinct ($p < 0.005$) from only $5.9 \pm 1.5\%$ (n=11, *versus* $100.0 \pm 7.9\%$) in the colon. That of C20:5 ω 3 averaged $14.9 \pm 0.8\%$ (n=14) in the duodenum-jejunum, as distinct ($p < 0.04$ or less) from $24.1 \pm 3.2\%$ and $20.5 \pm 2.6\%$ (n=11 in both cases) in the caecum and colon, respectively. Last, that of C22:5 ω 3 represented no more than $14.5 \pm 0.9\%$ (n=14) in the duodenum-jejunum and $18.3 \pm 2.9\%$ (n=11) in the colon, as distinct ($p < 0.001$) from $32.1 \pm 1.9\%$ (n=11) in the caecum.

When the 7-week-old rats were exposed for 3 or 7 months to the sunflower lipid-containing diet, no C18:3 ω 3, C20:5 ω 3 or C22:5 ω 3 could anymore be detected in any segment of the intestinal tract, except in one out of 126 determinations (Table I). In the ω 3D rats, the fractional contribution of C22:6 ω 3 to the phospholipid total fatty acid content, when expressed relative to that found at the same age and at the

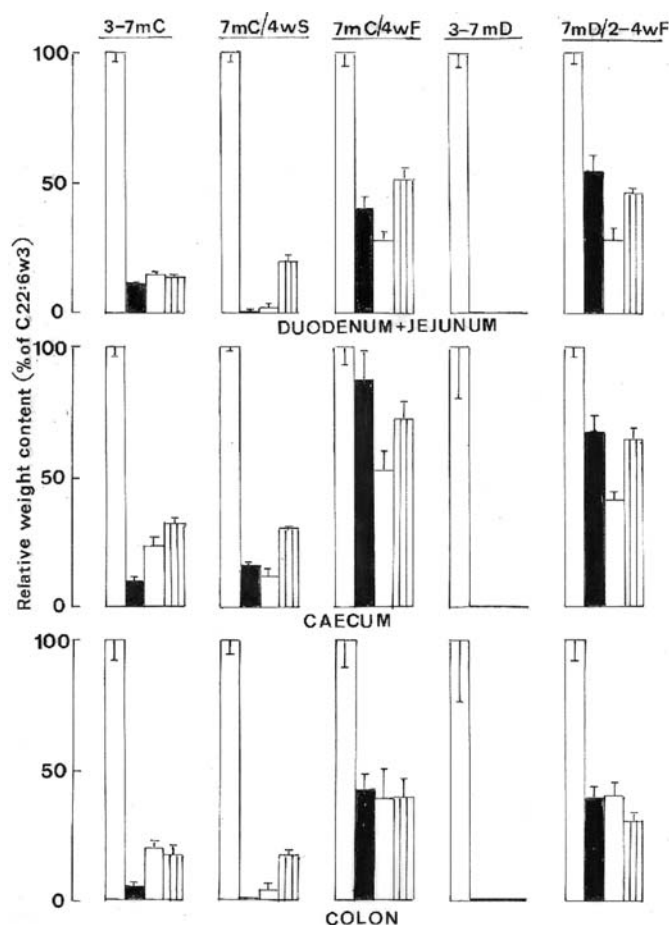


Figure 1. Relative weight content of C18:3 ω 3 (black columns), C20:5 ω 3 (middle open columns) and C22:5 ω 3 (vertically hatched columns), all expressed as a percentage of the mean corresponding C22:6 ω 3 relative weight content (left open columns), in the phospholipids of duodenum and jejunum (top), caecum (middle) and colon (bottom) of control animals examined during the first 7 months of the present experiments (3-7mC) or exposed thereafter for 4-5 weeks to either a soybean oil-enriched diet (7mC/4wS) or a flaxseed oil-enriched diet (7mC/4wF), and of ω 3D rats examined during the first 7 months of the present experiments (3-7mD) or exposed thereafter for 2 to 4-5 weeks to a flaxseed oil-enriched diet (7mD/2-4wF). Mean values (\pm SE) refer to 6-24 individual determinations.

same level of the intestinal tract in the control animals, decreased from the reference values of $100.0 \pm 4.9\%$ and $100.0 \pm 3.5\%$ (n=18 in both cases) in the control rats examined 3 and 7 months after the start of the present experiments to $25.1 \pm 3.2\%$ (n=18) and $17.3 \pm 2.0\%$ (n=24) after 3 and 7 months of dietary ω 3 fatty acid deprivation, respectively, the latter two mean percentages being significantly different ($p < 0.04$) from one another.

In the ω 3D rats given access for 2 to 4-5 weeks to the flaxseed oil-enriched diet, C22:6 ω 3 remained the most abundant long-chain polyunsaturated ω 3 fatty acids in the intestinal phospholipids. After 2 weeks exposure to the flaxseed oil-enriched diet, the relative phospholipid content in C22:6 ω 3 already represented $81.1 \pm 4.1\%$ (n=24) of the mean corresponding reference values found at the same level of the intestinal tract in the control rats examined 7 months after the onset of the present experiment. Nevertheless, the former percentage remained significantly lower ($p < 0.005$) than the latter reference value ($100.0 \pm 3.5\%$; n=18). Such was no more

Table I. Relative weight content (per thousand) of long-chain polyunsaturated ω 3 fatty acids in intestinal phospholipids.

