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Ingestion of Epilactose, a Non-digestible Disaccharide, Improves Postgastrectomy Osteopenia and Anemia in Rats through the Promotion of Intestinal Calcium and Iron Absorption

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Gastrectomy often results in osteopenia and anemia because of calcium (Ca) and iron (Fe) malabsorption. Here, we investigated the effects of feeding epilactose, a non-digestible disaccharide, on gastrectomy-induced osteopenia, anemia, and Ca and Fe malabsorption in male Sprague−Dawley rats. Totally gastrectomized or sham-operated rats were fed the control or epilactose (50 g/kg) diets for 30 days. Gastrectomy severely decreased intestinal Ca and Fe absorption, femoral bone strength, Ca content, hemoglobin concentration, and hematocrit. These decreases were partly or totally restored by feeding epilactose. Feeding epilactose increased the cecal tissue weight and the soluble Ca concentration and short-chain fatty acid pools of the cecal contents. Collectively, the increases in cecal mucosal area and/or soluble Ca concentration of the cecal contents, resulting from short-chain fatty acid production by intestinal microbes, are thought to be responsible for the epilactose-mediated promotion of Ca and Fe absorption in the gastrectomized rats.

KEYWORDS: Epilactose; gastrectomy; osteopenia; anemia

INTRODUCTION

Osteopenia and anemia are common manifestations in patients after gastrectomy (*1*, *2*). One of the most important factors in the onset of gastrectomy-induced osteopenia and anemia is the intestinal malabsorption of calcium (Ca) and iron (Fe) because of the lack of gastric acid. Gastric acid plays a crucial role in small intestinal Ca and Fe absorption through the solubilization of insoluble dietary Ca (*3*) and Fe (*4*). It has been reported that total gastrectomy dramatically decreases intestinal Ca and Fe absorption in rats (*5*−*8*). Therefore, the promotion of Ca and Fe absorption is considered to be an effective mean to prevent postgastrectomy osteopenia and anemia.

Non-digestible carbohydrates, such as dietary fibers, oligosaccharides, and resistant starch, exhibit various beneficial effects on our health, including the promotion of intestinal Ca and Fe absorption. We previously reported that hydrolyzed guar gum, difructose anhydride III (DFAIII), and water-soluble soybean fiber improved postgastrectomy osteopenia and anemia through the restoration of Ca and Fe absorption (*5*−*10*). Although the mechanisms underlying their effects have not been fully established, the fermentation of these saccharides in the large intestine is thought to be responsible for this restoration.

Epilactose (4-*O*-*β*-galactopyranosyl-D-mannose) is a rare non-digestible disaccharide. A considerable amount of epilactose is known to be produced from cow's milk by heating and alkali treatments (*11*); however, the biological activity remains to be investigated. Recently, we developed a method for the preparation of epilactose from the ruminal strain *Ruminococcus albus* NE1 through the use of cellobiose 2-epimerase (EC 5.1.3.11) (*12*). We have reported that epilactose promotes intestinal Ca absorption in rats and that the increase is greater than that induced by fructooligosaccharide (FOS), which is also known to enhance Ca absorption (*13*, *14*). One mechanism responsible for this increase is the solubilization of Ca salts by acids produced through the microbial fermentation of the saccharides in the large intestine, as described above (*13*). Another mechanism is the promotion of the paracellular Ca transport through the direct stimulation of the intestinal epithelium in the small intestine by the intact saccharide (*14*). Whereas the effect of epilactose on the intestinal Fe absorption has not been investigated to date; however, the promotion of Fe absorption by epilactose is expected on the basis of the physiological and physical characteristics of epilactose.

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Table 1. Composition of Test Diets

ingredient	control diet (g/kg of diet)	epilactose diet (g/kg of diet)
casein[a]	200	200
dextrin[b]	429.5	379.5
sucrose[c]	200	200
soybean oil	70	70
choline bitartrate	2.5	2.5
L-cystine	3	3
mineral mixture[d]	35	35
vitamin mixture[d]	10	10
cellulose[e]	50	50
epilactose	0	50

[a] Casein (ALACID, New Zealand Daily Board, Wellington, New Zealand). [b] Dextrin (TK-16, Matsutani Chemical Industry Co., Ltd., Itami, Japan). [c] Sucrose (Nippon Beet Sugar Manufacturing Co., Ltd., Tokyo, Japan). [d] Mineral and vitamin mixtures were prepared according to the AIN-93G formulation. [e] Crystallized cellulose (Ceolus PH102, Asahi Chemical Industry, Tokyo, Japan).

