Biochimica et Biophysica Acta, 984 (1989) 158–166
Elsevier

BBAMEM 74531

Lipid permeability of the intestinal microvillus membrane may be modulated by membrane fluidity in the rat

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(Received 16 February 1989)

Key words: Lipid permeability; Membrane fluidity; Microvillus membrane; Cholesterol; 7-Dehydrocholesterol; (Rat)

Fatty acids and cholesterol permeate across the intestinal microvillus membrane at rates dictated by the hydrophobicity of the permeating lipid and the permeability properties of the microvillus membrane. A theory has evolved suggesting that the chemical composition and physical properties of the microvillus membrane are important in determining microvillus membrane lipid permeability in vivo. This communication reports a test of this hypothesis. To compare in vivo membrane lipid permeability within the same intestinal region, but under conditions where membrane physical properties were radically altered, rats were fed an inhibitor of cholesterol synthesis. This resulted in the replacement of 87–90% of membrane cholesterol with its' precursor, 7-dehydrocholesterol. Marked changes in membrane physical properties were observed, including a reduction in the static and dynamic componenent of membrane fluidity within the jejunal microvillus membrane. These changes were limited primarily to the outer regions of the bilayer. Associated with these alterations was a pronounced reduction in membrane lipid permeability. Therefore, microvillus membrane lipid permeability, in vivo, appears to be correlated with physical properties of the bilayer, especially those of the superficial regions.

Introduction

Lipids cross the intestinal microvillus membrane by a diffusional process [1,2], therefore, it is reasonable to suggest that their permeability coefficients be determined by physical properties of the microvillus membrane. In turn, membrane physical properties are dependent upon the chemical composition of the bilayer, a parameter increasingly amenable to dietary or pharmacological manipulation [3]. Recently, methods have become available to quantitate certain physical properties of membranes and correlate these with membrane lipid composition and various aspects of membrane function. Using a variety of lipid soluble fluorescent probes, estimates of membrane lipid fluidity have been obtained within several membrane microenvironments. Alterations in the physical properties of these environments have been associated with changes in the activity of membrane bound enzymes or receptors including the Na^+/K^+-dependent ATPase, and the beta-adrenergic receptor [4–7]. Furthermore, membrane transport processes have also been demonstrated to be sensitive to changes in the lipid fluidity of the plasma membrane. Sodium-dependent glucose transport across the microvillus membrane of the intestine or renal tubule may be modulated by alterations in the lipid fluidity of these membranes. Rates of glucose transport decrease with increasing membrane fluidity [8] induced either in vitro or in vivo by renal is ischaemia [9]. The permeability of the plasma membrane for molecules that cross by a carrier-independent process also appears to vary with changes in the physical properties of the bilayer [10–12]. However, data concerning the relationship between membrane lipid permeability and lipid fluidity are presently unavailable. It is recognized that the plasma membrane discriminates between permeating solutes on the basis of both the size and hydrophobicity of the probe molecule. Furthermore, it has been argued on theoretical grounds that the major membrane permeability barrier to hydrophobic solutes, such as fatty acids or cholesterol, is found in the outer third of the bilayer [13]. Since methods are now available to selectively determine membrane physical properties in different regions of the bilayer these predictions can be experimentally tested. In preliminary work from this laboratory [14] it has been demonstrated that the ileum is less permeable to fatty acids than the jejunum. Using fluo-

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rescent probes that reflect the dynamic component of membrane fluidity at different depths in the bilayer it was apparent that increased jejunal lipid permeability correlated with increased fluidity in the outer third of jejunal microvillus membrane. Further experiments have demonstrated that this pattern is present not only in adult animals, but is also reproduced in the developing jejunum. During the suckling period in the rat, the jejunal microvillus membrane is highly lipid permeable and very fluid; with weaning lipid permeability decreases and the membrane becomes progressively more rigid [15]. These data supported the theoretical predictions of Stein [13] and suggested that intestinal lipid permeability could be modulated by manipulating physical properties in this region of the microvillus membrane.