Rats	Intestinal segments	C18:3 ω 3	C20:5 ω 3	C22:5 ω 3	C22:6 ω 3
3mC	Duodenum + jejunum	5.34 \pm 0.53 (6)	7.15 \pm 0.39 (6)	6.18 \pm 0.57 (6)	50.27 \pm 3.30 (6)
	Caecum	5.04 \pm 1.08 (6)	12.36 \pm 2.10 (6)	13.42 \pm 1.47 (6)	42.96 \pm 3.08 (6)
	Colon	2.92 \pm 0.93 (6)	9.57 \pm 1.62 (6)	7.39 \pm 2.18 (6)	41.13 \pm 5.08 (6)
3mD	Duodenum + jejunum	0 \pm 0 (6) ^e	0 \pm 0 (6) ^e	0.24 \pm 0.24 (6) ^e	13.94 \pm 1.30 (6) ^e
	Caecum	0 \pm 0 (6) ^e	0 \pm 0 (6) ^e	0 \pm 0 (6) ^e	10.61 \pm 2.26 (6) ^e
	Colon	0 \pm 0 (6) ^d	0 \pm 0 (6) ^e	0 \pm 0 (6) ^c	9.39 \pm 3.36 (6) ^e
7mC	Duodenum	4.89 \pm 0.48 (3)	7.60 \pm 0.46 (3)	9.37 \pm 0.16 (3)	47.91 \pm 1.16 (3)
	Jejunum	5.40 \pm 0.57 (5)	6.46 \pm 0.91 (5)	6.02 \pm 0.26 (5)	42.28 \pm 3.11 (5)
	Caecum	3.95 \pm 1.07 (5)	8.80 \pm 1.19 (5)	15.85 \pm 0.74 (5)	47.83 \pm 2.16 (5)
	Colon	1.95 \pm 0.85 (5)	7.56 \pm 1.26 (5)	8.24 \pm 0.78 (5)	44.01 \pm 4.66 (5)
7mD	Duodenum	0 \pm 0 (6) ^e	0 \pm 0 (6) ^e	0 \pm 0 (6) ^e	9.98 \pm 0.64 (6) ^e
	Jejunum	0 \pm 0 (6) ^e	0 \pm 0 (6) ^e	0 \pm 0 (6) ^e	8.31 \pm 1.08 (6) ^e
	Caecum	0 \pm 0 (6) ^d	0 \pm 0 (6) ^e	0 \pm 0 (6) ^e	5.65 \pm 2.02 (6) ^e
	Colon	0 \pm 0 (6) ^a	0 \pm 0 (6) ^e	0 \pm 0 (6) ^e	7.45 \pm 2.59 (6) ^e
7mC/4wS	Duodenum	0.73 \pm 0.73 (6)	2.57 \pm 1.69 (6)	13.01 \pm 1.35 (6)	53.82 \pm 2.64 (6)
	Jejunum	0 \pm 0 (6)	0 \pm 0 (6)	10.69 \pm 0.65 (6)	59.27 \pm 3.49 (6)
	Caecum	6.50 \pm 0.48 (6)	4.83 \pm 1.05 (6)	12.03 \pm 0.53 (6)	39.98 \pm 0.84 (6)
	Colon	0 \pm 0 (6)	1.68 \pm 1.06 (6)	6.81 \pm 0.83 (6)	39.91 \pm 2.31 (6)
7mC/4wF	Duodenum	22.58 \pm 3.80 (6) ^e	18.12 \pm 2.03 (6) ^e	28.41 \pm 2.33 (6) ^e	49.08 \pm 4.69 (6)
	Jejunum	17.63 \pm 3.21 (6) ^e	9.94 \pm 1.15 (6) ^e	23.52 \pm 2.94 (6) ^e	51.79 \pm 3.88 (6)
	Caecum	31.42 \pm 3.90 (6) ^e	18.92 \pm 2.73 (6) ^e	26.19 \pm 2.44 (6) ^e	36.05 \pm 2.64 (6)
	Colon	17.83 \pm 2.28 (6) ^e	16.27 \pm 4.82 (6) ^b	16.38 \pm 3.01 (6) ^b	41.15 \pm 4.41 (6)
7mD/2wF	Duodenum	28.13 \pm 5.82 (6)	15.90 \pm 2.87 (6)	19.00 \pm 1.21 (6)	39.30 \pm 2.90 (6)
	Jejunum	23.52 \pm 2.78 (6)	8.93 \pm 1.67 (6)	16.55 \pm 1.55 (6)	39.17 \pm 2.64 (6)
	Caecum	19.49 \pm 1.97 (6)	13.50 \pm 2.07 (6)	25.03 \pm 2.98 (6)	36.81 \pm 3.89 (6)
	Colon	12.52 \pm 2.59 (6)	14.44 \pm 3.40 (6)	10.49 \pm 1.98 (6)	32.04 \pm 4.93 (6)
7mD/4wF	Duodenum	16.59 \pm 6.86 (6)	11.10 \pm 5.98 (6)	20.49 \pm 1.03 (6)	45.26 \pm 5.92 (6)
	Jejunum	22.62 \pm 6.28 (6)	10.91 \pm 5.16 (6)	22.28 \pm 3.53 (6)	45.88 \pm 5.13 (6)
	Caecum	27.17 \pm 3.11 (6)	15.42 \pm 1.53 (6)	20.61 \pm 1.33 (6)	33.35 \pm 3.42 (6)
	Colon	16.32 \pm 2.10 (6)	14.85 \pm 1.23 (6)	11.81 \pm 1.71 (6)	40.51 \pm 2.92 (6)

