Effect of Dietary Vitamin D₃ and Cadmium on the Lipid Composition of Rat Intestinal Brush Border Membranes

Fukiko TSURUKI,¹ Sachiko MORIUCHI,² and Norimasa HOSOYA¹

¹*Department of Nutrition, School of Health Science, Faculty of Medicine,
University of Tokyo, Bunkyo-ku, Tokyo 113, Japan*

²*Department of Food and Nutrition, School of Home-Economics,
Japan Women's University, Bunkyo-ku, Tokyo 112, Japan*

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Summary Vitamin D deficiency in cadmium-exposed rats was observed along with enhanced tissue cadmium accumulation. In relation to the barrier function, the changes in the lipid composition have been studied in the intestinal brush border membranes prepared from rats raised on diets differing in vitamin D status in the absence or presence of cadmium. In an analysis of lipid composition, vitamin D₃ treatment resulted in an increase of phospholipid content, and cadmium ingestion resulted in a decrease of cholesterol and glycolipid contents in the duodenal brush border membranes. On the other hand, vitamin D₃ and cadmium showed no significant effect on the lipid composition of the jejunal brush border membranes.

Further analysis of the fatty acid composition in duodenal brush border membrane lipids showed that vitamin D₃ treatment led to an increase of the proportion of fatty acids (18:1 and 18:2 in the total and phospholipid fraction) and also shorter chain fatty acids in neutral lipid fractions in the absence of cadmium. However, vitamin D₃ treatment in the presence of cadmium led to a decrease of the proportion of fatty acid (18:2 in the total and phospholipid fraction) and also shorter chain fatty acids in neutral lipid fractions.

Vitamin D-dependent alterations of the membranes might act as a barrier in cadmium-exposed rats.

Key Words vitamin D₃, cadmium, lipid composition, fatty acid composition, rat intestine, brush border membranes, sucrase, alkaline phosphatase

It has been indicated that the toxicity of cadmium is elevated by dietary deficiencies, such as calcium, phosphorus and protein (1-4). Recently, we have shown that the tissue accumulation of cadmium was markedly increased in

¹ 鶴木富紀子, ² 森内幸子, ¹ 細谷憲政

vitamin D deficiency, irrespective of calcium or phosphorus dietary contents (5,6). Moreover, using the everted gut sac technique, cadmium absorption in rat duodenum was shown to be enhanced by vitamin D deficiency (7). Presumably, vitamin D acts as a barrier or as a reducing factor for intestinal cadmium absorption.

The precise cellular sites of vitamin D actions in the intestine have not been established, but present evidence indicates that the microvillar membrane of the brush border of the intestinal epithelium is one of these sites (8). Vitamin D may affect the permeability characteristics of the brush border membranes. Max *et al.* reported vitamin D-induced alterations of lipid and protein composition in chick intestinal brush border membranes (9).

On the other hand, vitamin D-stimulated intestinal calcium transport was inhibited by the presence of cadmium (10). Further, much remains to be studied about the underlying effects of cadmium on intestinal epithelium and those of vitamin D on intestinal cadmium absorption.

The present study was conducted to investigate the effects of dietary vitamin D₃ and cadmium on the lipid composition of rat intestinal brush border membranes in relation to the barrier function.

MATERIALS AND METHODS

Animals. Female weanling albino Wistar strain rats weighing 40–50 g were raised on either a vitamin D-deficient diet (11) or a vitamin D-deficient diet supplemented with 200 ppm cadmium in a dark room. CdO was used as the Cd source. After 5 weeks, each group was divided into either a vitamin D-deficient or a vitamin D₃-treated group for an additional 2 weeks. Doses of either 100 IU of vitamin D₃ dissolved in 0.1 ml of propylene glycol and ethanol solution (9:1, v/v) or vehicle alone were given orally 5 times during the 2 weeks. Twenty-four hours after the last dose, the animals were sacrificed by decapitation. The small intestine (from the pyloric end to the ileocecal junction) was removed. The proximal 10 cm and proximal half of the remaining intestine were defined as the duodenum and the jejunum, respectively.

