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The metabolic syndrome of ω3-depleted rats. V. Intestinal phospholipid ω6 fatty acids

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Abstract. This study aims mainly at investigating the effects of a dietary deprivation and replenishment of ω3 PUFA upon the phospholipid pattern of ω6 PUFA in the duodenum, jejunum, caecum and colon of rats exposed for 3-7 months to an ω3-depleted diet and then eventually exposed for 2-4 weeks to an ω3-rich diet. In control rats, the relative weight content of all ω6 fatty acids differed in the proximal and distal intestinal segments. In the ω3-depleted rats the C18:2ω6, C20:2ω6 and C20:3ω6 content was decreased whilst that of C20:4ω6 and C22:4ω6 was increased. Significant correlations were found in the caecum or colon between the C18:2ω6 or C20:4ω6 content of intestinal phospholipids and their C22:6ω3 content, an increase in the latter content coinciding with an increase in C18:2ω6 and decrease of C20:4ω6. Such was also the case for C20:4ω6, but not C18:2ω6, in the duodenum and jejunum. At these proximal intestinal levels, exposure of the ω3-depleted rats to a flaxseed oil-enriched diet indeed decreased the C18:2ω6 phospholipid content, an effect possibly attributable to the much lower content of C18:2ω6 in the latter diet, as distinct from the sunflower diet offered to the ω3-depleted rats during the first 7 months. However, at more distal intestinal levels, and like in the liver, a deficiency in ω3 fatty acids apparently favours the stepwise conversion of C18:2ω6 to C20:4ω6 and C22:4ω6.

Introduction

In the preceding study in this series, the relative weight content of long-chain polyunsaturated ω3 fatty acids in the phospholipids of the duodenum, jejunum, caecum and colon was examined in rats exposed from the seventh week after birth and for the following 3 to 7 months either to a control diet or an ω3-depleted diet (1). The time course for the repletion of ω3 fatty acids in the intestinal phospholipids was also investigated during exposure of the ω3-deficient rats for 2 to 4-5 weeks to a flaxseed oil-enriched diet. For the sake of comparison, the control rats were also given access for the last 4-5 weeks before sacrifice to either a soybean or flaxseed oil-enriched diet. The present study extends this information to the five long-chain polyunsaturated ω6 fatty acids identified in the intestinal phospholipids of these 8 groups of rats.

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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 (2). 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 (2). The small and large bowel segments were removed from mesenteric and vascular connections and sequentially removed from the peritoneum. Segments used for intestinal mucosa fatty acid analysis were the duodenum (5 cm distal to the pylorus), jejunum (20 cm distal from duodenum), caecum and 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 (3), separated by thin-layer chromatography (4), and their fatty acid pattern determined by gas-liquid chromatography (5).

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 ω3-depleted rats (ω3D) 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 diet, and 7mD/2wF and 7mD/4wF for the ω3D rats eventually exposed for 2 or 4-5 weeks to the flaxseed oil-enriched diet.

All results are presented as mean values (± 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

Methodological considerations. The individual absolute values for both the C18:2ω6, i.e. the most abundant long-chain polyunsaturated ω6 fatty acids, and total fatty acid content of intestinal phospholipids displayed a rather high individual scatter. For instance, for the 7 sets of data collected in the control animals during the first 7 months of the present experiments (Table I), the coefficient of variation averaged 33.2±7.0% and 39.2±7.4% (n=7 in both cases) for the total and C18:2ω6 results, respectively. The two sets of variation coefficients were tightly correlated (r=0.9620; p<0.001), with a C18:2ω6/total paired ratio of 121.4±5.9% (n=7; p<0.02 *versus* unity). As expected from these findings, the coefficient of variation for the weight content of C18:2ω6 expressed relative to the total fatty acid content of phospholipids, which averaged 11.9±2.8% (n=7), was much lower (p<0.02 or less) than that found for the absolute values of either the C18:2ω6 or total fatty acid content of intestinal phospholipids. It represented no more than 43.1±11.1% (p<0.005 *versus* unity) and 33.9±8.1% (p<0.001 *versus* unity) of the paired value found for the total fatty acid content of phospholipids and their C18:2ω6 absolute content, respectively. Hence, the relative weight content of each fatty acid was used in all further analyses. This approach was further validated by the finding that, in the same 7 sets of data, no significant correlation (r=0.2443; n=36; p>0.1) was found between the individual values for the C18:2ω6 relative weight content and total fatty acid content of intestinal phospholipids, both variables being expressed relative to their respective mean value within each of these 7 sets of data in order to avoid interference of any group effect. Using the same analytical procedure, an even lower correlation coefficient (r=0.0790; n=36) was found, in the same 7 sets of data, between the individual values for the relative weight content of C20:4ω6, the second most abundant long-chain polyunsaturated ω6 fatty acid in intestinal phospholipids, and the total fatty acid content of such phospholipids.

Long-chain polyunsaturated ω6 fatty acid content in intestinal phospholipids. In the control rats, the relative weight content of C18:2ω6, which was the most abundant long-chain polyunsaturated ω6 fatty acid in intestinal phospholipids, averaged in the duodenum and jejunum 34.2±0.8% (n=14) as distinct (p<0.001) from 24.0±1.4% (n=11) in the caecum, being further decreased (p<0.02) to 19.6±0.9% (n=11) in the colon. In the ω3D rats, a comparable hierarchy was observed, the measurements made in the caecum and colon representing, respectively 59.2±3.6 and 49.9±1.2% (n=11 in both cases) of those recorded at the same age in the duodenum and jejunum (100.0±5.2%; n=18). Thus, in these ω3D rats, the relative weight content of C18:2ω6 was indeed lower (p<0.001) in the caecum than in the duodenum and jejunum, and further decreased (p<0.025) in the colon.

The relative weight content of C18:2ω6 in intestinal phospholipids was lower in ω3D rats than in control animals both examined during the first 7 months of the present experiments. In the duodenum and jejunum, caecum and colon, it averaged in the control animals and ω3D rats, respectively, 32.44±0.76% (n=14) *versus* 27.58±1.51% (n=18; p<0.02), 24.01±1.36% (n=11) *versus* 16.17±1.13% (n=12; p<0.001), and 19.60±0.91% (n=11) *versus* 13.55±0.34% (n=12; p<0.001). Already after 3 months of dietary ω3 deprivation, the C18:2ω6 relative weight content of intestinal phospholipids was significantly lower (p<0.005 or less) in ω3D rats than in control animals, whether in the duodenum and jejunum, caecum or colon. After 7 months of dietary ω3 deprivation, the relative weight content of C18:2ω6 in phospholipids averaged in the ω3D rats 84.1±3.9% (n=24; p<0.004) of the mean corresponding values found at the same age and same level of the intestinal tract (duodenum, jejunum, caecum and colon) in the control animals (100.0±2.2%; n=18). The relative magnitude of such a decrease (15.9±4.9%; df=40) was less pronounced (p<0.02), however, than that recorded 3 months after the start of the present experiments (34.3±4.9%; df=34).