Since physical properties in the outer region of the membrane are largely determined by membrane sterol, the replacement of cholesterol with a sterol having different physical properties would be predicted to significantly alter the permeability of the microvillus membrane to fatty acids and other lipids. The testing of this prediction forms the basis for the present communication. HCG-917 potently inhibits the final step in cholesterol biosynthesis. Previous work has demonstrated that feeding this agent to rats leads to a rapid replacement of membrane cholesterol with its immediate precursor, 7-dehydrocholesterol, and a dramatic reduction in plasma sterol concentration (unpublished observations). The intestinal microvillus membrane of animals treated with this agent would, therefore, be expected to have different physical properties than the microvillus membrane of control animals, where cholesterol is the predominant sterol. Thus, these animals represent an ideal model in which to test the hypothesis that rates of intestinal lipid permeation are determined by physical properties in the outer third of the intestinal microvillus membrane.

Methods

Chemicals. Unlabelled fatty acids were obtained from either Sigma (St. Louis, MO) or NuChek (Elysian, MN) and their purity established by gas-liquid chromatography (GLC). Any lots found to be less than 99% pure were discarded. [^{3}H]Polyethylene glycol (PEG) and [^{14}C]dodecanoic acid (12:0) were obtained from New England Nuclear (Boston, MA) and used as supplied. The fluorescent probes 1,6-diphenyl-1,3,5-hexatriene (DPH), six isomers of (anthroyloxy)stearic acid and 16-(9-anthroyloxy)palmitic acid were purchased from Molecular Probes (Junction City, OR) and also used as supplied. All other reagents and chemicals were of the highest grade available.

Animals. Female Sprague-Dawley rats (200–225 g) were used for all experiments. Animals were housed in light cycled rooms and allowed free access to water and a ground diet (Wayne Labs.). In the treated group HCG-917 was added to the diet at a concentration of 100 mg/kg and fed for two weeks, a dose found sufficient in preliminary studies to produce a change in membrane sterol composition in both plasma and hepatic microsomes.

Perfusion studies. Rates of fatty acid absorption were measured by a recently established technique [14] based on a previously published method [16]. Briefly, following anesthesia with pentobarbital (65 mg/kg i.p.) a 10–15 cm loop of intestine was fashioned either just distal to the ligament of Treitz or just proximal to the ileocecal valve in female Sprague-Dawley rats. The ends of the loop were cannulated with teflon tubing and returned to the abdominal cavity. During an equilibration period of 20 min, the loop was perfused with buffer alone (mM): NaCl (150), KCl (5), Hepes (50), pH 7.5. The infusate was then changed to a solution identical to the buffer but containing eight fatty acids (μM), 5:0 (800), 7:0 (400), 8:0 (400), 9:0 (200), 10:0 (180), 11:0 (150), 13:0 (60), and trace quantities of [^{14}C]12:0. The perfusion continued for 48 min at a rate of 5 ml/min and aliquots of the perfusate were regularly obtained for the determination of fatty acid concentrations as well as of the nonabsorbable marker, [3H]PEG [16]. Rates of fatty acid absorption for each probe were calculated from the linear fall in concentration of each fatty acid during the course of the perfusion. Absorption rates were normalized to both the length of the perfused loop and the driving concentration gradient, assumed to be the geometric mean concentration of the probe during the perfusion, as described by Winne and Markgraf [2]. The resultant value is defined as the apparent permeability coefficient, * P, and represents the nmoles of probe absorbed per minute per cm length of intestine per mM concentration gradient. Diffusion barrier resistance was measured by the diffusion-limited probe method [16,17]. This value, established in each animal, allowed derivation of the true membrane permeability coefficient, P, for fatty acid probes 5:0 through 10:0 which were then used to characterize the lipid permeability properties of the microvillus membrane (MVM). The interfacial concentration, C_2, of each probe during the course of the perfusion was calculated and assumed to approximate the transmembrane concentration gradient [16,18].