Preparation of intestinal brush border membranes. Brush border membranes of rats raised on various diets were prepared according to the method of Kessler *et al.* (12). Mucosal scrapings from rat intestine were suspended in 30 vol of ice cold 50 mM mannitol in 2 mM Tris-HCl buffer (pH 7.1) and homogenized in a Waring blender at maximum speed for 2 min. Solid CaCl₂ was added to the homogenate to give a final concentration of 10 mM. After standing for 20 min in ice, the suspension was centrifuged at $3,000 \times g$ for 15 min, and the resulting supernatant was centrifuged at $27,000 \times g$ for 30 min. The pellet was then resuspended in distilled water. After removing aliquots of each preparation for enzyme assays, the brush border membranes were used for lipid extraction.

Extraction of lipids. The lipids were extracted according to a slight modification of the method of Folch *et al.* (13). The membrane suspensions (10 mg hy-

droquinone/g tissue added as antioxidant) were mixed with 17 vol of chloroform-methanol (2:1, v/v) and heated in a water bath at 64°C for 10 min and then kept overnight in a cold room. The residue was collected by filtration and washed with 2 vol of chloroform-methanol (2:1, v/v). The residue on the filter was reextracted with 5 vol of chloroform-methanol (2:1, v/v). The combined extract was partitioned three times with 0.2 vol of 0.2% HCl. The resulting lower layer was evaporated to dryness *in vacuo* and dissolved in a small amount of chloroform. The weight of the lipids was determined by weighing dried aliquots of the extract.

Separation of neutral lipids, phospholipids and glycolipids. Lipids dissolved in chloroform were applied to a column of silicic acid-Hyflo Super-Cel (2:1) equilibrated with chloroform. Approximately 1 g of silicic acid (200 mesh, activated at 100°C for 24 h before use) was used per mg of lipids. Neutral lipids were eluted with 8 column vol of chloroform-methanol (49:1, v/v), next glycolipids with 40 column vol of acetone and finally phospholipids with 10 column vol of chloroform-methanol (1:4, v/v) (14).

Analysis of fractions. Cholesterol in the neutral lipid fraction was determined by the Zak *et al.* method (15). The amount of glycolipids in the acetone fraction was estimated from the hexose contents which were determined with an anthrone reagent (16). Glucose was used as a standard. Calculations were made assuming that the mixed glycolipids had a molecular weight of 846 (17). Phospholipid contents in the chloroform-methanol (1:4, v/v) fraction were determined after hydrolysis using 10 N H₂SO₄ and 30% H₂O₂ by the method of Chen *et al.* (18), and these calculations were made assuming that the average phospholipids had a molecular weight of 780 (19).

Preparation of fatty acid methyl esters. To the dry residue of total lipids, phospholipid and neutral lipid fractions in Folch extracts, 2 ml of methanolic 2% H₂SO₄ and 1 ml of benzene were added and sealed under a N₂ atmosphere. Methanolysis formed overnight at 60°C. The tube contents were cooled and diluted with 1 ml of distilled water. The fatty acid methyl esters were extracted three times with 3 ml of petroleum ether (bp 38–40°C), and the extracts were collected in a tube and evaporated to dryness under N₂ gas. This residue was dissolved in 0.5 ml of petroleum ether and stored at –20°C under N₂ for further gas-liquid chromatographic analysis (20).

Gas-liquid chromatography of fatty acids. Fatty acids were analyzed using a Shimadzu Gas Chromatograph Model GC-6A, with 10% EGSS-X on gas chrome P (mesh 100–120). The flow rate of the carrier gas (N₂) was 40 ml/min, and the temperature of the column, injector and detector were 180°C, 200°C, and 200°C, respectively. Fatty acids were identified with methyl ester standards and a semi-logarithmic plot of carbon number *versus* retention time (21).

