VITAMIN D_3 INDUCED ALTERATION OF MICROVILLAR MEMBRANE LIPID COMPOSITION

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Summary: The lipid composition of microvillar membranes prepared from vitamin D_3-treated and vitamin D_3-deficient chick intestine has been investigated. Vitamin treatment results in an increase in the proportion of phospholipid in this target membrane as well as an alteration in the fatty acid composition of both membrane phospholipids and cholesterol esters. An increase in the weight percentage of several classes of phospholipid long chain polyunsaturated fatty acids results from vitamin treatment. The primary effect of vitamin treatment on cholesterol ester fatty acids appears to be in shorter chain species.

Vitamin D_3 administration has been shown to increase calcium transport across the intestine. This response has been thought to be mediated by a primary effect of the vitamin on either nucleic acid or protein synthesis (1,2), because an increase in both the content of a calcium binding protein in the intestinal mucosa (3) and the activity of an intestinal microvillar Ca^{++}ATPase-alkaline phosphatase (4,5,6) has been demonstrated after vitamin D treatment of D-deficient animals. The notion that the lipid composition of the target microvillar membrane might also be altered by vitamin treatment was suggested by the observations that: (a) addition of filipin, a lipophilic polyene antibiotic, to isolated chick intestine stimulates calcium transport across D-deficient, but not D-treated preparations (7); and (b) aldosterone, a hormone that stimulated sodium transport across the toad urinary bladder, alters the fatty acid composition of its target cell membranes (8). In this communication we report that vitamin D_3 treatment of vitamin D-deficient chicks alters the lipid composition of the subsequently isolated intestinal microvillar membrane.

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METHODS

Four week old vitamin D-deficient chicks (9) were used for these studies. Fourty hours after a single oral, physiological, dose of 50 I. U. of vitamin D_3, intestinal microvillar membranes were isolated from control and treated chicks by a modification of the procedure of Forstner et al. (10). After removing aliquots of each preparation for protein determination (11), the membranes were centrifuged and the pellet stored at -20° under an N_2 atmosphere. The lipids from these membrane pellets were subsequently extracted for 2 hours at 40° C in chloroform-methanol (2:1 v/v) and the extract washed three times with 0.2 volumes of 0.05 M NaCl. The weight of the lipid was determined by weighing dried aliquots of the extract. The phospholipid content of the extract was determined after 10N H_2SO_4 hydrolysis utilizing the Baginski, Foa, and Zak phosphate assay (12) and phosphatidylethanolamine as a standard. Cholesterol was determined by the method of Zlatkis et al. (13) and lipid hexose by anthrone reagent (14). Phospholipid classes were separated on a thin layer of silica gel H (15), eluted from the silica with methanol, and quantitated as described above. Fatty acid methyl esters were prepared and analyzed by previously published techniques (8).

RESULTS AND DISCUSSION

In this study data are reported on the lipid composition of pooled microvilli from 10 vitamin D-deficient chicks and from 12 vitamin D-treated animals. Results similar to those reported below have been observed in the two other microvilli preparations examined. As shown in Table 1 vitamin D treatment leads to an increase in the lipid:protein ratio in the microvillar membrane. More detailed analysis reveals that this increase is due specifically to phospholipids. In the microvilli prepared from D-treated animals the percentage of the total lipid present as phospholipid is increased. There appears to be no change in the proportion of cholesterol or glycolipid in the membrane. This result contrasts with the previously reported data of Adams et al. that showed no difference in the lipid:protein ratio in the brush border

TABLE 1

Lipid Composition of Microvilli From Vitamin D-Deficient and Vitamin D-Treated Chick Small Intestine[a]

	Vitamin D-Treated	Vitamin D-Deficient
mg lipid/mg protein	0.197 ± 0.015	0.164 ± 0.009
mg phospholipid/100 mg protein	9.912 ± 0.748	7.394 ± 0.550
mg cholesterol/100 mg protein	3.637 ± 0.283	4.054 ± 0.217
mg glycolipid/100 mg protein	5.993 ± 0.456	6.636 ± 0.309
Molar Ratio cholesterol:phospholipid[b]	0.741 ± 0.025	1.108 ± 0.073

a. Each number represents the average ± standard deviation of at least 4 determinations.
b. An average phospholipid molecular weight of 780 has been assumed.

60 hours after 500 I. U. of vitamin D_3 and an increase in the amount of cholesterol in the D-treated membrane preparation. When the cholesterol: phospholipid molar ratio is calculated from the present data, it is apparent that vitamin D treatment has induced a profound alteration in the lipid composition of the microvillar membrane. The consequences of this alteration

TABLE 2

Phospholipid Content of Microvillar Membrane[a]

	Vitamin D-Treated	Vitamin D-Deficient
Phosphatidylethanolamine	48.4 ± 7.2[b]	52.3 ± 5.4
Phosphatidylserine	12.1 ± 2.2	12.4 ± 1.7
Phosphatidylcholine	37.0 ± 5.4	32.5 ± 4.1
Sphingomyelin	1.4 ± 0.6	1.8 ± 0.2
Lysophosphatidylcholine	1.2 ± 1.4	1.0 ± 0.7

a. Data represent the mean ± standard deviation of three separate thin layer chromatographic separations.
b. Percentage of total phospholipid recovered in each class.

in the relative proportion of cholesterol and phospholipid might be expected to increase the permeability of the vitamin treated membrane (16,17,18). Although detailed studies of permeability have not been carried out with this biological membrane, the calcium permeability of the microvillus has been shown to increase after vitamin D treatment (7,19).