$$C_2 = C_1 - \frac{Jd}{DS_a} \quad (1)$$

In this expression C_1 represents the concentration of probe in the bulk phase, J represents the absorption rate of the probe, D its aqueous diffusion coefficient, d the average thickness of the diffusion barrier and S_a its surface area. The ratio d/S_a reflects the resistance of

this barrier. The concentration of unlabelled fatty acids were determined by gas-liquid chromatography (GLC) while [^{14}C]12:0 and [^{3}H]PEG concentrations were quantitated by standard liquid scintillation techniques. All GLC analyses were performed using a Hewlett-Packard 5890 GLC system (Hewlett-Packard, Avondale, PN). Free fatty acids from the perfusion were separated on a packed column containing GP 10% SP-216-PS (Supelco, Bellafonte, PA) and quantitated with a Hewlett-Packard 3392 integrator coupled to a FID detector. Besides quantitating the permeability properties of the MVM to fatty acids these data can also be used to calculate the relative hydrophobicity of the MVM. There exists a log-linear relationship between the membrane permeability coefficients of a series of lipid probes and the hydrophobicity of the probe, i.e., chain length for a series of fatty acids [1,13,16,18]. This relationship holds true for the longer chain length members of the fatty acid series. However, in most studies the shorter chain length members appear to be anomalously permeable suggesting the presence of accessory transmembrane pathways. In the present system it has previously been shown that fatty acid probes 9:0 and 10:0 are not anomalously permeable so that the permeability coefficients for these probes can be used to define the change in incremental free energy that occurs when a methylene group partitions from the aqueous luminal environment into the membrane, $\delta\Delta F_{w\to l}$ [16,18].

$$\delta\Delta F_{w\to l} = -RT \ln\left(\frac{P^+}{P^-}\right) \quad (2)$$

In this equation R represents the gas constant, T the absolute temperature, and P^+ or P^- the permeability of the probe with and without the additional methylene group, respectively. The units become cal/mole and a more hydrophobic membrane, i.e., one having a steeper relationship between permeability and chain length, would have a more negative value for this term.

Membrane isolation. Microvillus membrane vesicles were prepared from both jejunal and ileal mucosal scrapeings using a previously published method [19]. Purification was assessed by the concentration of sucrase-specific activity [20]. Basolateral contamination was evaluated by the marker enzyme Na^+/K^+-ATPase [22]. Compositional analysis was normalized to the protein content of the vesicles determined by the method of Bradford [21]. Membrane or plasma lipids were extracted by the method of Folch et al. [23]. Total cholesterol content was quantitated by GLC using a Supelco SP2250 packed column and stigmasterol as an internal standard. The total phospholipid content of the same aliquot was determined by the method of Ames and Dubin [24]. Total brush-border fatty acids were methyl transesterified using the method of Morrison and Smith [25]. The resulting fatty acid methyl esters were separated by GLC using a Supelcowax 10 capillary column and a temperature programmed run from 170 °C to 240 °C. Under these conditions the range of fatty acids identified from standard mixtures ranged from 14:0 to 22:6. Authentic standards were used to identify all compounds quantified by GLC.

Fluorescence polarization studies. Eight fluorescent probes were used: 1,6-diphenyl-1,3,5-hexatriene (DPH), six isomers of stearic acid labelled with 9-anthroyloxy groups at either carbon numbers 2, 3, 6, 7, 9 or 12 and 16-(9-anthroyloxy)palmitic acid. Steady-state polarization measurements were performed using a SLM polarization spectrofluorometer (SLM-Aminco, Urbana, IL). Previously published methods [26] were used to load the vesicles with the fluorescent probe and obtain the measurements [14]. Data are reported as the steady-state anisotropy parameter, r_s. It has been shown by Van Blitterswijk et al. [27] that the limiting hindered anisotropy, r_∞, can be calculated from this measurement using an empirical relationship. Since 'the dynamic component' of membrane fluidity varies as a function of depth in the bilayer it was determined with seven isomers of anthroyloxy stearic or palmitic acid that differed in the carbon labelled with the fluorescent group. The use of *n*-(9-anthroyloxy)stearic or palmitic fatty acids to measure fluidity gradients in membranes has been extensively reviewed [29,30]. These probes orient themselves in a vertical direction in the bilayer with the labelled group placed progressively deeper in the membrane in a graded fashion. It is important to note that fluorescent lifetime and relative quantum yield increase as the fluorescent group is moved further into the membrane interior. Furthermore, the rotational movement of these probes in liquids of well characterized viscosity are not identical [30] and, therefore, the interpretation of data obtained from such experiments must be made with caution. However, these probes are useful in determining relative fluidity gradients in a variety of membrane preparations [29–32]. It should be mentioned that for the probes used in this study the measured anisotropy of each in glycerol at 50 °C was remarkably similar. The interprobe variation did not exceed 3% (data not shown).