Assays. Sucrase activity was assayed by the Dahlqvist method (22). Enzymatic activity was expressed as μ mol sucrose hydrolyzed/mg protein/h. Alkaline phosphatase activity was assayed using *p*-nitrophenylphosphate as a substrate at pH 10.0 (23). Enzymatic activity was expressed as μ mol of *p*-

nitrophenol produced/mg protein/min. Protein concentration was determined by the Lowry *et al.* method using bovine serum albumin as the standard (24).

Chemicals. CdO was purchased from Kishida Chem. Ltd.; Tris was obtained from Sigma Chem. Co.; hydroquinone from Nakarai Chem. Ltd.; silicic acid from Malinckrodt Chemicals; Hyflo Super-Cel from Manville Corporation. Glucose oxidase was from Worthington Biochem. Co.; *p*-nitrophenylphosphate and other chemicals from Wako Pure Chem. Ind., Ltd. Petroleum ether (boiling range 38–40°C) was redistilled before use. All reagents were of analytical grade. Vitamin D₃ was kindly supplied by Dr. Katsui (Eisai Co.).

RESULTS

1. The effects of dietary vitamin D₃ and cadmium on rat intestinal brush border membranes

Initially the adequacy of the method to isolate the dietary modified brush border membranes had been investigated with special reference to sucrase and alkaline phosphatase as marker enzymes. The method developed by Kessler *et al.* was adapted to isolate intestinal brush border membranes from rats raised on a vitamin D-deficient or vitamin D₃-treated diet in the presence or absence of cadmium.

The recovery and enrichment factors of sucrase activity were not influenced by dietary factors. The preparation represents a 20–25-fold purification of intestinal sucrase. Sucrase activity in the duodenum was not influenced by vitamin D₃ but was inhibited by cadmium. Sucrase activity in the jejunum was approximately 2-fold higher than that in the duodenum. Alkaline phosphatase activity in the duodenal brush border membranes was significantly increased by vitamin D₃ treatment, but significantly decreased in the presence of cadmium. Recovery and enrichment factors of this enzyme were not changed either in the duodenum or the jejunum. The preparation represents a 15-fold purification of intestinal alkaline phosphatase. Alkaline phosphatase activity in the duodenum was 20–30-fold higher than that in the jejunum in the absence of cadmium (Tables 1 and 2).

These results reveal that the preparations of brush border membranes from rats raised on diets differing in vitamin D status and cadmium showed good response to these dietary factors, and they share many common properties, such as the recovery and enrichment factors of brush border marker enzymes. Therefore, they should serve as a valuable source of materials for further analysis of intestinal lipid composition.

2. The effects of dietary vitamin D₃ and cadmium on the lipid composition of rat intestinal brush border membranes

The intestinal brush border membrane preparations from rats raised on diets differing in vitamin D status and cadmium were used as the source for lipid composition analysis.

Table 1. Recoveries of enzymes during preparation of rat duodenal brush border membranes.

Group	Diet (D, Cd)	Protein	Homogenate		Brush border membranes		Sucrase*		Alkaline phosphatase**	
		Recovery (%)	Sucrase (Activity)	Al-Pase (Activity)	Sucrase (Activity)	Recovery (%)	Enrichment (×)	Recovery (%)	Enrichment (×)	
1	(-, -)	2.8 ± 0.6	1.5 ± 0.3	6.4 ± 0.9	27.6 ± 3.6	88.1 ± 7.4	45.1 ± 7.7	18.4 ± 2.7	26.8 ± 4.0	13.8 ± 2.7
2	(+, -)	2.3 ± 0.6	1.4 ± 0.2	15.8 ± 1.2 ^a	26.0 ± 5.7	268.8 ± 42.0 ^a	42.5 ± 3.3	18.5 ± 4.5	27.0 ± 3.4	17.0 ± 1.8
3	(-, +)	2.4 ± 0.4	1.5 ± 0.2	1.6 ± 0.1	27.3 ± 4.5	28.1 ± 4.4 ^c	50.2 ± 4.0	18.1 ± 2.4	27.6 ± 2.5	17.6 ± 2.1
4	(+, +)	2.2 ± 0.3	1.4 ± 0.3	3.8 ± 0.2 ^{ac}	26.4 ± 3.1	63.0 ± 7.4 ^{ab}	50.7 ± 2.5	18.9 ± 3.0	27.3 ± 3.3	16.6 ± 1.8

