Modulation in Δ9, Δ6, and Δ5 fatty acid desaturase activity in the human intestinal CaCo-2 cell line

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Abstract We report the influence of media lipids, growth in lipid-poor medium, and cell differentiation on Δ9, Δ6, and Δ5 desaturase activity in the human CaCo-2 enterocyte cell line. We also describe the level of incorporation of palmitic (16:0), linoleic (18:2n-6), and eicosapentaenoic (EPA) acids (20:5n-3) and their higher homologues into cytosolic and membrane lipids during long-term (10 days) medium supplementation in fully differentiated 16- to 18-day-old cultures. CaCo-2 monolayers reached confluency by day 6 with subsequent development of microvilli and maximal expression of microvillus membrane sucrose, alkaline phosphatase, and γ-glutamyltransaminase occurring between days 16 and 23 after plating. There was evidence of the presence and modulation of Δ9, Δ6, and Δ5 desaturase activity (Δ9 > Δ6 > Δ5). Δ6 Desaturase activity decreased approximately 2-fold between days 6 and 24 of culture and when the fetal bovine serum concentration was increased from 0.5% to 25%; in contrast, when cells were starved for 72 h, activity increased 5.4-fold. When the media was supplemented with either linoleic acid and/or EPA, both Δ6 and Δ5 desaturase activities were inhibited, the greatest reduction of Δ5 desaturase activity occurring with EPA. Incorporation of media fatty acids plus their desaturase and elongase products was highly dependent on medium composition with the homologues of Δ9 > Δ6 > Δ5. Supplementation of cellular media with 100 μM EPA for 10 days decreased membrane phosphatidylethanolamine arachidonic acid level from 13.2 to 8.9%. From these results we conclude that enterocyte membrane fatty acid composition and desaturase enzyme activity are regulated by both dietary fat intake and cell maturation. The clinical relevance of these observations on lipid dietary modification for the management of chronic inflammatory bowel disease is still uncertain but these observations suggest that the beneficial effects of EPA supplements on human ulcerative colitis may be due to a reduction in enterocyte arachidonic acid content by down-regulation of Δ6 and Δ5 desaturase activity.—**Dias, V. C., and H. G. Parsons.** Modulation in Δ9, Δ6, and Δ5 fatty acid desaturase activity in the human intestinal CaCo-2 cell line. *J. Lipid Res.* 1995. **36:** 552–563.

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Phospholipids are integral to all cell membranes and a variety of fatty acids are required for phospholipid synthesis. Fatty acid chain desaturation is a necessary step in the production of the polyunsaturated fatty acids (PUFA) of membrane phospholipids (1). The demand for phospholipid in intestinal epithelium is expected to be high as it has the fastest rate of turnover of any healthy body tissue (2). There are few studies on dietary fatty acid modulation of fatty acid desaturation in the enterocyte; none have examined desaturase activity in the colonocyte. Garg et al. (2) demonstrated that dietary linoleic acid (LA) increased jejunal mucous and Δ6 desaturase activity but fasting decreased it. The long term viability and reproducibility of the CaCo-2 cell line derived from a human colorectal carcinoma has been used to date in many qualitative and quantitative studies as an in vitro intestinal epithelial cell model (3). In culture it spontaneously differentiates to express many characteristic biochemical and morphological features of adult differentiated small intestinal enterocytes as well as 15-week fetal colonocytes (4). We originally reported the presence of Δ6 and Δ5 desaturase activity in the CaCo-2 enterocyte cell line when it was fully differentiated and polarized (5). Chen and Nilsson (6) examined Δ6 and Δ5 desaturase in this cell line after confluency but before maturation and polarization.

The requirement of fatty acids for cell membranes (7) as well as our observation that a human colonic CaCo-2 enterocyte cell line was capable of significant leukotriene B_4 (LTB_4) synthesis (a major pro-inflammatory agent that originates from phospholipid arachidonic acid) (8, 9) prompts questions regarding regulation of arachidonic acid synthesis in the enterocyte.

Abbreviations: EPA, eicosapentaenoic acid; PUFA, polyunsaturated fatty acid; LA, linoleic acid; LTB_4, leukotriene B_4; CoA, coenzyme A; FBS, fetal bovine serum; PA, palmitic acid; MVM, microvillus membrane; ALP, alkaline phosphatase; GGT, γ-glutamyltransaminase; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PLP, phospholipid; TG, triacylglycerol; FFA, free fatty acid; CE, cholesteryl ester; SFA, saturated fatty acid; DHA, docosahexaenoic acid; UI, unsaturation index; C, cholesterol.

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Arachidonic acid is synthesized from LA, an essential fatty acid. Two enzymes involved in the synthetic pathway are Δ6 desaturase, which converts linoleoyl-coenzyme A (CoA) to γlinolenyl-CoA, and Δ5 desaturase, which converts dihomo-γlinolenoyl-CoA to arachidonoyl-CoA. Clinical studies have shown that diet supplementation with fish oil has led to symptomatic improvement of active ulcerative colitis (10, 11). One hypothetical mechanism to explain these observations is that eicosapentaenoic acid (EPA) and arachidonic acid compete as precursors of eicosanoid synthesis (12). EPA, by being incorporated into tissue, will be released from membrane phospholipids during cell activation and transformed into eicosanoid analogues with diminished biological activities when compared with arachidonic acid derivatives (13). There is evidence that increased eicosanoid generation is a pathophysiological feature of inflammatory bowel disease (14–16). Little is known about the mechanism by which EPA regulates arachidonic acid enterocyte tissue levels.

The objectives of our present study relate to the CaCo-2 enterocyte cell line: *1)* to demonstrate the level of Δ9, Δ6, and Δ5 desaturase activity in fully differentiated cell monolayers (18 days after plating); *2)* to assess the modulation of Δ6 desaturase activity under a number of conditions such as CaCo-2 cell differentiation, supplementation of media with fetal bovine serum (FBS), and starvation; and *3)* to assess the influence of exogenous fatty acids, i.e., palmitic (PA), LA, and EPA on Δ6 and Δ5 desaturase enzyme activity, and on membrane phospholipid arachidonate composition and membrane cholesterol/phospholipid (mol/mol) ratio.

MATERIALS AND METHODS

All tissue culture materials were purchased from Gibco Canada (Toronto, ON). Other reagent grade chemicals, including PA, LA, and EPA, were purchased from Sigma Chemical Co. (St. Louis, MO). [1-^{14}C]-16:0, [1-^{14}C]18:2n-6, and [1-^{14}C]20:3n-6 were purchased from NEN (Dupont Canada, Markham, ON).

Cell culture and lipid analysis

For all experiments CaCo-2 cell monolayers were grown in 6-well Nunclon plates (Nunc Canada, Mississauga, ON). Cell culture techniques and methods have been described previously (8). Total lipids were extracted from cell homogenates by the method of Folch, Lees, and Sloane Stanley (17). Lipid free cholesterol was determined using an enzymatic-colorimetric method (18). Total phospholipids were separated from neutral lipids by one-dimensional thin-layer chromatography on silica gel G plates using a solvent system comprised of hexane–diethyl ether–acetic acid 80:30:1 (v/v/v) by the method of Skipski et al. (19). Phospholipid subclasses were separated similarly on silica gel H plates using a solvent system comprised of chloroform–methanol–acetic acid–water 50:30:8:4 (v/v/v/v) by the method of Fine and Sprecher (20). Phospholipid and media fatty acids were transesterified by the method of Lepage and Roy (21). The resulting fatty acid methyl esters were separated and quantitated as previously described (8).