Statistical analysis. Unless stated otherwise all data are presented as the mean ± S.E. Comparisons between groups were assessed by the Mann-Whitney U test with the level of significance set at $P < 0.05$. All statistical calculations were performed on a microcomputer using a commercially available statistical software package.

Results

Animals fed a ground diet plus drug fared as well as animals receiving the control diet, illustrated in Table I. Weight gain was equivalent in both groups over the two week test period but a systemic effect of the drug was

TABLE I

Growth rates and plasma sterol composition

Values are means ± S.E. for 20 rats in each group fed the control and experimental diet for 14 days.

	Weight (g)		Plasma sterol (μM)		
	initial	final	7-DC	cholesterol	total
Control ($n = 20$)	224 ± 2	232 ± 2	0	1825 ± 187	1825 ± 187
Treated ($n = 20$)	225 ± 3	235 ± 3	840 ± 41 *	87 ± 3 *	927 ± 45 *

* $P < 0.05$ controls vs. treated.

TABLE II

Microvillus membrane chemical composition

Values represent the means ± S.E. from four separate membrane preparations. Each preparation represents material isolated from the pooled mucosal scrapings of four animals.

Intestinal segment	Group	Sucrase concn. (×-fold)	Membrane sterols (nmol/mg protein)			Phospholipid	Ratio sterol/phospholipid (mol:mol)
			7-DC	cholesterol	total		
Jejunum	control	20.6 ± 2.2	0	266 ± 15	266 ± 15	247 ± 2	1.08 ± 0.07
Jejunum	treated	20.8 ± 1.4	332 ± 11 *	47 ± 1 *	371 ± 11 *	405 ± 20 *	0.92 ± 0.07 *
Ileum	control	23.7 ± 1.9	0	333 ± 19	333 ± 19	274 ± 14	1.22 ± 0.13
Ileum	treated	20.5 ± 2.1	283 ± 10 *	47 ± 1 *	327 ± 13	307 ± 21	1.07 ± 0.12

* $P < 0.05$ controls vs. treated.

TABLE III

Fatty acid composition of microvillus membrane phospholipids

Values represent the means ± S.E. from the same four membrane preparations described in Table II.

Intestinal segment	Group	Fatty acid (mol%)								
		14:0	16:0	16:1	18:0	18:1	18:2	20:4	22:5	22:6
Jejunum	control	0.9 ± 0.1	25.3 ± 2	0.9 ± 0.1	19.3 ± 1	19.4 ± 1	24.9 ± 2	7.1 ± 1	0.9 ± 0.1	1.3 ± 0.1
Jejunum	treated	0.7 ± 0.2	25.7 ± 1	0.4 ± 0.3	23.4 ± 2	13.7 ± 2 *	19.5 ± 1 *	13.7 ± 2 *	1.2 ± 0.1	1.8 ± 0.2
Ileum	control	0.9 ± 0.1	28.1 ± 1	0.5 ± 0.2	23.5 ± 1	16.7 ± 1	17.3 ± 1	9.8 ± 1	1.4 ± 0.1	1.8 ± 0.1
Ileum	treated	0.9 ± 0.1	25.5 ± 1	0.5 ± 0.1	25.3 ± 1	18.8 ± 1	16.0 ± 1	9.9 ± 1	1.4 ± 0.1	1.7 ± 0.2

* $P < 0.05$ controls vs. treated.

apparent upon analysis of plasma sterols. Animals receiving the drug had close to a 50% reduction in total plasma sterol concentration and, furthermore, 7-dehydrocholesterol represented almost 90% of total plasma sterol.

Microvillus membrane composition

Microvillus membrane was isolated from both the jejunum and ileum as described. Tables II and III outline the chemical composition of this membrane fraction. Isolates were of equivalent purity irrespective of intestinal region or whether the animals had received the drug, as judged by equal concentration of sucrase specific activity. Contamination with basolateral membrane was negligible evidenced by the lack of concentration of Na^+/K^+-ATPase activity in these fractions (data not shown). Treatment with the cholesterol synthesis inhibitor induced a profound change in the sterol composition of the microvillus membrane. In both ileum and jejunum, 7-dehydrocholesterol was the dominant sterol in treated animals, comprising 87–90% of the total. Total sterol content was not different in the ileum of treated animals but in the jejunum accumulation of 7-dehydrocholesterol was associated with a significant increase in total membrane sterol.

