Polyunsaturated fatty acids as differentiation markers of rat jejunal epithelial cells: a modeling approach

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Fatty acid compositions of intestinal cell phospholipids were compared in six groups of rats fed miscellaneous dietary oils (corn, rapeseed, peanut, hydrogenated palm, hydrogenated coconut, and salmon oils). Diets were representative of different supplies of essential polyunsaturated fatty acids (n-6 and n-3). Epithelial cells were isolated according to a gradient descending from the villus tip to the crypt base, and fatty acid compositions in total cell phospholipids were reported relative to the level of differentiation. The standard differentiation pattern of linoleic acid was profiled after computer processing of the data. The amount of linoleic acid in the phospholipids of crypt stem cells represented about 50% of the maximum amount found in mature villus cells. A minimum linoleic acid threshold of 6–7% of total fatty acids in the crypt stem cells was necessary to reach a maximum level of 13–15% in the upper villus cells. Hydrogenated coconut and salmon oils did not meet this minimum requirement. When diets supplied sufficient amounts of n-6 fatty acids, arachidonic acid accretion paralleled that of linoleic acid so that 20:4n-6 contents rose up to 20–26% of total fatty acids in mature cells. Dietary deficiency in total polyunsaturated fatty acids (hydrogenated vegetable oils) and a relative excess of n-3 fatty acids (salmon oil) induced major alterations of the accretion profile of arachidonic acid. Concomitantly, eicosatrienoic acid appeared in both hydrogenated vegetable oil groups, while n-3 fatty acids dramatically increased in salmon oil-fed rats. In all groups the sum of total dimethylacetals (representative of total plasmalogens in cell phospholipids) decreased as stem cells were translocated toward the villus base. These data indicate that enterocyte differentiation involves an increasing incorporation of n-6 fatty acids, which is controlled by the diet. The main physiological features directly concerned are membrane biogenesis, remodelling, and functions on the one hand, and intestinal metabolism of eicosanoids on the other hand.

Keywords: differentiation; enterocyte; dietary lipids; polyunsaturated fatty acids

Introduction

Changes in lipid composition were previously shown to occur during differentiation and migration of cells from crypt base to villus tip of the small intestine.[1] Regarding phospholipid fatty acid composition, the most striking changes consisted of an increasing incorporation of linoleic and arachidonic acids into total phospholipids,[2] while alkenyl groups of phosphatidylethanolamine-plasmalogens decreased from crypt stem cells to lower villus cells.[3] Studying a human colon adenocarcinoma cell line (HT29), Reynier et al.[4] recently showed that changes in the phospholipid class composition were closely related to the differentiation state, i.e., significant differences between plasma membrane and total cell phospholipid profiles appeared in differentiated HT29 cells, whereas in undifferentiated cells phospholipid profiles of plasma membranes were identical to those of total homogenates. Increased contents of polyunsaturated fatty acids (PUFA)(18:2n-6, 20:4n-6, and 18:3n-3) in phosphatidylcholine and phosphatidylethanolamine were also

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found in differentiated culture cells in comparison with their undifferentiated counterparts.

In rat brush border membranes the cholesterol to phospholipid ratio increased and the phospholipid content of oleic acid decreased while ascending the villus column.[5] Changes in lipid composition were correlated with an alteration of membrane fluidity, suggesting that differences in lipid composition and fluidity of brush border membranes of villus and crypt enterocytes could have a functional significance.[5–6] However, the contribution of membrane lipids to the process of cell differentiation is still unclear. The relevance of these data is uncertain inasmuch as lipid compositional changes depend on the dietary lipid supply. Thus, essential fatty acid deficiency was shown to damage the ultrastructure of differentiated cells,[7] to alter the fatty acid composition of the whole mucosa,[8] and to modify the normal crypt villus evolution of lipids linked to intestinal cell differentiation.[2] Dietary trans-fatty acids were recently shown to induce lengthening of villi in association with a reduction of the migration rate of rat enterocytes.[9] However, the respective influence of each PUFA series, n-6 and n-3 fatty acids, remains unknown. In the present study, we compare the crypt villus evolution of phospholipid fatty acid compositions in relation to the balance of n-6 and n-3 fatty acids in dietary lipids. The aim of this investigation was to outline a final differentiation pattern of PUFA, including nutritional status. To that end, salmon oil and five different vegetable oils were used as lipid supply to six groups of rats. Epithelial cells were isolated according to their crypt villus position, and phospholipid fatty acid compositions were reported relative to the level of differentiation. Special emphasis was placed on n-6 and n-3 fatty acids of total cell phospholipids. The compilation of data showed a general trend for linoleic and arachidonic acids as relevant lipid markers of intestinal cell differentiation.

