Biochimica et Biophysica Acta, 979 (1989) 177–186
Elsevier

BBA 74325

Dietary triacylglycerol modulates sodium-dependent D-glucose transport, fluidity and fatty acid composition of rat small intestinal brush-border membrane

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(Received 23 August 1988)

Key words: D-Glucose; Brush-border membrane; Fish oil; Butter fat; Lipid fluidity; Fatty acid; (Rat small intestine)

Rats were maintained on nutritionally complete diets enriched in unsaturated (menhaden fish oil) or saturated (butter fat) triacylglycerols. After 4 weeks, the animals were killed, proximal small intestinal brush-border membranes were prepared, and examined and compared with respect to their lipid composition, molecular species of phosphatidylcholine, lipid fluidity and sodium-dependent D-glucose transport. Membranes prepared from the two dietary groups were found to possess similar ratios of cholesterol/phospholipid (mol/mol), sphingomyelin/phosphatidylcholine (mol/mol), and protein/lipid (w/w). In contrast to these findings, however, striking differences were noted in the total fatty acid compositions of these membranes. Plasma membranes prepared from animals fed the fish oil diet possessed higher percentages of saturated fatty acids as well as ($n-3$) unsaturated fatty acids and lower percentages of monounsaturated and ($n-6$) unsaturated fatty acids than those prepared from animals fed the butter fat diet. Analysis of the molecular species of phosphatidylcholine by HPLC, moreover, revealed that membranes from rats fed fish oil had higher levels of 16:0-20:5, 16:0-22:6 and 18:0-20:5 and lower levels of 18:0-18:2 and 16:0-18:1 than their butter fat counterparts. As assessed by steady-state fluorescence polarization, differential polarized phase fluorometric and excimer/monomer fluorescence intensity techniques using various fluorophores, the lipid fluidity of membranes from rats fed fish oil was also found to be significantly lower compared to membranes from rats fed butter fat. Finally, comparison of the kinetic parameters of Na^+-dependent D-glucose transport revealed that fish oil-membrane vesicles had a higher maximum velocity (V_{max}) than butter fat membrane vesicles but a similar K_m for glucose.

Introduction

Considerable evidence exists that many functions of biological membranes are influenced by the composition and physical state of the membrane lipids [1–3]. Over the past several years, our laboratory has examined and characterized the lipid–protein interactions of the brush-border membranes of rat small intestinal epithelial cells (for review, see Ref. 4). These studies have revealed that a number of plasma membrane activities, including certain transmembrane transport processes such as Na^+-dependent D-glucose transport appear to be influenced by the lipid composition and/or lipid fluidity * of these membranes [4–8].

Although evidence suggests the existence of regulatory mechanisms which maintain the lipid composition and fluidity of specific plasma membranes and membrane organelles, the precise nature of these mechanisms is largely unknown [6,9]. Accordingly, the present studies were initiated to examine the role of membrane fatty acids in the regulation of lipid fluidity and Na^+-dependent D-glucose transport in rat proximal small intestinal brush-border membrane vesicles. Specifically, we explored the hypothesis that rats fed diets enriched in fish oils or butter fat would have alterations in their brush-border membrane fatty acid composition which, in turn, would influence membrane lipid fluidity and Na^+-dependent D-glucose transport. Several lines

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* The term 'lipid fluidity' as applied to natural membranes is used in this paper to denote the relative motional freedom of the lipid molecules or substituents thereof. A more detailed description has previously been published [7].

of evidence point to these possibilities. Studies from a number of laboratories [10–13] have shown that feeding animals diets enriched in fish oils and/or saturated fats markedly affected the fatty acid composition of phospholipids in various cellular membranes. Philbrick et al. [12] and Hock et al. [10], moreover, suggested that these compositional alterations might be expected to alter membrane fluidity in rat brain and myocardium, respectively. Storlien et al. [14] have also shown that dietary fish oil supplementation prevented insulin resistance induced by high-fat feeding in rats. Lardinois [15] has suggested that chronic fish oil ingestion might positively influence carbohydrate metabolism and improve glucose homeostasis and, thereby, account for the rarity of diabetes mellitus seen in Alaskan and Greenland Eskimos [16,17]. In this regard, however, Glauber et al. [18] and Friday et al. [19] have recently shown adverse effects of fatty acid dietary supplementation on serum glucose levels in non-insulin dependent diabetic patients.

Our working hypothesis was tested by maintaining rats on nutritionally complete diets supplemented with 20% fish oil (menhaden oil) or butter fat for 4 weeks and then examining these biophysical and biochemical membrane parameters. The results described below demonstrate that the fish oil as compared to the butter fat dietary regimen increased the $(n-3)$ fatty acids of the brush-border membranes, decreased lipid fluidity and, concomitantly, increased Na^+-dependent D-glucose transport. These results as well as a discussion of their physiological significance serve as the basis for this report.