Alterations in membrane composition were not limited to the sterol fraction. Total phospholipid content increased significantly in the jejunum of treated animals, producing a significant reduction in the sterol/phospholipid ratio. The phospholipid content of ileal microvillus membrane did not vary with drug treatment. Nine major fatty acids were identified in the brush-border fraction of both the ileum and jejunum. The molar percentage of each is tabulated in Table III. It is

apparent that, in terms of this parameter, no significant differences existed between ileal microvillus membrane isolated from control or treated animals. However, once again differences were noted in the jejunal microvillus membrane. The most prominent of these was a significant reduction in the membrane content of linoleic acid (18:2) with a corresponding increase in arachidonate (20:4). The molar percentage of oleic acid (18:1) was also significantly reduced in these membranes.

In conclusion, the only difference demonstrated in the ileal microvillus membrane of treated animals was a radical change in sterol species. In the jejunum, however, this change was accompanied by alterations in the total amount of membrane sterol and in both the quantity of phospholipids and their fatty acid composition.

Membrane physical properties

Of the eight fluorescent probes used to evaluate the physical properties of these membranes one, DPH, can be used to estimate the static component of membrane fluidity. The other seven probes can be used to estimate the dynamic component of membrane fluidity as a function of depth in the bilayer. Table IV illustrates the changes induced in the static component of membrane fluidity by treatment with HCG-917. A significant change was evident in the jejunal microvillus membrane but not in microvillus membrane isolated from the ileum. Jejunal microvillus membrane from treated animals was more rigid than the microvillus membrane obtained from control animals and this alteration translated into a significantly greater order parameter (S) for these membranes (Table IV).

Data reflecting the dynamic component of membrane fluidity are presented in Fig. 1. These measurements were obtained using stearic or palmitic acid probes labelled with a fluorescent anthroyloxy group at either carbon 2, 3, 6, 7, 9, 12 or 16. From Fig. 1 it is apparent that the dynamic component of membrane fluidity varies in a reproducible fashion with depth in

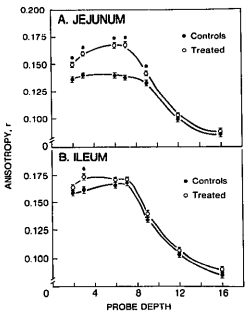

Fig. 1. The dynamic component of membrane fluidity. Values represent the means ± S.E. from four separate determinations obtained with each of the four membrane fractions described in Table II. The vertical axis reflects the steady-state anisotropy of the fluorescent label while the horizontal axis indicates the carbon on stearic acid or palmitic acid to which the fluorescent group is attached. Panel A illustrates the data derived from experiments with jejunal microvillus membrane while Panel B shows the data from ileal microvillus membrane.

the bilayer. Rotational freedom of the probe is lowest in the superficial portion of the bilayer, and increases towards the core of the membrane. In jejunal microvillus membrane obtained from treated animals there was a significant reduction in motional freedom of these probes. Furthermore, this loss of fluidity was limited to the superficial regions of the bilayer (Panel A). Microvillus membrane isolated from the ileum of either treated or control animals were equivalent in terms of the dynamic component of membrane fluidity, although ileal microvillus membrane demonstrated higher anisotropies for superficial probes than those found in the jejunal membrane. This is in agreement with previous work suggesting that ileal microvillus membrane has a more rigid lipid environment than jejunal microvillus membrane [14,26].

TABLE IV

The static component of membrane fluidity

Values represent the means ± S.E. from four separate determinations obtained with each of the four membrane fractions described in Table II. The limiting hindered anisotropy and the order parameter are calculated from the steady-state anisotropy value as described in the text.

Intestinal segment	Group	Steady-state anisotropy (r_s)	Limiting hindered anisotropy (r_∞)	Order parameter (S)
Jejunum	control	0.262 ± 0.001	0.249 ± 0.002	0.826 ± 0.003
Jejunum	treated	0.268 ± 0.001 *	0.257 ± 0.002 *	0.839 ± 0.003 *
Ileum	control	0.265 ± 0.002	0.253 ± 0.002	0.833 ± 0.003
ileum	treated	0.266 ± 0.001	0.255 ± 0.001	0.835 ± 0.02

* $P < 0.05$ control vs. treated.