Preparation of cell membrane, and characterization of cell differentiation

CaCo-2 cell differentiation and maturation were characterized by quantitating microvillus membrane (MVM) sucrase, alkaline phosphatase (ALP) and γ-glutamyltransaminase (GGT) activity on days 6, 12, 24, and 30 after plating. Other cellular membranes were precipitated from MVM using 10 mM calcium chloride as described by Kessler et al. (22). Homogenates, calcium precipitated membranes, and MVM preparations were assayed for sucrase activity using the method of Dahlqvist (23). The ALP and GGT activities were measured using colorimetric/kinetic assays, in which the respective substrates, *p*-nitrophenylphosphate and L-γ-*p*-nitroanalide, were hydrolyzed. Activity was assayed using the Astra 8 automated system equipped with a continuous monitoring, temperature-controlled cuvette, and a dual-beam UV/VIS spectrophotometer (Beckman Instruments, Brea, CA).

Desaturase assay

Before measuring desaturase activity, the culture medium was always removed and cell monolayers were gently rinsed 3 times with sterile 0.2% albumin-RPMI at 37°C to minimize contamination from surface-bound but not incorporated fatty acids and from dilution of the 1-^{14}C-labeled fatty acid.

The Δ9, Δ6, and Δ5 desaturase activities were determined by the rate of desaturation of the fatty acid substrates [1-^{14}C]16:0, [1-^{14}C]18:2n-6, and [1-^{14}C]20:3n-6 to their respective radiolabeled monoenes, triene, and tetraene products by a modification of the method of Cook and Spence (24). The assay medium contained in a final volume of 1.0 ml: 0.2% albumin-RPMI and 40 μM cold fatty acid substrate with 0.1 μCi each of [1-^{14}C]16:0 (130 dpm/pmol), [1-^{14}C]18:2n-6 (110 dpm/pmol), or [1-^{14}C]20:3n-6 (106 dpm/pmol). The final concentration of 1-^{14}C-labeled fatty acid was 2 μM. In 'control' incubates the monolayers were pre-fixed (for 15 min), with 1.0 ml ice-cold methanol before incubation in the assay medium. 'Control' and 'test' incubates were placed in a metabolic shaker (Haake, Fisher Scientific) for 2 h at 37°C in air. The incubation time for desaturase activity was determined under these conditions to be linear for a fatty concentration 0–40 μM (data not shown). The medium was then removed and the monolayers were quickly rinsed in ice-cold 0.2% albumin–phosphate-

buffered saline (2°C) and fixed with 250 μl ice-cold methanol. The final volume was increased to 1 ml using phosphate-buffered saline, the cells were homogenized, and aliquots were taken for measurement of total cellular radioactivity, protein content (25), and extraction of total lipids (17).

Total fatty acid methyl esters were prepared from lipid extracts and known standards were spotted onto 10% silver nitrate-impregnated silica gel H thin-layer chromatography plates. For the Δ9 and Δ6 desaturase assays, the plates were developed in hexane–diethyl ether 85:15 (v/v), and for the Δ5 desaturase assay plates were developed in hexane–diethyl ether 60:40 (v/v) (26). Saturated, monoene, triene, and tetraene bands were identified, recovered into scintillation vials containing 10 ml of scintillation fluor, and counted with an efficiency of more than 90% (Beckman Beta Counter, CA). Desaturase activity was expressed as pmol of 1-^{14}C-labeled product fatty acid synthesized per h per mg of total cellular protein after correcting for background activity in control incubates. Protein was quantitated according to the method of Bradford (25).

Factors influencing desaturase activity and membrane lipid composition

Δ9, Δ6, and Δ5 desaturase activities. Mature CaCo-2 cells (18 days of culture in RPMI + 2.5% FBS) were incubated for 2 h with Δ9, Δ6, and Δ5 substrates. Desaturase activities were reported as the sum of the rate of conversion to their respective products along with the product incorporation into phospholipids, triglycerides, cholesterol esters, and free fatty acids (FFA).

Fetal bovine serum. The effect of FBS on Δ6 desaturase activity was investigated by growing cells in RPMI-media supplemented to different final concentrations (0.5%, 2.5%, 15%, and 25%) of FBS for 16 days after subculture. The influence of FBS concentration on total cellular phospholipid fatty acid composition was also examined. The same lot number of FBS was used for all experiments. The fatty acid concentration of the FBS was 2.3 ± 0.3 mmol/l; the fatty acid composition is shown in **Table 1.**

Cell differentiation. Δ6 desaturase activity was measured in cell monolayers as a function of cell growth and differentiation on days 6, 12, 18, and 25 after subculture. The culture medium for the CaCo-2 cell monolayers was supplemented with 2.5% FBS to optimize desaturase activities. Cellular phospholipid fatty acid composition was also examined at the successive timed intervals for measurement of Δ6 desaturase activity.

Starvation. Short term effects of starvation on Δ6 activity were investigated. Cell cultures were grown in 15% fetal serum-supplemented RPMI (for 16 days after subculture, as described above), 'fed' once (time 0), and at successive timed intervals from 4 h to 72 h the whole cell Δ6 desaturase activity was measured. Cellular phospholipid fatty acid composition was also examined.

Fatty acid supplementation. Cells were grown until confluence, day 6 after subculture. On day 6 and for 10 days thereafter, fatty acids were added to enrich the culture medium as previously described (4, 5). The final concentrations of fatty acids used were 100 μM of PA (16:0), LA (18:2n-6), or EPA (20:5n-3). The fatty acid mole % of each medium is shown in Table 1. The Δ6 and Δ5 desaturase activities were measured in control and fatty acid-supplemented whole-cell cultures on day 16. On day 16 using Ca^{2+}-precipitated membranes, the effects of fatty acid supplementation on phosphatidylethanolamine (PE) and phosphatidylcholine (PC) membrane fatty acid composition and the cholesterol/phospholipid membrane (mol/mol) ratio were also examined.

Statistical analysis

All data are expressed as mean ± standard error. Comparisons between groups were performed using a one-way analysis of variance and by the Newman-Keuls multiple comparisons test (Quickstat 2.0, Microsoft Corporation). With all statistical analyses, an associated probability (P value) of <0.05 was considered statistically significant.