On the basis of this evidence, we expected that epilactose could improve postgastrectomy osteopenia and anemia through the promotion of intestinal Ca and Fe absorption in rats.

The purposes of the present study are to investigate the ameliorative effects of epilactose on Ca and Fe malabsorption, osteopenia, and anemia in totally gastrectomized rats. To examine the effect of epilactose on Ca malabsorption and osteopenia, intestinal Ca absorption, plasma Ca concentrations, femur strength, and Ca amounts in the femur were compared among the sham-operated and gastrectomized rats fed the control and epilactose diets. To examine the effect of epilactose on Fe malabsorption and anemia, intestinal Fe absorption, plasma Fe concentration, blood hemoglobin concentration, and blood hematocrit were measured. In addition, we evaluated the pH, soluble Ca and Fe concentrations, and organic acid pools in the cecal contents to explore the role of intestinal fermentation in the epilactose-mediated effects, because epilactose is known to be fermented by the large intestinal microbes in rats (*13*).

MATERIALS AND METHODS

Animals and Diets. Male Sprague−Dawley rats (4 weeks old, $n = 32$, Clea Japan, Tokyo, Japan) were housed in individual cages in a room with controlled temperature (22 ± 2 °C), relative humidity (40−60%), and lighting (light 0800−2000 h) throughout the study. The rats were fed the control diet (AIN-93G formula; **Table 1**) for an acclimation period of 5 days and were divided into two groups, the gastrectomy and sham groups, on the basis of body weight. After food deprivation for 8 h, 18 rats in the gastrectomy group were subjected to total gastrectomy, as described previously (*7*−*10*), and the 14 rats in the sham group were subjected to laparotomy. Both surgeries were performed under the same anesthetic procedure (Nembutal, sodium pentobarbital, 40 mg/kg of body weight, Abbott Laboratories, North Chicago, IL). All rats were deprived of food and water for 16 h post-surgery and were then fed cow's milk for 3 days. The rats in each surgical group were divided into two subgroups, the control and epilactose diet groups, on the basis of body weight. The epilactose diet contained 5% epilactose by weight (**Table 1**). The actual Ca and Fe measurements were 4.96 g of Ca and 33.6 mg of Fe/kg in the control diet and 4.90 g of Ca and 32.9 mg of Fe/kg in the epilactose diet. Epilactose was synthesized from lactose using a recombinant cellobiose 2-epimerase obtained from *R. albus* and purified, as described previously (*12*). On days 1 and 2 of the feeding period, the rats were given 5 and 10 g of diet/day, respectively; thereafter, they were given 15 g of diet/day for 28 days because we previously observed that the gastrectomized rats did not consume more than 15 g of diet/day (unpublished observations). Therefore, all rats, including the sham rats, were given 15 g of diet/day to standardize food intake among all groups. Vitamin B-12 (0.5 mg/kg of body weight, Wako Pure Chemical Industries, Tokyo, Japan) was supplied subcutaneously every 2 weeks to prevent pernicious anemia. All rats were allowed free access to deionized water. Body weight and food intake were measured every day. Feces were collected for 3 consecutive days from day 28 of the feeding period and were freeze-dried to evaluate net Ca and Fe absorption. Blood was collected from the tail vein on days 0, 15, and 30 to measure hematocrit and hemoglobin concentration. At the end of the experiment (on day 30), blood was collected from aorta under anesthesia (Nembutal, sodium pentobarbital, 40 mg/kg of body weight, Abbott Japan Co.) to measure plasma Ca and Fe. The rats were then killed by exsanguination, and the femurs, livers, and cecum were removed. The cecal contents were collected and weighed by cutting open the cecal wall and then washing out the wall with saline.