In the duodenum and jejunum, the relative weight content of C18:2ω6 was markedly decreased when the control rats were exposed for 4-5 weeks to either the soybean or flaxseed oil-enriched diet, the values recorded in the latter rats averaging, respectively 65.5±3.8% and 62.8±2.7% (n=12 and p<0.001, in both cases) of the corresponding mean values found at the same level of the intestinal tract in the control rats examined 7 weeks after the start of the present experiments (100.0±2.8%; n=8). Such a decrease did not occur in the caecum and colon, which yielded after exposure to the soybean and flaxseed oil-enriched diet, respectively, results representing 103.3±4.6% and 95.4±4.7% (n=12 and p>0.4 in both cases) of those recorded at the same level of the intestinal tract in the control rats examined just before such an exposure (100.0±3.5%; n=10). Likewise, in the ω3D rats exposed for 2 weeks to the flaxseed oil-enriched diet, the C18:2ω6 relative content of duodenal and jejunal phospholipids decreased (p<0.05) to 82.0±4.3% of the corresponding values found at the same level of the intestinal tract just before such an exposure (100.0±7.4%; n=12) and further diminished (p<0.01) to 64.5±3.7% (n=12) of the same mean reference values after 4-5 weeks exposure to the flaxseed oil-enriched diet. Once again, such a decrease was not observed in the caecum and colon, in which case the measurements made in the ω3D rats after 2 and 4-5 weeks exposure to the flaxseed oil-enriched diet yielded, on the contrary, an overall mean value (115.4±3.7%; n=24) significantly higher (p<0.01) than the reference value derived from the measurements made at the same level in the ω3D rats examined after 7 months of dietary ω3-deprivation (100.0±3.6%; n=12). Even so, however, after 4-5 weeks exposure to the flaxseed oil-enriched diet, the mean relative weight content of C18:2ω6 in intestinal phospholipids remained, at all levels of the intestinal tract, somewhat lower in ω3D rats than in control animals. Such a difference failed, however, to achieve statistical significance (p<0.09), the results

Veight content (%) of long-chain polyunsaturated ω6 fatty acids in intestinal phospholipids.

Rats	Intestinal segments	C18:2ω6	C20:2ω6	C20:3ω6	C20:4ω6	C22:4ω6
3mC	Duodenum + Jejunum	33.45±1.21 (6)	0.55±0.03 (6)	0.84±0.08 (6)	14.32±0.78 (6)	0.37±0.03 (6)
	Caecum	25.05±2.13 (6)	1.27±0.09 (6)	1.73±0.10 (6)	17.12±1.31 (6)	1.40±0.17 (6)
	Colon	20.02±1.71 (6)	1.40±0.15 (6)	2.47±0.36 (6)	17.57±1.56 (6)	1.50±0.44 (6)
3mD	Duodenum + Jejunum	25.97±0.73 (6)	0.78±0.03 (6)	1.20±0.07 (6)	19.32±0.32 (6)	1.32±0.13 (6)
	Caecum	13.33±0.97 (6)	0.67±0.14 (6)	1.40±0.17 (6)	23.98±0.82 (6)	4.97±0.42 (6)
	Colon	13.27±0.41 (6)	1.00±0.22 (6)	1.80±0.39 (6)	18.38±1.54 (6)	1.78±0.12 (6)
7mC	Duodenum	30.57±0.58 (3)	0.61±0.01 (3)	1.25±0.13 (3)	12.67±1.27 (3)	0.47±0.03 (3)
	Jejunum	32.34±1.47 (5)	0.57±0.04 (5)	0.83±0.07 (5)	15.08±1.36 (5)	0.50±0.04 (5)
	Caecum	22.76±1.63 (5)	1.26±0.21 (5)	1.77±0.06 (5)	16.82±0.93 (5)	1.72±0.20 (5)
	Colon	19.10±0.29 (5)	1.26±0.09 (5)	2.63±0.20 (5)	16.02±0.78 (5)	1.44±0.22 (5)
7mD	Duodenum	26.67±2.00 (6)	0.61±0.03 (6)	1.07±0.05 (6)	20.88±1.10 (6)	1.07±0.11 (6)
	Jejunum	30.10±4.09 (6)	0.55±0.05 (6)	0.71±0.07 (6)	18.22±1.35 (6)	1.58±0.82 (6)
	Caecum	19.00±1.22 (6)	1.16±0.13 (6)	1.21±0.41 (6)	22.08±0.75 (6)	3.30±0.39 (6)
	Colon	13.83±0.55 (6)	1.25±0.08 (6)	2.26±0.16 (6)	20.13±1.31 (6)	2.50±0.19 (6)
7mC/4wS	Duodenum	20.20±1.72 (6)	0.79±0.10 (6)	1.16±0.16 (6)	17.45±1.78 (6)	1.73±0.51 (6)
	Jejunum	21.02±1.85 (6)	1.09±0.10 (6)	1.29±0.09 (6)	13.28±1.56 (6)	1.80±0.32 (6)
	Caecum	25.82±1.21 (6)	1.42±0.09 (6)	1.92±0.10 (6)	14.60±0.25 (6)	1.58±0.07 (6)
	Colon	17.80±0.94 (6)	1.53±0.07 (6)	2.76±0.27 (6)	14.07±0.58 (6)	1.37±0.08 (6)
7mC/4wF	Duodenum	20.17±0.96 (6)	0.65±0.14 (6)	1.21±0.11 (6)	12.53±0.69 (6)	0.80±0.08 (6)
	Jejunum	19.27±1.37 (6)	0.73±0.06 (6)	1.19±0.12 (6)	14.88±1.40 (6)	1.25±0.32 (6)
	Caecum	23.08±1.60 (6)	1.00±0.05 (6)	1.75±0.11 (6)	12.87±0.60 (6)	1.02±0.21 (6)
	Colon	17.07±1.13 (6)	1.14±0.24 (6)	2.27±0.24 (6)	12.65±1.60 (6)	1.07±0.35 (6)
7mD/2wF	Duodenum	22.92±1.99 (6)	0.56±0.05 (6)	1.04±0.08 (6)	13.62±1.21 (6)	0.73±0.13 (6)
	Jejunum	23.52±1.36 (6)	0.76±0.05 (6)	1.10±0.05 (6)	12.03±0.91 (6)	0.75±0.07 (6)
	Caecum	19.40±0.99 (6)	1.13±0.08 (6)	1.61±0.08 (6)	15.93±0.66 (6)	1.58±0.08 (6)
	Colon	17.73±1.37 (6)	1.25±0.17 (6)	2.44±0.19 (6)	16.18±1.42 (6)	0.93±0.12 (6)
7mD/4wF	Duodenum	19.52±1.07 (6)	0.22±0.15 (6)	0.41±0.19 (6)	12.62±1.31 (6)	0.45±0.24 (6)
	Jejunum	16.80±1.15 (6)	0.72±0.05 (6)	0.93±0.06 (6)	11.43±1.56 (6)	1.72±0.35 (6)
	Caecum	21.07±1.17 (6)	0.92±0.08 (6)	1.32±0.06 (6)	14.98±1.15 (6)	1.40±0.12 (6)
	Colon	16.65±0.39 (6)	1.19±0.05 (6)	2.02±0.19 (6)	13.60±0.96 (6)	0.73±0.15 (6)