Materials and Methods

Materials

Fatty acids, fatty acid methyl esters and GLC columns were all purchased from Applied Science Corp. (State College, PA), Supelco (Bellefonte, PA), and/or Nu-Check Prep (Elysian, MN). Phospholipid standards were obtained from Sigma Chemical Co. (St. Louis, MO), Serdary Research Labs, Inc. (Port Huron, MI) and Avanti Polar Lipids, Inc. (Delham, AL). L-[^{3}H]Glucose (10.7 Ci/mmol), D-[^{14}C]glucose (304.7 mCi/mmol) and ^{22}Na (24.1 Ci/mmol) were obtained from New England Nuclear (Boston, MA). All other chemicals were obtained from Sigma Chemical Co. or Fisher Chemical Co. (Fair Lawn, NJ), unless otherwise indicated.

Animal experiments

Albino male rats of the Sherman strain weighing 275–300 g were fed a standard powdered diet of Purina Rat Chow (Ralston Purina Co., St. Louis, MO, U.S.A.) and water ad libitum. Rats were maintained in a controlled environment for at least 1 week before use. Thereafter, the rats were fed a nutritionally complete powdered diet (Bio-Serve, Inc., Frenchtown, NJ) of the following composition (% by weight): protein 17.6; carbohydrate 46.2; ash 3.2; fiber 10; and lipid 20, in the form of either butter fat (Bio-Mix 2183) or menhaden fish oil (Bio-Mix 2181). Each of the diets was stored at 4°C until use, changed daily and contained 0.02% *t*-butylhydroquinone to avoid autoxidation [20]. The fatty acid composition of the two diets is given in Table I. Rats were maintained on the diets with water ad libitum for 4 weeks. The final weights of the animals fed the butter fat diet (351 ± 16 g, $N = 26$) was found to be similar to those fed the fish oil diet (334 ± 18 g, $N = 26$). After 4 weeks, 6–8 rats from each group were fasted for 18 h with free access to water, killed rapidly by cervical dislocation and their proximal one-half of the small intestine excised.

Membrane preparations

Brush-border membranes were prepared from the proximal half of the small intestine, by using 10 mM $MgCl_2$ instead of 10 mM $CaCl_2$ as the precipitating agent as previously described [5]. Purity and comparability of the preparations as well as contamination by microsomal, mitochondrial and basolateral membranes were assessed by using appropriate marker enzymes [5]. All plasma membranes were purified 18–26-fold compared with original homogenates as assessed by the specific activities of sucrase and showed minimal and comparable contamination by intracellular and basolateral membranes, as described previously [5]. Protein was measured by the method of Lowry et al. [21], with bovine serum albumin as standard.

Chemical determinations

Lipid was extracted from the membrane samples as described by Folch et al. [22]. The composition of the lipid extracts was examined by TLC as described by Katz et al. [23]. Total phospholipid was measured by the method of Ames and Dubin [24], and cholesterol by the procedure of Zlatkis et al. [25]. Derivatives of fatty acids of the total lipid extract were prepared by the procedure described by Gaitner and Vahouny [26]. Fatty acid methyl esters were analyzed on a Hewlett-Packard 5790A gas-liquid chromatograph equipped with a flame ionization detector and interfaced with a Hewlett-Packard 3390A integrator. These analyses were performed using a glass column ($6' \times 1/8''$) packed with GP 10% SP 2330 on 100/120 chromosorb WAW (Supelco, Bellefonte, PA). Nitrogen was used as a carrier gas at a flow rate of 20 ml/min. Initial oven temperature was 165°C for the first 6 min, then increased to 225°C at a rate of 5.5 C°/min and held at 225°C for another 20 min. Authentic fatty acid methyl esters were used to identify retention times [26].

Separation of phospholipid classes and phosphatidylcholine species

Phosphatidylcholine was separated from other classes of phospholipid by a high performance liquid chromatographic (HPLC) technique using an IBM LC/9533 Ternary Gradient Liquid Chromatograph with a variable UV wavelength detector (Nicolet Instrument Corp., Madison, WI) and an Altex Ultrasphere 5 μm silica column, 25 cm × 4.6 mm (Altex Scientific, Inc., Berkeley, CA). Peaks were detected at 205 nm and area recorded with a 3390 A Hewlett-Packard integrator (Avondale, PA). One mg of lipid extracted from proximal small intestinal brush-border membranes was dissolved in 100 μl of hexane/2-propanol/water (40:54:6, v/v) and injected in the column via a 500 μl loop. An isocratic solvent system composed of acetonitrile/methanol/0.1 M ammonium acetate buffer (pH 4.0) (82:10:8, v/v), pumped at a flow rate of 2 ml/min at 23–25°C, resulted in elution of phosphatidylcholine between 17 and 21 min, well-separated from other phospholipid classes. The phosphatidylcholine fraction was collected and evaporated with a rotary evaporator to assay for species.

