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Variations in dietary triacylglycerol saturation alter the lipid composition and fluidity of rat intestinal plasma membranes

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Rats were maintained on nutritionally complete diets enriched in unsaturated (corn oil) or saturated (butter fat) triacylglycerols. After 6 weeks, significant differences in the lipid composition and fluidity of a number of intestinal membranes were observed. The corn oil diet (enriched mainly in linoleic acid) increased the overall unsaturation of the acyl chains and enhanced the lipid fluidity, as assessed by the fluorescence anisotropy of 1,6-diphenyl-1,3,5-hexatriene, of enterocyte microvillus and basolateral membranes and of colonocyte basolateral membranes. Concomitantly, the cholesterol content and the cholesterol/phospholipid molar ratio were increased in the microvillus but not in the basolateral membranes. The increased cholesterol in ileal microvillus membranes can result from enhanced cellular biosynthesis, since ileal slices from rats fed the unsaturated diet incorporated [^{14}C]octanoate more rapidly into digitonin-precipitable sterol. Increased fluidity of the enterocyte microvillus and basolateral membranes, respectively, enhanced the enzyme specific activities of *p*-nitrophenylphosphatase and ($Na^+ + K^+$)-dependent adenosine triphosphatase. The results indicate that the lipid composition, fluidity and enzyme activities of intestinal plasma membranes can be altered by dietary means. Moreover, rat enterocytes possess regulatory mechanisms which modulate the cholesterol content of the microvillus membranes so as to mitigate changes in lipid fluidity.

Introduction

The plasma membrane of the rat enterocyte, the major absorptive cell of the small intestinal mucosa, is differentiated into a luminal (microvillus) and a contraluminal (basolateral) region, and these antipodal membranes differ in their lipid composition [1–5] and 'fluidity' [5–7].

The term 'lipid fluidity' as applied to model bilayers and natural membranes is used throughout this report to express the relative motional freedom of the lipid molecules or substituents thereof. When assessed by the estimation of steady-state fluorescence anisotropy of the fluorophore 1,6-diphenyl-1,3,5-hexatriene, changes in the fluorescence anisotropy may be due to alterations in the correlation time and/or maximal hindered anisotropy of the probe. As described previously [5], we use the terms 'lipid fluidity' or 'motional freedom' to designate both kinds of alterations.

While there is considerable evidence that the functions mediated by the intrinsic membrane pro-

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Abbreviations ($Na^+ + K^+$)-ATPase, (sodium plus potassium)-dependent adenosine triphosphatase, Mg^{2+}-ATPase, magnesium-dependent adenosine triphosphatase, 16 0, palmitic acid, 18 1, oleic acid, 18:2, linoleic acid; 20 4, arachidonic acid

teins of these organelles are influenced by the lipids [5,8–10], the cellular mechanisms which regulate the lipid composition and fluidity are poorly understood. We reported recently [10] that one such mechanism in rat ileal enterocytes is the modulation of microvillus membrane cholesterol content and fluidity by changes in the cellular biosynthesis of cholesterol. Enhanced biosynthesis owing to feeding cholestyramine, a bile-salt-binding resin, or to biliary ligation increased the cholesterol content and decreased the fluidity of the ileal microvillus membranes. Conversely, decreased biosynthesis owing to fasting reduced the cholesterol content and increased the fluidity of the membranes. The lipid changes so induced in the microvillus membranes, moreover, were selective and not observed in the antipodal basolateral membrane.

To explore the foregoing mechanism further and to develop a regimen for altering the lipid composition and fluidity of intestinal plasma membranes, we maintained rats on nutritionally complete diets supplemented with either saturated (butter fat) or unsaturated (corn oil) triacylglycerols. The results described below demonstrate that the unsaturated as compared to the saturated dietary regimen increased the lipid fluidity and decreased the saturation index of enterocyte microvillus and basolateral membranes and colonic mucosal cell (colonocyte) basolateral membranes. Concomitantly, the enzyme activities of two intrinsic membrane proteins, enterocyte microvillus membrane *p*-nitrophenylphosphatase and basolateral membrane $(Na^+ + K^+)$-dependent adenosine triphosphatase, $(Na^+ + K^+)$-ATPase, were increased and the rate of cholesterol biosynthesis in the ileal mucosa enhanced.

Methods

Animal experiments. Albino male rats of the Sherman strain weighing 50–75 g were fed a standard powdered diet of 20 g/day of Purina Rat Chow (Ralston Purina Co., St. Louis, MO) and water ad libitum. Rats were grouped in gang cages and maintained in a controlled environment with alternating 12-h periods of light and darkness for at least 1 week before use. Thereafter, the rats were fed 20 g/day of a nitritionally complete, powdered diet (Bio-Serve Inc., Frenchtown, NJ) of the following composition (% by weight): protein, 17.6; carbohydrate, 31.8; cholesterol, 0.12; fiber, 5.0; and lipid 37.0, in the form of either butter fat (Bio-Mix 0845K) or corn oil (Bio-Mix 0846K). The fatty acid composition of the two diets is given in Table I. Rats were maintained on the diets with water ad libitum for 3 and 6 weeks. The animals gained an average of 5 g per week, with no significant differences between the two diets. At appropriate times, groups of five rats were fasted for 18 h with free access to water, killed at the mid-dark point of the cycle and the small intestine and colon excised.

