Effects of dietary fat on lipid composition and iron uptake of intestinal brush-border membrane of rats

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Bioavailability of dietary iron is affected by many factors in the diet. But the mechanism of iron translocation from intestinal lumen to the mucosa cell and its regulation are obscure. Previous research has shown a possible role of fatty acids in the brush-border membrane as an iron carrier. To investigate the influence of dietary fats on the composition of fatty acids of the intestinal brush-border membrane and the relationship of fatty acids to iron uptake, three different dietary fats, safflower oil, olive oil and liquid lard, were fed to rats for 4 weeks. The intestinal brush-border membrane was isolated and assayed for fatty acid composition. Also iron uptake by these membrane vesicles was determined. The data show that rats fed safflower oil had a higher proportion of linoleic acid (18:2) in their intestinal brush-border membrane, but those fed olive oil possessed more oleic acid (18:1) in the brush-border membrane. Iron uptake by the brush-border membrane vesicles was highest in the safflower oil group. These results indicate a modification of fatty acid composition in the intestinal brush-border membrane by dietary fat and a possible correlation of changes in iron uptake.

Keywords: brush-border membrane; iron uptake; fatty acids; dietary fat

Introduction

Iron balance in animals is mainly regulated by intestinal iron absorption.[1] In anemic animals iron retention is enhanced to meet the needs of the body.[2] However, the mechanism controlling the adaptation of iron absorption is still not clear. A hypothesis that transferrin is the key mediator of mucosal iron uptake had been proposed.[3] But since no transferrin synthesis has been found in intestinal mucosa and no transferrin receptor is present on the brush border membrane, this postulation has become less likely.[4,5]

Since a saturable uptake of iron by everted intestine suggested a carrier-mediated transport, the possibility of a protein carrier had been investigated. However, prior boiling of rabbit brush-border membrane vesicles did not affect their iron uptake,[6] and iron uptake by mouse brush-border membrane vesicles was enhanced by heat treatment.[7] Moreover, pretreated brush-border membrane vesicles with protein-modification reagents such as iodoacetate did not inhibit iron uptake. All of this evidence was in conflict with the theory of protein-carrier–mediated uptake.

On the other hand, cholate extract from rabbit brush-border membrane can bind iron, the binding component being identified as lipids.[8] Furthermore, fatty acids were considered to be the most possible candidate in mediating iron transport through brush-border membrane, due to the enhanced iron uptake by loading oleic acid to the brush-border membrane vesicles[7] and the strong in vitro binding capacity of fatty acids to iron.[9]

So far, few studies on iron uptake have been done on rat brush-border membrane, and the enhancing effect of fatty acids on iron uptake has only been tested on in vitro–altered membrane vesicles. Thus, the present study aimed to approach this postulation of lipid-mediated iron uptake via modifying brush-border membrane composition in vivo and determining iron uptake of these membranes. The effects of three dietary fats differing in fatty acid content on rat intestinal brush-border membrane and their iron uptake rates are reported.

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Table 1 Composition of test diets

Ingredients	g/kg
Casein	200
DL-Methionine	3
Fat[a]	100
Corn starch	602
Salt mixture[b]	35
Vitamin mixture[c]	10
Cellulose	50

[a] Either safflower oil, olive oil, or liquid lard.
[b] AIN mineral mixture 76, ICN Biochemicals, Cleveland, OH.
[c] Vitamin diet fortification mixture, ICN Biochemicals, Cleveland, OH.

Table 2 Composition of fatty acids in the dietary fats (%)

		Unsaturated fatty acids		
Fat	Saturated fatty acids	Oleic acid	Linoleic acid	Linolenic acid
Safflower oil	9.4	11.9	73.3	0.5
Olive oil	14.2	76.5	8.2	0.7
Liquid lard	33.1	48.0	16.3	1.1

Materials and methods

Animals and diets

Male Sprague-Dawley rats, weighing 200 ± 5 g, were housed individually in wire cages. They were divided into three groups of 10 animals each. Each group was fed a diet containing 10% fat of either safflower oil, olive oil, or liquid lard. The diet composition is shown in *Table 1*, and fatty acid composition of each oil is shown in *Table 2*. Food and water were provided ad libitum. Body weight and food intake were recorded every other day.