The statistical indices (^a p <0.05; ^b p <0.02; ^c p <0.01; ^d p <0.005; ^e p <0.001) refer to the differences from the preceding group of rats (e.g. 3mD versus 3mC).

the case (p >0.2) after 4-5 weeks exposure of the ω 3D rats to the ω 3-rich diet, at which time the measurements of C22:6 ω 3 averaged 91.2 \pm 5.5% (n =24) of their corresponding reference values (100.0 \pm 3.5%; n =18).

The situation found in the ω 3D rats given access to the ω 3-rich diet differed, however, in other respects from that otherwise prevailing in control animals. Thus, as illustrated in Fig. 1, the relative abundance of other ω 3 fatty acids than C22:6 ω 3 was vastly different in these two groups of rats. For instance, in the duodenum-jejunum, the C18:3 ω 3 content of phospholipids, expressed relative to the mean corresponding amount of C22:6 ω 3, was about 5 times higher (p <0.001) in the ω 3D rats given access to the flaxseed oil-enriched diet (54.4 \pm 6.7%; n =24) than in the control animals (11.3 \pm 0.7%;

n =14). The C20:5 ω 3 content of phospholipids in the duodenum-jejunum, expressed in the same manner, was also significantly higher (p <0.05) in the former rats (27.9 \pm 4.7%; n =24) than in the control rats (14.9 \pm 0.8%; n =14). Last, in the case of C22:5 ω 3, the value found in the ω 3D rats exposed to the flaxseed oil-enriched diet (46.1 \pm 2.3%; n =24) was thrice higher (p <0.001) than in the control rats (14.5 \pm 0.9%; n =14). A comparable situation prevailed in the caecum, the amounts of C18:3 ω 3 (67.3 \pm 6.7%; n =12), C20:5 ω 3 (41.5 \pm 3.7%; n =12) and C22:5 ω 3 (64.9 \pm 4.4%; n =12) found in the phospholipids of ω 3D rats exposed for 2 to 4-5 weeks to the flaxseed oil-enriched diet, all expressed relative to the mean corresponding amount of C22:6 ω 3 (100.0 \pm 7.0%; n =12) being respectively 6-7 times higher (p <0.001), almost twice higher (p <0.005)

Table II. Paired ratio between selected long-chain polyunsaturated ω 3 fatty acids in intestinal phospholipids.

Rats	Intestinal segments	C20:5 ω 3/C18:3 ω 3	C22:6 ω 3/C20:5 ω 3	C22:5 ω 3/C22:6 ω 3
3mC	Duodenum + jejunum	1.419 \pm 0.196 (6)	7.125 \pm 0.599 (6)	0.123 \pm 0.006 (6)
	Caecum	2.308 \pm 0.101 (5)	4.322 \pm 1.053 (6)	0.315 \pm 0.029 (6)
	Colon	2.426 \pm 0.481 (4)	5.524 \pm 2.450 (6)	0.208 \pm 0.017 (5) ^a
7mC	Duodenum	1.567 \pm 0.076 (3)	6.350 \pm 0.431 (3)	0.196 \pm 0.007 (3)
	Jejunum	1.211 \pm 0.171 (5)	7.170 \pm 1.160 (5)	0.145 \pm 0.010 (5)
	Caecum	1.942 \pm 0.332 (4)	5.848 \pm 0.447 (5)	0.333 \pm 0.017 (5)
	Colon	2.076 \pm 0.355 (3)	7.595 \pm 2.899 (5)	0.190 \pm 0.014 (5)
7mC/4wS	Duodenum	2.152 (1)	6.790 \pm 2.322 (2)	0.242 \pm 0.023 (6)
	Jejunum	-	-	0.182 \pm 0.011 (6)
	Caecum	0.889 \pm 0.062 (5) ^b	7.028 \pm 0.506 (5)	0.301 \pm 0.012 (6)
	Colon	-	6.677 \pm 0.213 (2)	0.173 \pm 0.022 (6)
7mC/4wF	Duodenum	0.859 \pm 0.088 (6)	2.909 \pm 0.433 (6)	0.615 \pm 0.092 (6)
	Jejunum	0.623 \pm 0.079 (6)	5.694 \pm 0.928 (6)	0.477 \pm 0.082 (6)
	Caecum	0.621 \pm 0.060 (6)	2.221 \pm 0.542 (6)	0.732 \pm 0.065 (6)
	Colon	0.989 \pm 0.128 (5) ^c	2.409 \pm 0.564 (5)	0.400 \pm 0.053 (6)
7mD/2wF	Duodenum	0.635 \pm 0.129 (6)	3.137 \pm 0.768 (6)	0.491 \pm 0.033 (6)
	Jejunum	0.377 \pm 0.042 (6)	5.227 \pm 1.051 (6)	0.439 \pm 0.062 (6)
	Caecum	0.700 \pm 0.080 (6)	3.218 \pm 0.865 (6)	0.716 \pm 0.108 (6)
	Colon	1.192 \pm 0.173 (5)	1.665 \pm 0.219 (5)	0.334 \pm 0.044 (6)
7mD/4wF	Duodenum	1.206 \pm 0.352 (3) ^d	2.757 \pm 1.490 (3)	0.511 \pm 0.096 (6)
	Jejunum	0.418 (1) ^e	6.241 (1)	0.520 \pm 0.096 (6)
	Caecum	0.509 \pm 0.061 (6)	2.342 \pm 0.377 (6)	0.674 \pm 0.124 (6)
	Colon	0.940 \pm 0.063 (6)	2.862 \pm 0.359 (6)	0.298 \pm 0.044 (6)