Membrane preparations. The small and large intestine of each rat were divided into proximal and distal halves and the corresponding segments from five rats were pooled as starting material. Microvillus and basolateral membranes were prepared from the enterocytes of the small intestine as described by Brasitus et al. [6] and basolateral membranes were isolated from colonocytes as reported by Brasitus and Keresztes [11]. Purity and comparability of the microvillus and basolateral

TABLE I

FATTY ACID PROFILES OF THE EXPERIMENTAL DIETS

Values were calculated from data supplied by the manufacturer (Bio-Serve, Inc., Frenchtown, NJ). Fatty acids were designated by number of carbon atoms number of double bonds

Component	% by weight of total fatty acids	
	Saturated diet (butter fat)	Unsaturated diet (corn oil)
Fatty acid		
14.0	7.8	0.1
16 0	25 5	11 6
16.1	0	0
18.0	8.8	2.2
18 1	31 7	26 7
18·2	6.1	57 6
18 3	1 9	1.1
20.4	0.2	0
others	18 0	0.7
Classes		
Saturated	48.0	14.0
Monoenoic	41.4	27 5
Polyenoic	10.6	58.5

membranes, respectively, were assessed by estimations of the specific activities of the marker enzymes *p*-nitrophenylphosphatase and $(Na^+ + K^+)$-ATPase. All preparations were purified from 12- to 20-fold as compared to the starting homogenates, and comparable purification was observed for each membrane type isolated from rats on the two diets. In addition, specific activity ratios (membranes)/(homogenates) for NADPH-cytochrome *c* reductase and succinate dehydrogenase, marker enzymes for microsomal and mitochondrial membranes, respectively, were in the range 0.10–0.30.

Enzyme assays The activities of $(Na^+ + K^+)$-ATPase, *p*-nitrophenylphosphatase (at pH 9.5), NADPH-cytochrome *c* reductase, succinic dehydrogenase and magnesium-dependent adenosine triphosphatase (Mg^{2+}-ATPase) were estimated as described previously [6]. Assay conditions were chosen to assure excess of substrate (maximal velocity) and linear kinetics throughout the test period. Generally, no more than 5% of a given substrate was utilized in the reaction period. Arrhenius plots of *p*-nitrophenylphosphatase activity at pH 9.5 were constructed from the results of assays under maximal velocity (excess substrate) conditions as reported previously [8].

Fluorescence polarization. Lipid fluidity was assessed by estimations of the fluorescence anisotropy, *r*, of 1,6-diphenyl-1,3,5-hexatriene. Methods used to load the membranes with this probe and to quantify the steady-state polarization of fluorescence in an SLM (Urbana, IL) polarization spectrofluorometer have been described [8,12]. For Arrhenius studies, values of *r* were estimated over the range of 0–40°C [12]. Changes in excited-state lifetime were monitored by estimations of the total fluorescence intensity as previously described [12]. In comparisons of the various membrane preparations described below, changes in fluorescence anisotropy of diphenylhexatriene were not accompanied by changes in fluorescence lifetime.

Membrane composition studies. Membrane lipids were extracted by the method of Folch et al. [14]. Under conditions of the extraction, glycolipids were not obtained quantitatively and were not estimated. Total neutral and phospholipids were examined by thin-layer chromatography according to the method of Katz et al. [15]. Fatty acids of the neutral plus phospholipid fraction were derivatized by the method of Gartner and Vahouny [16], and the resulting methyl esters were quantified on a JEOL JGX-20K gas chromatograph equipped with a flame-ionization detector and interfaced with a Hewlett-Packard 3390A integrator. Authentic fatty acid methyl esters were used to identify retention times. Cholesterol and total phospholipids were also estimated by the methods of Zlatkis et al. [17] and Ames and Dubin [18], respectively. Protein was estimated by the method of Lowry et al. [19] using bovine serum albumin as the standard.

Sterol synthesis Cholesterol biosynthesis was assessed by quantification of the incorporation in vitro of [1-^{14}C]octanoate into digitonin-precipitable neutral sterols, using a modification of the method of Andersen and Dietschy [20]. After an overnight fast, rats were anesthetized with diethyl ether and exsanguinated via the abdominal aorta. Intestinal segments approx 1 cm in length were excised from the proximal jejunum (just distal to the ligament of Treitz), ileum (just proximal to the ileocecal valve), ascending colon (just distal to the cecum), and rectum (just proximal to the anus). The segments were washed with ice-cold 0.154 M NaCl to remove lumenal contents, and longitudinal slices, 8–10 mm in width, were cut and placed in silanized 25-ml Erlenmeyer flasks containing 5 ml Krebs-Ringer bicarbonate buffer supplemented with 5 mM D-glucose, 2 mM sodium octanoate and 6 μCi/ml [1-^{14}C]octanoate (New England Nuclear; 52.5 mCi/mmol). The incubation mixture was equilibrated with O_2/CO_2 (95%/5%) for 15 min at 37°C prior to addition of the tissues and flushed thoroughly with the same gas mixture for 15 s after the tissues were included. The flasks were sealed with rubber caps, shaken at 125 oscillations per min at 37°C, and flushed with the same O_2/CO_2 mixture every 30 min. After 90 min of incubation, the flasks were chilled in ice and 4 ml 4 M NaOH added to each. Approx. 75000 cpm [7-^{3}H]cholesterol were added as internal standard and the entire sample saponified at 80°C for 1 h. An equal volume of ethanol was added to each and saponification continued for the further 90 min. After cooling, the samples were extracted four times with light petroleum ether and the pooled extracts concentrated by evaporation and