Preparation of brush-border membrane vesicle

After 4 weeks of feeding, the rats were killed in the fasting state; the upper small intestine was excised and rinsed with ice-cold 0.9% NaCl solution. It was then cut open longitudinally, and the mucosa were removed with a thin flat spatula. The brush-border membrane vesicle was isolated from the mucosal scrapings by Mg^{2+} precipitation technique[10] modified from the method of Kessler et al.[11] The mucosal scrapings were weighed, placed in 20 volumes of buffer I (50 mM mannitol/2 mM HEPES, pH 7.1), and homogenized. $MgCl_2$ was added to the homogenate, and the mixture was allowed to stand for 20 min, then centrifuged at 3000*g* for 10 min. After the pellets were discarded, the supernatant was recentrifuged at 27000*g* for 30 min. The resulting sediment was resuspended in 5 mL buffer II (0.1 M mannitol/0.1 M NaCl/0.1 mM $MgSO_4$/20 mM HEPES–NaOH, pH 7.4, filtered through a 0.45 μm Millipore filter before use). The resuspension was centrifuged at 6000*g*, and the sediment was discarded. The supernatant was again centrifuged at 27000*g* for 30 min. The final pellet containing brush-border membrane vesicles was resuspended in 0.5 mL buffer II. All operations were performed at 4° C.

Iron uptake studies

Iron uptake by the brush-border membrane vesicles was determined by the Millipore filtration method described by Simpson and Peters[10] modified from the method of Hopfer et al.[12] The incubation medium was prepared by mixing $^{55}FeCl_3$(50 μCi/μmol Fe), $FeCl_3$, and trisodium nitrilotriacetate (NTA) in buffer III (0.1 M mannitol/0.1 M NaCl/20 mM HEPES, pH 7.4). Incubation was performed at 37° C for 30 min and initiated by adding 20 μL of brush-border membrane vesicles preparation to 200 μL of incubation mixture. After incubation, the mixture was filtered through 0.22 μm Millipore filter followed by rinsing the filter with 6–10 mL ice-cold washing solution (0.15 M NaCl/0.1 mM Fe^{3+}/0.22 mM NTA, pH 4.5). The filter was air-dried and transferred to counting vials containing filter solubilizing scintillation fluid (Filtron-X, National Diagnostics, Manville, NJ, USA). After resting overnight, the sample vials were counted with a liquid scintillation counter (LS5000TA, Beckman Instruments, Fullerton, CA, USA).

Fatty acid composition

The lipids of the brush-border membrane vesicle were extracted by 20 volumes of chloroform:methanol (2:1) according to the method of Folch et al.[13] The organic phase was evaporated under N_2 and redissolved in methanol:hexane (4:1). The fatty acids were methylated with acetylchoride[14] and assayed by a gas chromatograph (438A, Packard, Rockville, MD, USA) with a 5% DEGS–PS coated column.

Electron microscopy

The final brush-border membrane pellets were fixed without resuspension with 3% phosphate buffered glutaraldehyde (pH 7.3) in a microtube. The fixed pellet was postfixed with 1% OsO_4 and stained with uranyl acetate.[15] After dehydrating with acetone, the sample was embedded in Spur resin. The thin-cut section was mounted on a copper-grid and examined with an electron microscope (JEM 1200EX2, Jeol, Tokyo, Japan) operated at 80 kv.

Enzyme assay

Disaccharidase, a marker for brush-border membrane, was assayed with glucose oxidase-peroxidase reagent as described by Dahlqvist.[16] Activities of cytochrome C oxidase, a marker for mitochondria, were determined by a procedure based on that of Wharton and Tzagoloff.[17]

Protein assay

The protein content of homogenate and brush-border membrane vesicles was determined by a protein assay (Bio-Rad, Richmond, CA, USA) according to Bradford,[18] with bovine serum albumin as standard.