Including zero values: ^a0.173 \pm 0.037 (6); ^b0.741 \pm 0.157 (6); ^c0.824 \pm 0.195 (6); ^d0.905 \pm 0.391 (4); ^e0.209 \pm 0.209 (2).

and twice higher ($p < 0.001$) than the values for C18:3 ω 3, C20:5 ω 3 and C22:5 ω 3 otherwise found in control rats (see above). Likewise, in the colon of the ω 3D rats exposed to the ω 3-enriched diet, the amounts of C18:3 ω 3 (39.7 \pm 4.6%; $n=12$), C20:5 ω 3 (40.9 \pm 5.5%; $n=12$) and C22:5 ω 3 (30.9 \pm 3.6%; $n=12$), always expressed relative to the mean amount of C22:6 ω 3 (100.0 \pm 8.1%; $n=12$), were respectively 6-7 times higher ($p < 0.001$), twice higher ($p < 0.005$) and 1.7 times higher ($p < 0.02$) than the corresponding values otherwise found in the control rats (see above).

For the sake of simplicity, the results illustrated in Fig. 1 refer to pooled data collected in the control and ω 3D rats examined 3 and 7 months after the onset of the present experiments and ω 3D rats exposed for 2 and 4-5 weeks to the flaxseed oil-enriched diet. It should be stressed, however, that the mean results found after only two weeks exposure to the latter diet were on occasion higher than those recorded after 4-5 weeks exposure to the ω 3-rich diet. Such was the case for the amounts of C18:3 ω 3 in the duodenum (71.6 \pm 14.8 *versus* 36.7 \pm 15.2%; $n=6$ in both cases) and jejunum (60.1 \pm 7.1% *versus* 49.3 \pm 13.7%; $n=6$ in both cases) and the amount of C20:5 ω 3 in the duodenum (40.5 \pm 7.3% *versus* 24.5 \pm 13.2%; $n=6$ in both cases). Although none of these difference achieved

statistical significance, they yielded an overall 2 weeks/ 4-5 weeks ratio of 1.61 \pm 0.29 ($df=34$; $p < 0.05$ *versus* unity). Such a difference was not observed for C18:3 ω 3 and C20:5 ω 3 in lower segments of the intestinal tract or for C22:5 ω 3 in any of the four segments of the intestinal tract here under consideration.

In most respect, the situation found in the ω 3D rats exposed to the flaxseed oil-enriched diet was duplicated when the control animals were also given access for 4-5 weeks to a flaxseed oil-enriched diet. First, C22:6 ω 3 remained the most abundant ω 3 fatty acid in the intestinal phospholipids. Its relative weight content in these phospholipids represents 98.5 \pm 4.9% ($n=24$; $p > 0.8$) of the mean corresponding values found at the same level of the intestinal tract in the control animals examined 7 months after the onset of the present experiments (100.0 \pm 3.5%; $n=18$). Second, relative to the mean corresponding values for the weight percentage of C22:6 ω 3, those of C18:3 ω 3, C20:5 ω 3 and C22:5 ω 3 were always higher in the control animals exposed to the flaxseed oil-enriched diet than in the control animals examined during the first 7 months of the present experiments (Fig. 1). Such a difference was highly significant ($p < 0.005$) in all cases, except for the C20:5 ω 3 content of colon phospholipids ($p < 0.06$). Third, there