Analysis of individual phospholipid classes (Table 2) reveals that the chick intestinal microvillar membrane, like that of the rat (20), contains predominantly phosphotidylethanolamine and phosphatidylcholine. The relative proportion of the phospholipids in the brush border is not, however, altered by vitamin treatment. Although experimental conditions are not comparable, the present data confirm a previous report showing an effect of vitamin D on

TABLE 3

Fatty Acid Composition of Microvillar Phospholipids[a]

Fatty Acid	Weight % Fatty Acid in Vitamin D-Treated	Vitamin D-Deficient
12:0	0.43 ± 0.07	0.81 ± 0.16
14:0	2.15 ± 0.20	2.35 ± 0.10
14:1	trace	2.35 ± 0.10
16:0	5.96 ± 0.09	4.86 ± 0.08
16:1	8.36 ± 0.15	8.29 ± 0.77
16:2	0.82 ± 0.10	0.97 ± 0.03
18:0	9.18 ± 0.71	9.55 ± 0.70
18:1ω9	4.18 ± 0.16	4.26 ± 0.30
18:2ω6	13.19 ± 0.21	14.15 ± 0.05
20:0	0.17 ± 0.03	0.25 ± 0.02
18:3ω3	6.10 ± 0.25	6.46 ± 0.22
18:4ω3	1.11 ± 0.05	1.15 ± 0.09
20:2ω6	1.08 ± 0.29	0.93 ± 0.10
20:3ω9	1.45 ± 0.14	0.92 ± 0.01
20:3ω6	0.38 ± 0.10	0.31 ± 0.03
20:4ω6	28.52 ± 0.25	28.42 ± 0.40
20:4ω3	0.39 ± 0.07	0.90 ± 0.11
20:5ω3	1.43 ± 0.17	1.37 ± 0.24
22:4ω6	1.60 ± 0.12	1.81 ± 0.28
22:5ω6	2.05 ± 0.09	3.56 ± 0.48
22:5ω3	2.46 ± 0.60	1.29 ± 0.23
24:4ω6	3.56 ± 0.12	2.79 ± 0.45
22:6ω3	5.44 ± 0.36	2.23 ± 0.40

a Values are the mean +/- standard deviation of three separate determinations.

intestinal phospholipid metabolism, i.e., in rats 3 hours after 2,000 I. U. of vitamin D_3 ^{32}P incorporation into phospholipids is increased, but no specific class of phospholipid is preferentially labeled (21).

When the fatty acid composition of the phospholipids is analyzed (Table 3), it is clear that vitamin D treatment leads to an increase in the weight percentage of the long chain polyunsaturated fatty acids 20:3ω9, 22:5ω3, 24:4ω6 and 22:6ω3 and to a decrease in the weight percentage of fatty acids 12:0, 14:1, 20:0, 20:4ω3 and 22:5ω6. In contrast to the predominant effect of vitamin D on phospholipid long chain polyunsaturated fatty acids, analysis of the fatty acids derived from cholesterol esters shows that vitamin treatment effects the weight percentage of short chain species (Table 4). An increase is seen after vitamin treatment in the weight percentage of 14:0 and 16:1 fatty acids as well as in the weight percentage of one fatty acid class presumed to be an hydroxy fatty acid, while a decrease is observed in the weight percentage of 12:0, 18:0 and 18:1ω9 fatty acids. Although the precise biochemical mechanism responsible for the vitamin induced alteration in membrane fatty acid composition remains to be elucidated, it should be noted that similar but not identical increases in the weight percentage of phospholipid long chain polyunsaturated fatty acids is seen in the toad bladder after treatment with aldosterone (8).

To date the primary effort in elucidating the mechanism of action of vitamin D in the intestine has been centered on the role of protein synthesis. The present results, however, suggest that lipid metabolism must also now be considered. The vitamin-induced increase in the activity of microvillar Ca^{++}ATPase-alkaline phosphatase could result from an alteration in the lipid environment of this membrane-bound enzyme without any increase in the actual number of enzyme catalytic units. Alternatively, new lipid might be incorporated into the microvillar membrane concomitant with the incorporation of newly synthesized enzyme. Further studies are required to decide between these two possibilities.

TABLE 4

Fatty Acid Composition of Microvillar Cholesterol Esters[a]

Fatty Acid	Weight % Fatty Acid in Vitamin D-Treated	Vitamin D-Deficient
12:0	0.92 ± 0.26	1.61 ± 0.01
A[b]	1.55 ± 0.06	0.86 ± 0.67
14:0	5.25 ± 1.01	1.81 ± 0.35
14:1	1.57 ± 0.09	1.59 ± 0.21
B[b]	2.12 ± 0.29	1.47 ± 0.08
16:0	21.64 ± 1.98	19.64 ± 1.39
16:1	6.99 ± 0.51	4.16 ± 0.34
16:2	1.59 ± 0.10	1.48 ± 0.25
18:0	8.92 ± 2.47	17.28 ± 1.17
18:1ω9	16.49 ± 2.85	21.41 ± 2.65
18:2ω6	5.01 1.04	5.98 ± 0.34
20:0	0.88 ± 0.57	1.53 ± 0.64
18:3ω3	2.19 ± 1.13	3.52 ± 1.89
18:4ω3	0.55 ± 0.09	0.82 ± 0.36
20:2ω6	3.47 ± 3.25	1.13 ± 0.13
20:3ω9	3.55 ± 3.25	1.03 ± 0.38
20:4ω6	14.13 ± 4.48	12.33 ± 2.91
20:4ω3	1.13 ± 0.30	1.35 ± 0.09
20:5ω3	1.13 ± 0.16	2.51 ± 0.74
24:1	0.89 ± 0.30	1.94 ± 0.97

a Values are the mean +/- standard deviation of three separate determinations.
b These fatty acids are presumed to be hydroxy fatty acids with 12 and 14 carbons respectively.

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