Each value represents the mean $\pm$ SE of 6 rats. * Enzymatic activities were expressed as either μ mol substrate hydrolyzed/mg protein/h for sucrase, or ** μ mol *p*-nitrophenol produced/mg protein/min for Al-Pase (alkaline phosphatase). The enrichment is the ratio of the specific activities in the final brush border membranes. ^a Significantly different from respective vitamin D-deficient group at $p < 0.001$. ^b Significantly different from respective non-Cd-exposed group at $p < 0.01$, or ^c at $p < 0.001$. D -, vitamin D deficient; D +, 100 IU vitamin D was given orally 5 times in 2 weeks. Cd -, Cd not added; Cd +, 200 ppm Cd was added to the diet fed for 7 weeks.

Table 2. Recoveries of enzymes during preparation of rat jejunal brush border membranes.

Group	Protein	Homogenate		Brush border membranes		Sucrase*		Alkaline phosphatase**	
		Sucrase	Al-Pase	Sucrase	Al-Pase	Recovery (%)	Enrichment (×)	Recovery (%)	Enrichment (×)
Diet (D, Cd)	Recovery (%)	(Activity)	(Activity)	(Activity)	(Activity)	(%)	(×)	(%)	(×)
1 (—, —)	2.6±0.2	2.8±0.3	0.4±0.1	56.1±6.4	5.1±0.5	51.9±4.0	19.7±1.7	29.4±1.2	12.1±1.2
2 (+, —)	2.4±0.3	2.6±0.4	0.5±0.1	58.0±5.8	8.0±1.2	52.5±4.0	22.5±1.3	32.2±1.8	14.8±0.9
3 (—, +)	3.2±0.4	2.9±0.8	0.5±0.2	54.5±4.2	5.6±0.3	51.4±4.3	18.8±0.9	35.2±4.3	13.8±2.3
4 (+, +)	3.0±0.3	2.5±0.2	0.5±0.1	51.4±4.7	6.1±0.6	56.5±3.1	20.1±1.9	34.0±4.1	13.0±2.2

Each value represents the mean ± SE of 6 rats. * Enzymatic activities were expressed as either μmol substrate hydrolyzed/mg protein/h for sucrase, or ** μmol *p*-nitrophenol produced/mg protein/min for alkaline phosphatase (Al-Pase). The enrichment is the ratio of the specific activities in the final brush border membranes. D —, vitamin D-deficient; D +, 100 IU vitamin D was given orally 5 times in 2 weeks. Cd —, Cd not added; Cd +, 200 ppm Cd was added to the diet fed for 7 weeks.

Table 3. Lipid composition of rat duodenal brush border membranes.

Group Diet (D, Cd)	mg Lipid mg Protein	mg Cholesterol 100 mg Protein	mg Phospholipid 100 mg Protein	mg Glycolipid 100 mg Protein
1 (–, –)	0.69 ± 0.07	16.3 ± 1.2	20.7 ± 1.2	21.8 ± 1.9
2 (+, –)	0.74 ± 0.06	17.6 ± 1.9	25.2 ± 1.6 ^a	22.0 ± 2.0
3 (–, +)	0.51 ± 0.05 ^b	11.7 ± 0.8 ^c	19.9 ± 0.6	14.1 ± 1.7 ^c
4 (+, +)	0.57 ± 0.05 ^b	12.9 ± 1.0 ^b	22.3 ± 1.0 ^a	14.5 ± 1.3 ^c

Each value represents the mean ± SE of 6 rats. ^aSignificantly different from respective vitamin D-deficient group at $p < 0.05$. ^bSignificantly different from respective non-Cd-exposed group at $p < 0.05$, or ^c at $p < 0.01$. D–, vitamin D-deficient; D+, 100 IU vitamin D₃ was given orally 5 times in 2 weeks. Cd–, Cd not added; Cd+, 200 ppm Cd was added to the diet fed for 7 weeks.