Choline was then removed from phosphatidylcholine using phospholipase C (grade I, from *Bacillus cereus*; Boehringer, Mannheim, Indianapolis, IN), and the resulting diradylglycerols derivatized to the dinitrobenzoyl esters as previously described by Kito et al. [27] and Takamura et al. [28]. Separation of diradylglyceroldinitrobenzoyl derivatives was achieved by HPLC using the two solvent systems on a C_{18}-bonded silica column as reported by Kito et al. [27] and Takamura et al. [28]. The derivatives were detected at 254 nm. Since all had the same peak area per mol, computation of percent composition for species was possible [27,28]. Molecular species were confirmed by GLC analysis of the fatty acid composition eluted from HPLC and/or by comparison of elution times of standards.

Glucose transport studies

The kinetics of stereospecific uptake of D-glucose by small intestinal brush-border membrane vesicles prepared from each group were estimated as previously described by our laboratory using L-[^{3}H]glucose, D-[^{14}C]glucose and a Millipore filtration technique [5].

To assess intravesicular volumes of the membrane vesicles as well as their sodium permeability, the uptake of ^{22}Na was measured at 26°C by a Millipore filtration technique as previously described by our laboratory [7,29].

Fluorescence measurements

Excitation and emission spectra and estimation of total fluorescence intensity were obtained with a Perkin-Elmer 650-40 spectrofluorometer equipped with a multitemperature cuvette holder, using the lipid-soluble fluorophore pyrenedecanoic acid (Molecular Probes, Inc., Eugene, OR) at 25 and 37°C. Since similar differences were noted in the excimer/monomer ratios of the preparations studied (see below), only values obtained at 25°C are reported in this manuscript. Samples and buffer were thoroughly deoxygenated and saturated with N_2 prior to examination, and a nitrogen atmosphere was maintained within the sample chamber. The samples were loaded with pyrenedecanoate as previously described [30]. The content of the fluorophore in each preparation was estimated fluorometrically as described by Cogan and Schachter [30]. Final molar ratios of probe/lipid ranged from 0.001 to 0.003 and the excimer/monomer differences noted in the samples (see below) could not be ascribed to differences in the probe concentration in the preparations. Excimer and monomer fluorescence intensities were determined at an excitation wavelength of 345 nm and emission wavelengths of 397 nm (monomer) and 465 nm (excimer). Both excitation and emission slits were set at 4 nm. Measured intensities were corrected as necessary for background fluorescence of the buffer and/or unlabeled samples [31]. Each measurement was repeated at least three times. For a given sample, the precision in the ratio of the excimer to monomer fluorescence intensities was better than ±4%. Excimer and monomer lifetimes of the excited state for pyrenedecanoate in each preparation, using phase and modulation values determined at 6 MHz modulation frequency relative to a POPOP reference solution [32], were determined at 25°C as described by Hresko et al. [31] with an SLM 4800 spectrofluorometer (SLM-Aminco, Urbana, IL). No significant differences in the excited-state lifetimes of the probe in the various preparations were noted in the present studies (not shown).

Steady-state fluorescence polarization studies were also performed with an SLM-4800 spectrofluorometer in the T format using the lipid-soluble fluorophores 1,6-diphenyl-1,3,5-hexatriene (DPH) (Aldrich Chemical Co., Milwaukee, WI) and DL-12-(9-anthroyl)stearic acid (12-AS) (Molecular Probes, Inc., Eugene, OR). The methods used to load the membranes with each of these probes and quantification of fluorescence have been previously described by our laboratory [5,33,34]. In this regard, it should be noted that as evaluated by steady-state fluorescence polarization of lipid fluorophores, fluidity has usually been assessed by the fluorescence anisotropy, r, without further resolution of the components that determine r. Recent time-resolved fluorescence anisotropy decay measurement, however, have revealed that the rotation of certain fluorophores, such as DPH, are restricted by the molecular packing of biological and model membrane lipids [35–37]. Therefore, the fluorescence anisotropy of such a fluorophore is not adequately described by the Perrin equation but rather by a modified relationship [38,39]:

$r = r_\infty + (r_0 - r_\infty)[T_c/(T_c + T_F)]$ where r is the fluorescence anisotropy, r_0 is the maximum anisotropy, r_∞ is the limiting hindered anisotropy, T_c is the correlation time and T_F is the lifetime of the excited-state. In natural and artificial membranes, therefore, r_∞ values of DPH are high and largely determine r [9,40]. Unlike DPH, anthroyloxy probes, such as 12-AS reflect mainly T_c, the speed of rotation [41,42]. Final molar ratios of each probe/lipid ranged from 0.002 to 0.003 and the anisotropy (r) differences noted in these studies could not be ascribed to differences in the probe concentrations of these preparations. Corrections for light scattering (membrane suspensions minus probe) and for fluorescence in the ambient medium (quantified by pelleting the membranes after each estimation) were made routinely as previously described [33]. The lifetimes of DPH and 12-AS in each preparation were estimated by phase fluorometry at 30 mHz in an SLM 4800 polarization spectrophotometer as previously described by our laboratory [34]. No significant differences in the excited-state lifetimes of each of the probes in the various preparations were noted in the present experiments (not shown).