applied to columns of neutral alumina as described by Popjak [21]. Following removal of the fraction containing squalene, the entire neutral sterol fraction was eluted by four washes of 5 ml acetone/ethyl ether (1 : 1, v/v) and the pooled effluent dried under nitrogen. In initial studies, the neutral sterol mixture was resolved by thin-layer chromatography on silica-gel G in a solvent system of hexane/ethyl ether/glacial acetic acid (40 : 60 : 1.5, v/v), and the cholesterol bands scraped into vials and counted in 10 ml of a liquid scintillation mixture (Ultrafluor; National Diagnostics, Somerville, NJ). The results showed that the percent of the total ^{14}C in the neutral sterols which cochromatographed with authentic cholesterol was 46, 51 and 59% in three experiments with small intestine, but only 23 and 14% in two experiments with the colon. Accordingly, in all studies, cholesterol biosynthesis was evaluated by estimation of the incorporation of [1-^{14}C]octanoate into the digitonin-precipitable fraction of the neutral sterols. The calculations and corrections were used as described by Andersen and Dietschy [20] and the values were corrected for recovery of the internal standard. Sterol digitonides prepared from extracts of the small intestine have been shown to consist almost entirely of cholesterol [22].

Results

Membrane lipid fluidity

Groups of rats were maintained on the diets supplemented, respectively, with saturated or unsaturated triacylglycerols, and six types of membranes (proximal and distal enterocyte microvillus and basolateral membranes; proximal and distal colonocyte basolateral membranes) were isolated and the lipid fluidity assessed by estimation of the fluorescence anisotropy, r, of diphenylhexatriene. No consistent effects of the diet were observed after 3 weeks on the regimen. After 6 weeks, however, values of the fluorescence anisotropy were significantly lower, i.e., fluidity was enhanced, in all the membrane preparations isolated from rats fed the unsaturated as compared to the saturated triacylglycerols (Table II). The greatest reduction in fluorescence anisotropy was observed in the distal enterocyte microvillus membranes ($P < 0.005$), with lesser reductions ($P < 0.05$ for each type) in the other membranes. Values of the fluorescence anisotropy of diphenylhexatriene in proximal microvillus membranes prepared from rats on the saturated and unsaturated diets were also estimated over the temperature range of 0–40°C. Arrhenius plots were constructed to compare each

TABLE II

FLUORESCENCE POLARIZATION OF DIPHENYLHEXATRIENE IN MEMBRANES ISOLATED FROM RATS FED SATURATED AND UNSATURATED TRIACYLGLYCEROLS

Values are means ± S.E. for 4 different preparations of each membrane Rats were maintained on the diet for 6 weeks Fluorescence anisotropy was estimated at 25°C.

Membrane type	Fluorescence anisotropy		
	Saturated diet	Unsaturated diet	P
Microvillus			
Proximal small intestine	0 274 ± 0.003	0.265 ± 0.006	< 0.05
Distal small intestine	0.282 ± 0 004	0.264 ± 0 001	< 0 005
Basolateral			
Proximal small intestine	0.226 ± 0.003	0 215 ± 0.004	< 0.05
Distal small intestine	0.245 ± 0.005	0.231 ± 0.005	< 0 05
Proximal colon	0.232 ± 0.005	0 216 ± 0.005	< 0.05
Distal colon	0 246 ± 0.004	0.235 ± 0.004	< 0.05

of three pairs of membrane preparations, and Fig. 1 illustrates one such comparison. A reduction in fluorescence anisotropy owing to ingestion of the unsaturated triacylglycerols was evident at each temperature tested. In confirmation of prior studies [5,6,12], a change in slope indicative of the lower critical temperature of a broad thermotropic transition was observed in each plot. This transition temperature occurred at 24.5 ± 0.9 and 20.0 ± 1.1°C ($P < 0.05$), respectively, in the saturated and unsaturated diet groups, consistent with an increase in fluidity in the latter group.

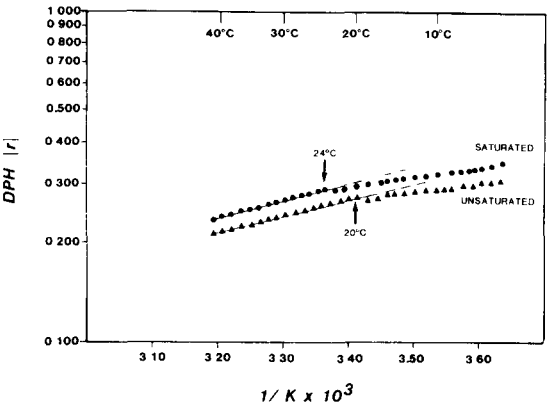

Fig 1 Arrhenius plots of the diphenylhexatriene fluorescence anisotropy, r, in proximal enterocyte microvillus membranes prepared from rats fed diets enriched in saturated (●) or unsaturated (▲) triacylglycerols for 6 weeks Values are for one pair of preparations and are representative of three such comparisons DPH, diphenylhexatriene

Membrane lipid composition

Rats were maintained on the experimental diets for 6 weeks, each of the six membrane types were then isolated, and lipid extracts were prepared in order to characterize the alterations in lipid composition which underly the fluidity change. Table III summarizes the values obtained for lipid classes in extracts of the enterocyte membrane lipids. A number of significant changes in composition owing to the diet were observed in the microvillus but not in the basolateral membrane extracts. Microvillus membrane lipids prepared from rats on

TABLE III

LIPID COMPOSITION OF SMALL INTESTINAL MICROVILLUS AND BASOLATERAL MEMBRANES PREPARED FROM RATS FED DIETS ENRICHED IN SATURATED AND UNSATURATED TRIACYLGLYCEROLS FOR 6 WEEKS