This study was approved by the Hokkaido University Animal Committee, and the rats were maintained in accordance with the Hokkaido University guidelines for the care and use of laboratory animals (approval number 08-0137).

Analytical Methods. Blood hemoglobin and plasma Ca and Fe concentrations were measured using commercial assay kits (hemoglobin B-test, calcium C-test, and Fe C-test, Wako Pure Chemical Industries).

The amounts of Ca and/or Fe in the diets, feces, right femurs, and cecal contents (total and soluble amounts) were measured. Diets, freeze-dried feces, and freeze-dried femurs were dry-ashed at 550 °C in an electric furnace (EYELA TMF-3200, Tokyo Rikakikai, Tokyo, Japan), as described previously (*7*, *8*). The cecal contents were diluted with 4 volumes of deionized water and then homogenized with a Teflon homogenizer. For the measurement of total Ca and Fe in the cecal contents, the homogenates were dry-ashed and centrifuged (16000*g*, 20 min, 4 °C) and the resultant supernatants were deproteinized by the addition of 0.5 mol/L perchloric acid. The Ca and Fe concentrations in these preparations were determined by atomic absorption spectrometry (Z-5310, Hitachi, Tokyo, Japan) after appropriate dilution. The Ca concentrations were measured in the presence of 1000 ppm lanthanum chloride to overcome the interference of phosphate.

The pH in the homogenates of the cecal contents was measured with a semiconducting electrode (ISFET pH sensor 001015C, Horiba, Kyoto, Japan) as the pH of cecal contents. Organic acid (acetic, propionic, butyric, and succinic acids) concentrations in the homogenates of the cecal contents were measured using a high-performance liquid chromatography (HPLC) (LC-10ADvp, Shimadzu Corp., Kyoto, Japan) system equipped with two Shim-pack SCR-102H columns (8 mm inner diameter/30 cm long, Shimadzu Corp.) and an electroconductibility detector (CDD-6A, Shimadzu Corp.) as described previously (*7*, *8*).

A three-point bending test was performed using a rheometer (RE-3305 Rheoner, Yamaden, Tokyo, Japan) to measure bone strength (*7*, *9*). The center of the left femur was fractured under the following conditions: 1 cm sample space, 30 mm/min plunger speed, and 20 kg load range. The maximum breaking force was recorded as the bone strength.

Calculations and Statistical Analyses. Net Ca and Fe absorption was calculated using the following formula: net Ca absorption = 100(total Ca or Fe intake − fecal Ca or Fe excretion)/(total Ca or Fe intake). All values are expressed as mean ± standard error of the mean (SEM). Statistical analyses were performed by two-way analysis of variance (ANOVA) followed by Duncan's multiple range test. A difference with $p < 0.05$ was considered to be significant. The result of the plasma Fe concentration was logarithmically transformed before statistical analyses, because heteroscedasticity was present within data. Statistical analyses were performed using the general linear model procedure of the SAS program (version 6.07, SAS Institute).

RESULTS

Gastrectomy lowered initial body weight and body weight gain, with the values for both parameters lower in the gastrectomized rats than in the sham rats (two-way ANOVA, $p < 0.05$ for surgery; **Table 2**). There were no significant differences in food intake among groups, although all sham rats consumed all of the diet provided, whereas some gastrectomized rats did not.

Gastrectomy markedly impaired intestinal Ca and Fe absorption in the rats (two-way ANOVA, $p < 0.05$ for surgery) (**Figure 1**). Feeding epilactose enhanced Ca and Fe absorption rates, and an interaction between diet (epilactose feeding) and surgery (gastrectomy) was also observed according to the two-way ANOVA ($p < 0.05$ for diet and interaction). Feeding epilactose partially recovered the