Statistical analysis of data was done with analysis of variance and Scheffe's test.[19]

Results

All rats gained around 120–130 g during the 4-week feeding period, but no significant differences were shown in either food intake or body weight gain among the three groups (*Table 3*). A typical appearance of the final brush-border membrane preparation can be seen in *Figure 1*. Many membranes assumed a vesicular form. Morphologically, the brush-border membrane preparations from rats of three groups looked similar. The prepared rat brush-border membrane vesicles showed about a 12-fold increase in the specific activity of disaccharidase compared to the homoge-

Table 3 Effect of dietary fat on body weight gain and food intake of rats

Dietary fats	Body weight gain (g)	Total food intake (g)
Safflower oil	121.1 ± 3.8	418.6 ± 6.4
Olive oil	127.3 ± 5.3	440.2 ± 7.6
Liquid lard	130.4 ± 5.5	415.9 ± 10.7

Note. Values are mean ± SEM; $n = 10$.

nate. No detectable activity of cytochrome C oxidase was present in the final pellets.

The relative fatty acid compositions of brush-border membrane vesicles are shown in *Table 4*. The type of dietary fat did not affect the relative content of saturated fatty acids in the brush-border membrane of rats. However, the brush-border membrane obtained from rats fed olive oil showed the greatest percentage of oleic acid among the three groups. Also, rats fed safflower oil possessed a higher proportion of linoleic acid (18:2) than rats fed olive oil or liquid lard. The total polyunsaturated fatty acids in the brush border membrane were significantly higher in the rats fed safflower oil than the olive oil group, but did not significantly differ from the liquid lard group. Results of iron uptake studies are shown in *Table 5*. Brush-border membrane vesicles of rats fed safflower oil had significantly higher iron uptake rates compared to those of the other two groups.

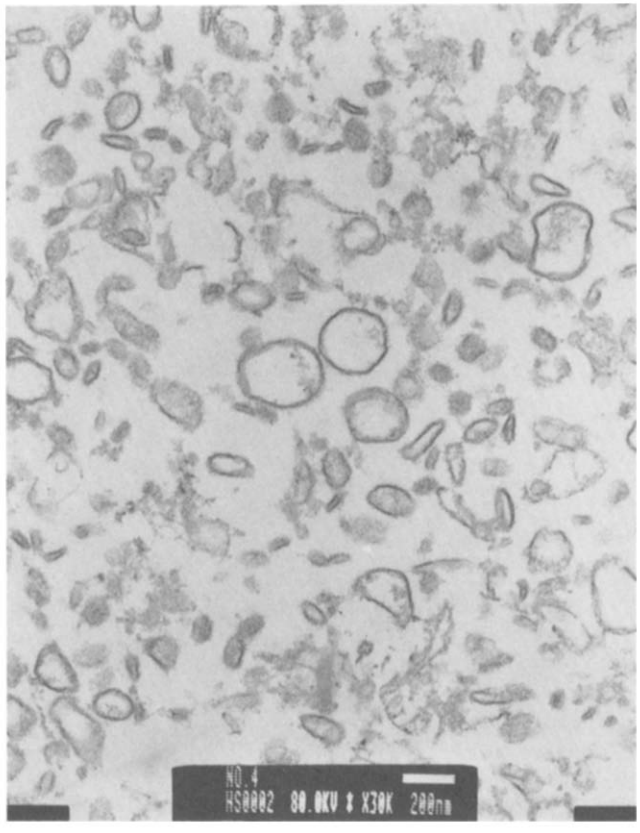

Figure 1 Electron micrograph of positively stained purified brush-border membrane of rat intestine (× 30,000).

Table 4 Effect of dietary fat on fatty acid composition of rat intestinal brush border membranes

Fatty acid	Dietary fat		
	Safflower oil	Olive oil	Liquid lard
	(%)		
16:0	15.70 ± 0.74*	16.36 ± 0.98	14.69 ± 0.79
16:1	3.86 ± 0.42	4.44 ± 0.52	4.17 ± 0.50
18:0	22.64 ± 1.10	22.49 ± 0.50	23.60 ± 0.71
18:1	12.69 ± 1.68[a]	21.89 ± 0.70[b]	16.77 ± 0.37[c]
18:2	20.15 ± 0.64[a]	14.87 ± 0.55[b]	17.52 ± 0.67[c]
18:3	0.45 ± 0.15	0.44 ± 0.15	1.23 ± 0.51
20:1	1.88 ± 0.26[a]	2.70 ± 0.40[ab]	3.36 ± 0.47[b]
20:4	19.49 ± 1.64	16.03 ± 1.16	17.33 ± 0.65
Polyunsat'd† fatty acids	40.09 ± 2.17[a]	31.34 ± 1.46[b]	36.08 ± 1.04[ab]

* Values are mean ± SEM; $n = 10$. Values in a row with different letter superscripts are significantly different at $P < 0.05$.
† Polyunsaturated fatty acids = 18:2 + 18:3 + 20:4.