Lipid permeability properties

As outlined in Methods, it is impossible to obtain accurate membrane permeability coefficients for fatty acids unless diffusion barrier resistance is first established. This was achieved using the diffusion-limited probe method [16,17]. By plotting the ratio $* P/D$ as a function of chain length for a homologous series of fatty acids a maximal value of this ratio is approached (i.e., a diffusion-limited situation) in both the jejunum and ileum of control and treated animals. Fig. 2 demonstrates that diffusion-limited situations were established

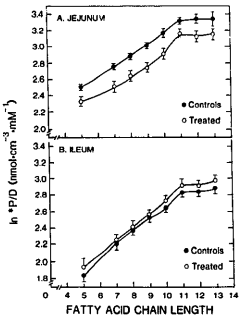

Fig. 2. Intestinal fatty acid absorption rates. Values represent the means ± S.E. of eight separate perfusions of either jejunum (Panel A) or ileum (Panel B) in either control or treated animals. The apparent permeability coefficient, * P, for each probe has been normalized to that probes aqueous diffusion coefficient, D, and the ratio plotted on the vertical logarithmic axis. The horizontal axis reflects the chain length of the probe (hydrophobicity). A clear breakpoint occurs in all curves with the value of * P/D being equal for fatty acids 11:0, 12:0 and 13:0. The interpretation of these data is discussed in the text.

Discussion

It has been suggested that the fatty acid permeability properties of the intestinal microvillus membrane are determined by its' physical properties [13,14]. The data presented in this study lend support to this hypothesis. Membrane permeability coefficients for all fatty acids were greatest in the jejunum of control animals (Fig. 3, Panels A and B) and this group had the most fluid microvillus membrane. All three situations in which fatty acid permeability was reduced from values found in control jejunum (control ileum, treated ileum or treated jejunum) were associated with a reduction in both the static and dynamic component of membrane fluidity. Furthermore, in all cases, when the dynamic component of membrane fluidity was studied at various depths within the bilayer loss of motional freedom was limited to the superficial region of the bilayer; i.e., proximal to the twelfth carbon of stearic acid (Fig. 1, Panels A and B). Therefore, the initial hypothesis of this study; that physical properties within the outer third of the microvillus membrane dictate fatty acid permeability in vivo, is strongly supported. A second important question is also raised by such studies. Whenever a functional change in some membrane event is observed in conjunction with a change in membrane fluidity it is important to consider whether it was precipitated by the

and identifies three diffusion-limited probes 11:0, 12:0 and 13:0. The ratio $D/*P$ for these probes represents the resistance of the diffusion barrier in each treatment group. It is apparent that this value was different between control jejunum and ileum. The ileum had a significantly greater diffusion barrier resistance than jejunum (0.056 ± 0.004 vs. 0.034 ± 0.001). In animals treated with HCG-917 diffusion barrier resistance increased slightly in the jejunum (0.034 ± 0.001 vs. 0.042 ± 0.003, control vs. treated) but did not change significantly in the ileum. With the resistance of the diffusion barrier firmly established, membrane permeability coefficients for all non-diffusion-limited fatty acids can be calculated. These are presented in Fig. 3 and suggest two conclusions. First, fatty acid permeability coefficients in control animals were far greater in the jejunum than the ileum. For instance the permeability coefficient for fatty acid 10:0 was 72 ± 10 nmol/min per cm per mM in the jejunum compared to 39 ± 3 in the ileum. Secondly, in animals treated with HCG-917, jejunal permeability to fatty acids was significantly reduced, and approached that found in control ileum (e.g., the permeability coefficient for fatty acid 10:0 was 41 ± 4 in the jejunum of the treated animals. Ileal fatty acid permeability was not significantly affected by treatment with this drug.