Table 2. Initial Body Weight, Body Weight Gain, Food Intake, Cecal Tissue Weight, Cecal Content Weight, and Cecal pH of Sham-Operated and Gastrectomized Rats Fed the Control and Epilactose Diets for 28 Days[a]

surgery	diet	initial body weight (g)	body weight gain (g/day)	food intake (g/day)
sham	control	118 ± 2 a	4.9 ± 0.1 a	14.6 ± 0.1
	epilactose	118 ± 1 a	4.9 ± 0.1 a	14.6 ± 0.2
gastrectomy	control	106 ± 2 b	4.0 ± 0.2 b	13.9 ± 0.5
	epilactose	105 ± 2 b	4.3 ± 0.1 b	14.1 ± 2
ANOVA	surgery (S)	<0.01	<0.01	0.58
	diet (D)	0.75	0.35	0.10
	S × D	0.75	0.30	0.13

[a] Mean ± SEM (n = 7 or 9), p < 0.05 according to Duncan's multiple range test.

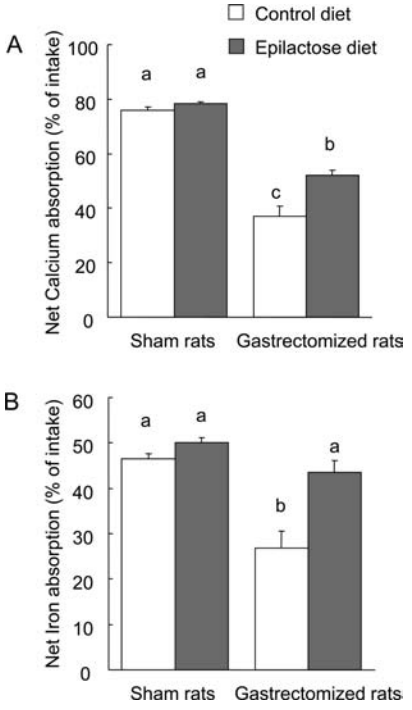

Figure 1. Net (A) Ca and (B) Fe absorption rates in a balance of study of sham-operated and gastrectomized rats (on days 28−30 of the feeding period). Values are mean ± SEM (n = 7 or 9), and means not sharing a letter differ significantly according to Duncan's multiple range test (p < 0.05). p values of two-way ANOVA were <0.01, <0.01, and 0.03 for surgery, diet, and their interaction for Ca absorption, respectively, and <0.01, <0.01, and 0.02 for surgery, diet, and their interaction for Fe absorption, respectively.

postgastrectomy Ca malabsorption, and the Ca absorption rate in the gastrectomized rats fed the epilactose diet was higher than that in the sham rats fed the control diet. Feeding epilactose almost completely restored the gastrectomy-impaired Fe absorption, with the Fe absorption rate in the gastrectomized rats fed the epilactose diet higher than that in those rats fed the control diet and comparable to those for the sham rats.

Gastrectomy and the interaction between diet and surgery had significant influences on plasma Ca and Fe concentrations according to the two-way ANOVA (**Table 3**). The plasma Ca concentration in the gastrectomized rats fed the control diet was slightly but significantly lower than that in the sham rats fed the same diet, and the decrease was reversed by epilactose feeding. Gastrectomy markedly decreased the plasma Fe concentration, and feeding epilactose partially but significantly recovered the decrease in Fe concentration induced by gastrectomy.

Table 3. Plasma Ca and Fe Concentrations of Sham-Operated and Gastrectomized Rats Fed the Control and Epilactose Diets for 28 Days[a]

surgery	diet	Ca (mg/dL)	Fe (mg/dL)
sham	control	9.40 ± 0.11 a	332 ± 17 a
	epilactose	9.18 ± 0.08 ab	312 ± 13 a
gastrectomy	control	8.86 ± 0.09 b	17.5 ± 1.4 c
	epilactose	9.22 ± 0.14 a	26.2 ± 2.7 b
ANOVA	surgery (S)	0.04	<0.01
	diet (D)	0.52	0.05
	S × D	0.01	0.01

[a] Mean ± SEM (n = 7 or 9), p < 0.05 according to Duncan's multiple range test.