Materials and methods

Animals and diets

Two weeks before mating, 12 female Wistar rats were divided into six groups. Each group of two females received a semi-synthetic meal containing casein (22%), cellulose (2%), starch (44%), saccharose (22%), adequate minerals and vitamins, and fat (5%) ad libitum. They had free access to water. Dietary fat was supplied by a vegetable oil or salmon oil (SO). Oils were representative of different n-6 to n-3 ratios. Peanut oil (PO), corn oil (CO), and a mixture of rapeseed and peanut oils in equal shares (PRO) supplied sufficient amounts of n-6 fatty acids, with an n-6 to n-3 ratio varying from 5 to 220 *(Table 1)*. Salmon oil (SO) brought about a relative excess of n-3 fatty acids (Σn-6 to Σn-3 ratio equal to 0.1). Hydrogenated coconut oil (HCO) and hydrogenated palm oil (HPO) were representative of low PUFA supplies with only traces of n-3 fatty acids *(Table 1)*. Moreover, HCO was particularly rich in saturated fatty acids with respect to all other oils. After delivery, females were caged individually and the litters were equalized to 10 pups each. The most significant difference between diets was that postnatal mortality (up to 3 days) decreased in group PRO in comparison with all other groups (3% versus 14–20%). During the suckling period, the growth rate was diminished (13%) in the case of low supplies of total polyunsaturated fatty acids (hydrogenated vegetable oils). After weaning (3 weeks of age), the young rats ingested their mothers' diets for 3 weeks. Male rats were sacrificed at 6 weeks of age for isolation of intestinal cells.

Isolation of intestinal cells

Epithelial cells were isolated from the jejunum of three male rats of the same dietary group using a combination of sequential incubations[10,11] and longitudinal vibrations.[12] These routine procedures already have been described and compared[13]; further minor modifications are indicated in the present study.

Overnight-fasted rats were killed at morning by decapitation between 9:00 and 10:00 a.m. The small intestine, with the omission of the duodenum, was immediately excised and flushed free of intestinal contents with 9 g/L cold NaCl. Thirty cm intestinal segments (proximal jejunum) were everted and mounted onto special stainless-steel rods. Careful handling of the tissue was essential as the mucosal surface was exposed. Tooled rods (0.3 × 40 cm) with a blunted tip were used to roll up the bowel and to keep it in position until complete scouring. Each extremity of the everted intestinal segment was tightly knotted to the rod, and each rod was vertically immersed into individual 35 cm glass tubes full of an appropriate buffer *(Table 2)*. The tubes were incubated at 37° C in a deep bathing vat equipped with a mobile platform. The tubes were gently stirred under slow rotary motion of the platform (30 cycles per minute). Ten to 13 cell fractions were isolated by successive incubations of the tubes followed by longitudinal vibrations of the rods. At the end of each incubation, the non-immersed tip of the intestinal rod was clipped to the mobile head of a vibration apparatus, which produced low-amplitude and short-lived vibrations with a frequency of 25 or 50 Hz. This procedure ensured complete scouring of the mucosa, but subepithelial cells could be present in the deeper fractions. Cells were collected in the tube and washed by centrifugation after the last incubation. Cells harvested from the simultaneous incubation and vibration of three individual bowels were pooled and subsequently treated as one unique cell fraction. Pelleted cells were homogenized in a preservation-glycerol buffer and stored at −80° C as previously described.[3]

Assaying samples were diluted into distilled water (10–20-fold) for measuring cell protein contents.[14] Specific activities of sucrase and alkaline phosphatase were routinely used as enzymatic markers of cell differentiation *(Figure 1)*.[3,15]

Phospholipid fatty acid composition of intestinal cells

Purification of phospholipids. Total cell lipids were extracted using chloroform/methanol (3:1, vol/vol) containing 0.01% (wt/vol) butylated hydroxytoluene (modified method).[16] One volume of each cell fraction (suspended in the preservation-glycerol buffer) was mixed with 4 volumes of solvent. The suspension was thoroughly homogenized and centrifuged at 4° C. Total lipids were collected in the bottom phase and evaporated under nitrogen. The dry matter was resuspended in 1 volume of 0.15 M NaCl before being extracted with chloroform/methanol 2:1 (vol/vol) to remove traces of glycerol. Phospholipids were separated from neutral lipids on a silicic acid column[17] as previously described.[2]