Table 4. Lipid composition of rat jejunal brush border membranes.

Group Diet (D, Cd)	mg Lipid mg Protein	mg Cholesterol 100 mg Protein	mg Phospholipid 100 mg Protein	mg Glycolipid 100 mg Protein
1 (–, –)	0.32 ± 0.04	5.2 ± 0.7	9.2 ± 0.8	14.4 ± 1.2
2 (+, –)	0.31 ± 0.03	5.3 ± 0.6	9.3 ± 0.9	13.3 ± 1.6
3 (–, +)	0.31 ± 0.04	5.2 ± 0.7	9.2 ± 0.3	13.0 ± 1.3
4 (+, +)	0.32 ± 0.03	5.0 ± 0.3	9.4 ± 0.3	13.3 ± 1.0

Each value represents the mean ± SE of 6 rats. D–, vitamin D-deficient; D+, 100 IU vitamin D₃ was given orally 5 times in 2 weeks. Cd–, Cd not added; Cd+, 200 ppm Cd was added to the diet fed for 7 weeks.

In duodenal brush border membranes from vitamin D₃-treated rats, the total lipid content was increased (Table 3). More detailed analysis clearly revealed the effects of dietary vitamin D₃ and cadmium on the contents of cholesterol, phospholipids and glycolipids. In duodenal lipid contents from vitamin D₃-treated rats, the phospholipid contents increased significantly in the absence of cadmium. The decrease of phospholipid contents in the presence of cadmium was partly recovered upon vitamin D₃ treatment. Cholesterol and glycolipid contents were decreased significantly in duodenal lipids from cadmium-exposed rats. There was no significant alteration in these lipids due to vitamin D₃ treatment. On the other hand, no dietary factor appeared to alter jejunal lipid composition (Table 4).

3. The effects of dietary vitamin D₃ and cadmium on the fatty acid composition of lipids from rat duodenal brush border membranes

The effects of dietary vitamin D₃ and cadmium on the fatty acid composition of

Table 5. Fatty acid composition of total lipids from rat duodenal brush border membranes.

Group Diet (D, Cd)	1 (-, -)	2 (+, -)	3 (-, +)	4 (+, +)
Fatty acid (%)				
14:0	1.7±0.5	1.0±0.3	1.5±0.5	0.6±0.2
14:1	0.9±0.2	0.8±0.2	0.8±0.2	0.7±0.2
14:2	1.0±0.3	0.5±0.1	0.6±1.0	0.6±0.1
16:0	19.7±1.1	19.5±1.0	19.8±1.5	19.3±0.8
16:1	2.1±0.2	2.2±0.1	1.5±0.2 ^c	1.6±0.1 ^d
16:2	0.9±0.1	0.7±0.1	0.7±0.1	0.8±0.3
18:0	20.6±0.6	19.1±1.1	20.9±1.1	21.6±1.1
18:1 ω 9	13.0±0.8	17.2±1.6 ^a	13.4±0.3	13.4±0.9 ^c
18:2 ω 6	12.9±0.4	15.2±1.0 ^a	14.7±0.6 ^c	12.0±0.4 ^{bd}
20:0	1.7±0.1	1.5±0.2	1.8±0.2	2.2±0.5
18:3 ω 3	1.5±0.3	1.0±0.2	1.3±0.1	1.1±0.1
20:3 ω 9	0.5±0.1	0.6±0.1	0.7±0.1	0.5±0.3
20:3 ω 6	0.9±0.2	1.0±0.1	1.6±0.4	1.2±0.2
20:4 ω 6	12.4±0.9	12.5±1.0	13.6±0.7	13.6±0.5
20:4 ω 3	0.3±0.4	0.3±0.1	0.3±0.0	0.4±0.0
20:5 ω 3	6.7±1.1	3.9±0.7 ^a	1.9±0.4 ^d	2.3±0.1 ^c
22:3 ω 9	1.4±0.3	1.5±0.4	0.9±0.3	0.8±0.1
22:4 ω 6	1.1±0.1	1.3±0.3	2.1±0.1 ^c	1.8±0.1 ^a
22:5 ω 6	1.5±0.1	1.3±0.2	1.8±0.4	1.8±0.2
22:5 ω 3	1.6±0.3	1.4±0.3	2.2±0.4	2.8±0.5 ^c
22:6 ω 3	0.8±0.1	0.8±0.1	1.1±0.1	1.1±0.1