recorded in the ω3D rats averaging 93.2±2.4% (n=24) of the corresponding mean values recorded at the same level of duodenum, jejunum, caecum, colon) in the control rats also exposed for 4-5 weeks to a flaxseed oil-enriched diet (100.0±3.6%; n=12).

A sizeable amount of C18:3ω6 (0.48±0.24%; n=7) was only detected in 7 out of 174 intestinal samples.

At variance with C18:2ω6, the C20:2ω6 content of phospholipids was, in the control animals, lower ($p<0.001$) in the duodenum and jejunum (0.57±0.02%; n=84) than in either the caecum (1.27±0.10%; n=11) or colon (1.33±0.09%; n=11). It failed to differ significantly ($p>0.3$) in control animals and ω3D rats, the results recorded in the latter rats averaging 93.5±5.2% (n=42) of the mean corresponding values found at the same age and same location of the intestinal tract in control animals (100.0±3.3%; n=36). When the control rats were exposed for 4-5 weeks to the soybean oil-enriched diet, the C20:2ω6 content of intestinal phospholipids increased ($p<0.002$) to 138.7±8.9% (n=24) the corresponding mean values found at the same level of the intestinal tract in the control rats examined just before such an exposure (100.0±5.0%; n=18). However, when the control rats were exposed for 4-5 weeks to the flaxseed oil-enriched diet, the C20:2ω6 content of intestinal phospholipids only represented 73.5±5.9% (n=24; $p<0.001$) of the mean corresponding values recorded at the same age and intestinal location in the control rats exposed for the same time to the soybean oil-enriched diet. In the ω3D rats exposed for 2 and 4-5 weeks to the flaxseed oil-enriched diet, the C20:2ω6 content of intestinal phospholipids averaged, respectively, 103.1±5.1% and 82.3±8.3% (n=24 in both cases) of the mean corresponding values recorded in the control animals also exposed to the flaxseed oil-enriched diet (100.0±7.5%). In other words, as already observed during the first 7 months of the present experiments, the results recorded in ω3D rats were somewhat lower, albeit not significantly so (p>0.1) than those found, under the same experimental conditions, in the control animals, i.e. after 4-5 weeks exposure to the flaxseed oil-enriched diet. Even when pooling together these two sets of observations, the difference between the reference values found in control animals (100.0±3.5%; n=60) and the results recorded in ω3D rats (89.4±4.5%; n=66) still failed to achieve statistical significance (p<0.08).

The results concerning the C20:3ω6 content of intestinal phospholipids resembled, in several respects, those just mentioned in the case of C20:2ω6.

First, the C20:3ω6 content of phospholipids was, in the control animals, lower (p<0.001) in the duodenum and jejunum (0.93±0.06%; n=14) than in the caecum (1.75±0.06%; n=11) and further increased (p<0.001) in the colon (2.54±0.19%; n=11). Second, it failed to be significantly lower (p<0.1) in the control and ω3D rats, the measurements collected in the latter rats averaging 88.8±5.6% (n=42) of the mean corresponding values recorded at the same age and intestinal location in the control animals (100.0±3.1%; n=36). Third, when the control rats were exposed for 4-5 weeks to the soybean oil-enriched diet, the C20:3ω6 content of phospholipids averaged 115.4±6.9% (n=24) of the mean corresponding values found at the same level of the intestinal tract shortly before such an exposure (100.0±3.3%; n=18). The difference between the two latter percentages failed, however, to achieve statistical significance (p<0.08). Fourth, the C20:3ω6 content of phospholipids represented, in the control animals exposed for 4-5 weeks to the flaxseed oil-enriched diet, 92.6±4.2% (n=24) of the mean corresponding values found at the same age and same intestinal location in the control animals fed the soybean oil-enriched diet, such a difference again failing to achieve statistical significance. Last, the C20:3ω6 relative weight content of phospholipids decreased in the ω3D rats exposed for 2 and 4-5 weeks to the flaxseed oil-enriched diet to respectively 94.3±3.5 and 69.1±6.2% (n=24 in both cases) of the corresponding mean values found at the same intestinal level in the control animals also exposed to the flaxseed oil-enriched diet (100.0±4.2%; n=24). When the latter results were taken into consideration together with those collected during the first seven months of the present experiments, the C20:3ω6 content of intestinal phospholipids averaged, in the ω3D rats, 81.7±4.3% (n=66; p<0.001) of the mean corresponding values found at the same age and intestinal location in the control animals (100.0±2.5%; n=60).

In the latter respect, the results obtained with C18:2ω6, C20:2ω6 and C20:3ω6 were quite similar, as illustrated in Fig. 1, yielding an overall mean value in the ω3D rats of 84.5±2.2% (n=198; p<0.001) of the mean corresponding reference values found in control animals (100.0±1.6%; n=180).

A mirror image was found for the last two other long-chain polyunsaturated ω6 fatty acids, namely C20:4ω6 and C22:4ω6 (Fig. 1).

In mirror image of the situation found for C18:2ω6, the relative weight content of intestinal phospholipids in C20:4ω6 was, in the control animals, lower (p<0.025) in the duodenum and jejunum (14.2±0.6%; n=14) than in the caecum (17.0±0.8%; n=11) or colon (16.9±0.9%; n=11).