RESULTS

After monolayers became confluent (5 to 6 days after plating) 'dome' formation increased rapidly in number

TABLE 1. Fatty acid composition of fetal bovine serum (FBS) and fatty acid-enriched cell culture media

	Mol % Composition				
Fatty Acid	FBS	Control	PA	LA	EPA
C14:0	1.6	2.0	1.0	1.0	0.4
C16:0	23.4	24.7	70.6	6.9	6.3
C18:0	23.4	24.5	6.1	3.8	4.3
C18:1n-9	19.5	16.6	4.8	4.4	4.6
C18:2n-6	6.5	5.6	2.6	70.1	3.1
C18:3n-3	0.7	0.8	0.1	0.3	0.1
C20:4n-6	8.9	7.2	2.3	1.6	1.7
C20:5n-3	0.6	0.4	0.3	0.2	72.4
C22:6n-3	1.8	1.8	1.2	1.1	1.4
Other	13.6	16.4	11.1	10.4	5.8
ΣSAT		53.3	79.1	14.1	11.6
Σn-9		18.1	5.5	4.9	5.2
Σn-6		15.2	2.8	72.9	4.9
Σn-3		4.7	4.5	2.2	75.4

The control medium contained RPMI with 2.5% fetal calf serum. The palmitic acid (PA, 16:0) oleic acid (OA, 18:1n-9), linoleic acid (LA, 18:2n-6), and eicosapentaenoic acid (EPA, 20:5n-3) media contained RPMI with 2.5% fetal calf serum supplemented with 100 μM fatty acid, respectively. Aliquots of culture media were analyzed for fatty acid composition by gas chromatography. Data shown are the mean relative percent composition of fatty acids from three different experiments; SEM was <5%.

and size, peaking by day 12. Thereafter the size and number of domes stabilized and remained constant (data not shown). **Figure 1** displays, over time, measurements of the activities of sucrase, ALP, and GGT in MVM preparations. The activities of all enzymes increased rapidly after confluence. ALP mass activity was the highest, followed by sucrase and then GGT. The rates of increase in enzyme activities with culture time were not parallel: sucrose activity crested on day 15; ALP and GGT crested on days 18 and 25, respectively.

Δ9, Δ6 and Δ5 desaturase activity and their products in cellular lipids

The capacity of CaCo-2 fully differentiated monolayers (day 18 of plating) to convert PA (16:0) to palmitoleic acid (16:1) by Δ9 desaturase, LA (18:2n-6) to γlinolenic acid (18:3n-6) by Δ6 desaturase, and dihomo-γ-linolenic acid (20:3n-6) to arachidonic acid (20:4n-6) by Δ5 desaturase is reported in **Fig. 2.** The results are expressed as pmol of substrate converted per h per mg of CaCo-2 protein. Cellular lipids were first separated by thin-layer chromatography into phospholipids (PLP), triacylglycerols (TG), cholesteryl esters and free fatty acid (CE+FFA) before identification of desaturation products. Relatively large differences occurred among the Δ9, Δ6, and Δ5 desaturation rates.

The rate of formation of desaturation products was Δ9>Δ6>Δ5. The distribution of the desaturation products was as follows: PLP>TG>CE+FFA. For Δ9 desaturase approximately 49%, 39%, and 12% of the products of desaturase activity was found in the PLP, TG, and CE+FFA fractions, respectively. For Δ6 desaturase approximately 58%, 32%, and 8% of the products of desaturase activity was found in the PLP, TG, and CE+FFA fractions, respectively. For Δ5 desaturase approximately 80%, 18%, and 2% of the products of desaturase activity was found in the PLP, TG, and CE+FFA fractions, respectively.

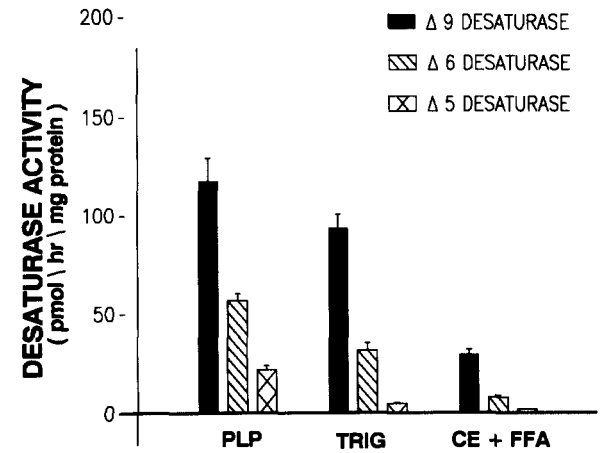

Fig. 2. Fatty acid desaturation in cellular lipids. Cells were cultured in media supplemented with 2.5% fetal bovine serum, and cultured to day 18 after subculture. The Δ9 (■), Δ6 (▨), and Δ5 (⊠) desaturase activities were measured for phospholipid, triglyceride, and cholesterol ester (CE) plus free fatty acid fractions. The desaturase activity is expressed as pmol of 1-^{14}C-labeled fatty acid product formed per h per mg of cell protein. Values represent mean ± SE from six separate cell passages 20-26.

Effect of fetal bovine serum

The impact of FBS supplementation of culture media on Δ6 desaturase activity is shown in **Fig. 3.** Compared with 0.5% FBS, in cells grown in media supplemented

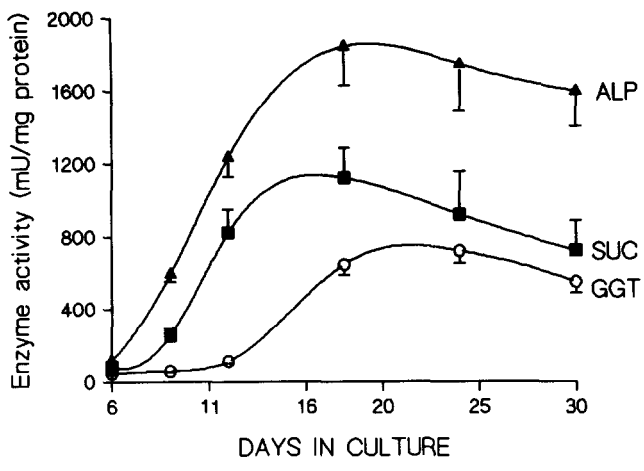

Fig. 1. Growth-related differentiation of microvillous membrane enzyme activity in CaCo-2 cells. Measurements of sucrase (SUC), alkaline phosphatase (ALP), and γ-glutamyltransaminase (GGT) activities in brush border microvillous preparations on days 6, 8, 12, 18, 24, and 30 after plating as described in Materials and Methods. Values represent means ± SE from six separate cell passages 20-26.

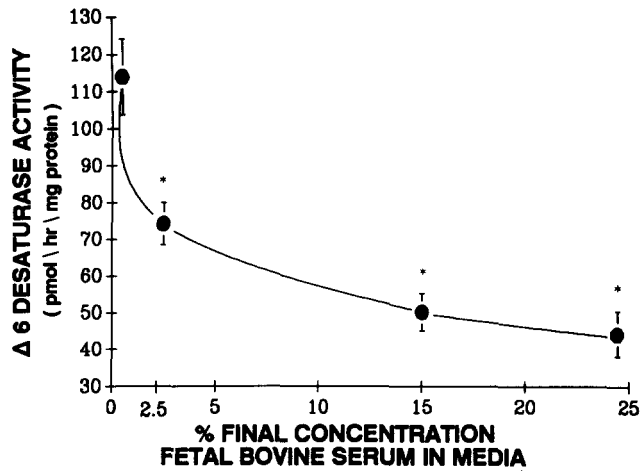

Fig. 3. Effect of fetal bovine serum supplementation of culture media on Δ6 desaturase activity in the CaCo-2 cell. Cells were grown in media supplemented with varying concentrations of fetal bovine serum (0.5%, 2.5%, 15%, and 25%), and cultured to day 18 after subculture. The Δ6 desaturase activity was measured in cell monolayers. Values represent mean ± SE from five separate cell passages 22-27. (*$P < 0.05$ versus 0.5% FBS).

with 2.5%, 15.0%, or 25% FBS Δ6 desaturase activity was significantly inhibited by 37.7%, 50.2%, and 74.2% respectively.