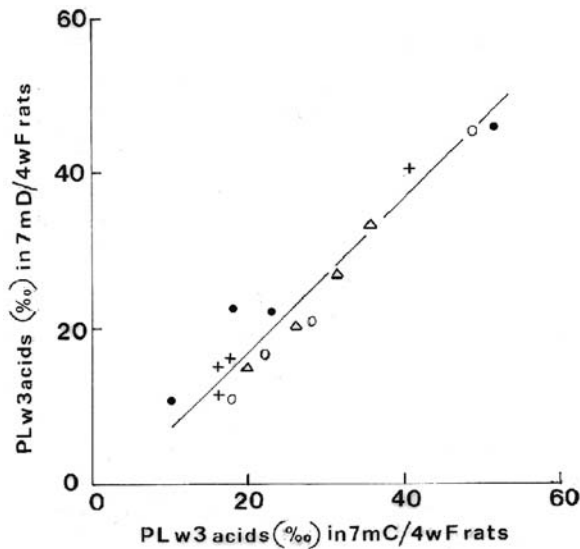


Figure 2. Correlation between the mean relative weight content (per thousand) of long-chain polyunsaturated ω 3 fatty acids (C18:3 ω 3, C20:5 ω 3, C22:5 ω 3 and C22:6 ω 3) in duodenum (open circles), jejunum (closed circles), caecum (triangles) and colon (crosses) phospholipids of control animals (abscissa) and ω 3D rats (ordinates) exposed for 4-5 weeks to a flaxseed oil-enriched diet. The oblique line corresponds to the regression line.

were highly significant positive correlations between the results recorded in the control animals and the ω 3D rats both exposed for 4-5 weeks to a flaxseed oil-enriched diet. Thus, the mean values for C18:3 ω 3, C20:5 ω 3 and C22:6 ω 3 in the four segments of the intestinal tract, all expressed relative to the mean corresponding values for C22:6 ω 3, yielded, when comparing control animals and ω 3D rats exposed for 4-5 weeks to the flaxseed oil-enriched diet, a correlation coefficient of 0.8860 (df=10; $p<0.001$), the results collected in the ω 3D rats averaging $93.8\pm 7.4\%$ ($n=72$; $p>0.4$) of the corresponding measurements made in the control animals ($100.0\pm 4.1\%$; $n=72$). Moreover, when considering the absolute values for the weight percentage of the four ω 3 fatty acids in the different segments of the intestinal tract, the correlation between control animals and ω 3D rats both exposed to a flaxseed oil-enriched diet yielded an even higher correlation coefficient ($r=0.9636$; df=14; $p<0.001$), the values found in the ω 3D rats now averaging no more than $88.3\pm 5.1\%$ ($n=96$; $p<0.06$) of the corresponding values recorded for the same ω 3 fatty acid at the same level of the intestinal tract in the control animals ($100.0\pm 3.3\%$; $n=96$). The latter correlation is illustrated in Fig. 2.

As illustrated in Fig. 1, the exposure of the control rats for 4-5 weeks to the soybean oil-enriched diet also affected the phospholipid content of C18:3 ω 3, C20:5 ω 3 and C22:5 ω 3 relative to that of C22:6 ω 3. Such differences were significant ($p<0.03$ or less), except as far as the C22:5 ω 3 content in caecum and colon is concerned ($p>0.4$ or more). In other words, the paired ratio between selected ω 3 fatty acids often differed in the control rats examined before and after exposure to the soybean oil-enriched diet (Table II).

Likewise, when the control animals were eventually exposed for 4-5 weeks to a soybean oil-enriched diet, the relative content of phospholipids in C18:3 ω 3 and C20:5 ω 3

was unexpectedly much lower than in the control animals examined just before such an exposure. It averaged $36.3\pm 8.5\%$ ($n=48$; $p<0.001$) of the mean corresponding reference values found at the same level of the intestinal tract in the control animals examined 7 months after the start of the present experiments ($100.0\pm 7.4\%$; $n=36$). This decrease appeared less pronounced in the caecum than in the duodenum, jejunum or colon, even missing in the case of C18:3 ω 3 in the caecum. In the case of C22:5 ω 3 and C22:6 ω 3, however, no significant difference ($p>0.07$) was found between these two groups of rats. Thus, the results recorded in the control animals exposed to the soybean oil-enriched diet averaged $112.7\pm 5.7\%$ ($n=48$) of the mean corresponding reference values found at the same level of the intestinal tract in the control animals examined 7 months after the start of the present experiments ($100.0\pm 2.3\%$; $n=36$). More precisely, however, the values for C22:5 ω 3 and C22:6 ω 3 represented $142.2\pm 8.6\%$ (df=38; $p<0.001$ versus unity) of their reference values in the duodenum and jejunum, as distinct from $83.2\pm 4.8\%$ (df=42; $p<0.005$ versus unity) in the caecum and colon.