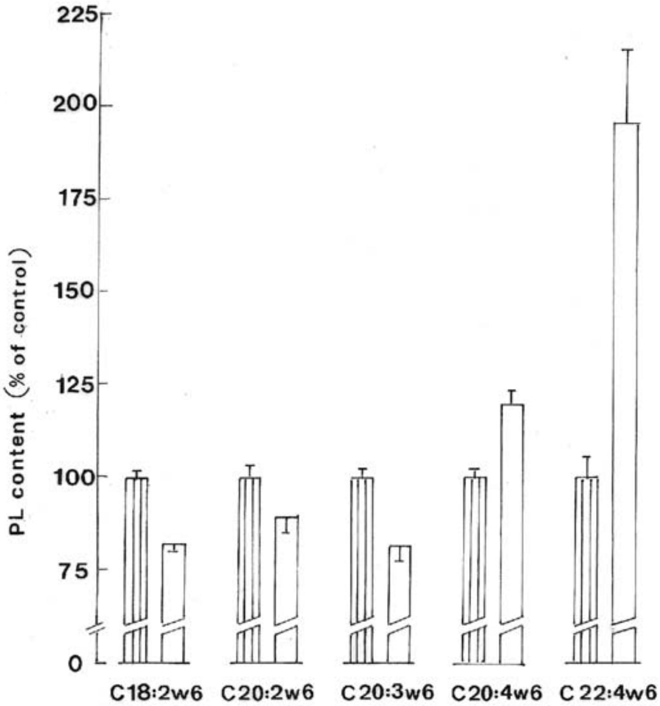

Figure 1. Relative weight content of long-chain polyunsaturated ω6 fatty acids in the intestinal phospholipids of control animals (vertically hatched columns) and ω3D rats (open columns). All results are expressed relative to the mean values found at the same intestinal level in control animals of the same age. Mean values (± SEM) refer to 60 (control) and 66 (ω3D rats) individual determinations in all cases.

Over the first 7 months of the present experiment, the mean relative weight content of C20:4ω6 was higher in the ω3D rats than in the control animals, whether in the duodenum and jejunum (p<0.001), caecum (p<0.001) or colon (p<0.11). In the duodenum and jejunum and in the caecum, it was already significantly increased (p<0.005 or less) after 3 months exposure to the ω3-deficient diet. In the colon, however, no significant difference (p>0.7) was found at this early time, a significant increase (p<0.04) being only recorded after 7 months exposure to the ω3-deficient diet. At that time, the relative magnitude of the increase in C20:4ω6 relative weight content of phospholipids appeared higher (p<0.05) in the duodenum (+64.8±14.3%; df=7) than in the jejunum (+20.0±12.8%; df=9), caecum (+31.3±7.0%; df=9) or colon (+25.7±10.1%; df=9).

When the control rats were exposed for 4-5 weeks to the soybean oil-enriched diet, the C20:4ω6 content of intestinal phospholipids averaged 100.1±6.2% (n=24; p>0.98) of the mean corresponding values found at the same level of the intestinal tract just before exposure to such a diet (100.0±3.3%; n=18). More precisely, the C20:4ω6 relative weight failed indeed to differ significantly (p>0.1) in duodenal phospholipids, whilst the measurements made in lower segments of the intestinal tract averaged 87.6±3.5% (n=18; p<0.02) of the corresponding reference values (100.0±3.6%; n=15). When the control rats were exposed for 4-5 weeks to the flaxseed oil-enriched diet, a modest but significant (p<0.05) decrease in the C20:4ω6 relative content of intestinal phospholipids was observed, down to 88.3±4.2% (n=24) of the mean corresponding values recorded at the same level of the

SPANDIDOS PUBLICATIONS Weight content (%) of C22:5ω6 in intestinal phospholipids.

Rats	Duodenum	Jejunum	Caecum	Colon
7mC	N.D. (3)	N.D. (5)	N.D. (5)	N.D. (5)
7mD	1.87±0.19 (6)	1.72±0.41 (6)	2.53±0.47 (6)	2.57±0.25 (6)
7mC/4wS	0.18±0.08 (6)	0.05±0.05 (6)	N.D. (6)	N.D. (6)
7mC/4wF	N.D. (6)	N.D. (6)	N.D. (6)	N.D. (6)
7mD/2wF	N.D. (6)	N.D. (6)	0.30±0.13 (6)	0.16±0.08 (6)
7mD/4wF	0.06±0.06 (6)	0.26±0.09 (6)	N.D. (6)	N.D. (6)

N.D., not detected.

intestinal tract in the control rats examined 7 months after the start of the present experiments (100.0±3.3%; n=18). When the ω3D rats were exposed to the flaxseed oil-enriched diet, a rapid and greater decrease in C20:4ω6 phospholipid content was observed, the values recorded after 2 and 4-5 weeks exposure to such a diet, which failed to differ significantly (p>0.1) from one another, averaging respectively 71.1±2.8% and 64.6±3.0% (n=24 and p<0.001 in both cases) of the mean corresponding values found at the same level of the intestinal tract in the ω3D rats examined 7 weeks after the start of the present experiments (100.0±2.7%; n=24). The relative extent of such a decrease was similar at all levels of the intestinal tract, the measurements made after 4-5 weeks exposure to the flaxseed oil-enriched diet, expressed relative to those made just before exposure to such a diet, averaging 60.4±6.3% (n=6) in the duodenum, 62.7±8.6% (n=6) in the jejunum, 67.8±5.2% (n=6) in the caecum and 67.6±4.8% (n=6) in the colon. Moreover, the data recorded in the ω3D rats after 4-5 weeks exposure to the flaxseed oil-enriched diet were no more different from those recorded in the control animals also exposed for 4-5 weeks to the flaxseed oil-enriched diet, the data collected in the ω3D rats averaging 100.4±5.4% (n=24; p>0.95) of the mean corresponding values recorded at the same level in the control animals (100.0±4.0%; n=24).