Values are means ± S.E. Numbers of determinations are indicated in parentheses

Component	% by weight of neutral lipids plus phospholipids							
	Microvillus membranes				Basolateral membranes			
	Proximal segment		Distal segment		Proximal segment		Distal segment	
	Saturated diet (8)	Unsaturated diet (5)	Saturated diet (4)	Unsaturated diet (3)	Saturated diet (5)	Unsaturated diet (4)	Saturated diet (5)	Unsaturated diet (4)
Cholesterol	18 2±1 2	26 8±1.8	16 8±1 6	25.1±0 1	14.5±0 7	14.3±1 3	15 1±0 7	14.2±1 1
Cholesterol esters	1 3±0.2	1.1±0.1	1.5±0 2	1.4±0.3	1 3±0.1	1 1±0 2	1 5±0 1	1 4±0.1
Triacylglycerols	1.4±0.2	1.5±0 2	1.5±0 1	1 5±0 2	1 4±0.2	1 4±0 2	1 5±0 1	1 4±0 2
Fatty acids	13 8±1.1	19.5±1 7	12.5±1 7	21.9±1.3	7 6±0.5	6 2±0 4	6.5±0 5	7 8±0 4
Total phospholipid	63.8±1 4	49.8±4.3	66.8±3.0	48.6±1 4	73 5±1.2	77 9±3 3	74 0±1 3	73 7±1 7
Phosphatidylcholine	28.0±1 8	25.7±2.1	33.9±2.9	30.9±1.2	26.6±1.1	27.5±1 9	20 0±1 3	20.5±2 4
Lysophosphatidylcholine	1.2±0 1	1.3±0 1	1 6±0.2	1 3±0 1	0 6±0.1	0 6±0 2	0 6±0.1	0.6±0 1
Phosphatidylethanolamine	17.9±1 6	10.5±1 1	19 0±1 2	12.1±0.5	17 0±0.4	16 0±1.5	20 0±1 0	16.6±1 8
Sphingomyelin	16 7±1.2	12 3±3 7	12.3±1 5	4.4±0.6	29.3±1 1	33.8±2.8	33 5±1.7	36.0±2.8

the unsaturated as compared to the saturated diet contained less total phospholipid ($P < 0.005$), less phosphatidylethanolamine ($P < 0.01$) and, in distal segment preparations only, less sphingomyelin ($P < 0.001$). Concomitantly, the microvillus membrane extracts of the unsaturated as compared to the saturated diet group contained more cholesterol ($P < 0.01$) and more fatty acids ($P < 0.005$). The decrease in sphingomyelin is noteworthy, since this change is expected to increase fluidity [13], and the distal microvillus membranes exhibited the greatest increment in fluidity owing to the unsaturated diet (Table II). The changes in cholesterol and total phospholipid content owing to the unsaturated diet significantly increase the cholesterol/phospholipid molar ratio (see Table VII). While this effect is expected to decrease lipid fluidity [23–25], it is not sufficient to overcome the fluidization owing to the decreases in sphingomyelin and saturation index (see below).

Similar composition studies of lipid extracted from colonocyte basolateral membranes are summarized in Table IV. The only significant differences in extracts prepared from the unsaturated as compared to the saturated diet groups were increases in phosphatidylethanolamine ($P < 0.025$) and, in proximal colonocyte preparations only, a decrease in sphingomyelin ($P < 0.01$).

Estimations of the acyl chain composition of the membrane lipids and of the sera of rats maintained for 6 weeks on the experimental diets are summarized in Tables V and VI. The unsaturated triacylglycerol diet is enriched in linoleic (18 : 2) acid (Table I), and increments in 18 : 2 owing to maintenance on this diet were observed in the serum ($P < 0.001$) and in all the intestinal membrane types ($P < 0.01$), except for the proximal colon basolateral membranes. Diet related changes in the content of oleic (18 : 1) and arachidonic (20 : 4) acids were also noted. Serum lipids (Table V) of rats fed the unsaturated as compared to the saturated diet contained 33% less 18 : 1 ($P < 0.001$) and 39% more 20 : 4 ($P < 0.01$). A similar pattern was observed in the small intestinal basolateral membranes (Table VI), but the relative changes were more dramatic. In the distal enterocyte preparations, for example, the 18 : 1 content was reduced by 54% ($P < 0.001$) and the 20 : 4 content was increased by 158% ($P < 0.001$). Basolateral membranes of the distal colonocytes (Table VI) showed changes in 18 : 1 and 20 : 4 similar to those in serum, but the basolateral mem-

TABLE IV

LIPID COMPOSITION OF BASOLATERAL MEMBRANES PREPARED FROM COLONOCYTES OF RATS FED DIETS ENRICHED IN SATURATED AND UNSATURATED TRIACYLGLYCEROLS FOR 6 WEEKS

Values are means ± S E for three different preparations of each membrane type

Component	% by weight of neutral lipids plus phospholipids			
	Proximal colon membranes		Distal colon membranes	
	Saturated diet	Unsaturated diet	Saturated diet	Unsaturated diet
Cholesterol	21 4 ± 0 8	23 2 ± 1 4	19.1 ± 4 1	20 5 ± 2 4
Cholesterol esters	1.3 ± 0 1	1 3 ± 0 1	1 2 ± 0.1	1.3 ± 0 1
Triacylglycerols	1.3 ± 0 1	1 3 ± 0 1	1 6 ± 0.1	1.4 ± 0 2
Fatty acids	15.5 ± 0.6	7 1 ± 0.1	8 7 ± 0.9	7.5 ± 0 1
Total phospholipid	57.8 ± 1.8	63 1 ± 3.3	67 5 ± 5.7	67.6 ± 1 9
Phosphatidylcholine	24 5 ± 2.3	30 7 ± 0.7	20 2 ± 3.4	17.1 ± 2 0
Lysophosphatidylcholine	0 5 ± 0.1	0.5 ± 0 1	0 6 ± 0.1	0.6 ± 0 1
Phosphatidylethanolamine	7.4 ± 0.1	18.0 ± 1.2	8 2 ± 0 9	13.0 ± 1.8
Sphingomyelin	25.3 ± 0.8	15.9 ± 1.7	38.6 ± 1 7	36.9 ± 1.7