Table 1 Fatty acid composition in dietary oils

Fatty acid composition (%, wt/wt)	Peanut/ Rapeseed oil* (PRO)	Corn oil (CO)	Peanut oil (PO)	Hydrogenated coconut oil (HCO)	Hydrogenated Palm (HPO)	Salmon (SO)
10:0				0.9		
12:0				44.5	3.1	
14:0	0.3	1.2		21.9	2.4	5.9
16:0	7.1	9.9	9.3	11.7	49.5	13.6
18:0	2.8	2.1	3.4	13.7	5.2	2.6
20:0	1.0	0.2	1.4	0.2		0.2
22:0	1.3	0.1	2.6			0.2
Σ SFA	**13.1**	**13.5**	**17.8**	**92.9**	**60.2**	**23.5**
16:1n-9		0.1	0.1		1.1	0.3
16:1n-7	0.2	0.1	0.2		0.3	9.4
18:1n-9	59.4	26.3	58.2	2.2	32	13.9
18:1n-7		0.7			4.4	3.9
20:1n-9	1.1	2.3	1.2			7.6
20:1n-7		0.3				0.5
22:1n-11		0.1	0.1			3.9
Σ MUFA	**60.7**	**29.9**	**60**	**2.2**	**37.8**	**41**
18:2n-6	21.6	53.5	22.1	4.7	2.0	2.3
Σ n-6	**21.6**	**55.5**	**22.1**	**4.7**	**2**	**4.1**
18:3n-3	4.6	1.1	0.1	0.2	tr	0.7
18:4n-3						2.4
20:4n-3						0.7
20:5n-3						12.2
22:5n-3						2.6
22:6n-3						12.8
Σ n-3	**4.6**	**1.1**	**0.1**	**0.2**	tr	**31.4**
Σ n-6/ Σ n-3	4.7	50.5	221	23.5	—	0.1
Σ n-6 + Σ n-3	26.2	56.6	22.2	4.9	2	35.5
Dietary supply (mg/100g)						
n-6	1015	2609	1039	221	94	193
n-3	216	52	5	9	tr	1476

*In equal amounts.

Table 2 Procedure for the isolation of intestinal cells

		Mature cells			Stem cells		Subepithelial cells
Number of incubation	Preincubation	1	2 to 4	5	6	7 to 9	10 and beyond*
Buffer (from ref. [10])	A	B	B	B	B	B	B
Incubation time (min)	10	3	3	5	7	10	15
Vibration time (sec)	none	1	1 to 3	5	5	5	10 to 15
% of isolated cells (total = 100)	(discarded)	3–10	10 to 17 (each)	10–15	9–11	5 to 8 (each)	1 to 3 (each)

Buffer A was composed of 1.5 mmol/L KCl, 96 mmol/L NaCl, 27 mmol/L sodium citrate, 5.6 mmol/L KH_2PO_4, pH 7.3 Buffer B was composed of 3.2 mmol/L KCl, 137 mmol/L NaCl, 1.5 mmol/L KH_2PO_4, 8.2 mmol/L Na_2HPO_4, 1.5 mmol/L EDTA, 1 mmol/L dithiothreitol, 0.1% bovine serum albumin (fatty acid free), pH 7.4.
*Maximum 13.

Fatty acid composition. The fatty acid methyl esters, obtained by transmethylation of purified phospholipids,[3,18] were analyzed using a Chrompack model CP 9000 (Chrompack International, The Netherlands) gas chromatograph equipped with a flame ionization detector and a CP Wax 52 CB bonded fused-silica capillary column (50 m with 0.2 mm inner diameter). Fatty acid methyl esters, solubilized with isooctane, were injected into a retention gap through an "on-column" injector. Hydrogen was the carrier gas with a flow rate of 1–2 mL/min. Oven temperature was programmed from 79 to 140° C at a heating rate of 5° C/min, and, after 5 minutes, from 140 to 210° C at a heating rate of 3° C/min. The detector

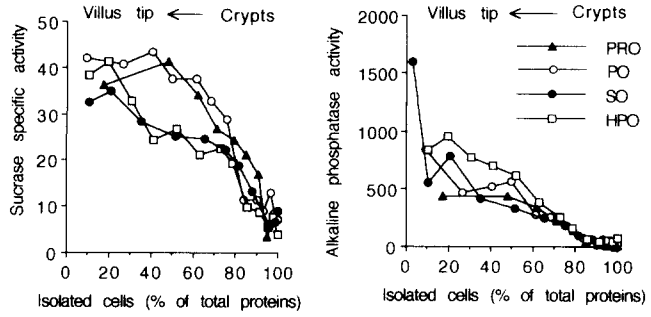

Figure 1 Crypt villus profiles of sucrase (A) and alkaline phosphatase (B) in cell homogenates of HPO-, PO-, PRO-, and SO-fed rats. Activities are expressed in nmole of glucose or p-nitrophenol released per minute and per mg of protein.

was set to 270° C. Fatty acid methyl esters and dimethylacetals (DMA) were automatically integrated and identified by comparison of equivalent chain lengths (ECL) with those of authentic compounds (standard DMA were obtained by transmethylation of bovine brain phosphatidylethanolamine purchased from Merck, Darmstadt, Germany). All fatty acid compositions were expressed as percentage (by weight) of total fatty acids.

