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Note

Effect of L-Lactic Acid on Calcium Absorption in Rats Fed Omeprazole

Osamu CHONAN,* Rie TAKAHASHI, Hisako YASUI and Masaaki WATANUKI

Yakult Central Institute for Microbiological Research, Kunitachi, Tokyo 186–0011, Japan

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Summary We examined the effect of L-lactic acid on calcium absorption in male Wistar rats made achlorhydric by dietary omeprazole, a proton pump inhibitor. The dietary omeprazole intake (0.03 g/100 g of diet) increased the gastric pH and decreased the apparent calcium absorption ratio. Dietary famotidine (0.03 g/100 g of diet), an H_2-receptor antagonist, and lower doses of omeprazole (0.005 or 0.01 g/100 g of diet) did not affect the gastric pH or the calcium absorption. In a second experiment, dietary lactic acid (0.5, 1.0, or 2.5 g/100 g of diet) increased the intestinal calcium absorption dose dependently in rats fed omeprazole (0.03 g/100 g of diet). The gastric pH was significantly decreased only in the rats fed higher doses of lactic acid (1.0, or 2.5 g/100 g of diet). In a third experiment, a dietary sour milk beverage containing lactic acid (0.5 g/100 g of diet) increased the intestinal calcium absorption, but did not affect the gastric pH in rats fed omeprazole (0.03 g/100 g of diet). Although the significance of gastric acid in terms of overall calcium absorption is not known, under the present experimental conditions, the inhibition of gastric acid secretion by dietary omeprazole decreased the apparent calcium absorption, and the dietary lactic acid prevented the calcium absorption in rats fed omeprazole.

Key Words lactic acid, calcium, absorption, omeprazole

It is generally accepted that gastric acid secretion is a prerequisite for $CaCO_3$ solubilization before its intestinal absorption in the ionic form (*1*). The importance of gastric acid secretion in dietary calcium absorption was described in human (*1*) and rat (*2*) studies. However, other reports have demonstrated a lack of effect of a high intragastric pH on dietary calcium absorption (*3*, *4*). The significance of gastric acid secretion regarding dietary calcium absorption is thus controversial.

If gastric acid affects dietary calcium absorption as a result of increased calcium solubility in the stomach, the intake of other inorganic and/or organic acids such

* To whom correspondence should be addressed.

as L-lactic acid (LA) may stimulate dietary calcium absorption, especially in gastrectomy and achlorhydric patients. LA is present in fermented products such as yogurt. Previous studies showed that the bioavailability of calcium from milk and from yogurt are not different (*5*, *6*), but these studies tested only normal subjects.

The purposes of the present study were to identify the role of gastric acid secretion in dietary calcium absorption in rats made achlorhydric by dietary omeprazole (OM), a proton pump inhibitor (*7*), and the effect of dietary LA on calcium absorption in achlorhydric rats. We also examined the effect of a sour milk beverage (SMB) containing LA on calcium absorption in achlorhydric rats.

Materials and methods

Materials. Omepral® in tablet form (OM 20 mg/tablet) was purchased from Astra Japan Ltd. (Osaka, Japan) and ground to a fine powder. Famotidine (FA), an H_2-receptor antagonist, was purchased from Sigma Chemical Co. (St. Louis, MO, USA). We prepared LA in a powder form for this study. LA solution (Wako Pure Chemicals, Osaka, Japan) and corn starch (Oriental Yeast Co., Tokyo, Japan) were mixed in the ratio of 1 to 2 (wt/wt), and a 9th volume of distilled water was then added. Subsequently the mixture was lyophilized and assayed for LA content with a Determina LA kit (Kyowa Medix Co., Tokyo, Japan). The LA content in this preparation was 19.8 g/100 g of LA preparation. The SMB was Yakult 65, a commercial product (Yakult Co. Ltd., Tokyo, Japan). It was prepared based on fermented milk by using *Lactobacillus casei* strain Shirota as a starter, and it contained (g/100 g): crude protein (N × 6.25) 1.2, lipid 0.1, and carbohydrate 16.2. We prepared the SMB in a powder form. The SMB and corn starch were mixed in a ratio of 10 to 1 (wt/wt), and the same volume of distilled water was added. The mixture was then lyophilized and assayed for LA content with a Determina LA kit. The LA, calcium, and phosphorus contents in this preparation were 1.56 g, 0.169 g, and 0.222 g/100 g of SMB preparation, respectively.

Diets. The ingredients of the diets are given in Table 1. In study 1, we prepared five types of diet. The standard diet was based on the AIN-76 formulation (*8*), but calcium carbonate and potassium dihydrogen phosphate were used as sources of calcium and phosphorus. We also prepared an FA diet, OM 0.005% diet, OM 0.01% diet, and OM 0.03% diet. These diets contained FA (0.03 g/100 g of the diet) and Omepral® (0.0358 g/100 g of the diet, 0.0715 g/100 g, and 0.2145 g/ 100 g) as a source of OM (0.005 g/100 g of the diet, 0.01 g/100 g, and 0.03 g/ 100 g, respectively).