Ratio between selected long-chain polyunsaturated ω 3 fatty acids in intestinal phospholipids. As expected from the results so far presented, the paired ratio between selected ω 3 fatty acids often differed in distinct segments in the intestinal tract in control animals (Table II). For instance, in the control rats, the C20:5 ω 3/C18:3 ω 3 ratio averaged 1.38 ± 0.11 ($n=14$) in the duodenum and jejunum, as distinct ($p<0.005$ or less) from 2.15 ± 0.16 ($n=9$) and 2.28 ± 3.0 ($n=7$) in caecum and colon, respectively. Inversely, in the control rats, the C22:6 ω 3/C20:5 ω 3 ratio was lower ($p<0.02$) in the caecum (5.02 ± 0.63 ; $n=11$) than in the duodenum and jejunum (6.97 ± 0.47 ; $n=14$), whilst the colon yielded an in-between value (6.47 ± 1.81 ; $n=11$) not significantly different ($p>0.4$ or more) from those recorded in either the duodenum and jejunum or caecum. Last, the C22:5 ω 3/C22:6 ω 3 ratio followed, in the control rats, the following hierarchy: duodenum and jejunum (0.146 ± 0.009 ; $n=14$) < colon (0.199 ± 0.011 ; $n=10$) < caecum (0.323 ± 0.017 ; $n=11$), the latter 3 mean values being all significantly different from one another ($p<0.002$ or less).

In the duodenum and jejunum of control rats examined during the first 7 months of the present experiments, the same C20:5 ω 3/C18:3 ω 3 ratio averaged 1.38 ± 0.11 ($n=14$). It could only be once estimated in the control rats exposed to the soybean oil-enriched diet, and was decreased ($p<0.001$) to 0.74 ± 0.07 ($n=12$) in the control rats exposed to the flaxseed oil-enriched diet. The values recorded in the ω 3D rats exposed to the flaxseed oil-enriched diet were somewhat lower, albeit not significantly so ($p<0.1$), averaging $71.9\pm 11.9\%$ ($n=18$) of the mean corresponding values recorded at the same level (duodenum or jejunum) in the control animals also given access to a flaxseed oil-enriched diet ($100.0\pm 7.7\%$; $n=12$). A comparable situation prevailed in the lower segments of the intestinal tract. In the caecum, the C20:5 ω 3/C18:3 ω 3 ratio indeed decreased ($p<0.001$) from a mean value of 2.15 ± 0.16 ($n=9$) in the control rats examined during the first 7 months to respectively 0.62 ± 0.06 ($n=6$) and 0.60 ± 0.06 ($n=12$) in the control animals and ω 3D rats both exposed to a flaxseed oil-enriched diet. Likewise, in the colon, the C20:5 ω 3/C18:3 ω 3 ratio was higher ($p<0.01$ or less) in the control rats examined

during the first 7 months of the present experiments (2.28 ± 0.30 ; $n=7$) than either the control animals (0.99 ± 0.13 ; $n=5$) or ω 3D rats (1.05 ± 0.09 ; $n=11$) both given access to a flaxseed oil-enriched diet.

In the control animals exposed to the soybean oil-enriched diet, the C20:5 ω 3/C18:3 ω 3 ratio could only be estimated once in the duodenum, but never in either the jejunum or colon. In the caecum, it averaged 0.89 ± 0.06 ($n=5$), a value also lower ($p < 0.001$) than that recorded in the control animals during the first 7 months of the present experiments.

In essence, comparable results were collected as far as the C22:6 ω 3/C20:5 ω 3 ratio is concerned. In the duodenum and jejunum phospholipids of control animals examined during the first 7 months of the present experiments, it averaged 6.97 ± 0.47 ($n=14$) and was decreased ($p < 0.001$) to 4.15 ± 0.43 ($n=28$) in the control and ω 3D rats exposed to the flaxseed oil-enriched diet. More precisely, in these rats, the values recorded in the duodenum (2.97 ± 0.42 ; $n=15$) and jejunum (5.52 ± 0.62 ; $n=13$) represented no more ($p < 0.005$) than $60.8 \pm 6.0\%$ ($n=28$) of the mean corresponding values found at the same level of the intestinal tract in the control animals examined just before exposure to the flaxseed oil-enriched diet ($100.0 \pm 9.9\%$; $n=8$). Likewise, in the caecum and colon, respectively, the C22:6 ω 3/C20:5 ω 3 ratio decreased ($p < 0.005$ or less) from 5.85 ± 0.45 and 7.59 ± 2.90 ($n=5$ in both cases) in the control animals examined after the first 7 months of the present experiments to 2.59 ± 0.36 ($n=18$) and 2.34 ± 0.36 ($n=16$) in the control and ω 3D rats exposed to a flaxseed oil-enriched diet. Whenever measurable, the values found in the control animals exposed to the soybean oil-enriched diet failed to differ significantly ($p > 0.5$ or more) from those recorded theretofore, whether in the duodenum (6.79 ± 2.32 ; $n=2$), caecum (7.03 ± 0.51 ; $n=5$) or colon (6.68 ± 0.21 ; $n=2$).