Each value represents the mean $\pm$ SE of 6 rats. ^aSignificantly different from respective vitamin D-deficient group at $p < 0.05$, or ^b at $p < 0.01$. ^cSignificantly different from respective non-Cd-exposed group at $p < 0.05$, or ^d at $p < 0.01$. D-, vitamin D-deficient; D+, 100 IU vitamin D₃ was given orally 5 times in 2 weeks. Cd-, Cd not added; Cd+, 200 ppm Cd was added to the diet.

lipids were observed in duodenal brush border membranes. The proportion of the deacylated fatty acids in the total lipids and the phospholipid and neutral lipid fractions were investigated. The fatty acid composition of total lipids showed significant alterations due to the influence of dietary factors (Table 5). Vitamin D₃ treatment led to an increase in the proportion of 18:1 and 18:2, and led to a decrease of 20:5 in the absence of cadmium. However, vitamin D₃ treatment in cadmium-exposed rats occasionally led to contrary effects compared with those in noncadmium-exposed rats. That is, vitamin D₃ treatment led to a significant decrease in the proportion of 18:2 and 22:4, and led to a slight increase of 20:5 and 22:5 ω 3 in the presence of cadmium.

Further analysis of the fatty acid composition of the phospholipid fraction

Table 6. Fatty acid composition of phospholipids from rat duodenal brush border membranes.

Group Diet (D, Cd)	1 (-, -)	2 (+, -)	3 (-, +)	4 (+, +)
Fatty acid (%)				
14:0	2.6±0.7	2.4±0.6	0.8±0.2 ^d	0.7±0.2 ^d
14:1	0.8±0.2	0.8±0.1	0.6±0.2	0.5±0.2
16:0	17.3±1.0	15.9±1.1	19.0±1.1	21.7±1.9 ^d
16:1	1.4±0.3	1.6±0.3	1.4±0.4	1.3±0.2
16:2	1.0±0.2	1.5±0.4	1.2±0.3	1.2±0.4
18:0	24.3±2.0	21.9±1.4	25.1±1.4	24.6±1.6
18:1 ω 9	7.2±0.2	9.3±0.4 ^c	11.6±0.6 ^f	11.5±0.6 ^e
18:2 ω 6	10.7±1.2	11.2±0.8	12.5±0.9	8.8±0.6 ^{be}
20:0	2.4±0.2	2.7±1.2	2.1±0.2	2.2±0.1
18:3 ω 3	2.1±0.2	3.2±0.6 ^a	1.7±0.2	1.5±0.3 ^e
20:3 ω 9	0.4±0.0	0.4±0.2	0.7±0.2	0.5±0.1
20:3 ω 6	0.6±0.1	1.2±0.2 ^a	1.1±0.2	1.5±0.3
20:4 ω 6	14.0±0.5	14.4±0.5	13.4±0.5	14.0±1.2
20:4 ω 3	0.7±0.2	0.7±0.3	0.4±0.1	0.4±0.1
20:5 ω 3	0.5±0.1	0.4±0.1	0.5±0.3	0.3±0.1
22:3 ω 9	1.2±0.3	1.1±0.3	0.6±0.1	0.7±0.1
22:4 ω 6	1.5±0.1	1.4±0.3	1.7±0.2	1.8±0.1
22:5 ω 6	2.0±0.3	2.3±0.3	1.6±0.2	1.9±0.3
X	1.3±0.1	2.4±0.3 ^b	1.4±0.3	1.4±0.3
22:5 ω 3	1.6±0.3	2.0±0.3	1.4±0.3	1.7±0.4
22:6 ω 3	1.5±0.3	1.3±0.3	1.2±0.1	1.0±0.1