The results concerning the C22:4ω6 content of intestinal phospholipids were, in several respects, similar to those obtained for their C20:4ω6 content. First, in the control animals, such a content was lower (p<0.001) in the duodenum and jejunum (0.44±0.03%; n=14) than in the caecum (1.55±0.13%; n=11) or colon (1.47±0.25%; n=11). Second, during the first 7 months of the present experiments, such a content was higher in the ω3D rats than in the control animals, whether in the duodenum and jejunum (p<0.01), caecum (p<0.001) or colon (p<0.05). Already after 3 months of dietary ω3 deprivation, the measurements made in the intestinal phospholipids averaged 276.8±30.8% (n=18; p<0.001) of the corresponding values recorded at the same level of the intestinal tract in the control animals of same age (100.0±10.3%; n=18). Third, when the control animals were exposed for 4-5 weeks to the soybean oil-enriched diet, the C22:4ω6 content of phospholipids increased (p<0.001) in the duodenum and jejunum from 0.44±0.03% (n=14) to 1.77±0.29% (n=12), whilst being not significantly affected (p>0.7 or more) in the caecum and colon. Fourth, when the control animals were exposed for 4-5 weeks to the flaxseed oil-enriched diet, the C22:4ω6 content of intestinal phospholipids was decreased (p<0.005) to 64.6±8.3% (n=24) of the corresponding mean values found at the same age and same location in the control animals exposed to the soybean oil-enriched diet (100.0±8.2%; n=14). Last, when the ω3D rats were exposed for 2 to 4-5 weeks to the flaxseed oil-enriched diet, the C22:4ω6 content of intestinal phospholipids rapidly decreased to a level representing 99.1±7.8% (n=48; p>0.9) of the mean corresponding values recorded at the same level in the control rats also exposed to a flaxseed oil-enriched diet (100.0±11.1%; n=24).

Despite the fact that the difference between control animals and ω3D rats, in terms of the C20:4ω6 and C22:4ω6 content of intestinal phospholipids, was more pronounced during the first 7 months of the present experiments than after exposure for 4-5 weeks to the flaxseed oil-enriched diet, the relative weight content of these two long-chain polyunsaturated ω6 fatty acids remained significantly higher (p<0.001) in ω3D rats than in control animals even when the latter data were also taken into account (Fig. 1). According to this analysis, the relative magnitude of the difference between control animals and ω3D rats was more pronounced (p<0.001) in the case of C22:4ω6 (+95.4±21.6%; df=124) than in the case of C20:4ω6 (+20.3±4.3%; df=124).

The results so far presented do not concern C22:5ω6, which was not measured in our routine analytical procedure. Table II summarizes the findings relative to the phospholipid relative weight content of C22:5ω6. This fatty acid remained below the limit of detection in all control rats examined during the first seven months of the present experiments, whatever the intestinal segment under consideration. It was invariably present in sizeable amounts in the ω3-depleted rats examined 3 or 7 months after the onset of the present experiments. Unexpectedly, it only represented after 7 months of dietary ω3 deprivation 75.5±6.1% (n=24; p<0.001) of the mean corresponding values found at the same intestinal level after only 3 months of dietary ω3 deprivation (100.0±5.0%; n=14). More precisely, such a difference achieved statistical significance (p<0.02) at the duodenal and/or jejunal level, in which case the relative weight content of C22:5ω6 in phospholipids averaged 2.67±0.11% (n=6) and 1.79±0.22% (n=12) after 3 and 7 months ω3 deprivation, respectively. At the caecal and colonic level, however, the results recorded after 7 months represented 84.0±9.6% (n=12; p>0.6) of the mean corresponding values found at the same intestinal level after only 3 months of dietary ω3 deprivation (100.0±8.5%; n=8). The results collected in the ω3-depleted rats averaged, at the caecal and colonic level, 130.2±9.8% (n=20; p<0.025) of the mean corresponding values found at the same age in the proximal intestinal segments (duodenum and/or jejunum), i.e. 100.0±8.1% (n=18).

When the control rats were exposed for 4-5 weeks to the flaxseed oil-enriched diet, the C22:5ω6 relative content of phospholipids remained below the limit of detection whether in the duodenum (n=6), jejunum (n=6), caecum (n=6) or colon (n=6). A comparable situation prevailed in the caecum (n=6) and colon (n=6) when the control rats were exposed for the same period of 4-5 weeks to the soybean oil-enriched diet. In the latter rats, a sizeable amount of C22:5ω6 in phospholipids was only detected in one (0.32%) out of 6 rats at the jejunal level and in 3 (0.36±0.02%; n=3) out of 6 rats at the duodenal level. These findings were not vastly different from those recorded in the ω3-depleted rats exposed for 2 to 4-5 weeks to the flaxseed oil-enriched diet. Thus, at the duodenal level, a sizeable amount of C22:5ω6 (0.33%) was only detected in one rat exposed for 5 weeks to the flaxseed oil-enriched diet, whilst such was not the case in the other 11 ω3-depleted rats exposed to the same diet. At the jejunal level, 4 rats exposed for 4-5 weeks to the flaxseed oil-enriched diet displayed sizeable amounts of C22:5ω6 in phospholipids (0.39±0.04%; n=4), whilst no C22:5ω6 could be detected in the other 8 rats exposed to the same diet. At the caecal level, only 3 rats exposed for 2 weeks to the flaxseed oil-enriched diet were found to contain a sizeable amount of C22:5ω6 (0.59±0.03%; n=3) in their phospholipids, whilst such was not the case in the other 9 ω3-depleted rats exposed to the same diet. Last, at the colonic level, a sizeable amount of C22:5ω6 in phospholipids was again only detected in 3 rats exposed for 2 weeks to the flaxseed oil-enriched diet (0.32±0.05%; n=3), whilst the phospholipid relative content of C22:5ω6 was below the limit of detection in the other 9 ω3-depleted rats exposed to the same diet. Pooling all available data, the C22:5ω6 relative content of phospholipids did not exceed 0.11±0.04% (n=24) and 0.08±0.03% (n=24) in the intestinal segments after 2 and 4-5 weeks exposure of the ω3-depleted rats to the flaxseed oil-enriched diet, respectively, as compared (p<0.001) to 2.17±0.18% (n=24) in the ω3-depleted rats examined just before exposure to this diet.

Ratio between selected long-chain polyunsaturated ω6 fatty acids. As expected from the data listed in Table I and illustrated in Fig. 1, the ratio between selected long-chain polyunsaturated ω6 fatty acids was affected by such factors as the intestinal location and dietary status of the animals (Table III).