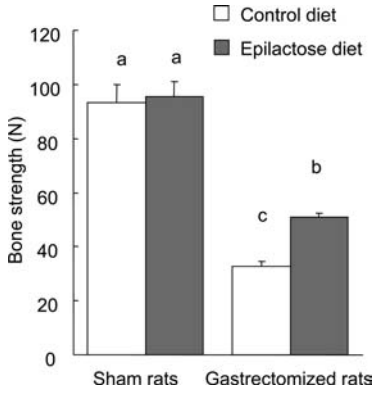

Figure 2. Femoral bone strength (maximum breaking force) in sham-operated and gastrectomized rats fed a control or epilactose diet for 28 days. Values are mean ± SEM (n = 7 or 9), and means not sharing a letter differ significantly according to Duncan's multiple range test (p < 0.05). p values of two-way ANOVA were <0.01, <0.02, and 0.8 for surgery, diet, and their interaction, respectively.

Table 4. Femoral Weight, Ca Content, and Ca Concentration in Sham-Operated and Gastrectomized Rats Fed the Control and Epilactose Diets for 28 Days[a]

surgery	diet	dry weight (mg/rat)	Ca content (mg/whole femur)	Ca concentration (mg/1 g of dry femur)
sham	control	309 ± 7 a	75.9 ± 2.0 a	246 ± 2
	epilactose	311 ± 6 a	78.4 ± 1.4 a	252 ± 3
gastrectomy	control	168 ± 8 c	40.8 ± 0.9 c	245 ± 8
	epilactose	203 ± 8 b	53.6 ± 1.2 b	267 ± 13
ANOVA	surgery (S)	<0.01	<0.01	0.42
	diet (D)	0.02	<0.01	0.11
	S × D	0.04	<0.01	0.38

[a] Mean ± SEM (n = 7 or 9), p < 0.05 according to Duncan's multiple range test.

Gastrectomy also severely impaired the bone strength (maximum breaking force) of the femurs (two-way ANOVA, p < 0.05 for surgery; **Figure 2**). Feeding epilactose influenced the bone strength according to the two-way ANOVA, with the strength in the gastrectomized rats fed the epilactose diet higher than that in those rats fed the control diet. Gastrectomy, feeding epilactose, and their interaction had significant effects on the dry weight and Ca content but not the Ca concentration of the femurs according to the two-way ANOVA (**Table 4**). In the gastrectomized rats, the dry weights and Ca contents were higher in the epilactose diet group than in the control diet group.

Hematocrits and hemoglobin concentrations in the blood at 15 and 30 days after the start of the feeding period were lower in the gastrectomized rats fed the control diet than in the sham rats fed the same diet (**Figure 3**). On day 15, the hematocrit and hemoglobin concentration in the gastrectomized rats fed the

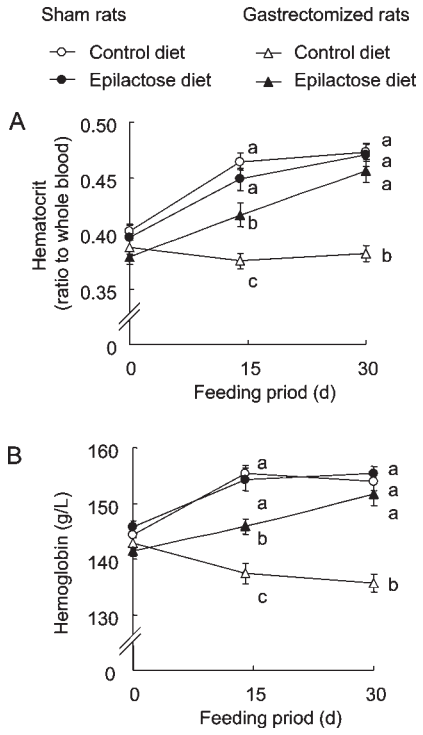

Figure 3. (A) Hematocrit and (B) hemoglobin concentration in sham-operated and gastrectomized rats on days 0, 15, and 30 of the feeding period. Values are mean ± SEM (n = 7 or 9), and means not sharing a letter differ significantly according to the Duncan's multiple range test (p < 0.05). p values of two-way ANOVA were all <0.01 for time, surgery, diet, time by surgery, time by diet, surgery by diet, and time by surgery by diet for hematocrit and all <0.01 for time, surgery, diet, time by surgery, time by diet, surgery by diet, and time by surgery by diet for hemoglobin concentration, respectively.

epilactose diet were higher than those in rats fed the control diet but lower than those in the sham rats fed the control and epilactose diets. On day 30, these two parameters in the gastrectomized rats fed the epilactose diet were recovered to levels comparable to those of the sham rats fed the control and epilactose diets.