Treatment of data and expression of results

The raw data were plotted relative to the level of differentiation, i.e., the crypt villus position of cells. It was estimated by the percentage of isolated cells expressed as proteins.[10] One hundred percent of isolated cells corresponded to the sum of all protein fractions. Because the percentage of cells harvested from one incubation fluctuated between experiments, the data were normalized to 10% of cell extraction as previously suggested.[19] This was performed using fourth order polynomial regression, which was automatically fitted from the raw data. Then, smooth curves could be drawn and the different groups compared at an equivalent step of extraction. From the crypt base to the villus extremity 11 standard positions were thus defined (100–0% of extraction with a 10% interval).

Results

Linoleic acid profiles

Figure 2 shows that whatever the source of dietary fat linoleic acid content in total cell phospholipids regularly increased all along the crypt villus unit. Depending on the lipid supply, LA phospholipid content in crypt stem cells varied from 2.2–4.2% of total fatty acids (HCO and SO, respectively) to 6.2–8.6% (PO, HPO, PRO, and CO) *(Figure 2A)*. To simplify the statement of results, the former group (characterized by its very low level of LA) was defined as "group 1" (HCO and SO), the latter being called "group 2" (PO, HPO, PRO, and CO). In both groups, the phospholipids of mature cells located in the upper quarter of the villus contained much more linoleic acid than stem cells. With respect to its initial value, LA level increased 1.6 fold in CO-fed rats, about two fold in PO-, HPO-, PRO-, and SO-fed rats, and three fold in HCO-fed rats. Upper villus cells harvested from group 1 contained less linoleic acid than their group 2 counterparts. In group 2 mature cells, linoleic acid rose to 13–16% of total fatty acids, versus 6.5–8% in group 1 *(Figure 2B)*. The low initial level of LA in crypt stem cells in group 1 could not be recovered along the crypt villus path. It is noteworthy that LA contents in group 1 mature cells were identical to those of group 2 stem cells *(Figure 2B)*. Thus, a minimum initial LA content of 6–7% in the stem cells seemed to be necessary for reaching the standard level of 13–16% in the upper villus cells. Hydrogenated coconut and salmon oils (group 1) did not meet this minimum requirement.

The second observation was that with the exception of group HCO, there was a trend of linoleic acid to change to the opposite in the terminal zone (Figures 2B and 2C). This means that phospholipids of extruding cells and crypt stem cells generally contained similar amounts of linoleic acid.

Computer-compiled data

Linoleic acid content was fitted to normalized intervals of cell extraction as described in Materials and methods *(Figure 2B)*. It is clear that all fatty acid compositions in group 2 converged on 12% linoleic acid at the mid-villus level. Thus, the LA maximum content was derived from the vertex of the corresponding curve, and LA changes in each group were computed relative to the highest value *(Figure 1C)*. In that way, distribution profiles of LA could be drawn along the crypt villus axis as a percentage of the maximum level and compared between both groups. Lastly, this standardized presentation of data displayed very similar LA profiles, with the exception of group 1 HCO, which deviated