TABLE V

FATTY ACID COMPOSITION OF SERUM AND SMALL INTESTINAL MICROVILLUS MEMBRANES OF RATS FED DIETS ENRICHED IN SATURATED AND UNSATURATED TRIACYLGLYCEROLS FOR 6 WEEKS

Values are means ± S.E. for four different preparations of each membrane type, five groups of serum samples on the saturated diet and six groups of serum samples on the unsaturated diet.

Fatty acid	% of total fatty acids					
	Serum		Microvillus membranes			
	Saturated diet	Unsaturated diet	Proximal segment		Distal segment	
			Saturated diet	Unsaturated diet	Saturated diet	Unsaturated diet
14·0	4.0±0.3	1 3±0 2	4 4±0.7	1.9±0.2	3 8±0 5	1 2±0 1
14:1	1 4±0 1	0.7±0 1	1 4±0.1	0 5±0.1	1 4±0 1	0 5±0 1
16 0	27.4±0.7	22.4±0 8	22 0±0.5	21 0±0.9	22.0±2 5	20 2±0 2
16 1	5.5±0.4	2.7±0 2	3.2±1.1	2 8±0.7	5.6±0 4	1 8±0 6
18·0	15.6±0.5	14 5±0 4	24.5±4.1	20 6±2.6	21 0±3.0	22.5±0 3
18 1	19.1±0.6	12.8±0.5	22 6±0 8	21 8±0.3	21 4±2 2	23 1±0.1
18:2	11.4±0 8	18 5±0.4	12.3±1 3	23 3±0.8	9 5±1 6	23 3±0.1
20·0	0	0	0.5±0 2	1 0±0 5	1 2±0 6	0 1±0 1
20 1	0	0	1.9±0 9	2 8±1.4	3 2±0 7	0 9±0 4
20·2	1.2±0.6	3 8±1 1	0 2±0 1	0 4±0 2	1 2±0 2	0 8±0.3
20 3	1.3±0.6	4 4±0.8	0 3±0 1	0 2±0 1	2 1±0 6	0 6±0 3
20 4	11.0±1.2	15 3±0 7	5.9±0 3	2 2±0 1	5 2±2 1	4 8±0.3

branes of the proximal colon did not. Lastly, the enterocyte microvillus membranes (Table V) exhibited no significant change in 18 : 1 and either a 63% reduction in 20 : 4 (proximal microvillus membranes; $P < 0.001$) or no change (distal membranes).

TABLE VI

FATTY ACID COMPOSITION OF INTESTINAL BASOLATERAL MEMBRANES PREPARED FROM RATS FED DIETS ENRICHED IN SATURATED AND UNSATURATED TRIACYLGLYCEROLS FOR 6 WEEKS

Values are means ± S.E. for four different preparations of each membrane type.

Fatty acid	% of total fatty acids							
	Proximal small intestine		Distal small intestine		Proximal colon		Distal colon	
	Saturated diet	Unsaturated diet	Saturated diet	Unsaturated diet	Saturated diet	Unsaturated diet	Saturated diet	Unsaturated diet
14:0	0 9±0.3	0.4±0 2	1 3±0 5	0.4±0 2	1.4±0.4	0.9±0.4	2.3±0 4	1 0±0 5
14:1	0 5±0.2	0.4±0 2	0 9±0.5	0.2±0.01	0.8±0.4	0 9±0 4	1.6±0 8	0 9±0.4
16.0	24.8±0.4	19.2±0.9	25 3±0 4	21.0±1.7	26.1±0.5	24 2±0.2	26 5±0 2	23 9±0.3
16 1	0 4±0.2	0.3±0 1	2 1±1.0	0.3±0 2	2.8±1.4	1 4±0.7	3.2±1 6	1 6±0.8
18·0	25 1±1.0	25 3±0.2	22 3±0.3	24.9±0.4	15.5±0.1	14.1±0 5	13 9±0.1	14 7±0.3
18·1	17 6±0 5	10 5±1.5	20 3±0 7	9.3±0 9	25.8±1 8	21.8±2 6	34.0±3.1	22 4±1.6
18.2	21.7±0.8	30.9±0.9	15.4±1 2	26.2±3 2	16.8±3 6	22 1±1 1	9.1±1 0	17 8±1.0
20:0	0 3±0 1	0.1±0.1	1.2±0.6	0	0	0	1.3±0 2	0 2±0.1
20 1	0 4±0 2	0	2.5±1.2	0	0.9±0.2	1.4±0 3	0 6±0 1	4 1±0.9
20·2	0	0	0.2±0 1	0	0.9±0 4	3 4±0 8	0 1±0 1	2 5±0.4
20.3	0.8±0.4	0.1±0.1	1.6±0 8	0	2.3±0 1	2 6±1 0	2.7±0 1	4 3±0.4
20 4	7.5±1.2	12.5±1 8	6 9±0 1	17 8±0.6	6.4±0 5	6 2±0 6	4 2±0 9	6 2±0.7