A mirror image prevailed in the case of the intestinal phospholipid C22:5 ω 3/C22:6 ω 3 ratio. In the duodenum and jejunum, such a ratio indeed increased ($p < 0.001$) from 0.146 ± 0.009 ($n=14$) in the control animals examined during the first 7 months of the present experiments to 0.546 ± 0.062 ($n=12$) and 0.490 ± 0.036 ($n=24$) in the control animals and ω 3D rats, respectively, both exposed to a flaxseed oil-enriched diet. Likewise, in the caecum, the same 3 values averaged 0.323 ± 0.017 ($n=11$) *versus* ($p < 0.001$) 0.732 ± 0.065 ($n=6$) and 0.695 ± 0.079 ($n=12$). In the colon also, the same 3 values averaged 0.199 ± 0.011 ($n=10$) *versus* ($p < 0.005$ or less) 0.403 ± 0.053 ($n=6$) and 0.316 ± 0.030 ($n=12$). Thus, the C22:5 ω 3/C22:6 ω 3 ratio was invariably higher in the control animals after then before exposure to the flaxseed oil-enriched diet. As a rule, it failed, however, to differ significantly ($p > 0.2$ or more) in the control animals examined before or after exposure to the soybean oil-enriched diet. In this respect, a significant difference ($p < 0.001$) was only observed in the duodenum and jejunum of control animals, in which the exposure to the soybean oil-enriched diet increased the C22:5 ω 3/C22:6 ω 3 ratio from 0.146 ± 0.009 ($n=14$) to 0.212 ± 0.015 ($n=12$).

Discussion

The data presented in this article and the two further companion reports (7,8) and concerning the female rats

examined 3 months after the start of the present experiments are in good agreement with those collected by Korotkova and Strandvik (9) in 6 rats of unspecified gender and age receiving for 7 weeks a control diet tightly comparable to that used in our study. Indeed, for the 14 fatty acids identified in both studies in the phospholipids of intestinal mucosa, the coefficient of correlation between the molar percentages of each fatty acid in the two studies amounted to $+0.9887$ and $+0.9582$ ($n=14$ and $p < 0.001$ in both cases) in the duodenum and/or jejunum and colon, respectively. The paired ratio between such percentages, as recorded in the present and prior study, averaged in the proximal and distal segments of the intestinal tract $98.0 \pm 11.2\%$ ($n=24$; $p > 0.8$ *versus* unity). Two fatty acids were excluded from the latter comparison, namely C12:0, because it yielded null values in our study, and C22:6 ω 3, for which our mean molar percentages unexpectedly represented in the duodenum-jejunum and colon almost thrice ($293.1 \pm 5.0\%$; $n=2$) the mean corresponding values reported by Korotkova and Strandvik (9). Even so, however, the difference between the mean molar percentages of each of the 14 fatty acids in the two studies under consideration did not exceed, ignoring their sign (positive or negative) $1.0 \pm 0.3\%$ ($n=14$) in the duodenum and/or jejunum and $1.7 \pm 0.6\%$ ($n=14$) in the colon. Moreover and most importantly, the differences in molar percentages between the data collected in the duodenum and/or jejunum and in the colon were also quite comparable in the two studies, yielding for all 14 fatty acids under consideration differences with the same sign (positive or negative) and a highly significant positive correlation ($r = +0.9795$; $n=14$; $p < 0.0001$).

Little information was previously available on the issue considered in the present report. Garg *et al* (10) reported that, in rats, jejunal microsomal phospholipids contained a higher level of C20:5 ω 3 but reduced level of C22:6 ω 3 when compared with those from the ileum. Higher $\Delta 4$, $\Delta 5$ and $\Delta 6$ desaturase activities were also found in ileal compared with jejunal enterocytes. Ruiz-Gutierrez *et al* (11) indicated that phospholipids account for 90% of the total lipid content of rat caecal mucosa, with a relative C22:6 ω 3 content of 11.1% in phosphatidylserine. Hess *et al* (12) reported that feeding one-day-old pigs a milk-based diet enriched with C20:5 ω 3 (5% of the total fatty acids, wt:wt) provokes within 8 days a close to 20-fold increase of this fatty acid content in jejunal mucosa phospholipids. Likewise, Garg *et al* (13) observed that feeding a diet rich in ω 3 fatty acids increases C20:5 ω 3 and C22:6 ω 3 levels in both jejunal and ileal rat microsomes.

The present study affords four major pieces of information. First, it documents that, even in control rats, the relative weight content of long-chain polyunsaturated ω 3 fatty acids in intestinal phospholipids, as well as the product/precursor ratio for such ω 3 fatty acids, often differs in distinct segments of the intestinal tract.

Second, it reveals that, within 3 months of dietary ω 3-deprivation, the intestinal phospholipid content of C18:3 ω 3, C20:5 ω 3 and C22:5 ω 3 reaches low values, below the limit of detection by the present experimental procedure. Even the C22:6 ω 3 relative weight content became, within 3 months of ω 3-deprivation much lower than in the control rats, and was further decreased after 7 months of dietary ω 3-deprivation.