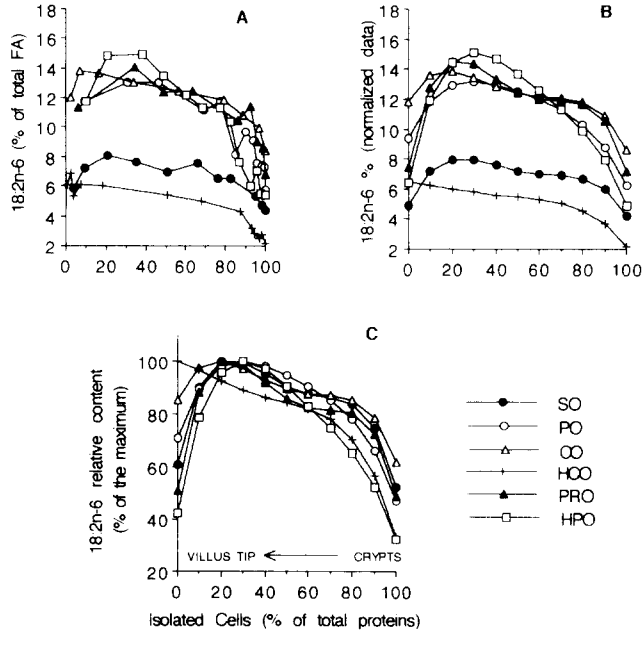

Figure 2 Evolution along the crypt villus axis of linoleic acid contents in relation to dietary oil; A) raw data; B) computer-normalized data to equivalent step of extraction (curves were drawn from a fourth-order polynomial regression); C) data were standardized to the maximum content of each set and all curves were superimposed.

from the standard pattern *(Figure 2C)*. All data have been compiled in *Figure 3*, with the exclusion of HCO-fed rats, and the common curve has been drawn from the mean value of the five remaining groups. The resulting profile gives the general tendency for crypt villus changes in the level of LA in intestinal cells. There were three zones. First, the phospholipid LA content increased from 50 to 80% of the maximum level in the lower quarter of the crypt villus unit. Then, it slightly increased up to the upper quarter of the villus, so that mature functional cells contained the maximum amount of linoleic acid. Lastly, cells located on the villus tip were characterized by a 30% mean decrease of the LA level *(Figure 3)*. However, this result was essentially theoretical because the terminal value, found by extrapolation to zero percent of cell extraction, was variable from one experiment to another (at zero percent of cell extraction, the computed value of LA content ranged from 55 to 90% of the maximum). The magnitude of decline was uncertain, but the general tendency was that phospholipids of extruding cells never contained the maximum amount of LA.

Arachidonic acid accretion into phospholipids

Arachidonic acid incorporation into phospholipids resulted from n-6 bioconversion, basal uptake from plasma lipoproteins, and acyltransferase activities. Accretion of arachidonic acid (AA) into cell phospholipids is shown in *Figure 4A*. As reported for linoleic acid, phospholipids of group 1 crypt stem cells had the lowest content of arachidonic acid (about 4% of total fatty acids). However, differences between dietary groups in arachidonic acid contents were more pronounced than those of linoleic acid, and the distinction between groups 1 and 2 was not valid anymore. In particular, AA level in group HPO broke down in comparison with unsaturated oil groups (CO, PO, and

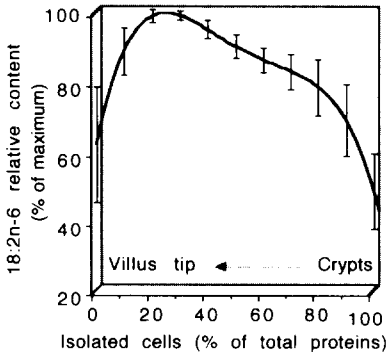

Figure 3 Proposal for the standard pattern of crypt villus changes of linoleic acid into cell phospholipids. Standardized data (see *Figure 1C*) were averaged with the exclusion of group HCO. The final curve was drawn from fourth-order polynomial regression fitted from the mean values (± S.D.). The model curve is described by equation 1.

$$Y = 62 + 3.8(X) - 0.13(X^2) + 0.0016(X^3) - 7.610^{-6}(X^4) \quad (1)$$

with, Y: cell phospholipid 18:2n-6 content expressed as a percent of the maximum, and X: cell position along the crypt villus axis, as assessed by the cumulated cell protein extract.

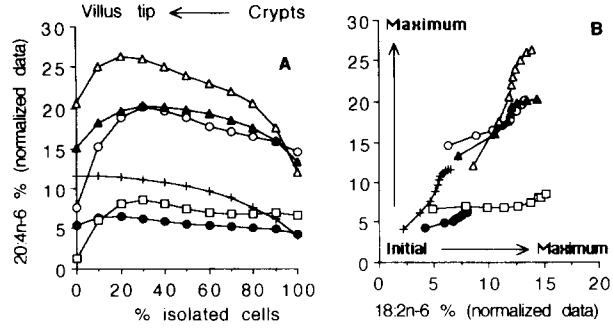

Figure 4 A) Evolution along the crypt villus axis of arachidonic acid contents in cell phospholipids of rat jejunum: normalized data were computed as reported in *Figure 2B*. B) Comparative evolution of arachidonic and linoleic acids: in each set of cell fractions, the AA contents *(Figure 4A)* were plotted relative to those of their LA precursor *(Figure 2B)*. Data comprised between the initial value (stem cells) and the maximum value (mature cells) were taken into account (i.e., excluding the descending part of the profiles).