Adding FBS to the culture medium also resulted in significant changes in the fatty acid composition of CaCo-2 phospholipids as shown in **Table 2.** At all levels of FBS, PA (16:0) and stearic (18:0) acid were the major saturated fatty acids (SFA). Although the FBS-supplemented media had little influence on the proportion of PA, stearic acid increased between 0.5% FBS and 2.5%, 15%, and 25% FBS supplementation. The changes in 18:0 did not affect the proportion of total SFA present which was approximately 40–43% of the total phospholipid fatty acid. Although there was little influence on the proportion of oleic acid (18:n-9) above 2.5% FBS, there was a significant increase between 0.5% FBS and 2.5% FBS. Cells grown in 0.5% FBS had substantially higher levels of Mead's acid (20:3n-9) than cells grown at all other FBS concentrations. As the proportion of 20:3n-9 decreased using 2.5% FBS, 18:1n-9 rose such that there was no change in the sum of n-9 fatty acids.

As expected from the decrease in Δ6 desaturase activity when FBS was added, n-6 polyunsaturated profiles changed similarly (Table 2). While LA (18:2n-6) increased with increasing concentrations of FBS above 2.5%, arachidonic acid (20:4n-6) levels plateaued at 2.5% FBS and were lower than those at 0.5% FBS. At all levels of FBS the predominant n-6 fatty acid was arachidonic acid. The 20:4n-6/18:2n-6 ratio, an index of total Δ6 and Δ5 desaturase activities (27), was high using 0.5% FBS, dropped 40% using 2.5% FBS, dropped further using 15% FBS, and then stabilized. DHA (22:6n-3), the main n-3 PUFA, decreased between 0.5% and 2.5% FBS supplementation and thereafter remained stable. The unsaturation index, a reflexion of Δ9, Δ6, and Δ5 desaturation activity, fell between 0.5% and 2.5% FBS supplementation and plateaued thereafter. No differences were noted in PE and PC fatty acid composition between 15% and 25% FBS supplementation.

Effect of maturation

The capacity of CaCo-2 cell monolayers to convert LA (18:2n-6) to γlinolenic acid (18:3n-6) by Δ6 desaturase during cell growth and maturation is shown in **Fig. 4.** Cells were grown in 2.5% FBS, the concentration that was shown to optimize Δ6 desaturase activity. After confluency (day 6 of culture), Δ6 desaturase activity decreased rapidly, paralleling the increase in microvillous membrane sucrase, ALP and GGT activity. The Δ6 desaturase activity decreased approximately 25% between days 6 and 12, 34% between days 6 and 18 and 40% between days 6 and 24 of culture.

TABLE 2. Effect of supplementation of fetal bovine serum to cell culture media on the fatty acid composition of CaCo-2 cellular phospholipids

	Mol % Composition			
Fatty Acid	0.5% FBS	2.5% FBS	15% FBS	25% FBS
C14:0	0.6 ± 0.1^a	1.3 ± 0.1^b	1.5 ± 0.1^b	0.7 ± 0.1^a
C16:0	28.0 ± 2.1^a	20.4 ± 1.4^a	24.7 ± 2.0^a	26.0 ± 2.3^a
C18:0	11.2 ± 1.4^b	15.6 ± 2.0^a	15.1 ± 2.2^a	15.9 ± 2.3^a
C18:1n-9	19.6 ± 2.7^b	26.1 ± 4.1^a	27.1 ± 4.5^a	29.1 ± 4.2^a
C18:2n-6	0.9 ± 0.1^a	1.2 ± 0.1^a	5.2 ± 0.4^b	6.3 ± 0.7^c
C20:0	0.8 ± 0.1^b	1.4 ± 0.1^c	0.2 ± 0.0^a	0.4 ± 0.0^a
C20:1n-9	1.1 ± 0.1^a	0.9 ± 0.1^{a,d}	2.0 ± 0.2^b	0.5 ± 0.0^{c,d}
C20:3n-9	4.2 ± 0.8^a	0.9 ± 0.1^b	<0.1	<0.1
C20:3n-6	2.7 ± 0.4^b	1.1 ± 0.1^c	0.8 ± 0.1^a	0.5 ± 0.0^a
C20:4n-6	13.1 ± 0.8^b	9.8 ± 0.7^a	9.8 ± 0.8^a	10.2 ± 0.8^a
C22:5n-3	1.2 ± 0.1^b	1.5 ± 0.2^c	0.9 ± 0.1^a	0.9 ± 0.1^a
C22:6n-3	5.0 ± 0.4^b	3.2 ± 0.2^a	3.4 ± 0.3^a	4.1 ± 0.3^a
Other	11.6 ± 2.0^a	16.6 ± 2.4^c	9.2 ± 1.5^{a,b}	5.4 ± 0.7^b
ΣSAT	40.7 ± 4.4^a	39.3 ± 4.1^a	42.3 ± 4.6^a	43.5 ± 4.0^a
Σn-9	25.9 ± 1.9^a	28.0 ± 2.4^a	29.1 ± 2.0^a	29.6 ± 3.6^a
Σn-6	17.1 ± 1.4^b	13.5 ± 1.3^a	13.6 ± 1.3^a	12.5 ± 1.2^a
Σn-3	6.5 ± 0.6^a	5.6 ± 0.4^a	5.0 ± 0.4^a	5.8 ± 0.6^a
Δ9I	1.8 ± 0.2^a	1.7 ± 0.2^a	1.8 ± 0.2^a	1.6 ± 0.2^a
Δ6I	13.4 ± 1.2^a	8.2 ± 0.7^b	1.3 ± 0.1^c	1.4 ± 0.2^c
UI	144.3 ± 11.6^b	121.1 ± 11.9^a	107.3 ± 11.8^a	117.7 ± 8.8^a

Values are mean ± SEM determined on four separate culture passages (21–25) for each group. Confluent cell monolayers were grown in culture media supplemented with varying final FBS concentrations. Cellular total phospholipid fatty acids were measured as described in Methods by gas-liquid chromatography. Other: includes the sum total of the following fatty acids: 20:0, 22:0, 16:1n-7, 18:1n-7, 20:1n-9, 18:3n-3, 20:2n-6, 22:4n-6. Plasmalogenic dimethylacetals were detected but not measured. UI, unsaturation index = Σ[(wt %) × (C=C bonds)]; Δ9I: is the ratio of 18:1n-9/18:0; Δ6I: is the ratio of 20:4n-6/18:2n-6.

$^{a-d}$Values without the same superscript are significantly different ($P < 0.05$).

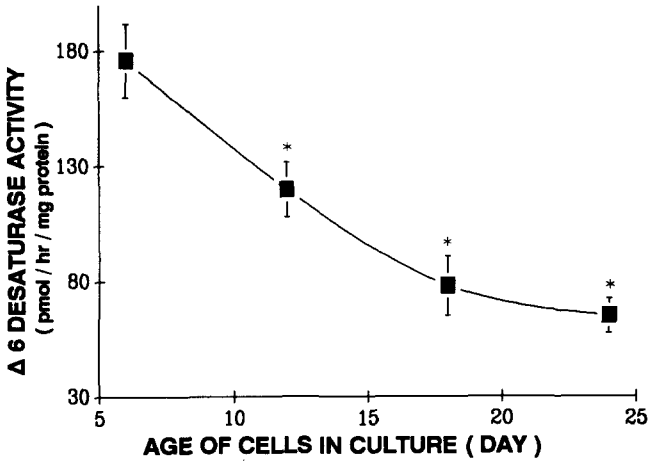

Fig. 4. Effect of cell maturation on Δ6 desaturase activity in the CaCo-2 cell. Cells were grown in media supplemented with 2.5% fetal bovine serum to optimize desaturase activity. At days 6, 12, 18, and 24 after subculture, the Δ6 desaturase activity was measured in cell monolayers. Values represent means ± SE from four separate cell passages 22-26. (*$P < 0.05$ versus day 6).