TABLE VII

CHOLESTEROL/PHOSPHOLIPID MOLAR RATIO AND SATURATION INDEX IN LIPID EXTRACTS OF MEMBRANES FROM RATS FED SATURATED AND UNSATURATED TRIACYLGLYCEROLS FOR 6 WEEKS

Values are means ± S E for four different preparations of each membrane type. *P* values were calculated from *t*-tests of independent means

Membrane type	Cholesterol/phospholipid molar ratio			Saturation index		
	Saturated diet	Unsaturated diet	*P*	Saturated diet	Unsaturated diet	*P*
Microvillus						
Proximal small intestine	0.90 ± 0 09	1.46 ± 0 13	< 0 01	0.68 ± 0 10	0 52 ± 0.05	< 0 05
Distal small intestine	0.81 ± 0 11	1.26 ± 0.05	< 0.01	0.68 ± 0.18	0 47 ± 0.01	< 0.05
Basolateral						
Proximal small intestine	0.60 ± 0.03	0 54 ± 0.04	n s	0 56 ± 0.05	0.38 ± 0 03	< 0.01
Distal small intestine	0.56 ± 0.02	0.60 ± 0.05	n.s	0.57 ± 0 04	0.35 ± 0.02	< 0.005
Proximal colon	0 76 ± 0.11	0 72 ± 0.10	n.s.	0.45 ± 0.03	0 35 ± 0.01	< 0.01
Distal colon	0 73 ± 0.08	0.66 ± 0.08	n.s	0.53 ± 0.01	0.37 ± 0 01	< 0.001

Owing largely to the foregoing changes in content of 18 : 2 and 20 : 4, the saturation index in each membrane type was decreased significantly by the unsaturated diet (Table VII), a change which is expected to increase lipid fluidity [26,27]. Table VII also lists values of the cholesterol/phospholipid molar ratio. A significant increase was observed in the microvillus but not in the basolateral membranes of rats fed the unsaturated diet. Such an increase is expected to reduce lipid fluidity and it is reasonable to propose that it could be a regulatory response designed to mini-

TABLE VIII

EFFECTS OF DIETS ENRICHED IN SATURATED AND UNSATURATED TRIACYLGLYCEROLS ON THE INCORPORATION OF [^{14}C]OCTANOATE INTO DIGITONIN-PRECIPITABLE STEROLS OF INTESTINAL SLICES IN VITRO

Values are means ± S.E. for groups of six rats on each diet at each time-point, number of determinations is shown in parenthesis. Each intestinal segment was 1 cm long and taken as follows. just distal to the ligament of Treitz (jejunum); just proximal to the ileocecal valve (ileum); just distal to the cecum (ascending colon); and just proximal to the anus (rectum) n s., not significant

Weeks on diet	Intestinal segment	Incorporation of [^{14}C]octanoate (nmol/mg protein per 90 min)		
		Saturated diet	Unsaturated diet	*P*
3	jejunum	3.6 ± 0.7 (10)	5.4 ± 1.4 (10)	n s
	ileum	28.8 ± 3.0 (6)	50 0 ± 4.9 (12)	< 0 02
	ascending colon	19.0 ± 3 2 (10)	23.5 ± 1 9 (12)	n.s
	rectum	7 5 ± 0.9 (10)	5 6 ± 0.7 (12)	n s
6	jejunum	4.8 ± 1.1 (10)	2 9 ± 0.5 (10)	n.s.
	ileum	21 9 ± 5 0 (10)	48.9 ± 4.6 (10)	< 0.001
	ascending colon	7.8 ± 1 0 (12)	7.2 ± 0.6 (12)	n.s.
	rectum	9 1 ± 0 8 (11)	5.6 ± 1 0 (12)	n s.

mize the fluidity change owing to the diet. To explore this hypothesis further, studies of sterol biosynthesis by intestine in vitro were undertaken.

Sterol biosynthesis

Rats were maintained on the experimental diets for 3 or 6 weeks and thereafter intestinal slices were prepared and incubated in vitro with [1-^{14}C]octanoate (methods). Incorporation into the digitonin-precipitable sterols was estimated and the results are summarized in Table VII. Irrespective of the diet, the highest rates of incorporation were observed with slices of the distal ileum as compared to those of the proximal jejunum, ascending colon or rectum. Maintenance on the unsaturated as compared to the saturated triacylglycerols, moreover, significantly increased the rate of incorporation in distal ileal slices. At 3 and 6 weeks, respectively, the relative increments owing to the unsaturated diet were 74% ($P < 0.02$) and 123% ($P < 0.001$). No significant change in incorporation owing to the diets was observed with slices of the proximal jejunum, ascending colon or rectum.

Intrinsic membrane enzymes

Prior studies demonstrate that a number of enterocyte plasma membrane enzymes are 'intrinsic' in that their activity is dependent on the fluidity and physical state of the membrane lipids [5,8,9]. Two such activities were examined to determine whether the diet-induced changes in lipid fluidity and composition described above can modulate the functions of enterocyte membrane proteins. The specific activity of *p*-nitrophenylphosphatase in proximal microvillus membranes was estimated as a function of temperature, and Fig. 2 illustrates the Arrhenius plots obtained in studies of four groups of rats maintained on each of the experimental diets. The