When considering pairs of fatty acids interconverted in a single reaction, only three of such ratios could be established in the majority of rats. First, the C20:2ω6/C18:2ω6 ratio, whilst refering to an elongase-catalyzed interconversion, was lower in the duodenum and jejunum than in the caecum or colon, yielding in control animals mean values of 17.8±0.9 10^{-3} (n=14) in the duodenum and jejunum, as distinct (p<0.001) from 55.2±6.5 10^{-3} (n=11) and 68.1±3.0 10^{-3} (n=11) in the caecum and colon, respectively. The C20:2ω6/C18:2ω6 ratio averaged in the ω3D rats 125.5±9.1% (n=42; p<0.02) of the mean corresponding values found, at the same intestinal level, in control animals of the same age (100.0±4.0%; n=36). When the control animals were exposed for 4-5 weeks to the soybean oil-enriched diet, the C20:2ω6/C18:2ω6 ratio increased (p<0.002) to 183.3±10.1% (n=24) of the mean corresponding values found at the same intestinal level in the control rats examined 7 months after the start of the present experiments (100.0±5.9%; n=18). In the control rats exposed to the flaxseed oil-enriched diet, however, this ratio only represented 76.3±6.4% (n=24; p<0.01) of that found at the same age and same intestinal level in the control rats exposed to the soybean oil-enriched diet (100.0±5.5%; n=24) and, as such, failed to differ significantly (p>0.06) from that found in the control animals just before exposure to the enriched diets. Likewise, in the ω3D rats exposed for 2 and 4-5 weeks to the flaxseed oil-enriched diet, the C20:2ω6/C18:2ω6 ratio failed to differ significantly (p>0.975) from that recorded shortly before exposure to this diet.

The C20:4ω6/C20:3ω6 ratio was higher in the duodenum and jejunum than in the caecum or colon. For instance, in the control animals, it averaged 16.5±1.4 (n=14) in the duodenum and jejunum as compared (p<0.001) to 9.9±0.7 (n=11) in the caecum, being further decreased (p<0.01) to 7.0±0.7 (n=11) in the colon. Already after 3 months of dietary ω3-deprivation, it averaged in the ω3D rats 130.8±12.1% (n=18; p<0.001) of the mean corresponding values found at the same age and same intestinal level in control animals (100.0±6.7%; n=18). After 7 months of ω3-deprivation, it represented 156.2±8.1% (n=22; p<0.001) of the mean corresponding values found at the same age and same intestinal level in the control animals (100.0±4.4%; n=18). In the control rats, it failed to be significantly affected by exposure to either the soybean or flaxseed oil-enriched diet, the values recorded after 4-5 weeks exposure to those two diets averaging, respectively, 101.0±16.7% (n=24; p>0.95) and 89.6±8.8% (n=24; p>0.3) of the mean corresponding values found in the control animals shortly before such an exposure (100.0±4.4%; n=18). In the ω3D rats exposed for 2 and 4-5 weeks to the flaxseed oil-enriched diet, however, the C20:4ω6/C20:3ω6 ratio decreased, respectively, to 65.6±4.7% (n=24; p<0.001) and 71.7±6.2% (n=24; p<0.001) of the mean corresponding values recorded at the same intestinal level in the ω3D rats examined shortly before such an exposure (100.0±4.4%; n=22). The two above percentages found in the ω3D rats after 2 and 4-5 weeks exposure to the ω3-enriched diet failed to differ significantly (p>0.4) from one another.

In mirror image of the C20:4ω6/C20:3ω6 ratio, the C22:4ω6/C20:4ω6 ratio was lower in the duodenum and jejunum than in the caecum or colon, averaging in the control animals 31.6±2.6 10^{-3} (n=14) in the proximal segments of the intestinal tract as distinct (p<0.001) from 89.5±5.5 10^{-3} (n=11) in the caecum and 84.5±9.4 10^{-3} (n=11) in the colon. The C22:4ω6/C20:4ω6 ratio was significantly higher in ω3D rats than in control animals, whether in the duodenum and jejunum (p<0.01), caecum (p<0.001) or colon (p<0.04), averaging in the ω3D rats 187.3±16.6% (n=42; p<0.001) of the mean corresponding values found at the same age and same intestinal level in the control animals (100.0±4.4%; n=36). Exposure of the control animals for 4-5 weeks to the soybean oil-enriched diet augmented (p<0.005) the C22:4ω6/C20:4ω6 ratio to 214.0±29.7% (n=24) of the mean corresponding values found

Paired ratio between selected long-chain polyunsaturated ω6 fatty acids in intestinal phospholipids.

Rats	Intestinal segments	C20:2ω6/C18:2ω6 $(x10^3)$	C20:4ω6/C20:3ω6	C22:4ω6/C20:4ω6 $(x10^3)$
3mC	Duodenum + Jejunum	16.7±1.6 (6)	17.9±2.0 (6)	25.4±3.2 (6)
	Caecum	53.8±8.3 (6)	10.2±1.2 (6)	80.3±5.5 (6)
	Colon	69.8±4.1 (6)	7.7±1.0 (6)	81.1±15.3 (6)
3mD	Duodenum + Jejunum	30.2±0.6 (6)	16.4±1.1 (6)	69.2±6.7 (6)
	Caecum	51.8±10.9 (6)	18.4±2.6 (6)	206.8±17.3 (6)
	Colon	71.5±17.8 (6)	9.3±0.4 (5)	102.0±12.9 (6)
7mC	Duodenum	20.2±1.3 (3)	10.2±1.3 (3)	41.2±5.9 (3)
	Jejunum	17.7±1.4 (5)	18.6±2.1 (5)	33.3±3.0 (5)
	Caecum	56.9±11.4 (5)	9.5±0.2 (5)	100.5±8.2 (5)
	Colon	66.0±4.5 (5)	6.3±0.8 (5)	88.5±11.4 (5)
7mD	Duodenum	23.4±1.9 (6)	19.7±1.2 (6)	53.3±5.1 (6)
	Jejunum	24.0±8.5 (6)	26.4±2.8 (6)	77.4±31.0 (6)
	Caecum	62.6±8.1 (6)	13.6±2.4 (4)	150.2±16.8 (6)
	Colon	91.2±7.2 (6)	8.9±0.3 (6)	127.8±13.7 (6)
7mC/4wS	Duodenum	41.1±7.3 (6)	18.4±5.7 (6)	96.7±21.1 (6)
	Jejunum	53.1±6.1 (6)	10.8±2.1 (6)	134.4±14.0 (6)
	Caecum	55.6±4.6 (6)	7.7±0.3 (6)	108.1±5.4 (6)
	Colon	87.3±5.7 (6)	5.3±1.1 (6)	97.6±8.2 (6)
7mC/4wF	Duodenum	31.9±7.6 (6)	11.0±1.9 (6)	63.5±6.2 (6)
	Jejunum	39.0±4.5 (6)	13.4±2.4 (6)	81.5±14.9 (6)
	Caecum	44.4±4.1 (6)	7.7±1.0 (6)	78.2±13.5 (6)
	Colon	64.8±14.6 (6)	6.1±1.6 (6)	80.7±16.1 (6)
7mD/2wF	Duodenum	25.9±4.1 (6)	14.1±2.8 (6)	52.2±7.5 (6)
	Jejunum	32.6±2.8 (6)	11.2±1.2 (6)	63.7±5.1 (6)
	Caecum	59.0±5.6 (6)	10.0±0.4 (6)	99.8±8.6 (6)
	Colon	77.4±22.3 (6)	6.7±0.6 (6)	64.6±17.3 (6)
7mD/4wF	Duodenum	10.5±6.7 (6)	16.3±5.5 (3)	31.7±20.0 (6)
	Jejunum	43.7±3.7 (6)	12.5±2.0 (6)	146.5±13.1 (6)
	Caecum	45.0±5.5 (6)	11.6±1.5 (6)	94.2±8.7 (6)
	Colon	71.5±3.6 (6)	6.8±0.4 (6)	49.9±11.2 (6)