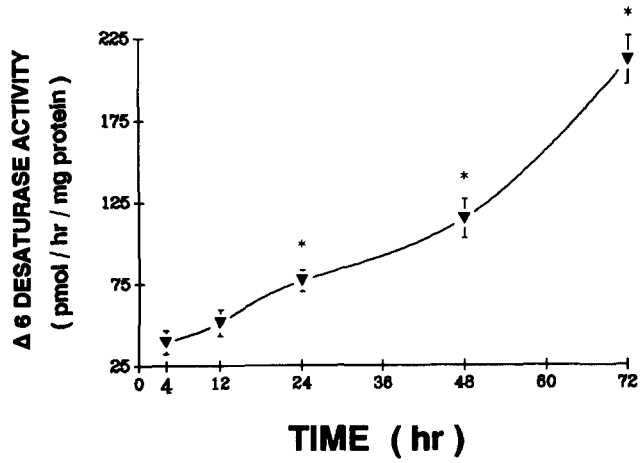

Fig. 5. Effect of 'starvation' media on Δ6 desaturase activity in the CaCo-2 cell. Cells were grown in media supplemented with 15% fetal bovine serum and cultured to day 18 after subculture. Fresh culture medium was replaced at time 0, and at successive time intervals—4 h, 12 h, 24 h, 48 h, and 72 h—the Δ6 desaturase activity was measured in cell cultures. Values represent mean ± SE from five separate cell passages 22-27. (*$P < 0.05$ versus 4 h).

Effect of 'starvation'

Mature CaCo-2 cells were fed media containing 15% FBS at time 0 and their capacity to convert LA (18:2n-6) to γlinoleic acid (18:3n-6) (Δ6 desaturase activity) was measured at 4, 12, 24, 48, and 72 h after feeding (**Fig. 5**). The 4 h and 12 h Δ6 desaturase activities were not found to be significantly different ($P < 0.05$). However, Δ6 desaturase activity was increased 2-, 3-, and 5.4-fold above the 4 h activity at 24, 48, and 72 h respectively.

The effects of 'starvation' on CaCo-2 phospholipid fatty acid composition is shown in **Table 3.** PA (16:0) and stearic (18:0) acid, the major saturated fatty acids, were not changed by 'starvation'. Not unexpectedly, the sum of saturated fatty acids did not change. 'Starvation' had little effect on monounsaturated fatty acid until 72 h when oleic (18:1n-9) acid decreased; in contrast, the proportion of Mead's acid (20:3n-9) rose substantially. The decrease in 18:1n-9 at 72 h was offset by the increase in 20:3n-9 such that the sum of n-9 fatty acids did not change.

TABLE 3. Effect of 'starvation' on the fatty acid composition of CaCo-2 cellular phospholipids

	Mol % Composition					
Fatty Acid	0 h	4 h	12 h	24 h	48 h	72 h
C16:0	26.7 ± 1.9^a	27.5 ± 3.3^a	26.0 ± 3.8^a	27.5 ± 2.7^a	26.0 ± 2.1^a	26.3 ± 2.3^a
C18:0	12.7 ± 1.1^a	15.4 ± 1.0^a	13.8 ± 1.7^a	13.9 ± 1.4^a	12.9 ± 1.3^a	11.2 ± 1.0^a
C18:1n-9	23.1 ± 1.9^{a,b}	28.5 ± 2.5^a	32.4 ± 3.9^a	30.0 ± 3.2^a	29.1 ± 3.2^a	19.1 ± 1.5^b
C18:2n-6	6.7 ± 0.6^b	3.7 ± 0.4^c	2.2 ± 0.3^a	1.8 ± 0.2^a	2.1 ± 0.2^a	2.2 ± 0.2^a
C20:1n-9	0.9 ± 0.1^a	0.7 ± 0.1^a	2.1 ± 0.1^b	2.0 ± 0.2^b	2.0 ± 0.2^b	2.8 ± 0.3^c
C20:3n-9	0.5 ± 0.0^a	0.7 ± 0.1^a	0.6 ± 0.1^a	0.5 ± 0.1^a	1.1 ± 0.1^b	5.2 ± 0.5^c
C20:3n-6	1.3 ± 0.1^b	0.7 ± 0.1^a	0.6 ± 0.1^a	0.7 ± 0.1^a	1.7 ± 0.2^b	2.7 ± 0.2^c
C20:4n-6	3.0 ± 0.3^a	4.0 ± 0.4^a	4.2 ± 0.2^a	4.7 ± 0.6^a	6.8 ± 7^b	10.5 ± 1.1^c
C22:6n-3	1.9 ± 0.2^a	1.8 ± 0.1^a	2.1 ± 0.2^a	1.6 ± 0.2^a	1.5 ± 0.2^a	4.7 ± 0.5^b
Other	23.2 ± 2.2^a	17.8 ± 2.4^a	16.5 ± 2.2^a	17.5 ± 2.5^a	16.8 ± 2.9^a	15.3 ± 2.8^a
ΣSAT	42.0 ± 5.6^a	45.8 ± 3.9^a	41.6 ± 4.5^a	44.0 ± 6.4^a	41.5 ± 5.0^a	39.3 ± 3.0^a
Σn-9	24.5 ± 3.5^a	29.9 ± 2.2^a	35.1 ± 3.0^a	32.5 ± 4.5^a	32.2 ± 4.3^a	27.1 ± 2.3^a
Σn-6	12.3 ± 1.8^a	9.1 ± 0.5^{a,b}	7.3 ± 0.6^b	8.3 ± 0.7^{a,b}	11 ± 1.6^{a,b}	17.3 ± 1.4^c
Σn-3	3.5 ± 0.5^a	4.1 ± 0.6^a	4.4 ± 0.4^a	3.2 ± 0.3^a	3.1 ± 0.5^a	7.3 ± 0.7^b
Δ6I	0.4 ± 0.0^a	1.1 ± 0.1^a	1.9 ± 0.1^b	2.6 ± 0.2^{b,c}	3.2 ± 0.4^c	4.8 ± 0.5^d
Δ9I	1.8 ± 0.3^a	1.9 ± 0.2^a	2.3 ± 0.2^a	2.2 ± 0.1^a	2.3 ± 0.3^a	1.7 ± 0.2^a
UI	94 ± 13.6^a	93 ± 12.4^a	89 ± 7.8^a	88 ± 9.1^a	99 ± 8.0^a	143 ± 17.4^b

Values are means ± SEM determined on four separate culture passages (21-25) for each group. Cell monolayers were grown in 15% FBS-supplemented RPMI culture medium until day 18 after subculture. Culture medium was replaced only once (0 h) and total cellular phospholipid fatty acids were measured at successive time intervals.

$^{a-d}$Values without the same superscript are significantly different ($P < 0.05$).