Differential polarized phase fluorometry was also used to determine the rotational relaxation rate (R, in radians per second) and limiting hindered anisotropy (r_∞) of DPH in membranes from each group as previously described by Lakowicz et al. [36]. R and r_∞ for DPH in membranes of both groups were calculated from the measurements of steady-state anisotropy (r), phase lifetime (T_p) and differential tangent (tan Δ) measured at 30 mHz and r_0, the anisotropy in the absence of rotational motion. An r_0 value of 0.390 was used for DPH [36].

Statistical methods

All results are expressed as mean values ± S.E. Paired or unpaired t-tests were used for all statistical analysis. $P < 0.05$ was considered significant.

Results

Effects of butter fat and fish oil diets on brush-border membrane lipid composition

As shown in Table I, the butter fat diet was enriched in saturated and monounsaturated fatty acids compared to the fish oil diet. The latter diet, however, contained approximately 6-fold and 15-fold greater amounts of polyunsaturated and $(n-3)$ fatty acids, respectively, than the butter fat diet.

In agreement with previous studies performed in other tissues [10,13], neither diet was found to alter the neutral lipid or phospholipid composition of rat proximal small intestinal brush-border membranes (Table II). Similarly, as shown in Table III, feeding these diets for four weeks did not appear to significantly alter the ratios of cholesterol/phospholipid (mol/mol), sphingomyelin/phosphatidylcholine (mol/mol), and protein/lipid (w/w) in the small intestinal luminal plasma membranes prepared from animals of each group.

In contrast to these findings, however, striking differences were noted in the total fatty acid compositions of these membranes (Table IV). As shown in this table, brush-border membranes prepared from animals fed the fish oil diets contained significantly greater amounts of

TABLE I

Fatty acid profiles of the experimental diets

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

Fatty acid	% by weight of total fatty acids	
	butter fat diet	fish oil diet
14:0	8.2	7.3
14:1	2.6	–
16:0	25.6	23.6
16:1($n-7$)	4.6	8.9
18:0	9.1	2.6
18:1($n-9$)	32.2	17.0
18:2($n-6$)	4.9	1.2
18:3($n-3$)	2.0	–
18:4($n-3$)	–	4.1
20:2($n-6$)	–	0.3
20:3($n-3$)	–	0.2
20:4($n-6$)	–	3.4
20:5($n-3$)	–	15.2
22:5($n-3$)	–	1.7
22:6($n-3$)	–	9.1
Other	10.8	5.4
($n-3$)	2.0	30.3
($n-6$)	4.9	3.7
Saturated	53.7	33.6
Monounsaturated	39.4	25.9
Polyunsaturated	6.9	39.4

TABLE II

Analysis of relative percentages of individual neutral lipids and phospholipids extracted from small intestinal brush-border membranes prepared from animals fed the butter fat and fish oil diets

Results are means ± S.E. for four individual preparations of each membrane.

Lipid	Percentage (w/w) of total lipid	
	butter fat membranes	fish oil membranes
Cholesterol	27.4 ± 0.4	27.8 ± 0.7
Cholesterol esters	2.5 ± 0.5	2.0 ± 0.4
Triacylglycerol	2.4 ± 0.5	2.6 ± 0.7
Non-esterified fatty acids	7.7 ± 0.5	7.3 ± 0.5
Phosphatidylethanolamine	13.9 ± 0.5	14.1 ± 0.5
Phosphatidylserine	9.9 ± 0.9	10.1 ± 0.7
Phosphatidylinositol	6.2 ± 0.6	5.5 ± 0.7
Phosphatidylcholine	17.9 ± 0.8	18.5 ± 0.9
Sphingomyelin	12.0 ± 0.6	12.1 ± 0.6

TABLE III

Compositional parameters of proximal small intestinal brush-border membrane of rats fed butter fat and fish oil diet for 4 weeks

Values represent means ± S.E. for four separate preparations of each membrane. The saturation index was calculated as $a/(bc)$, where a is the total number of saturated residues, b is the sum of the number of each type of unsaturated residue and c is the number of double bonds in the residue.

Parameter	Butter fat membranes	Fish oil membranes
Molar ratio		
Cholesterol/phospholipid	0.91 ± 0.03	0.92 ± 0.05
Sphingomyelin/phosphatidylcholine	0.67 ± 0.04	0.65 ± 0.04
Saturation index	0.52 ± 0.02	0.48 ± 0.05
Protein/lipid (w/w) ratio	1.16 ± 0.17	1.03 ± 0.19

TABLE IV

Fatty acid composition of proximal small intestinal brush-border membranes prepared from rats fed butter fat and fish oil diets for 4 weeks

Values are means ± S.E. for four separate preparations of each membrane. * $P < 0.05$ or less compared with butter fat-membrane values.