at the same intestinal level in the control animals examined shortly before such an exposure (100.0±5.0%; n=18). When the control animals were exposed to the flaxseed oil-enriched diet, however, the C22:4ω6/C20:4ω6 ratio only represented 70.3±5.9% (n=24; $p<0.001$) of the mean corresponding values found at the same intestinal level in the control rats of same age exposed, also for 4-5 weeks, to the soybean oil-enriched diet. As a matter of fact, the C22:4ω6/C20:4ω6 ratio in intestinal phospholipids was no more significantly higher ($p<0.07$) in the control animals exposed to the flaxseed oil-enriched diet (142.0±18.4%; n=24) than in the control animals examined shortly before such an exposure (100.0±6.1%; n=24) and used as reference in this comparison of data collected at the same levels of the intestinal tract. When the ω3D rats were exposed for 2 to 4-5 weeks to the flaxseed oil-enriched diet, the C22:4ω6/C20:4ω6 ratio failed to be significantly affected ($p>0.7$) at the duodenal and jejunal level, averaging 106.7±14.4% (n=24) of the mean corresponding values found at the same level in the ω3D rats examined shortly before such an exposure (100.0±19.7%; n=12). However, in the caecum and colon, the C22:4ω6/C20:4ω6 ratio found after exposure of the ω3D rats to the flaxseed oil-enriched diet was decreased ($p<0.001$) to 54.7±4.8% (n=24) of the corresponding mean values recorded at the same level in the ω3D rats examined at the 7th month of the present experiments (100.0±7.4%; n=12).

Correlations between the relative weight content of C22:6ω3 and either C18:2ω6 or C20:4ω6 in intestinal phospholipids. Two of the most abundant long-chain polyunsaturated ω6 fatty acids present in intestinal phospholipids, i.e. C18:2ω6 and C20:4ω6, were examined in terms of the possible correlation between their respective relative weight content and that of C22:6ω3 in intestinal phospholipids.

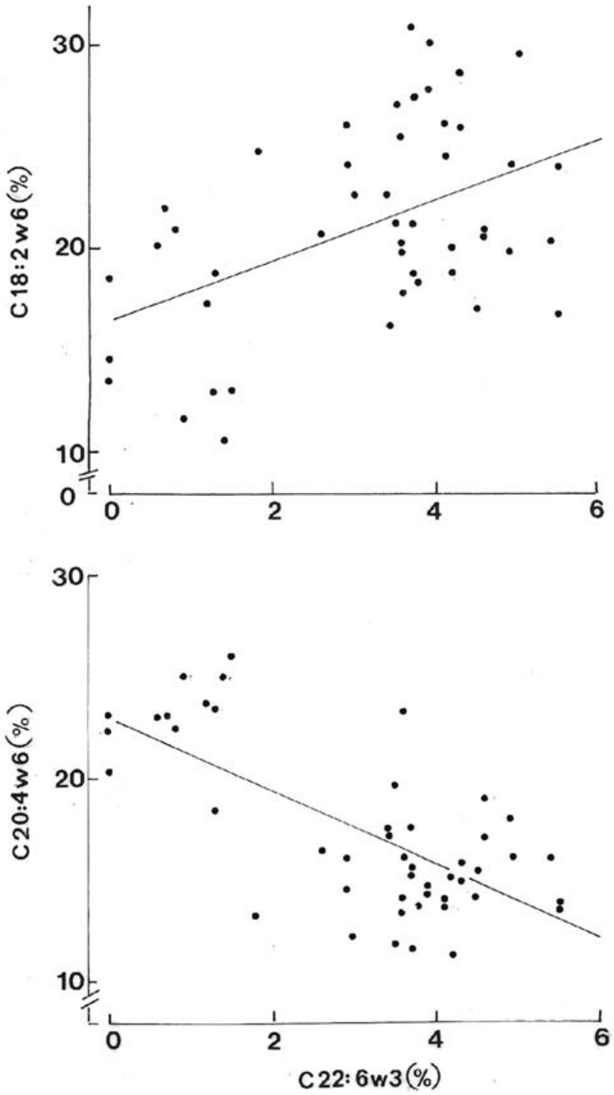

Figure 2. Comparison between the C18:2ω6 (upper panel) or C20:4ω6 (lower panel) and C22:6ω3 relative content of phospholipids in the caecum of all 47 rats examined in the present study. The oblique line corresponds to the regression line.

In the duodenum and jejunum, a significant correlation ($r=0.3532$; $n=32$; $p<0.05$) was observed, during the first 7 months of the present experiments, between the relative weight content of C18:2ω6 and C22:6ω3, all individual data being expressed relative to the mean corresponding value found at the same level of the intestinal tract (i.e. the duodenum and/or jejunum). In the caecum and colon, the correlation coefficients between the relative weight content of C18:2ω6 and C22:6ω3 amounted, respectively, to 0.4662 ($n=47$; $p<0.001$) and 0.2746 ($n=47$; $p<0.06$), even when all data collected in the 8 groups of rats examined in this study were taken into account.

Likewise, taking into account all available data, highly significant negative correlations between the relative weight content of C20:4ω6 and C22:6ω3 were found in the duodenum and jejunum ($r= -0.4475$; $n=80$; $p<0.001$), caecum ($r= -0.6889$; $n=47$; $p<0.001$) and colon ($r= -0.3992$; $n=47$; $p<0.01$). Fig. 2 illustrates such correlations between C22:6ω3 and either C18:2ω6 or C20:4ω6 in the caecal phospholipids of the 47 rats examined in the present study.