Table 5 Effect of dietary fat on iron uptake of intestinal brush border membrane vesicles

Dietary fat	Iron uptake
	(nmol Fe^{3+}/(mg protein · min))
Safflower oil	7.77 ± 1.50*[a]
Olive oil	2.69 ± 0.54[b]
Liquid lard	3.27 ± 0.81[b]

* Values are mean ± SEM; $n = 10$. Values with different letter superscripts are significantly different at $P < 0.05$.

Discussion

In the present study, when dietary fat level and caloric content were kept constant, varying the relative content of fatty acids in the diet did not affect the food intake or body weight gain of rats. However, the dietary treatment did alter the fatty acid composition of the intestinal brush-border membrane. Olive oil, containing the highest oleic acid, resulted in a significantly greater percentage of oleic acid in the brush-border membrane of these rats. On the other hand, the content of linoleic acid in the brush-border membrane was highest in the safflower oil–fed rats. But the relative content of saturated fatty acids (16:0 and 18:0) was not affected by the dietary treatment, which might be attributed to the unaltered de novo synthesis of saturated fatty acids. Similar modifications on membrane composition by dietary fats have been reported for erythrocytes,[20] heart muscle,[21] and adipocytes.[22] The alteration in composition was considered to be associated with changes in membrane fluidity and functions.[23,24]

The fluidity of pig intestinal brush-border membrane decreased in dietary essential fatty acid defi-

ciencies.[25] On the other hand, the corn oil diet was found to enhance the fluidity of rat intestinal membranes.[26] The altered transport of glucose and fatty acids in the rat intestinal brush-border membrane has been demonstrated via variation of dietary fat saturation.[27] Also reported was the fact that these alterations in transport were not associated with changes in intestinal morphology or membrane content of protein, cholesterol, or phospholipids. In contrast, it might be more closely related to fatty acid composition of membrane lipids.[28]

This study showed that rats fed safflower oil possessed the highest linoleic acid content in the intestinal brush-border membrane and also showed the greatest iron uptake rate. This result is consistent with the findings done with artificial lipid bilayers.[29] Among the several fatty acids tested, linoleic acid was most effective in enhancing iron transport when incorporated into the liposomes. The association between fatty acid composition of brush-border membrane and ion transport has also been demonstrated in calcium uptake.[30] The uptake of calcium was stimulated in rabbit brush-border membrane exposed to exogenous unsaturated fatty acids, whereas saturated fatty acids had no marked effect. Whether this enhancing effect of unsaturated fatty acids to iron and calcium uptake is common to all of the mineral ions remains to be clarified.

Questions may arise as to whether the iron uptake of the brush-border membrane reflected the physiological behavior of intestinal mucosa in vivo. Since luminal iron uptake by the brush border cannot be examined independently in intact animals, this question may never be answered satisfactorily. However, studies with isolated mucosal cells[31] and tied off segments of intestine in live animals[32] indicate that iron transport of purified brush-border membrane is quantitatively similar to those observed with intact cells or intestinal segments in vivo. Thus, isolated brush-border membrane can be an adequate tool for examining the luminal iron uptake.

This study confirmed the important role of fatty acids in iron uptake of the brush-border membrane by introducing a diet-induced alteration of brush-border membrane composition. The enhanced effect of polyunsaturated fatty acid might be attributed to the forming of Fe^{3+} (fatty acid)$_x$ complex in facilitating iron transport as suggested by Simpson et al.[7] The physiological significance of this increased iron uptake during ingestion of high polyunsaturated fatty acid requires further investigation.

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