Component	Composition (%, by mass)	
	butter fat	fish oil
Fatty acid		
14:0	4.7 ± 0.2	4.2 ± 0.2
16:0	24.5 ± 0.6	24.1 ± 0.8
16:1($n-7$)	2.3 ± 0.1	6.6 ± 0.3 *
18:0	19.7 ± 0.7	24.5 ± 0.9 *
18:1($n-9$)	23.9 ± 0.3	15.8 ± 0.5 *
18:2($n-6$)	10.1 ± 0.1	4.2 ± 0.1 *
18:3($n-3$)	1.0 ± 0.1	0.9 ± 0.1
20:4($n-6$)	8.8 ± 0.3	5.9 ± 0.4 *
20:5($n-3$)	0.6 ± 0.2	6.6 ± 0.7 *
24:0	1.7 ± 0.2	1.2 ± 0.4
22:5($n-3$)	0.6 ± 0.2	1.9 ± 0.3 *
22:6($n-3$)	1.2 ± 0.2	3.8 ± 0.4 *
Classes		
Saturated	51.1 ± 0.5	54.0 ± 1.3 *
Monoenoic	26.7 ± 0.3	22.7 ± 0.7 *
Polyenoic	22.3 ± 0.6	23.4 ± 1.8
($n-3$)	3.4 ± 0.2	13.2 ± 0.4 *
($n-6$)	18.9 ± 0.2	10.1 ± 0.3 *
Ratios		
($n-3$)/($n-6$)	0.2 ± 0.03	1.3 ± 0.1 *
20:5/20:4	0.1 ± 0.02	1.2 ± 0.2 *

16:1($n-7$) (palmitoleic), 18:0 (stearic), 20:5($n-3$) (eicosapentaenoic), 22:5($n-3$) (timnodonic) and 22:6($n-3$) (docosahexaenoic) acids and lesser amounts of 18:1($n-9$) [oleic], 18:2($n-6$) [linoleic] and 20:4($n-6$) (arachidonic) acids than their butter fat-fed membrane counterparts. Moreover, membranes prepared from animals fed the fish oil diet for 4 weeks were found to possess a higher percentage of ($n-3$) unsaturated fatty acids, a higher percentage of saturated fatty acids and a lower percentage of monounsaturated and ($n-6$) fatty acids than those prepared from animals fed the butter fat diet (Table IV). The percentages of polyunsaturated fatty acids in these membranes (Table IV) as well as their saturation indices (Table III), however, were similar.

TABLE V

Quantification of major peaks representing different molecular species of phosphatidylcholine from brush-border membranes of rats fed butter fat or fish oil

Peak numbers correspond to those shown in Fig. 1. Molecular species were confirmed by GLC analysis of the fatty acid composition in peaks eluted from HPLC, and/or by comparison to elution times of standards.

Peak No.	Species	Butter fat (% of total ± S.E., $n = 3$)	Fish oil (% of total ± S.E., $n = 3$)	P value
2	16:0-20:5 trace 18:1-22:6	0.33 ± 0.03	5.37 ± 0.43	0.001
3	16:0-22:6	0.27 ± 0.15	1.26 ± 0.10	0.01
7	18:1-20:4 16:0-20:4	3.20 ± 0.26	5.73 ± 1.19	0.10
9	18:0-20:5	0.47 ± 0.12	7.83 ± 1.34	0.01
11	16:0-18:2 18:0-22:6 [a]	15.23 ± 0.46	12.50 ± 1.01	0.10
14	18:0-20:4 16:0-14:0 [a]	4.57 ± 0.15	5.37 ± 0.09	0.01
17	18:0-18:2 16:0-18:1	28.93 ± 1.20	21.40 ± 1.57	0.02
18	16:0-16:0	10.67 ± 0.33	8.60 ± 0.76	0.10
24	16:0-18:0 18:0-16:0	12.10 ± 0.46	9.80 ± 0.80	0.10

[a] Present only in membranes from fish oil-fed rats.

In agreement with earlier studies by our laboratory [5], phosphatidylcholine was found to be the major phospholipid present in these plasma membranes. It was, therefore, of interest to examine and compare the molecular species of this phospholipid in the brush-border membranes prepared from the animals of the two dietary groups. As can be seen in Fig. 1, the HPLC chromatograms of the molecular species of phosphatidylcholine were found to differ in the membranes of each group. Table V summarizes the major differences in the molecular species of phosphatidylcholine found to exist in the brush-border membranes of each group. As can be seen in this table, membranes prepared from fish oil fed-animals were found to have significantly higher phosphatidylcholine molecular species 16:0-20:5, 16:0-22:6 and 18:0-20:5 than their butter fat-fed counterparts. In turn, membranes from butter fat-fed rats possessed significantly greater quantities of 18:0-18:2 and 16:0-18:1 as well as a tendency for higher 16:0-16:0 and 16:0-18:0 or 18:0-16:0 than the membranes from fish oil fed-rats.