Discussion

Restricted information was previously published on the distribution and regulation of long-chain polyunsaturated ω6 fatty acid content in intestinal phospholipids. Garg *et al* (6) first observed that the C18:2ω6 content of rat microsomal phospholipids is higher in the jejunum than in the ileum, with a mirror image for C20:4ω6. They also documented that the incorporation of [1-^{14}C]linoleic acid (C18:2ω6) into microsomal membranes was significantly higher in jejunal than in ileal microsomal lipid fractions. Likewise, Korotkova and Strandvik (7) observed, in fair agreement with the present study that, in normal Sprague-Dawley rats, the C18:2ω6 content of phospholipids is twice higher in the jejunal mucosa than in the ileal or colonic mucosa, whilst that of C20:4ω6 is slightly lower in the jejunal mucosa than in the ileal or colonic mucosa. Duranthon *et al* (8) reported that, in weaning pigs fed an essential fatty acid-deficient diet for 12 weeks, the C18:2ω6 content of intestinal mucosa cell phospholipids was largely reduced, with only a slight decrease of C20:4ω6. Garg *et al* (9) then documented, in rats fasted for 24 h or fed a diet high in C18:2ω6, increased Δ6-desaturase activity in jejunal mucrosal microsomes. After fasting, Δ6-desaturase activity was also enhanced in the ileum. Following fasting, the C18:2ω6 content of jejunal microsomal phospholipids was indeed decreased with a concomitant increase of C20:4ω6. These authors also observed that feeding a diet rich in ω3 fatty acids lowered the C20:4ω6 content of both jejunal and ileal microsomal phospholipids. Last, Hess *et al* (10) reported that feeding one-day-old pigs a milk-based formula containing 0.5 to 5.0% C20:4ω6 (wt:wt) increased within 8 days, in a dose-dependent manner the C20:4ω6 content of jejunal and ileal mucosa phospholipids, with a reciprocal decrease of C18:2ω6.

The present study affords mainly four pieces of information.

First, it documents that the profile of long-chain polyunsaturated ω6 fatty acids in phospholipids differs vastly in distinct segments of the intestinal tract. Such was the case for all 8 variables under consideration, with highest values in the duodenum and jejunum for the C18:2ω6 relative weight content and C20:4ω6/C20:3ω6 ratio and a mirror image for the six other variables.

Second, the present experiments reveal that, within 3 to 7 months, the dietary deprivation of ω3 fatty acids also affects the profile of long-chain polyunsaturated ω6 fatty acids in intestinal phospholipids, with a decrease of C18:2ω6, C20:2ω6 and C20:3ω6 and an increase of C20:4ω6 and C22:4ω6. All relevant ratios between selected ω6 fatty acids, i.e. the C20:2ω6/C18:2ω6, C20:4ω6/C20:3ω6 and C22:4ω6/C20:4ω6 ratios, were all also increased in the ω3D rats, as already observed in the liver of the same rats (2).

Third, highly significant negative correlations were found, under the present experimental conditions and including all available data, between the C20:4ω6 and C22:6ω3 content of phospholipids in either the duodenum and jejunum, the caecum or the colon. This finding is compatible with a possible cause-to-effect link between the changes in C22:6ω3 relative content of intestinal phospholipids orresponding changes in C20:4ω6 relative content. roposal is also compatible with the finding that the exposure of the ω3D rats for 2 to 4-5 weeks to an ω3-rich diet, i.e. a flaxseed oil-enriched diet, lowered the C20:4ω6 content of phospholipids at all intestinal levels. Such was also the case for the C22:4ω6 content of phospholipids, as well as the C20:4ω6/C20:3ω6 and C22:4ω6/C20:4ω6 ratios, at least at the level of caecum and colon for the latter variable. Even in the control animals exposed to the flaxseed oil-enriched diet, the C20:4ω6 and C22:4ω6 relative content of intestinal phospholipids, as well as C22:4ω6/C20:4ω6 ratio, were also lower than in the control animals exposed for the same period of time to a soybean oil-enriched diet.

Last, although the C18:2ω6 content of phospholipids appeared to be positively related to that of C22:6ω3 in the caecum and colon, the relationship between these two fatty acids was less obvious in the duodenum and jejunum. At these proximal levels of the intestinal tract, a significant correlation was only observed in the control animals and ω3D rats examined during the first seven months of the present experiments. Moreover, when the ω3D rats were exposed for 2 to 4-5 weeks to the flaxseed oil-enriched diet, opposite changes in the C18:2ω6 content of phospholipids were recorded in the duodenum and jejunum, as compared to caecum and colon.

Such findings suggest a multifactorial regulation of the C18:2ω6 content of intestinal phospholipids. For instance, the much higher relative content of the sunflower oil, as compared to flaxseed oil-enriched diet, in C18:2ω6 may well account, in part at least, for the opposite changes in the phospholipid content of C18:2ω6 at the proximal *versus* distal levels of the intestinal tract recorded when the ω3D rats were exposed for 2 to 4-5 weeks to the flaxseed oil-enriched diet. Moreover, and as recently revealed (11), individual data may, on occasion, differ vastly from those recorded in an otherwise homogeneous group of animals. For instance, such was the case in one control animal examined 3 months after the start of the present experiments. This animal displayed, at the level of the colon, a C22:6ω3 relative weight content in phospholipids of 6.31%, as distinct ($p<0.002$) from 3.68±0.33% in the other 5 control animals of the same age. Yet, in this animal, the C18:2ω6 (11.66%) and C20:4ω6 (24.59%) relative content of colic phospholipids were, respectively, much lower ($p<0.001$) and much higher ($p<0.001$) than the mean values recorded in the other 5 control animals of same age for C18:2ω6 (21.68±0.50%) and C20:4ω6 (16.16±0.82). This situation mainly accounted for the lower level of statistical significance reached in the colon, as distinct form caecum, for the correlations between the C22:6ω3 and either C18:2ω6 or C20:4ω6 content of phospholipids. The xy product in this single control animal indeed caused a 20.0±1.2% (n=2) reduction of the sum of the xy products recorded in the other 46 rats.

In conclusion, the present study extends to long-chain polyunsaturated ω6 fatty acids knowledge recently acquired in the case of long-chain polyunsaturated ω3 fatty acids and documenting both vast differences in their pattern at distinct levels of the intestinal tract and the dramatic and rapid modifications of such a pattern in response to changes in their dietary supply (1).

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