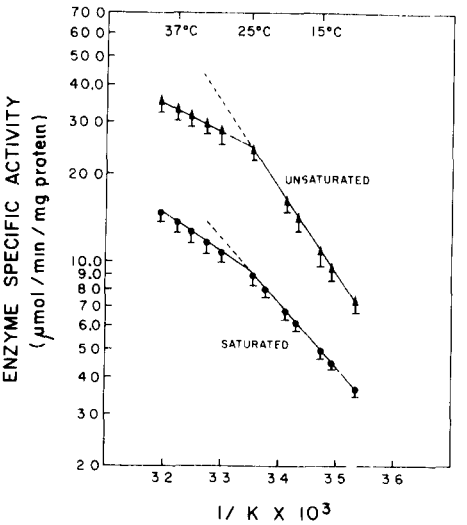

Fig. 2 Arrhenius plots of the enzyme specific activity of *p*-nitrophenylphosphatase in proximal enterocyte microvillus membranes prepared from rats fed diets enriched in saturated (●) or unsaturated (▲) triacylglycerols for 6 weeks Means ± S.E for four preparations are plotted Enzyme activity was assayed at pH 9.5 under conditions where the substrate was never rate-limiting

TABLE IX

EFFECTS OF DIETS ENRICHED IN SATURATED AND UNSATURATED TRIACYLGLYCEROLS ON ($Na^+ + K^+$)-ATPase OR Mg^{2+}-ATPase OF INTESTINAL BASOLATERAL MEMBRANES

Values are means ± S.E for four preparations of each membrane type Rats were maintained on the diets for 6 weeks

Basolateral membrane source	Enzyme specific activity (μmol/mg protein per min)			
	($Na^+ + K^+$)-ATPase		Mg^{2+}-ATPase	
	Saturated diet	Unsaturated diet	Saturated diet	Unsaturated diet
Proximal enterocyte	0 23 ± 0 02	0.52 ± 0 05 [a]	0 71 ± 0 02	0 79 ± 0 04
Distal enterocyte	0 33 ± 0 05	0.48 ± 0 01 [a]	0 86 ± 0 06	0 82 ± 0.06
Proximal colonocyte	0 54 ± 0 11	0 68 ± 0.14	0 70 ± 0 03	0 74 ± 0.03
Distal colonocyte	0 38 ± 0 03	0 41 ± 0.07	0 86 ± 0 14	0 78 ± 0 07

[a] $P < 0.01$ for difference between diet groups by *t*-test of independent means

increase in fluidity owing to the unsaturated diet was associated with a 2- to 3-fold increase in enzyme specific activity throughout the temperature range studied. Similar to prior results [8], a change in slope was observed at $26 \pm 1°C$ in both diet groups. The calculated apparent energies of activation above the transition temperature of 26°C were 4.7 ± 0.8 and 6.3 ± 0.7 kcal/mol, respectively, for the unsaturated and saturated diet groups ($P < 0.05$); the corresponding values below the transition temperature, 16.0 ± 1.5 and 14.3 ± 1.8 kcal/mol, were not significantly different.

Basolateral membrane $(Na^+ + K^+)$-ATPase and Mg^{2+}-ATPase were also assayed in preparations from each diet group, and the results are listed in Table IX. The specific activity of the $(Na^+ + K^+)$-ATPase in enterocyte basolateral membranes isolated from rats on the unsaturated as compared to the saturated diet was 128% greater ($P < 0.01$) in proximal segment preparations and 45% greater ($P < 0.01$) in distal preparations. Smaller mean differences between the corresponding colonocyte preparations were not statistically significant, and the diet did not affect the Mg^{2+}-ATPase values.

Discussion

The foregoing results demonstrate that maintenance of rats for 6 weeks on nutritionally complete diets enriched in unsaturated (corn oil) as compared to saturated (butter fat) triacylglycerols alters the lipid composition and fluidity of enterocyte microvillus and basolateral membranes and of colonocyte basolateral membranes. The composition changes are consonant with prior studies in vivo which have shown that diets enriched in corn oil, i.e., mainly in 18 : 2 acyl residues, increase the 18 : 2 content of rat [28] and human [29] erythrocyte membrane lipids. Further, the acyl chain composition of Ehrlich ascites tumor cell membranes is altered by growth of the tumor in host mice fed different dietary fats [30–32]. Modification in vitro of the membrane lipids of microorganisms [33–36] and of various mammalian cell lines in culture [37–43] is induced by changes in the fatty acid composition of the culture medium.

While there is general agreement that the acyl chain composition of biological membranes can be modulated by the procedures described above, the resulting effects on lipid fluidity have been questioned by some authors. *cis*-Unsaturation of phospholipid acyl chains is known to increase the molecular packing area in monolayers [44,45] and in bilayer liposomes [44] and to enhance the fluidity of model bilayer membranes [26,27]. Corresponding increases in the lipid fluidity of biological membranes owing to increases in the content of unsaturated acyl chains have been observed by some investigators [31,36,40,46] but not by others [41–43]. To characterize the reasons for this apparent inconsistency, it is instructive to compare the two groups of studies in relation to the present results. When our animals were examined after 3 weeks on the respective diets, some increase in the 18 : 2 content of membranes prepared from rats on the unsaturated diet was found (data not shown), but the overall saturation index and the lipid fluidity were not altered consistently. After 6 weeks on the unsaturated diet, in contrast, the fluorescence anisotropy of diphenylhexatriene was lowered significantly (Table II) and the degree of acyl chain unsaturation, expressed as the double bond index, was increased from 13 to 49% (average 29%) in the different membrane types (data of Tables V–VII). Similarly, King et al. [31] and King and Spector [40], who studied plasma membranes isolated from Ehrlich ascites cells grown in mice, reported significant differences in lipid fluidity associated with changes in acyl chain double bond index of approx. 18 and 33%. Storch and Schachter [46] observed significant increases in the fluidity of rat hepatocyte plasma membranes when the animals were maintained on a starve-refeed regimen which increased the double bond index of the membranes by approx. 20%. Cossins and Prosser [47] reported positive correlations between diphenylhexatriene fluorescence polarization and the ratios of saturated/unsaturated fatty acids in the choline and ethanolamine phosphoglycerides of synaptosomal membranes of various animal species. From their data, we calculate that a change in diphenylhexatriene fluorescence polarization of approx. 3% in rat membranes would require a change in the ratio of saturated/unsaturated fatty acids of approx. 10–12%.