Each value represents the mean±SE of 6 rats. ^aSignificantly different from respective vitamin D-deficient group at $p<0.05$, ^bat $p<0.01$ or ^cat $p<0.001$. ^dSignificantly different from respective non-Cd-exposed group at $p<0.05$, ^eat $p<0.01$ or ^fat $p<0.001$. D-, vitamin D-deficient; D+, 100 IU vitamin D₃ was given orally 5 times in 2 weeks. Cd-, Cd not added; Cd+, 200 ppm Cd was added to the diet.

showed alterations similar to those observed in total lipids (Table 6). Vitamin D₃ treatment in the absence of cadmium led to a significant increase in 18:1, 18:3 and 20:3, and led to a slight decrease in 16:0. In cadmium-exposed rats the proportion of 14:0 decreased and 18:1 increased significantly. On the other hand, vitamin D₃ treatment in cadmium-exposed rats mainly led to a decrease in 18:2.

The fatty acid composition of the neutral lipid fraction showed that dietary factors influenced the proportion of shorter chain species, in contrast with the dominant effects on the proportion of longer chain species in the phospholipids fraction (Table 7). Vitamin D₃ treatment in the absence of cadmium led to a significant increase in 12:0, 12:A, 14:B and 18:0. On the other hand, in cadmium-exposed rats the proportion of shorter chain fatty acids such as 12:0 and 12:A

Table 7. Fatty acid composition of neutral lipids from rat duodenal brush border membranes.

Group Diet (D, Cd)	1 (-, -)	2 (+, -)	3 (-, +)	4 (+, +)
Fatty acid (%)				
12:0	0.1±0.1	0.4±0.1 ^a	0.7±0.3 ^d	0.3±0.1
12:A	1.1±0.9	2.0±0.4 ^a	2.7±1.5 ^d	2.0±0.3
14:0	1.4±0.4	1.5±0.4	1.5±0.5	0.9±0.1 ^a
14:1	0.7±0.4	0.6±0.1	0.8±0.2	0.2±0.1 ^a
14:B	0.3±0.0	0.6±0.1 ^a	0.4±0.0	0.5±0.1
16:0	23.7±0.8	22.5±1.5	21.2±2.0	22.9±1.7
16:1	2.5±0.2	2.5±0.4	2.9±0.9	2.4±0.3
16:2	0.5±0.1	0.9±0.2	0.8±0.1	0.8±0.2
18:0	15.1±1.0	20.3±1.5 ^a	16.6±1.5	20.9±1.0 ^a
18:1 ω 9	15.8±1.0	14.4±0.6	13.0±2.0	13.1±0.5
18:2 ω 6	12.3±2.0	10.6±1.3	9.7±0.2 ^d	11.7±1.3
20:0	2.0±0.2	1.5±0.2	1.3±0.3	1.2±0.3
18:3 ω 3	2.3±0.9	2.4±0.7	1.9±0.3	1.9±0.3
20:3 ω 9	0.4±0.1	0.7±0.3	1.0±0.1 ^f	0.5±0.1 ^b
20:3 ω 6	0.9±0.2	0.8±0.2	1.1±0.0	0.7±0.2
20:4 ω 6	11.6±0.4	11.0±2.5	11.5±0.3	13.9±1.0
20:4 ω 3	0.6±0.2	0.7±0.3	0.9±0.4	0.3±0.0
20:5 ω 3	5.6±2.5	5.4±0.8	3.9±1.5	4.6±0.9
22:3 ω 9	1.0±0.3	2.5±0.7	3.5±0.6 ^e	1.1±0.1
22:5 ω 6	1.5±0.3	1.3±0.2	2.6±1.7	1.6±0.1

Each value represents the mean±SE of 6 rats. ^aSignificantly different from respective vitamin D-deficient group at $p<0.05$, ^bat $p<0.01$ or ^cat $p<0.001$. ^dSignificantly different from respective non-Cd-exposed group at $p<0.05$, ^eat $p<0.01$ or ^fat $p<0.001$. D-, vitamin D-deficient; D+, 100 IU vitamin D₃ was given orally 5 times in 2 weeks. Cd-, Cd not added; Cd+, 200 ppm Cd was added to the diet.

increased, and 18:2 decreased significantly. However, vitamin D₃ treatment in cadmium-exposed rats led to a significant decrease in 14:0 and 14:1, and led to a significant increase in 18:0.