Fluorescence studies

In view of the differences noted in the fatty acid composition of the brush-border membranes of the

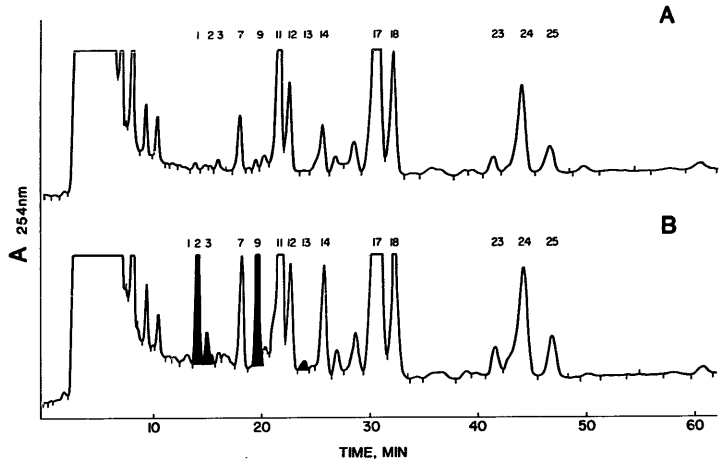

Fig. 1. Representative HPLC chromatograms of dinitrobenzoyl derivatives of phosphatidylcholine from rat intestinal brush-border membranes. (A) Rats fed butter fat. (B) Rats fed fish oil. The acetonitrile/2-propanol (80:20, v/v) system of Kito et al. [27] and Takamura et al. [28] was used, under identical chromatographic conditions. Peaks were quantitated as area and expressed as percentage of total. The shaded peaks are species found to occur almost exclusively in the fish oil group, comprising less than 0.5% in the butter fat group.

animals fed the two diets (see above), it was of interest to examine and compare the lipid fluidity of these membranes. The physical state of the lipids of these membranes was, therefore, assessed by the rotational mobilities of the probes DPH and 12-AS as well as by the translational mobility of the fluorophore pyrenedecanoic acid [9].

In the present studies, as shown in Table VI, the r and r_∞ values of DPH were similar in both membrane groups. R values, however, were significantly higher in butter fat-membranes, indicating that at least by this 'dynamic' parameter of DPH, butter fat membranes were more fluid than fish oil-membranes [9]. In this regard, it is important to note that these findings are in basic agreement with Stubbs et al. [43], using liposomes of purified lecithins with varying degree of unsaturation and DPH to assess the fluidity of these preparations. These investigators showed that while changes in the

TABLE VI

Fluorescence studies of proximal small intestinal brush-border membranes of rats fed butter fat and fish oil diets using DPH, 12-AS and pyrenedecanoate

Values represent means ± S.E. of four separate preparations of each membrane obtained at 25°C. * $P < 0.05$ compared to values of membranes prepared from animals fed the butter fat diet.

Preparation	Probe	Anisotropy (r)	Limiting hindered anisotropy (r_∞)	Rotational rate (R)	Excimer/monomer ratio
Butter fat-membranes	DPH	0.288 ± 0.001	0.280 ± 0.001	0.165 ± 0.009	–
	12-AS	0.147 ± 0.003	–	–	–
	pyrenedecanoate	–	–	–	0.258 ± 0.008
Fish oil-membranes	DPH	0.290 ± 0.001	0.280 ± 0.001	0.143 ± 0.004 *	–
	12-AS	0.161 ± 0.005 *	–	–	–
	pyrenedecanoate	–	–	–	0.206 ± 0.008 *

rotational relaxation time * of DPH secondary to alterations in fatty acid unsaturation are, in general, small, large changes in the rotational relaxation time can be seen within approximately 10 C° of their liquid-crystalline to solid transition. In the present studies R values for DPH were obtained at 25 and 37°C, i.e., temperatures within the broad small intestinal brush-border membrane lipid thermotropic transition previously observed by our laboratory using fluorescence polarization and differential scanning calorimetric techniques (23–39°C) [5,44]. Similarly, the r values of 12-AS indicated that brush-border membranes prepared from butter fat fed-animals were more fluid than their fish oil-fed counterparts (Table VI).

Moreover, in the present experiments, the ratio of excimer to monomer intensities of pyrenedecanoate was found to be significantly higher in membranes prepared from butter fat-fed animals compared to their fish oil-fed counterparts (Table VI), again indicating that the former membranes were more fluid as assessed by this technique [9].