'Starvation', in addition to up-regulating Δ6 desaturase activity, not unexpectedly altered n-6 PUFA profiles. At 4 h the proportion of LA (18:2n-6) was significantly lower and remained lower than at 0 h. The decrease in 18:2n-6 was offset at 48 h by an increase in arachidonic acid (20:4n-6) followed by a further rise at 72 h. At 48 h, the increase in 20:4n-6 had returned the sum of n-6 fatty acid levels to baseline (0 h) level resulting in a significant rise in the proportion of n-6 PUFA at 72 h. The 20:4n-6/18:2n-6 ratio, an index of total Δ6 and Δ5 desaturase activities as stated earlier, had increased at 24 h compared with 0 h and had further increased at 48 and 72 h. Starvation had a similar effect on n-3 PUFA but a longer latency period. The sum of n-3 PUFA showed an increase at 72 h that consisted almost entirely of a significant rise in 22:6n-3.

TABLE 4. Effect of media enrichment with different fatty acids on the Δ6 and Δ5 desaturase activities of the CaCo-2 cell

	Desaturase Activity			
	Control	Medium + PA	Medium + LA	Medium + EPA
	pmol/mg/h			
Δ6	77.9 ± 4.1^{b}	74.1 ± 3.1^{b}	31.5 ± 3.6^{a}	30.4 ± 3.0^{a}
Δ5	36.7 ± 1.1^{a}	37.7 ± 2.0^{a}	26.6 ± 4.0^{b}	15.1 ± 0.7^{c}

Values are the mean ± SEM determined on four to six separate culture passages (21-27) for each group. Cells were grown for 10 days in 2.5% fetal calf serum-supplemented RPMI culture medium (control), enriched with 100 μM final concentration of fatty acid; PA, palmitic acid (16:0); LA, linoleic acid (18:2n-6); EPA, eicosapentaenoic acid (20:5n-3). Δ6 and Δ5 desaturase activities were measured.

$^{a-d}$Values without the same superscript are significantly different ($P < 0.05$).

Fatty acid supplementation

Modification of the two desaturase enzymes involved in the conversion of LA to arachidonic acid (Δ6 and Δ5 desaturase) by the addition of fatty acid to the CaCo-2 cell medium is shown in **Table 4.** Control and fatty acid-supplemented media contained 2.5% FBS to optimize desaturase activity. Adding the SFA PA to the medium on day 6 of CaCo-2 cell plating for 10 days caused no change in the activities of either enzyme compared to control cells. Similar direction changes were found upon adding LA or EPA. The suppression in Δ6 desaturase activity was approximately 2.4-fold for LA- or EPA-supplemented cells compared to controls. Similarly, Δ5 desaturase activity was not significantly different in cells cultured in control or PA-enriched medium. In cells grown in LA-enriched medium, the Δ5 desaturase activities were decreased 1.4-fold. In comparison, in EPA-supplemented medium Δ5 desaturase activity was diminished 2.4-fold compared to control cells.

The fatty acid composition of PE and PC CaCo-2 cell membranes is shown in **Table 5** and **Table 6.** In control cells, before fatty acid supplementation, the unsaturation index (UI) was 2-fold higher in PE compared to PC. PE contained higher amounts of the products of fatty acid

TABLE 5. Effect of fatty acid supplementation on phosphatidylethanolamine (PE) fatty acid composition

	Mol % Composition			
Fatty Acid	Control	PA	LA	EPA
C16:0	9.6 ± 0.5^{a}	18.5 ± 1.2^{c}	8.5 ± 0.5^{a}	12.1 ± 0.8^{b}
C18:0	13.9 ± 0.9^{a}	20.2 ± 1.4^{b}	16.4 ± 1.5^{a,b}	20.7 ± 2.1^{b}
C18:1n-9	30.4 ± 3.3^{a}	31.1 ± 3.8^{a}	13.2 ± 1.7^{b}	13.3 ± 1.5^{b}
C18:2n-6	1.7 ± 0.1^{a}	1.9 ± 0.2^{a}	19.8 ± 1.5^{c}	1.8 ± 0.1^{a}
C20:3n-9	3.2 ± 0.2^{d}	2.5 ± 0.2^{c}	<0.1	0.8 ± 0.1^{b}
C20:3n-6	0.8 ± 0.1^{a,b}	0.4 ± 0.1^{c}	1.7 ± 0.2^{d}	0.6 ± 0.1^{b,c}
C20:4n-6	13.2 ± 0.7^{b}	15.5 ± 0.6^{a}	10.2 ± 1.0^{c}	8.9 ± 0.6^{c}
C20:5n-3	0.2 ± 0.0^{a}	0.2 ± 0.1^{a}	0.2 ± 0.1^{a}	10.2 ± 0.5^{b}
C22:5n-3	2.7 ± 0.2^{a}	1.9 ± 0.2^{b}	2.3 ± 0.2^{a,b}	10.4 ± 0.7^{c}
C22:6n-3	3.9 ± 0.2^{a}	3.7 ± 0.3^{a}	3.4 ± 0.3^{a}	3.5 ± 0.4^{a}
Other	20.5 ± 2.0^{a,b}	4.0 ± 0.3^{c}	24.3 ± 2.2^{a}	17.6 ± 1.7^{b}
ΣSAT	25.0 ± 1.8^{a}	39.9 ± 2.3^{b}	26.7 ± 1.7^{a}	37.1 ± 3.0^{b}
Σn-9	33.6 ± 2.9^{a}	33.6 ± 1.9^{a}	13.3 ± 0.9^{b}	14.1 ± 0.9^{b,c}
Σn-6	15.9 ± 1.5^{a}	12.8 ± 1.2^{a}	45.9 ± 4.7^{c}	12.2 ± 1.4^{a}
Σn-3	6.9 ± 0.9^{a}	2.3 ± 0.4^{a}	6.1 ± 0.4^{a}	24.9 ± 1.8^{b}
Δ9I	2.2 ± 0.1^{a}	1.5 ± 0.1^{c}	0.8 ± 0.1^{b}	0.6 ± 0.1^{b}
Δ6I	7.8 ± 0.7^{c}	5.4 ± 0.5^{a}	0.8 ± 0.1^{b}	4.9 ± 0.3^{a}
UI	156.2 ± 13.3^{a}	107.8 ± 9.1^{a}	192.5 ± 12.1^{b}	197.5 ± 15.9^{b}

Values are mean ± SEM determined on six separate culture passages (21-26) for each group. Cells were grown for 10 days in 2.5% FBS-supplemented RPMI culture medium (control), enriched with 100 μM final concentration of fatty acid; PA, palmitic acid (16:0); LA, linoleic acid (18:2n-6); EPA, eicosapentaenoic acid (20:5n-3). Ca^{2+}-precipitated membranes were recovered from cell homogentaes as described in Methods. Cellular phospholipid subclasses were separated by thin-layer chromatography and quantitated by gas-liquid chromatography.

$^{a-d}$Values without the same superscript are significantly different ($P < 0.05$).