DISCUSSION

Intestinal membranes, like other membranes, have two physiological roles: they create a barrier to free entry and exit of molecules and provide a matrix in which biochemical reactions take place. It is understood that the rates of both transport and enzymatic activities are affected by membrane lipid fluidity (25). It has been well established that the permeability of biological membranes is modified by temperature shift or exogenous supplement of lipids or hormones (26-29).

The present study was undertaken to elucidate the role of dietary vitamin D₃ and cadmium on the lipid composition in rat intestinal brush border membranes. It was shown that vitamin D₃ increased the activity of membrane alkaline phosphatase which was inhibited by dietary cadmium, specially in the duodenum. This observation is in agreement with the results observed in chick intestinal brush border membranes (8). Although we have not determined marker enzymes in other organelles, the analysis of DNA contents (0.14% of initial homogenate) and brush border marker enzymes suggested that other potential membrane contamination has been reduced to a relatively low level.

The lipid composition in intestinal brush border membranes clearly showed the effects of dietary vitamin D₃ and cadmium. Forstner *et al.* reported that rat intestinal microvillus membranes contain 103 μ g of phospholipids/mg protein and 53 μ g cholesterol/mg protein (30). These data are in agreement with our present data on jejunal membrane lipids (Table 4). Phospholipid or cholesterol content in duodenal membranes was approximately 2–3-fold higher than that in the jejunum, respectively.

In agreement with the findings of Max *et al.* (9), total duodenal lipid contents were increased by vitamin D₃ treatment due to an increase in phospholipid contents. On the other hand, cholesterol and glycolipid contents decreased significantly in the presence of cadmium.

It is understood that cholesterol in biological membranes plays an essential structural role as a stabilizer. As the incorporation of cholesterol in membranes results in decreased permeability (31), the removal of cholesterol from membranes may result in an increase in permeability, as was observed in our cadmium-exposed rats.

Our results have shown that vitamin D₃ acts in different ways in order to regulate the brush border membranes by modulating the fatty acid composition. It is currently thought that unsaturated fatty acids act more like a fluidizing factor than saturated fatty acids (32), and shorter chain saturated fatty acids act more like a fluidizing factor than longer ones in biological membranes (33). The two major vitamin D-dependent changes in the absence of cadmium were: an increase in the proportion of unsaturated fatty acids in the phospholipid fraction and thereby a compensating decrease in saturated fatty acids; and in the neutral lipid fraction there was an increase in the proportion of shorter chain species compensating for a decrease in longer ones.

In contrast, in the presence of cadmium, two other major vitamin D-dependent changes occurred: in the phospholipid fraction there was an increase in the proportion of saturated fatty acids compensating for a decrease in unsaturated fatty acids; and in the neutral lipid fraction longer chain species increased such as 18:0, 18:2 and 20:4, compensating for a decrease in shorter chain species.

Although we have no direct data for the explanation of the fluidizing effects of lipids, membranes deteriorated by both vitamin D deficiency and cadmium might not act as a barrier in consequence of the decrease in lipid contents and the increase

in polyunsaturated fatty acids in the phospholipid fraction, as well as due to the increase in shorter chain fatty acids in the neutral lipid fraction. Dietary vitamin D₃ and cadmium could affect the characteristics of fluidity, and consequently the permeability of brush border membranes. Therefore vitamin D₃ has dual effects on membrane lipids due to differences in dietary environment or circumstances. One is more fluidizing and the other is more rigidifying on membranes which are already fragile or damaged.

Increased cadmium absorption in vitamin D deficiency might be related to the decreased lipid contents and also to the altered fatty acid composition in brush border membranes. Vitamin D-dependent alterations of brush border membranes might act as a lipid barrier in cadmium-exposed rats.

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