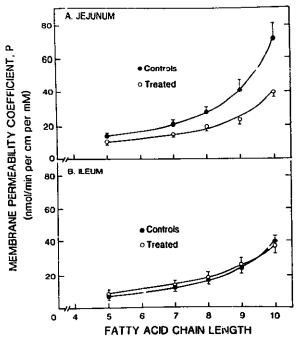

Fig. 3. Membrane permeability coefficients for non-diffusion-limited fatty acid probes. Values represent the means ± S.E. These data are derived from the values presented in Fig. 2 following appropriate correction for diffusion barrier effects (described in Methods). The vertical axis represents the microvillus membrane permeability coefficient of each fatty acid probe identified on the horizontal axis by its' chain length. Data derived from the jejunum (Panel A) are separated from the similar data obtained in the ileum (Panel B).

alteration in fluidity or associated with a specific lipid that also changed.

Alterations were induced in the composition of the intestinal microvillus membrane by HCG-917 (Tables II and III), but the changes were different in the two regions of the intestine studied. In the ileum a simple qualitative conversion of membrane sterol was achieved. Microvillus membrane isolated from the ileum of treated animals had a normal total sterol content, however, 87% of the sterol was 7-dehydrocholesterol. No significant differences were observed in these membranes in terms of total phospholipid or phospholipid fatty acids. Therefore, in the ileum, treatment with HCG-917 produced an isolated qualitative change in membrane sterol composition. Despite this alteration in membrane sterol, differences were not observed in either the static or dynamic component of membrane fluidity. This suggests that 7-dehydrocholesterol and cholesterol, at least in ileal microvillus membrane, are physically equivalent as assessed by these techniques. Since ileal fatty acid permeability did not change it appears that cholesterol, per se, does not dictate the lipid permeability properties of the microvillus membrane; it can be replaced with an equivalent sterol, provided the resultant physical properties of the membrane remain constant.

In the jejunum the same qualitative change in membrane sterol was observed. However, several other differences were noted making interpretation of these data more difficult. Both total sterol and total phospholipid content increased significantly in jejunal microvillus membrane isolated from treated animals. Since the percentage increase in membrane phospholipid was slightly greater than the increase in total sterol, the sterol/phospholipid ratio decreased. In addition to quantitative changes in membrane phospholipids, their fatty acyl composition differed in the treated animals. A significant reduction in linoleic acid content was associated with an increase in the arachidonate fraction, a correlation previously reported [33] suggesting increased formation of arachidonic acid from its precursor, linoleate, in the jejunum of treated animals. However, the microvillus membrane of treated animals also had a significant decrease in the molar percentage of oleic acid. These alterations in the jejunal microvillus membrane of treated animals were associated with significant differences in membrane physical properties. Jejunal microvillus membrane obtained from treated animals was less fluid than microvillus membrane from control animals, with probes measuring both the static and dynamic component of membrane fluidity. Differences in the dynamic component of lipid fluidity were limited to the proximal third of the bilayer; i.e., proximal to the twelfth carbon of stearic acid (Fig. 1). These changes were physiologically significant as demonstrated by the observation that fatty acid permeability of the jejunal microvillus membrane in treated animals was markedly reduced. In fact, the lipid permeability properties of treated jejunum were identical to control or treated ileum. It is important to note that this similarity extended to physical properties of the membrane as assessed by probes that measure the dynamic component of membrane fluidity. The anisotropy values for these probes were virtually identical in treated jejunum, control ileum and treated ileum at all depths in the bilayer. This suggests that lipid permeability properties of the microvillus membrane are correlated with motional freedom of the *n*-(9-anthroyloxy)stearic or palmitic acid probes where *n* varies between 2 and 9.

The observed differences in membrane lipid permeability could not be explained by changes in membrane surface area for two reasons. First, examination of intestinal histology by light microscopy revealed no difference between treated and control animals, although detailed morphometric studies were not performed. Secondly, these changes were associated with a change in the hydrophobicity of the jejunal microvillus membrane in the treated animals. This parameter is independent of surface area [16,34] and, therefore, reflects an in vivo functional property of the microvillus membrane itself. From the membrane permeability coefficients of fatty acid 9:0 and 10:0 the incremental free energy change that occurs when a methylene group partitions into the membrane ($\delta\Delta F_{w\to l}$) could be calculated as -421 ± 29 cal/mol in control jejunum. In the jejunum of treated animals this parameter had a value that was significantly different, -324 ± 23 cal/mol, implying that the jejunum of treated animals had a more polar microvillus membrane. In fact, this value is virtually identical to that found in the ileum of control animals of -312 ± 44. Therefore, these changes in membrane composition made the jejunal microvillus membrane of treated animals less fluid, less permeable to fatty acids and more polar as assessed by a measure of their in vivo hydrophobicity ($\delta\Delta F_{w\to l}$).