PRO). According to this criterion, HPO-fed rats rejoined groups HCO and SO. The plot of arachidonic acid content versus linoleic acid content might give some indications as to the AA accretion with respect to its dietary precursor *(Figure 4B)*. In this representation, AA and LA converged on a common composition of 17% AA and 11% LA when rats were fed unsaturated vegetable oils rich in linoleic acid (i.e., more than 1 g of lineolic acid per 100 g of diet). This standard composition was generally representative of the lower villus cells. By contrast, the other three groups (HCO, HPO, and SO) departed from this pattern. Owing to the very low n-6 to n-3 ratio, salmon oil led to the lowest content of AA in upper villus cells (6.5% versus 20–26% in unsaturated vegetable oils). Clearly, the relative excess of n-3 fatty acids in salmon oil inhibited the crypt villus accretion of n-6 fatty acids into cell phospholipids. Both hydrogenated vegetable oils, low in total polyunsaturated fatty acids (less than 0.25 g of total PUFA per 100 g of diet), led to an intermediate level of arachidonic acid, which did not pass beyond 12% of total fatty acids. The mechanisms by which the polyunsaturated fatty acid deficiency induced the arachidonic acid level to decrease were probably different within both groups of hydrogenated vegetable oils. The arachidonic acid content in intestinal cells of HPO-fed rats plateaued to 7–9% all along the villus, whereas linoleic acid increased two fold at the same time *(Figure 4B)*. By contrast, in group HCO arachidonic acid paralleled its precursor and increased three fold in mature cells as compared with stem cells.

Other fatty acids

In the vegetable oil-fed groups, n-3 fatty acids in total cell phospholipids were mainly represented by docosahexaenoic acid (DHA), while eicosapentaenoic acid (EPA) was the major n-3 fatty acid in group SO. We failed to observe regular crypt villus changes for n-3 fatty acids (data not shown); consequently, DHA and EPA contents within each set of cell fractions were

averaged *(Figure 5A)*. Clearly, total mucosal n-3 fatty acids increased dramatically when rats were fed fish oil in comparison with vegetable oils. Dietary long chain PUFA of the n-3 series substituted for biosynthesis and accretion of arachidonic acid. By contrast, the high n-6 to n-3 ratio in peanut oil resulted in a very low amount of 22:6n-3 in PO phospholipids *(Figure 5A)*. Deficiency in total polyunsaturated fatty acids (n-6 and n-3) was well evidenced in groups HPO and HCO; i.e., the mean content of neosynthesized trienoic acid (20:3n-9) rose to 7–9% of total fatty acids, whereas it did not exceed 0.6% in other groups *(Figure 5B)*. Biosynthesis of oleic acid (18:1n-9) also increased in HCO-fed rats *(Figure 5B)* receiving a large amount of saturated fatty acids.

Dimethylacetals

Dimethylacetals (DMA) were considered in the present study because it has been previously suggested in the piglet intestine that the phospholipid proportion of total alkenylacyl glycerophospholipids (plasmalogens) decreased as cells were translocated along the crypt villus axis.[3] Total DMA were obtained from transmethylation of the plasmalogen fraction present in cell phospholipids. Individual species of alkenyl groups were not taken into account in the present study. Regarding total amounts of DMA in rat intestinal cell phospholipids, no significant effect related to diet could be detected (data not shown). Thus, a mean value was computed from the DMA contents in each of the 11 standard cell positions, irrespective of the lipid supply. Although the mean proportion of DMA was much lower in rat than in infant piglet intestinal cells, *Figure 6* shows that it markedly decreased in the lower quarter of the crypt villus path.

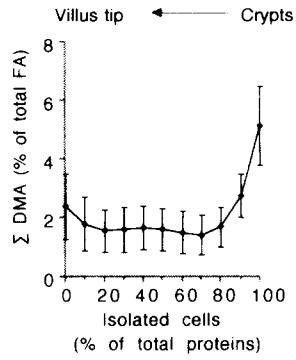

Figure 6 Evolution along the crypt villus axis of total DMA in cell phospholipids (normalized data). Raw contents of DMA were computed to normalized intervals (same procedure as described in *Figure 1A and 1B*). Total DMA contents were not significantly different between diets (data not shown). The data were averaged between the six diets (3 rats per diet) and the graph was fitted from the mean values (± SD).

Discussion

The crypt villus gradient of n-6 fatty acids

The present study was designed to outline the differentiation pattern for n-6 fatty acids in intestinal cell phospholipids. The data demonstrate that in rats receiving different amounts of dietary PUFA, phospholipid accretion of linoleic acid increased in relation with increasing level of cell differentiation. The standardization of data allowed us to work out the definite crypt villus profile for linoleic acid.