Gastrectomy and epilactose feeding influenced cecal tissue weights, cecal content weights, and the pH of the cecal contents according to the two-way ANOVA (**Table 5**). The cecal tissue and content weights of the epilactose diet groups were higher than those in the control group in each surgical group, whereas the pH of the cecal contents in the epilactose diet group was lower than that in the control group in each surgical group.

Gastrectomy increased the total Ca concentration and decreased the soluble Ca concentration in the cecal contents (two-way ANOVA, $p < 0.05$ for surgery in both comparisons; **Table 6**). An interaction between diet (feeding epilactose) and surgery (gastrectomy) was observed for the soluble but not total Ca concentration (two-way ANOVA, $p < 0.05$ for interaction), and the soluble Ca concentration in the gastrectomized rats fed the epilactose diet was higher than that in those rats fed the control diet. Gastrectomy increased total and soluble Fe concentrations in the cecal contents (two-way ANOVA, $p < 0.05$ for surgery in both comparisons). Feeding epilactose increased the soluble but not total Fe concentration (two-way ANOVA, $p < 0.05$ for diet).

Both gastrectomy and epilactose feeding increased the pools of acetic, propionic, and butyric acids and total short-chain fatty acids (SCFAs; sum of these three acids) according to the two-way ANOVA. An interaction between gastrectomy and feeding

Table 5. Cecal Tissue Weight, Cecal Content Weight, and pH of Cecal Content in Sham-Operated and Gastrectomized Rats Fed the Control and Epilactose Diets for 28 Days[a]

surgery	diet	cecal tissue (g/100 g of body weight)	cecal content (g/rat)	pH
sham	control	0.31 ± 0.02 c	2.39 ± 0.28 c	7.46 ± 0.12 a
	epilactose	0.47 ± 0.02 b	5.01 ± 0.31 b	7.19 ± 0.21 bc
gastrectomy	control	0.36 ± 0.01 c	3.26 ± 0.39 c	7.36 ± 0.22 ab
	epilactose	0.58 ± 0.02 a	6.90 ± 0.48 a	6.97 ± 0.17 c
ANOVA	surgery (S)	<0.01	<0.01	0.10
	diet (D)	<0.01	<0.01	<0.01
	S × D	0.09	0.20	0.089

[a] Mean ± SEM (n = 7 or 9), $p < 0.05$ according to Duncan's multiple range test.

epilactose was found in the pools of acetic acid and total SCFAs (two-way ANOVA, $p < 0.05$ for interaction in both comparisons). Further, in the epilactose diet groups, the pools of acetic, propionic, and butyric acids and total SCFAs in the gastrectomized rats were higher than those in the sham rats (**Table 7**). There were no differences in succinic acid pools among the groups.

DISCUSSION

We previously demonstrated that epilactose enhanced the intestinal Ca absorption in normal rats (*13*) and that other non-digestible and fermentable saccharides restored postgastrectomy osteopenia and anemia through the promotion of intestinal Ca and Fe absorption (*5*−*10*). Here, we first showed the ameliorative effects of epilactose, a rare non-digestible disaccharide, on Ca and Fe malabsorption, osteopenia, and anemia in totally gastrectomized rats. The epilactose-mediated promotion of intestinal Ca and Fe absorption resulted in improvements in osteopenia and anemia in the gastrectomized rats. Our previous studies showed that the promotion of Ca and Fe absorption by DFAIII and hydrolyzed guar gum was largely prevented by the surgical resection of cecum (*15*, *16*). Therefore, we suggest that the large intestinal fermentation of epilactose has a major role in the epilactose-mediated promotion of the Ca and Fe absorption.