Effect of diets on Na^+-dependent D-glucose transport

Previous studies have suggested that alterations in membrane fluidity may influence the uptake of Na^+-dependent D-glucose into rat small intestinal [5,6,45] and renal [46] brush-border membrane vesicles. It was, therefore, of interest to examine and compare the kinetic parameters of Na^+-dependent D-glucose transport in the brush-border membranes prepared from the two groups of animals. As shown in Fig. 2, membrane vesicles prepared from fish oil-fed animals were found to possess a significantly higher ($P < 0.05$) maximum velocity (V_{max}) than their butter fat-fed counterparts (603.1 ± 10.6 versus 460.8 ± 15.6 pmol/mg protein per 6 s, respectively, $N = 3$ for each). The K_m for glucose of the membrane vesicles prepared from butter fat (90.6 ± 1.2 μM) and fish oil (95.3 ± 2.7 μM) fed animals, however, were similar.

Accumulations of ^{22}Na at equilibrium (60 min) in membrane vesicles prepared from animals fed the butter fat and fish oil diets were similar in the vesicles of both groups (butter fat, 0.76 ± 0.11; fish oil, 0.69 ± 0.12 nmol/mg protein, $N = 3$, $P > 0.05$). As previously discussed [47], the latter finding indicates that the average intravesicular volumes of vesicles isolated from both animal groups were comparable, suggesting that the difference in the rates of uptake of Na^+-dependent D-glucose (see above) were not due to alterations in vesicle size. Since similar aliquots of membrane proteins were used in the transport experiments, this also rules out changes in vesicular number as being responsible for the D-glucose transport differences seen in the present studies.

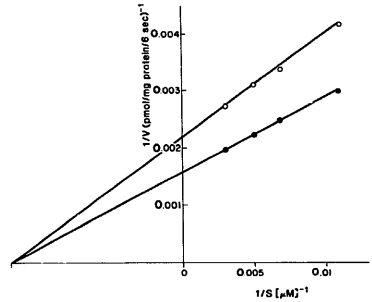

Fig. 2. A representative double-reciprocal plot of Na^+-dependent D-glucose transport versus glucose concentrations is shown for fish oil (●) and butter fat (○) membrane vesicles. See Materials and Methods as well as Results for further details.

Additionally, when ^{22}Na influx studies were performed in membrane vesicles of both groups, using 1 mM NaCl in the absence of a pH gradient (pH 7.5 inside and outside), no differences were noted between butter fat-vesicles (0.26 ± 0.02 nmol/mg protein, $N = 3$) and fish oil-vesicles (0.24 ± 0.03, $N = 3$), indicating that the differences in Na^+-dependent D-glucose transport in these vesicles did not appear to be secondary to differences in Na^+ permeability in these preparations [47].

Discussion

The present data demonstrate for the first time that feeding nutritionally complete diets supplemented with 20% fish oil or butter fat for 4 weeks results in alterations in the fatty acid composition, lipid fluidity and Na^+-dependent D-glucose transport of rat proximal small intestinal brush-border membranes. Each of these observations deserves further comment.

In agreement with earlier studies in rat myocytes [10], rat brain, sciatic nerve and retinal cells [12], rat hepatocytes [13] and monkey neutrophils [11], the present studies of rat proximal small intestinal brush-border membranes demonstrate that dietary fish oil supplementation had no effect on membrane cholesterol or phospholipid composition but had a rather marked effect on the fatty acid composition. Compared to membranes prepared from rats fed the dietary butter fat regimen, membranes prepared from dietary fish oil fed-animals had significantly higher $(n-3)/(n-6)$ and 20:5/20:4 ratios. As noted in earlier studies of other

* The rotational rate (R, in radians per second) can be converted to a rotational relaxation time (in nanoseconds) as follows: rotational relaxation time = $(6R)^{-1}$ [35,36].

membranes [10,13,48], the predominant $(n-3)$ fatty acids incorporated in brush-border membranes prepared from fish oil fed-animals were 20:5, 22:5 and 22:6 with concomitant decreases in 18:2 and 20:4 compared to butter fat-membranes. In contrast to these findings, however, Philbrick et al. [12] failed to detect notable increases in 22:6 in the phospholipids of neural tissues of rats fed a high fish oil diet. Taken together, these observations would suggest that dietary fish oil supplementation can influence the fatty acid composition of membranes of various organs but in a tissue specific manner. Moreover, changes resulting from these various dietary regimens may be very complex as demonstrated by our analysis of the molecular species of phosphatidylcholine isolated from brush-border membranes of the two groups of animals.

The mechanism(s) underlying these fatty acid compositional differences in butter fat- and fish oil-membranes have not been specifically addressed in the present studies. In this regard, however, previous studies have suggested that dietary effects on fatty acid desaturation, deacylation-reacylation reactions and availability of various acyl coenzyme A esters in the cellular pool may be involved in these membrane lipid compositional alterations [12,13]. Further studies will, therefore, be necessary to address this issue.

As assessed by steady-state fluorescence polarization, differential polarized phase fluorometric and excimer/monomer fluorescence intensity techniques, the lipid fluidity of membranes prepared from fish oil fed animals was significantly lower compared to their butter fat fed counterparts. Thus, fluidity as assessed by both the rotational and translational mobilities of the various probes in the fish oil-membrane lipid was decreased [9].