What remains unclear is the biochemical basis for the alterations observed in the physical properties of the jejunal microvillus membrane. Although it was originally hypothesized that the change in sterol would alter the properties of the microvillus membrane this did not appear to be the case. In the ileum, where the only alteration was a change in sterol, both physical and functional properties of the microvillus membrane remained constant. Since the decreased membrane fluidity observed in treated jejunal microvillus membrane was not due to the change in sterol or the sterol/phospholipid ratio other possibilites to be considered include a change in membrane phospholipid composition, an increase in the membrane protein/lipid ratio, or an increase in saturation of the phospholipid acyl chains. It is apparent that in the treated animals the jejunal microvillus membrane protein/lipid ratio actually decreased, therefore, this was not the explanation

for the increased membrane rigidity. A change in phospholipid classes is a distinct possibility. It has been reported that an increase in the ratio of sphingomyelin or phosphatidylethanolamine to phosphatidylcholine increases membrane rigidity [35]. At least part of the reason that sphingomyelin increases membrane rigidity is that the acyl chain of this phospholipid usually contains a high proportion of saturated fatty acids [35], and thereby increases the overall saturation of the membrane. However, since the mol% of saturated fatty acids did not increase in microvillus membranes isolated from treated animals this possibility is less likely. Phospholipid subclasses were not measured in this study and, thus, the possibility of an increase in phosphatidylethanolamine cannot be directly addressed.

The overall saturation of the phospholipid fatty acids is a superficially simple question. Several algorithms exist defining either saturation indexes or double bond indexes but their relationship to membrane lipid fluidity is not entirely clear. The addition of double bonds to phospholipid fatty acids unequivocally increases the fluidity of membranes measured by many techniques. However, the majority of this effect occurs with the addition of the first double bond in an acyl chain [36]. In the jejunal microvillus membrane isolated from treated animals the major alteration was a decrease in linoleic acid (18:2) with a corresponding increase in arachidonic acid (20:4). This alteration will increase the double bond index of the membrane but probably has little effect on the physical properties of the membrane. The observed reduction in oleic acid (18:1) and the increase in stearate (18:0) could account for the observed differences in fluidity of the treated jejunum. Arachidonate content clearly increased but this change is unlikely to have decreased the fluidity of these membranes.

Therefore, it is clear that treatment with HCG-917 dramatically alters membrane sterol content in many membranes including the microvillus membrane of both the jejunum and ileum. By itself, this alteration did not appear to produce great changes in either the physical or functional properties of ileal microvillus membrane. However, in the jejunal microvillus membrane a major change in chemical composition occurred, presumably in response to the alteration in membrane sterol. The net result was a significant decline in both the static and dynamic component of membrane fluidity. Changes in the dynamic component of membrane fluidity were limited to the outer third of the bilayer and were associated with a profound reduction in the fatty acid permeability properties of this membrane. Thus, these data strongly support the initial hypothesis of this study; that physical properties in the outer regions of the microvillus membrane determine membrane lipid permeability. Since rates of lipid absorption across the microvillus membrane are a function of the hydrophobicity of the permeating lipid [34] it is apparent that a change in the microvillus membrane that reduces lipid permeability will result in reduced rates of absorption for all lipids. Therefore, successful strategies aimed at reducing permeation rates of cholesterol and other lipids across the intestinal microvillus membrane would be predicted to decrease the fluidity of the outer third of the bilayer. This can potentially be accomplished at the pharmacological level (as in this study) or by dietary manipulation [3].

Acknowledgements

This work was supported by a grant from the Alberta Heritage Foundation for Medical Research. HCG-917 was a generous gift from Dr. J.M. Dietschy, Dallas, Texas. The author also wishes to acknowledge the superb technical skills of S. Thiesen without which this work could not have been done, and the endless patience and secretarial skills of I. McGovern.

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