Third, it indicates that exposure of the ω 3D rats for 2 to 4-5 weeks to a flaxseed oil-enriched diet dramatically increases

the intestinal phospholipid content in all ω 3 fatty acids. In the case of C18:3 ω 3, C20:5 ω 3 and C22:6 ω 3, the values reached in the ω 3D rats exposed to the flaxseed oil-enriched diet even exceeded those otherwise found in rats maintained on the control diet. In the case of C22:6 ω 3, a progressive return towards the latter control values was also observed in the ω 3D rats exposed to the flaxseed oil-enriched diet.

Last, the ratio between selected ω 3 fatty acids was also markedly affected under the same experimental conditions. As a result of exposure to the flaxseed oil-enriched diet, with a C18:3 ω 3 relative weight content 4 to 6 times higher than that prevailing in the control diet or soybean oil-enriched diet, the C20:5 ω 3/C18:3 ω 3 ratio was markedly decreased at all levels of the intestinal tract and in both control and ω 3D rats. Such was not the case, however, in the control rats exposed to a soybean oil-enriched diet.

A comparable situation prevailed in the case of the C22:6 ω 3/C20:5 ω 3 ratio, whilst the C22:5 ω 3/C22:6 ω 3 provided a mirror image. Such a ratio indeed increased, in both control and ω 3D rats, in response to the exposure of these animals to the flaxseed oil-enriched diet. Once again, and except for a minor increase of the C22:5 ω 3/C22:6 ω 3 in the duodenum and jejunum, the latter ratio failed to display obvious changes when the control rats were exposed to the soybean oil-enriched diet.

These findings reinforce the view that, in the present animal model, a dietary deprivation of ω 3 fatty acids, initiated in female rats at the age of 7 weeks, is quite efficient to provoke a severe depletion of long-chain polyunsaturated ω 3 fatty acids in several organs, including the intestinal tract. Inversely, the exposure of the ω 3D rats for only 2 to 4-5 weeks to an ω 3-enriched diet is quite efficient to restore close-to-normal or even higher than control values for the relative weight content of the ω 3 fatty acids in intestinal phospholipids. At this point, it should be stressed that other changes in the fatty acid profile of intestinal phospholipids, especially in terms of their content in saturated and mono-desaturated fatty acids, as well as long-chain polyunsaturated ω 6 fatty acids, also occur under the present experimental conditions, as will be documented in further reports in this series.

Acknowledgements

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 and A. Dufour for technical assistance, and C. Demesmaeker for secretarial help.

References

1. Malaisse WJ, Bulur N, Zhang Y, Hacquebard M, Portois L, Sener A and Carpentier YA: The metabolic syndrome of ω 3-depleted rats. I. Liver data. *Int J Mol Med* 24: 111-123, 2009.
2. Sener A, Zhang Y, Bulur N, Louchami K, Malaisse WJ and Carpentier YA: The metabolic syndrome of ω 3-depleted rats. II. Body weight, adipose tissue mass and glycemic homeostasis. *Int J Mol Med* 24: 125-129, 2009.
3. Portois L, Hacquebard M, Malaisse WJ and Carpentier YA: The metabolic syndrome of ω 3-depleted rats. III. Brain phospholipids. *Int J Mol Med* 24: 269-278, 2009.
4. 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.
5. 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.
6. Lepage G and Roy CC: Direct transesterification of all classes of lipids in a one step reaction. *J Lipid Res* 27: 114-120, 1986.
7. Hacquebard M, Portois L, Malaisse WJ and Carpentier YA: The metabolic syndrome of ω 3-depleted rats. V. Intestinal phospholipid ω 6 fatty acids. *Int J Mol Med* 24: 867-875, 2009.
8. Carpentier YA, Hacquebard M, Portois L and Malaisse WJ: The metabolic syndrome of ω 3-depleted rats. VI. Intestinal phospholipid saturated and monodesaturated fatty acids. *Int J Mol Med* (In press).
9. Korotkova M and Strandvik B: Essential fatty acid deficiency affects the fatty acid composition of the rat small intestinal and colonic mucosa differently. *Biochim Biophys Acta* 1487: 319-325, 2000.
10. Garg ML, Keelan M, Wierzbicki A, Thomson AB and Clandinin MT: Regional differences in lipid composition and incorporation of saturated and unsaturated fatty acids into microsomal membranes of rat small intestine. *Can J Physiol Pharmacol* 66: 794-800, 1988.
11. Ruiz-Gutierrez V, Vazquez CM and Quintero FJ: Lipid composition, phospholipid profile and fatty acid of rat caecal mucosa. *Biochim Biophys Acta* 1128: 199-204, 1992.
12. Hess HA, Cort BA, Lin X, Jacobi SK, Harrell RJ, Blikslager AT and Odle J: Enrichment of intestinal mucosa phospholipids with arachidonic and eicosapentaenoic acids fed to suckling piglets is dose and time dependent. *J Nutr* 138: 2164-2171, 2008.
13. Garg ML, Keelan M, Thomson AB and Clandinin MT: Desaturation of linoleic acid in the small bowel is increased by short-term fasting and by dietary content of linoleic acid. *Biochim Biophys Acta* 1126: 17-25, 1992.