In summary, the foregoing, evidence supports the conclusion that increases in the lipid fluidity of biological membranes, as assessed by spin-labeling

or fluorescence polarization, can be detected when the double bond index increases by at least 10–13% or more and provided that other alterations in composition do not obscure the fluidity effect. A change in double bond index of this magnitude was not demonstrated in the studies which report no difference in fluidity secondary to acyl chain modulation. Thus, Stubbs et al. [41] observed no differences in diphenylhexatriene fluorescence polarization on comparison of plasma membrane fractions of calf thymus lymphocytes grown in media enriched with 18 : 2 or palmitic acid (16 : 0). As compared to control cultures, however, the phospholipid double bond index was increased by 9 and 5% respectively, in the media enriched with 18 : 2 and 16 : 0. The results of McVey et al. [42] and Poon et al. [43] are more difficult to evaluate, inasmuch as the double bond index cannot be calculated nor the purity of the plasma membrane fractions assessed quantitatively from the data reported.

Our results indicate both similarities and differences in the patterns of response to the diets of the luminal and contraluminal intestinal plasma membranes. All membrane types tested responded to the unsaturated diet with a fall in saturation index and increases in lipid fluidity and 18 : 2 content. Only in the enterocyte microvillus membranes, however, were these changes accompanied by an increment in cholesterol content and a reduction in total phospholipid (Tables II and IV). The resulting increase in cholesterol/phospholipid ratio is of particular interest, because it would act to decrease lipid fluidity [23–25] and might represent a compensatory response to the diet. Observations with a Chinese hamster ovary cell mutant led Sinensky [48] to suggest that "the role of the cholesterol-synthesizing enzymes of the mammalian fibroblast is to regulate membrane fluidity", and several subsequent studies support this hypothesis. Anderson et al. [49] noted that Chinese hamster ovary cells adapt to various growth temperatures by adjusting membrane fluidity via changes in cholesterol content. Chin et al. [50] reported increases in the cholesterol content of erythrocyte and brain synaptosomal membranes of ethanol-tolerant mice.

The selectivity with which the experimental diets alter the cholesterol content of the microvillus but not the basolateral membrane is similar to that observed previously by modulating the rate of synthesis of the sterol in ileal mucosa [10]. Moreover, the results of [^{14}C]octanoate incorporation experiments (Table VIII) support the conclusion that the unsaturated diet stimulates cholesterol synthesis in the ileal mucosa and thereby increases selectively the cholesterol content of the microvillus membranes. This hypothesis implies that the antipodal microvillus and basolateral membranes derive their cholesterol from different intracellular pools or, alternatively, that the turnover of the sterol differs in the two membranes. The basolateral membranes, for example, are exposed directly to lipid-associated lipoproteins transported out of the enterocyte or present in the extracellular fluid. It bears emphasis that a change in sterol synthesis need not be the sole mechanism of a diet-induced change in microvillus membrane cholesterol. Proximal enterocyte microvillus membranes responded to the unsaturated diet with an increase in cholesterol content (Table III) but no apparent increase in incorporation of [^{14}C]octanoate (Table VIII). These findings may be due to a difference in sampling, since only 1 cm of proximal jejunum was used to test for incorporation of [^{14}C]octanoate, whereas the entire proximal half of the small intestine was used as starting material for preparation of the proximal microvillus membranes.

In addition to the foregoing results, a number of lipid composition changes induced by the experimental diets point to differences in lipid metabolism of the various intestinal membranes. For example, in response to the unsaturated diet, the content of phosphatidylethanolamine decreased in enterocyte microvillus membranes, remained unchanges in enterocyte basolateral membranes and increased in colonocyte basolateral membranes (Tables III and IV). The same diet decreased the content of 18 : 1 and increased that of 20 : 4 in both serum (Table V) and enterocyte basolateral membranes (Table VI) but not in microvillus membranes.

The dietary regimens employed also lead to striking changes in the specific activity of enterocyte microvillus membrane *p*-nitrophenylphosphatase (Fig. 2) and basolateral membrane ($Na^+ + K^+$)-ATPase (Table IX). Despite the marked in-

crease in *p*-nitrophenylphosphatase activity owing to the unsaturated diet, Arrhenius plots showed no shift in the break-point temperature of the enzyme (Fig. 2) which would correspond to the change in break-point temperature of the fluorescence anisotropy of diphenylhexatriene (Fig. 1). This finding agrees with the prior results of enzyme delipidation-relipidation experiments [8] which indicate that the *p*-nitrophenylphosphatase break-point temperature is not altered by the nature of the bulk lipid used for relipidation. Inasmuch as both enzymes studied are known to be sensitive to their lipid environments [5,9], it is reasonable to suggest that the diet-induced changes in activity are due in part to direct alterations of the membrane lipid composition and fluidity. Indirect effects, however, may also be involved. Increases in fluidity of the enterocyte plasma membranes, for example are expected to increase the passive flux of Na^+ into the cell and hence to require increased pumping via the $(Na^+ + K^+)$-ATPase. Under these circumstances, an increase in the number of pump units might well occur. Regardless of the mechanisms involved, however, the experiments demonstrate the feasibility of modulating intrinsic intestinal membrane functions in vivo by means of dietary manipulation.

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