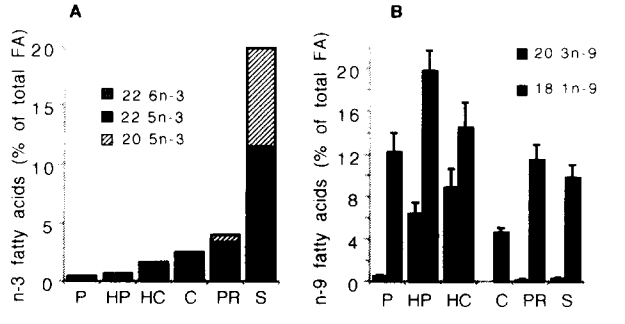

Figure 5 Mean fatty acid composition of intestinal cell phospholipids: A) n-3 fatty acids; B) trienoic and oleic acids (mean ± SD). Data representative of 3 rats per dietary group were averaged within each set of cell extraction (11 cell fractions per set). PO: peanut oil; HPO: hydrogenated palm oil; HCO: hydrogenated coconut oil; CO: corn oil; PRO: peanut-rapeseed oils; SO: salmon oil.

The linoleic acid crypt villus profiles established from HCO- and SO-fed rats (group 1) were distinct from those of group 2, although for different reasons. Hydrogenated coconut oil was very different from the other oils in that it contained about 93% of saturated fatty acids, especially short- and medium-chain fatty acids whose absorption and metabolism are different from longer chain fatty acids. Likely, differences in saturated, mono, and polyunsaturated fatty acid energy intake were responsible for the distinct pattern observed in the HCO-fed rats in comparison with other groups. This assumption is mainly founded on the comparison of HPO- and HCO-fed rats, the two polyunsaturated (essential) fatty acid-deficient groups. Although HPO, as well as HCO, supplied a very low level of dietary linoleic acid, it did not, contrary to HCO, induce any noticeable alteration in the LA level and crypt villus profile. Monounsaturated fatty acids are present in a much larger amount in HPO than in HCO (38% versus 2%), suggesting that the contribution of monounsaturated fatty acids (MUFA) could have a modulatory influence on the PUFA deficiency-induced alteration. Furthermore, the n-6 to n-3 ratio in dietary fat was of crucial importance for setting up the crypt villus gradient of linoleic acid; thus, the relative excess of n-3 fatty acids exerted an inhibitory effect on n-6 fatty acids because absolute amounts of LA were much lower in intestinal cell phospholipids of salmon oil-fed rats in comparison with those of rats fed the HPO diet. The latter was low in n-6 and very low in n-3 (total MUFA being equivalent in HPO and SO).

In a previous study,[2] we showed that essential fatty acid deficiency impaired the lipid compositional changes linked to intestinal cell differentiation (HCO versus CO). But we failed to observe any significant changes in the crypt villus phospholipid content of linoleic acid in HCO-fed rats. The apparent discrepancy with the

present results could be due to an improvement of the cell extraction technique, which now combines incubation and short longitudinal vibration on everted bowels instead of longer incubations, without vibration, of non-everted bowels as in the previous study (Weiser's method[10]). Also, the extractions were specifically made on proximal segments instead of on the whole intestine as in the previous work (actually, preliminary experiments not reported herein showed that ileal contents and profiles of n-6 fatty acids were different from those of jejunum). The incubation/vibration procedure could improve the delicacy of mucosal extraction, leading to well-staged cell fractions and more contrasted fatty acid compositions. All things considered, the possibility that crypt stem cell fractions could be occasionally contaminated by subepithelial cells cannot be excluded. But this would imply that the subepithelium contains lower levels of n-6 fatty acids than the epithelium, and this has not been investigated.

The overall data of both studies show that an increasing incorporation of linoleic acid into phospholipids is a general feature of intestinal cell differentiation that might be altered in different manners when unbalanced amounts of dietary polyunsaturated fatty acids are supplied. In the current study, a minimum threshold of 6–7% LA in crypt stem cells was necessary to reach the maximum level of 13–15% in upper villus cells. As shown in the CO-fed group, relative dietary excess of linoleic acid (54% of total fatty acids in corn oil) did not enhance this "ceiling level." These data suggest that a mean content of 13–15% LA in mature functional cells corresponds to an optimum level in the jejunum of the young rat.