TABLE 6. Effect of fatty acid supplementation on phosphatidylcholine (PC) fatty acid composition

Fatty Acid	Mol % Composition			
	Control	PA	LA	EPA
C16:0	27.6 ± 1.5[a]	27.0 ± 1.8[a]	24.8 ± 1.6[a]	29.5 ± 2.0[a]
C18:0	11.5 ± 0.7[a]	10.5 ± 0.7[a]	15.9 ± 1.5[b]	17.9 ± 1.8[b]
C18:1n-9	30.2 ± 3.2[a]	35.4 ± 4.2[a]	14.1 ± 1.8[b]	18.8 ± 2.1[b]
C18:2n-6	2.6 ± 0.2[a]	2.4 ± 0.2[a]	25.2 ± 1.9[b]	2.2 ± 0.2[a]
C20:3n-9	1.0 ± 0.1[a]	1.3 ± 0.1[a]	1.3 ± 0.1[a]	0.2 ± 0.0[b]
C20:3n-6	0.4 ± 0.1[a,b]	0.2 ± 0.0[a]	1.3 ± 0.1[d]	0.7 ± 0.1[c]
C20:4n-6	2.7 ± 0.1[a]	2.4 ± 0.1[a]	5.0 ± 0.3[b]	2.8 ± 0.2[a]
C20:5n-3	<0.1	<0.1	0.4 ± 0.1[a]	6.0 ± 0.7[b]
C22:5n-3	0.2 ± 0.0[a]	0.2 ± 0.0[a]	0.3 ± 0.0[a]	4.8 ± 0.8[b]
C22:6n-3	0.7 ± 0.1[a,b]	0.4 ± 0.1[d]	0.8 ± 0.2[a]	0.6 ± 0.2[b,c]
Other	23.0 ± 3.2[a]	20.0 ± 2.4[a]	11.0 ± 1.2[b]	16.6 ± 1.4[a,b]
ΣSAT	40.4 ± 6.5[a]	39.3 ± 5.1[a]	42.3 ± 5.9[a]	50.0 ± 9.0[a]
Σn-9	31.2 ± 5.9[a]	36.7 ± 4.8[a]	14.3 ± 2.1[b]	19.1 ± 2.7[b]
Σn-6	5.9 ± 1.2[a]	5.4 ± 1.1[a]	34.2 ± 7.9[c]	5.9 ± 1.5[a]
Σn-3	0.9 ± 0.3[a,b]	0.6 ± 0.1[a]	1.5 ± 0.2[b]	11.6 ± 1.9[c]
Δ9I	2.6 ± 0.4[b]	3.4 ± 0.5[c]	0.9 ± 0.2[a]	1.1 ± 0.2[a]
Δ6I	1.0 ± 0.2[a]	1.0 ± 0.2[a]	0.2 ± 0.0[b]	1.3 ± 0.2[a]
UI	77.4 ± 14.8[a]	76.9 ± 12.3[a]	112.0 ± 15.7[b]	109.6 ± 19.7[b]

Values are mean ± SEM determined on six separate culture passages (21–26) for each group.
[a–d]Values without the same superscript are significantly different ($P < 0.05$).

desaturation and elongation, i.e., 20:3n-9, 20:4n-6, and 22:6n-3. In contrast, the proportion of total fatty acids as SFA in PC was 40.4% and in PE it was 25%.

The fatty acid composition of PE and PC reflected changes in the medium fatty acid composition and desaturase activities. As expected, enrichment of the media with 100 μM PA, LA, or EPA significantly increased their concentrations in PE and PC fatty acids with LA being preferentially incorporated into PC and EPA into PE. The UI of PC was similar for control and cells fed PA and rose significantly upon LA or EPA supplementation. The trend in the UI of PE was similar to PC when LA or EPA was supplemented. The changes in both Δ6 and Δ5 desaturase activity were more evident in fatty acid changes in PE. Compared to control PE, the proportion of arachidonic acid decreased upon LA supplementation and there was a further significant reduction upon EPA supplementation. Supplementation with PA caused a slight increase in PE arachidonic acid content which is in keeping with the PA-supplemented Δ6 and Δ5 desaturase activities.

The effects of fatty acid-supplemented media on the Ca^{2+}-precipitated membrane lipid unesterified cholesterol (C)/phospholipid (PLP) (mol/mol) ratio are shown in **Table 7.** Due to limited sample size, Ca^{2+} precipitation membranes, which contain a homogenate of cell membranes including the endoplasmic reticulum but not brush border membranes, were examined in total. The total PLP content of cell membranes grown in fatty acid-enriched media was not significantly different from the control group. Compared to control cells, the membrane C content of cells cultured in media enriched with PA or

TABLE 7. Effect of media enrichment with different fatty acids on the cholesterol (C) and phospholipid (PLP) content of Ca^{2+}-precipitated CaCo-2 cell membranes

	Control	Medium + PA	Medium + LA	Medium + EPA
		nmol/mg protein		
C	62.6 ± 6.5[a]	76.4 ± 4.6[b]	73.7 ± 5.3[b]	54.6 ± 7.1[c]
PLP	565.4 ± 44.7[a]	587.4 ± 30.7[a]	670.0 ± 41.2[a]	607.8 ± 29.7[a]
		mol/mol		
C/P	0.11 ± 0.005[a]	0.13 ± 0.010[c]	0.11 ± 0.008[a]	0.09 ± 0.008[b]

Values are mean ± SEM determined on four separate culture passages (21–25) for each group. Cells were grown for 10 days in 2.5% FBS-supplemented RPMI culture medium (control), enriched with 100 μM final concentration of fatty acid: PA, palmitic acid (16:0); LA, linoleic acid (18:2n-6); EPA, eicosapentaenoic acid (20:5n-3). Ca^{2+}-precipitated membranes were recovered from cell homogenates as described in Methods, and membrane cholesterol (C) and phospholipid (PLP) concentrations were measured.
[a–d]Values without the same superscript are significantly different ($P < 0.05$).

LA contained significantly higher levels of C but were not different from each other. The membrane C of cells grown in media enriched with EPA had significantly less C than control PA or LA cell membranes. Changes in the C content were reflected by significant changes in the C/PLP ratio. The C/PLP (mol/mol) ratios of cells grown in PA or EPA were respectively significantly higher or lower than all others (Table 7).

DISCUSSION

Most of our knowledge on the regulation of desaturase activity in the enterocyte comes from animal studies. Garg et al. (26) have reported that in rats, intestinal ER activities of Δ9 and Δ6 desaturase are generally 2- to 4-fold lower than hepatic ER activities. While rat jejunal-ileal activities of Δ9 desaturase were not different, the Δ6 desaturase activities were 2.5- to 6-fold higher in the jejunum. In this study, whole cell CaCo-2 monolayer (day 18, after subculture) Δ9 desaturase activity was 1.7-fold higher, and Δ6 desaturase 3-fold lower than rat ileal ER desaturase activity; Δ5 desaturase activity was not reported. However, comparing whole cell activity with microsomal activity can be misleading. Garg, Thomson, and Clandinin (28) have recently shown that microsomal Δ6 activity was 4-fold higher than the corresponding activity in mucosal homogenates. In contrast to rats, Giron, Mataix, and Suarez (29), have shown that in dogs, duodenal Δ9 and Δ6 desaturase activity was markedly greater than hepatic activity.