We suggest that increases in the mucosal surface area of the cecum and the soluble Ca concentration in the cecal contents are responsible for the epilactose-mediated promotion of the intestinal Ca absorption in the gastrectomized rats. The cecal tissue weight of gastrectomized rats fed the epilactose diet was 1.6-fold higher than that in those rats fed the control diet. Our previous studies demonstrated that the mucosal surface area of the cecum was doubled by feeding DFAIII (*15*, *17*) and that the increase in the Ca absorption is associated with the enlargement of the surface area in the ligated cecum (*15*). Although we did not estimate the mucosal surface area of the cecum in this study, the increase in the cecal tissue weight induced by epilactose was comparable to that by DFAIII (*7*). The increase in the cecal tissue weight by epilactose would be attributed to the increase in the cecal pools of SCFAs, which are known to stimulate epithelial cell proliferation (*18*). The HPLC analysis revealed that epilactose feeding increased total SCFA pools by ∼2.6-fold in the cecal contents of the gastrectomized rats. Furthermore, epilactose feeding raised the soluble Ca concentration by 1.6-fold in the cecal contents of the gastrectomized rats. Intestinal Ca absorption occurs via both trans- and paracellular routes (*19*, *20*). The paracellular mechanism is non-saturable and diffusive, occurring throughout the intestines, and requires a gradient of Ca concentrations between the luminal and basolateral sides. The decrease in the pH of the cecal contents with the epilactose-induced increase in SCFA production increased the soluble Ca concentration in the cecal contents of the gastrectomized rats. The increase

Table 6. Soluble and Total Ca and Fe Concentrations in the Cecal Contents of Sham-Operated and Gastrectomized Rats Fed the Control and Epilactose Diets for 28 Days[a]

		Ca		Fe	
surgery	diet	total (μmol/1 g of cecal content)	soluble (μmol/1 g of cecal content)	total (μmol/1 g of cecal content)	soluble (μmol/1 g of cecal content)
sham	control	218 ± 14 c	15.4 ± 1.1 a	1.94 ± 0.19 b	0.069 ± 0.013 b
	epilactose	224 ± 9 c	13.4 ± 0.8 b	2.37 ± 0.14 ab	0.133 ± 0.007 b
gastrectomy	control	372 ± 27 a	2.9 ± 0.2 d	2.85 ± 0.17 a	0.278 ± 0.016 a
	epilactose	314 ± 11 b	4.6 ± 0.4 c	2.68 ± 0.11 a	0.328 ± 0.028 a
ANOVA	surgery (S)	<0.01	<0.01	<0.01	<0.01
	diet (D)	0.16	0.80	0.42	0.01
	S × D	0.08	0.01	0.06	0.95

[a] Mean ± SEM (n = 7 or 9), $p < 0.05$ according to Duncan's multiple range test.

Table 7. Pools of Acetic, Propionic, and *n*-Butyric Acids in the Cecal Contents of Sham-Operated and Gastrectomized Rats Fed the Control and Epilactose Diets for 28 Days[a]

surgery	diet	acetic acid (μmol/whole cecal content)	propionic acid (μmol/whole cecal content)	*n*-butyric acid (μmol/whole cecal content)	total SCFAs (μmol/whole cecal content)	succinic acid (μmol/whole cecal content)
sham	control	110 ± 32 c	37.7 ± 8.6 c	20.1 ± 6.6 c	168 ± 47 c	0.215 ± 0.12
	epilactose	185 ± 12 b	85.2 ± 8.0 b	42.8 ± 3.3 b	313 ± 21 b	1.39 ± 0.34
gastrectomy	control	129 ± 15 c	54.9 ± 3.8 c	32.6 ± 5.1 bc	216 ± 22 c	4.61 ± 0.90
	epilactose	365 ± 47 a	136 ± 14 a	60.0 ± 9.4 a	562 ± 67 a	31.3 ± 15.8
ANOVA	surgery (S)	<0.01	<0.01	0.04	<0.01	0.06
	diet (D)	<0.01	<0.01	<0.01	<0.01	0.12
	S × D	0.01	0.09	0.73	0.03	0.16

[a] Mean ± SEM (n = 7 or 9), $p < 0.05$ according to Duncan's multiple range test.

in the soluble Ca concentration enhances the Ca gradient across the intestinal epithelium and possibly facilitates the paracellular Ca absorption.