The question is raised about the physiological significance of the differentiation-related changes in n-6 fatty acids. Garg et al.[20] reported that linoleic acid was preferentially incorporated into microsomal membranes of rat intestine in comparison with palmitic acid. They suggested that linoleic acid-preferential membrane incorporation could be due to a higher specificity of microsomal acyltransferases for essential fatty acids. With these data in mind, it could be assumed that an increasing accretion of linoleic acid into total phospholipids reflects the settlement of intestinal acyltransferases in differentiating enterocytes. In a previous study, O'Doherty[21] showed that lysophosphatidyl acyltransferases specific for choline and ethanolamine exhibited a crypt villus gradient with four-times higher acyltransferase activities of the villus cell than that of the crypt cell enzymes. The capacity of fatty acid esterification into triglycerides has also been shown to be concentrated in the upper part of the villi.[22] All data suggest that cell membrane biogenesis and specific remodelling of the membrane fatty acid composition could account for linoleic acid accretion as the cells advanced in the differentiation phase.

Physiologic implications

The human Caco-2 cell line has recently been shown to synthesize arachidonic, eicosapentaenoic (20:5n-3), and docosapentaenoic (22:5n-3) acids from their respective precursors (18:2 n-6 and 18:3n-3), which were found to be preferentially incorporated into total cell phospholipids.[23] In another recent study, Reynier et al.[4] reported that undifferentiated HT29 cells were characterized by a low proportion of linoleic, arachidonic, and alpha-linolenic acids as compared with differentiated human colonic cells. Their observation seemed to be compatible with the present description of crypt villus changes in linoleic and arachidonic acids. They hypothesized that increased formation of leukotrienes and prostaglandins in undifferentiated HT29 cells could be responsible for the decreased content of eicosanoid precursors (arachidonic and both linoleic and alpha-linolenic acids) when compared with more differentiated cells. The enzyme responsible for liberating arachidonic acid from rat cellular phospholipids (PLA_2) was found to be concentrated within the subepithelium with significantly lower levels in the epithelium itself.[24] Within the rat epithelium PLA_2 activity was distributed according to a gradient descending from the crypt base to the villus tip.[24] On the other hand, prostaglandin synthesis was shown to be located predominantly in the subepithelium, whereas the capacity to degrade prostaglandins resided mainly in the epithelial cells, according to an ascending crypt-to-villus gradient.[25] It has been postulated that plasmalogens, whose physiological role is not fully determined, could act as a specific reservoir for eicosanoid precursors.[26] It is noteworthy that in the rat *(Figure 5)* as well as in piglet intestine,[3] a decreasing proportion of total DMA seemed to compensate for an increasing phospholipid content of n-6 fatty acids. The data as a whole lead to the concept that the subepithelium is the main site of prostaglandin intestinal synthesis, with high PLA_2 activity thereby releasing arachidonic acid from membrane phospholipids or plasmalogens, whereas prostaglandin catabolism occurs predominantly in the upper epithelium, the site of low PLA_2 activity, low eicosanoid synthesis, and hence, of increased content of n-6 precursors. According to this model, undifferentiated crypt cells would represent an intermediate level in the gradient of prostaglandin synthesis (or degradation) within the whole intestinal tissue.

The crypt villus profile of arachidonic acid clearly depended on the type of dietary fat. Hydrogenated vegetable oils (HPO and HCO) on the one hand, and salmon oil on the other hand, were inefficient for establishing the so-called arachidonic acid-optimum proportion in cell phospholipids. As discussed above, low levels of dietary n-6 and a relative excess of n-3 fatty acids were responsible for the alterations brought about by hydrogenated vegetable oils and salmon oil, respectively. In terms of intestinal eicosanoid production, the incidence of these dietary-induced alterations is not fully understood. In a number of studies, fish oil-enriched diets have been shown to exert a protective effect against experimentally induced damages to the gastrointestinal mucosa. Alteration of eicosanoid biosynthesis was assumed to be the most important process in mediating intestinal inflammatory activity. The protective effect of fish oil may occur either by

shifting the arachidonic acid cascade toward the synthesis of mucosal protective endogenous prostaglandins (cyclooxygenase pathway) at the expense of pro-inflammatory leukotrienes (lipoxygenase pathway), or by stimulating production of the less pro-inflammatory 5-series leukotrienes from eicosapentaenoic acid.[27-30]

Intestinal eicosanoids may be important not only as mediators of inflammation, but also in the regulation of secretory and absorptive functions[31] and in the process of cell-mediated immunity.[32] Further studies are still necessary for establishing the relationship between diet, the differentiation-related alterations in the cell level of polyunsaturated fatty acids, and the physiological events directly (or undirectly) related to the intestinal production of eicosanoids.

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