Previous studies in model and biological membranes [5,9,49,50] have shown that differences in membrane fluidity may be secondary to alterations in the ratios of cholesterol/phospholipid (mol/mol), sphingomyelin/phosphatidylcholine (mol/mol), protein/lipid (w/w) as well as in the fatty acid saturation index. In the present experiments, none of these parameters including the saturation index, were found to be significantly different in the two membrane groups. While a priori these findings are somewhat unexpected, it should be noted that as previously discussed by Stubbs and Smith (1984) [52], there is clearly no simple linear relationship between membrane physical properties and the number of fatty acid double bonds. Thus, it is now clear that the use of saturation or unsaturation indices fails to adequately address the complexity of membrane phenomena related to fluidity [51,52]. For example, the position of the double bond in the acyl chain as well as the linkage position of the acyl chain (molecular species) at times may be more important in terms of melting point than the actual number of double bonds [52]. Additionally, previous studies in model membranes [43] and rat renal brush-border membranes [53], using DPH and time-resolved fluorescence anisotropy to assess fluidity, have shown that the introduction of a single *cis*-double bond has the most pronounced effect on fluidity and that increasing the number of double bonds produces only small additional changes in the physical state of the membrane lipid.

Despite the marked increase in the $(n-3)$ class of unsaturated fatty aids in membranes prepared from fish oil-fed animals, the percentage of polyunsaturated fatty acids was similar in both membranes, whereas, the percentage of saturated and monounsaturated fatty acids was increased and decreased, respectively, in fish oil-brush-border membranes compared to their butter fat-membrane counterparts. The latter differences in membrane fatty acid composition would theoretically lead to a decrease in the fluidity of fish oil-membranes [52], which is consistent with our present findings.

As noted earlier, the levels of arachidonic acid were decreased in fish oil-membranes compared to their butter fat-counterparts. The accumulation of the $(n-3)$ fatty acids at the expense of arachidonic acid in cells has previously been implicated as a mechanism for the impaired conversion of this fatty acid to prostaglandins [54]. In this regard, recent studies by Deliconstantinos [55] have shown that prostaglandins increase the membrane fluidity of dog synaptasomal plasma membranes. Whether this phenomenon in small intestinal enterocytes plays any role in the decreased fluidity of fish oil-membranes is unclear at this time and will require further studies.

Concomitant with the changes in fatty acid composition and fluidity, Na^+-dependent D-glucose transport was found to be increased in brush-border membrane vesicles prepared from animals administered the fish oil diet. In contrast to these findings, previous studies from our laboratory [5,6], using other in vivo dietary manipulations, have demonstrated a direct correlation between fluidity and Na^+-dependent D-glucose transport in rat small intestinal brush-border membrane vesicles. Prior in vitro investigations [45–47], using the known fluidizer, benzyl alcohol, have suggested an inverse relationship between fluidity and D-glucose transport in a variety of membranes. Taken together, these results would strongly suggest that the differences in fluidity noted between the fish oil- and butter fat-membranes were not responsible for alterations in Na^+-dependent D-glucose transport seen in the present experiments.

While the exact mechanism(s) responsible for alterations in this transport process, therefore, remain to be elucidated, recent studies by Carruthers and Melchior [3] are of interest. In a series of elegant experiments, these investigators demonstrated that bilayer fluidity appeared to be a relatively unimportant determinant of the human erythrocyte hexose transporter's activity, whereas bilayer lipid composition was the major regu-

lator of protein-mediated hexose flux in reconstituted vesicle preparations. Since it is now clear that biological membranes [57], including rat small intestinal brush-border membranes [4], possess various lipid domains, while speculative, it would seem reasonable to suggest that changes in the fatty acid composition of the particular domain for D-glucose transporter, rather than fluidity per se, might be responsible for the increased Na^+-dependent D-glucose transport seen in the fish oil-membranes.

Regardless of the mechanism(s), however, it would appear that brush-border membrane vesicles prepared from animals fed a nutritionally complete diet supplemented with fish oils do have an increase in Na^+-dependent D-glucose transport compared to membrane vesicles prepared from butter fat fed-animals. Recently, our laboratory has utilized a nonspecific lipid transfer protein to vary the cholesterol/phospholipid molar ratio of rat small intestinal brush-border membranes in order to examine the role of cholesterol in modulating certain enzyme activities of this plasma membrane [58]. In the future, it may, therefore, be possible to use similar techniques to enrich intestinal brush-border membrane vesicles with the particular molecular species of phosphatidylcholine found to be increased in the fish oil-membranes noted in the present studies and to systematically assess their influence on Na^+-dependent D-glucose transport.

Acknowledgements

The authors would like to thank Alan Klitzke for his technical assistance and Lynn Nelson for her excellent secretarial support. This investigation was supported by PHS grants CA 36745, DK 35771 and DK 26678. T.A.B. is the recipient of a Merit Award from the N.C.I./N.I.H.

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