At present there are no data available on human colonic intestinal desaturase activity to make comparisons. The higher Δ9 and lower Δ6 desaturase activity was consistent with the finding of higher monounsaturated fatty acids (18:1n-9) and lower arachidonic acid concentrations in membrane phospholipids of CaCo-2 cells. A more active Δ9 desaturase activity, in comparison with Δ6 or Δ5 desaturation, has also been reported for human T-lymphocytes (30). Although desaturase activity has been demonstrated in the human CaCo-2 enterocyte cell line by Chen and Nilsson (6), comparisons with our data are not possible. They report only the percent conversion of 1-^{14}C-labeled substrate to 1-^{14}C-labeled product and the data were not normalized to the number of cells or amount of cell protein. A comparison of Δ6 or Δ5 desaturase activity at the time of confluency and maturity might have provided insight into desaturase activity levels in crypt versus villus cells. There are few studies comparing differences in the rates of activities between the major desaturase enzymes. Most studies suggest that rates of desaturase activity are different for each type of desaturase enzyme. More importantly, desaturase activity can be markedly dependent on the tissue of origin, species differences, and hormonal and metabolic conditions.

Studies in isolated ER have shown that, similarly to Δ9 desaturase (1), the activity of Δ6 was modified during development and aging. For example, in mice after birth the Δ6 desaturase activity in liver and brain decreases 12-fold and 9-fold, respectively, up to 21 days (31). In this study Δ6 desaturase was modulated by a number of factors such as levels of FBS in the culture medium (Fig. 3), and cell maturation (Fig. 4). Day 6 CaCo-2 cell cultures demonstrated higher Δ6 desaturase activity than fully differentiated day 18 cultures. It should also be noted that cell growth and proliferation at day 6 was much higher than at day 18, and because polyunsaturated fatty acids are necessary for membrane phospholipid synthesis, these findings would be consistent with increased Δ6 desaturase activity. These findings are consistent with recently reported studies showing that fatty acid desaturation-elongation systems are activated and markedly up-regulated in proliferating human T-lymphoblasts (30).

Consistent with previous studies using cultured human endothelial cells, the Δ6 desaturase activity was inhibited by supplementing the culture media with increasing concentrations of FBS (32). Growing cells in lipid-free medium increases Δ6 desaturase activity in many cell lines (33–35). Although 18:3n-6 and 20:4n-6 (products of Δ6 and Δ5 desaturation, respectively) will inhibit the synthesis of arachidonate from linoleate, they do so only at relatively high concentrations (36), suggesting that direct product inhibition is not a major aspect of the regulation of Δ6 desaturase activity. It is possible that supplementation of FBS at high concentrations provides sufficient arachidonic acid to at least partially inhibit endogenous synthesis. Similarly, enriching cell culture media with levels >100 μM of stearic acid, oleaic acid, linoleic acid, arachidonic acid, or *trans* fatty acids inhibits the desaturation of [^{14}C]linoleic acid in neuroblastoma cells (37), vascular endothelial cells (33), and fibroblasts (36). However, rat kidney cells (38) and 3T3 cells (39) appear to be less susceptible to inhibition by excess substrate (150 μM). In neuroblastoma cells, arachidonic acid was actually stimulatory with respect to the desaturation of [^{14}C]linoleic acid (37).

Similarly, in vitro data (Fig. 5) are compatible with Δ6 desaturase activity in short-term (24 h) fasted rats (2). After fasting, Δ6 desaturase activity increased in both jejunum (2.5-fold) and ileum; in contrast, the arachidonic acid content of microsomal total phospholipids increased in the jejunum with a concomitant decrease in LA content. The change in Δ6 desaturase activity was also consistent with the increased arachidonic acid content of membrane phospholipids. When CaCo-2 cells were grown in media supplemented with high concentrations of FBS (Fig. 3), or in media enriched with 100 μM concentrations of fatty acids (Table 4), the Δ6 desaturase activity was greatly attenuated. One possible explanation was that the intestinal desaturase activity was responsive

to the amount and possibly the type of dietary lipid, although growth factors and other hormones present in FBS may also have contributed to down-regulation.

Changes in dietary fat can lead to marked changes in the fatty acid composition of membrane phospholipids. In this study, fatty acid unsaturation of cell phospholipid (PE and PC) fatty acids was substantially modified when cells were cultured in media enriched with different polyunsaturated fatty acids (Table 5 and Table 6). In these cells, both Δ6 desaturase and Δ5 desaturase activity decreased (Table 4) as phospholipid fatty acid unsaturation was increased. For example, the increased membrane unsaturation of cells cultured in media enriched with LA or EPA was correlated with decreased desaturase activity. Increased fatty acid unsaturation of phospholipids may serve as a feedback mechanism to down-regulate desaturase activity by altering membrane fluidity (40). Other investigators (41, 42) have proposed that changes in desaturase activity are dependent on ER membrane fluidity. We did not directly measure membrane fluidity in this study; however, when cells were cultured in media enriched with EPA, the Δ6 and Δ5 desaturase activities were markedly decreased along with a decreased C/P molar ratio and increased membrane phospholipid unsaturation index, both of which contribute to fluidizing the cellular membrane. Similarly, when the membrane fluidity of rat liver endoplasmic reticulum was increased by increasing the molar C (43) content relative to the molar phospholipid content (mol:mol ratio), desaturase activity increased with decreasing membrane fluidity. Similarly, when the unsaturation of hepatic endoplasmic reticulum phospholipid polyunsaturated fatty acids was increased by high dietary intakes of n-3 or n-6 fatty acids, desaturase activity was decreased when compared with high dietary intakes of SFA (27, 43). Zevallos and Farkas (44), have shown that when the plasma membranes of rat lymphocytes were fluidized using synthetic phospholipids, Δ9 desaturation was inhibited. As membrane fluidity measurements were not directly measured in this study, it is unclear how this mechanism would regulate and modulate desaturase activity and maintain the polyunsaturated fatty acid content of membranes in the CaCo-2 cell. Presumably there exists a self-regulatory, compensatory control mechanism in the cell that senses the increased level of membrane phospholipid unsaturation and down-regulates desaturase activity; otherwise increased unsaturation would result (45).

CONCLUSION

This study confirms our previous observation of significant levels of desaturase activity in the fully differentiated CaCo-2 enterocyte cell line. The order of activity is as follows: Δ9 > Δ6 > Δ5. We also observed a pronounced decrease in Δ6 desaturation with cell differentiation as well as with the addition of FBS. In contrast, cell starvation increased Δ6 desaturase activity. In cells receiving long term media supplementation with PA, LA, or EPA, only EPA supplementation resulted in a decrease in phospholipid levels of arachidonic acid. Our results show that in long-term feeding PE, not PC, is the main reservoir for arachidonic acid. Finally, the present study also demonstrates that the reduction in tissue levels of arachidonic acid during EPA supplementation is characterized by a decrease in both Δ6 and Δ5 desaturase activities, the decrease in Δ5 desaturase being equal to Δ6 desaturase reduction.

In ulcerative colitis, increased arachidonic acid levels in colonic mucosa have been reported (46, 47). LTB_4, a potent chemotactic factor and a product of arachidonic acid metabolism, is present in high levels in inflamed colonic mucosa (48, 49). Treatments that reduce synthesis of leukotriene B_4 may be of benefit in ulcerative colitis. Several studies have claimed clinical benefits of fish oil (EPA) in ulcerative colitis (10, 11). A key finding in this study is that EPA disrupts arachidonic acid synthesis and hence is not available for incorporation into membrane phospholipids. Hillier et al. (11) have reported that EPA supplementation decreased arachidonic acid levels in colonic mucosa. The potential value of regulating eicosanoid synthesis by altering desaturase activities needs to be addressed.

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