It has been reported that ingestion of FOS induces the mRNA expression of the transient receptor potential vanilloid type 6 (TRPV6) and calbindin-D9k in the rat colon, which are involved in the transcellular mechanism, and that propionic and butyric acids enhance TRPV6 promoter activity in colonic epithelial Caco-2 cells (*21*). Our previous study showed that propionic and butyric acid pools in the cecal contents of rats fed epilactose were higher than and comparable to those fed FOS, respectively (*13*). The production of propionic and butyric acids from epilactose by intestinal microbes may also promote transcellular Ca absorption in the epilactose-fed rats.

The epilactose-mediated promotion of Fe absorption in the gastrectomized rats appears to result mainly from the expansion of cecal tissue. Our previous study demonstrated that Fe absorption capacity was higher in the ligated cecum of rats fed DFAIII, as is the case with Ca absorption, without any concomitant increases in divalent metal transporter-1 (DMT-1) transcript level (*15*), a major Fe transporter in the intestinal brush-boarder membrane (*22*). The soluble Fe concentrations in the cecal contents of gastrectomized rats fed the control and epilactose diets were similar (0.28 and 0.33 mmol/kg, respectively) and were much higher than the Michaelis constant for DMT-1, i.e., 1−2 μM (*23*), indicating that Fe transport by DMT-1 proceeded adequately in the cecum in both dietary groups.

Feeding epilactose did not exhibit any promotive effect on the Ca and Fe absorption in the sham-operated rats in the present study (*13*). This discrepancy between the present and previous studies would result from insufficient food intake in the sham-operated rats in the present study. All rats including the sham-operated rats were given ∼15 g of diet/day through the experimental period, because we previously observed that the gastrectomized rats did not consume more than 15 g of diet/day (unpublished observations). It seems that the Ca and Fe absorption was enough high in the sham-operated rats under this condition, and thereby, the promotive effects of epilactose were blunted.

Recently, we proposed another mechanism for the epilactose-mediated promotion of intestinal Ca absorption; epilactose directly stimulates the intestinal epithelial cells and promotes paracellular Ca transport in the small intestine (*13*, *14*). However, it is unlikely that this epilactose-mediated promotion of paracellular Ca transport in the small intestine occurs in the gastrectomized rats, because the Ca solubilization by gastric acid is essential for it.

The gastrectomy-induced osteopenia and anemia mainly resulted from intestinal Ca and Fe malabsorption. The lack of gastric acid results in insufficient solubilization of Ca and Fe and impairs Ca and Fe absorption in the small intestine. In this study, a water-insoluble calcium carbonate was used as a dietary Ca source. We have reported that the ingestion of calcium chloride, a water-soluble calcium salt, instead of calcium carbonate, completely recovers the postgastrectomy Ca malabsorption in rats (*5*). Ferric citrate, the Fe source in the test diets, dissolves very slowly in water, and the ingested ferric citrate appears to flow into the small intestine without sufficient solubilization in gastrectomized rats.

Our findings might be of benefit for patients with postgastrectomy osteopenia and anemia. It is known that a considerable amount of epilactose can be produced from cow's milk by heating and alkali treatments (*11*), and we did not find any pathological signs or gastrointestinal symptoms; for example, diarrhea, in rats fed the epilactose diets for 28 days. However, further study should be performed to confirm the safety of epilactose for human consumption.

In conclusion, ingestion of epilactose, a rare non-digestible disaccharide, ameliorates postgastrectomy osteopenia and completely restores anemia through the promotion of intestinal Ca and Fe absorption. The fermentation of epilactose by intestinal microbes possibly has a role in the epilactose-mediated promotion of Ca and Fe absorption in the gastrectomized rats.

ABBREVIATIONS USED

ANOVA, analysis of variance; DFA, difructose anhydride; DMT, divalent metal transporter; FOS, fructooligosaccharide; SCFA, short-chain fatty acid; TRPV6, transient receptor potential vanilloid type 6.

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