For study 2, five types of diets were prepared. The standard diet and the OM 0.03% diet were prepared as in study 1. An LA 0.5% diet, an LA 1.0% diet, and an LA 2.5% diet were also prepared. The LA preparation was added to the OM 0.03% diet at the levels of 2.53 g/100 g of the diet, 5.06 g/100 g, and 12.63 g/100 g as a source of LA (0.5 g/100 g of the diet, 1.0 g/100 g, and 2.5 g/100 g, respectively), and the LA contained in the preparation was replaced by an equal amount of sucrose.

For study 3, three types of diets were prepared. The standard diet and the OM 0.03% diet were prepared as in study 1. An SMB diet was also prepared. The SMB

Table 1. Composition of the experimental diets. (g/100 g of diet)

	Study 1					Study 2					Study 3		
	Standard	FA 0.03%	OM 0.005%	OM 0.01%	OM 0.03%	Standard	OM 0.03%	LA 0.5%	LA 1.0%	LA 2.5%	Standard	OM 0.03%	SMB
Casein	20	20	20	20	20	20	20	20	20	20	20	20	20
DL-Methionine	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Corn oil	5	5	5	5	5	5	5	5	5	5	5	5	5
Mineral mixture[1]	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Vitamin mixture[2]	1	1	1	1	1	1	1	1	1	1	1	1	1
Choline bitartrate	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Sucrose	6.99	6.96	6.9542	6.9185	6.7755	6.99	6.7755	6.2755	5.7755	4.2755	6.99	6.7755	6.2755
Corn starch	55	55	55	55	55	55	55	52.97	50.94	44.87	55	55	23.892
Cellulose powder	5	5	5	5	5	5	5	5	5	5	5	5	5
$CaCO_3$	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.113
KH_2PO_4	1.76	1.76	1.76	1.76	1.76	1.76	1.76	1.76	1.76	1.76	1.76	1.76	1.445
Famotidine	0	0.03	0	0	0	0	0	0	0	0	0	0	0
Omepral®	0	0	0.0358	0.0715	0.2145	0	0.2145	0.2145	0.2145	0.2145	0	0.2145	0.2145
Lactic acid preparation[3]	0	0	0	0	0	0	0	2.53	5.06	12.63	0	0	0
Sour milk beverage preparation[4]	0	0	0	0	0	0	0	0	0	0	0	0	32.06

[1] Mineral mixture was prepared according to the AIN-76 formulation, but $CaHPO_4$ was substituted for sucrose. [2] Vitamin mixture was prepared according to the AIN-76 formulation. [3] Made up in corn starch, supplying lactic acid 0.5, 1.0, 2.5 (g/100 g of diet), respectively. [4] Made up in corn starch, supplying lactic acid 0.5 (g/100 g of diet).

preparation was added to the OM 0.03% diet at a level of 32.06 g/100 g of the diet, as a source of LA (0.5 g/100 g of diet), and the LA content in the SMB preparation was replaced by an equal amount of sucrose. All diets were balanced for calcium and phosphorus with calcium carbonate and potassium dihydrogen phosphate, respectively (0.5 g of calcium and 0.4 g of phosphorus/100 g of diet).

Animals. Four-week-old male Wistar rats (Japan SLC, Inc., Shizuoka, Japan) were housed in individual stainless steel cages in a temperature-controlled (24 ± 1°C) room with 60 ± 5% humidity and a 12-h light12-h dark cycle. After a 6-d adaptation period in which all rats were fed the standard diet, they were separated into five groups of five animals each for studies 1 and 2 and three groups of six animals each for study 3 with similar mean body weights. Each group was fed one of the experimental diets and deionized water ad libitum for 7 d.

Sample collection and analyses. Fecal samples collected from day 4 for 3 d were cleaned of foreign adhering matter, dried by lyophilization, and ground to a fine powder. Between 1000 and 1100 h on day 7 of the experimental diet, all the rats were anesthetized by an intraperitoneal injection of sodium pentobarbital (40 mg/kg body weight), and the stomach was then removed. The pH of the gastric digesta was measured with a glass microelectrode inserted and held in the middle of the stomach.

The fecal samples were examined for calcium, after wet ashing as described previously (*9*), with the use of an inductive coupled plasma emission spectrometer (ICPS-2000; Shimadzu Ltd., Kyoto, Japan). The apparent absorption ratio of calcium was calculated as the calcium intake minus the fecal excretion and is expressed as the percentage of calcium intake.

These studies were approved by the ethical committee for animal experiments of Yakult Central Institute for Microbiological Research, and the animals were maintained in accordance with the guidelines for the care and use of laboratory animals of Yakult Central Institute for Microbiological Research.

Statistical analysis. All data were analyzed by one-way analysis of variance. When a significant F ratio was found, Tukey's test (*10*) was utilized to reveal significant differences among groups. The difference between means was considered significant at $p < 0.05$. All statistical analyses were conducted with the use of a statistical computer program (STATISTICA, Statsoft, Inc., Tulsa, OK, USA).

Results

Growth and gastric pH of the rats. The body weights, food consumption, and gastric pH of the rats are shown in Table 2. The growth rate and food intake were not significantly different among the groups. The gastric pH was significantly increased in the rats fed the OM 0.03% diet compared with the rats fed the standard diet (studies 1, 2, and 3). An increase in the LA content of the diet (LA 1.0 g/100 g of diet and 2.5 g/100 g of diet) resulted in a decrease of the gastric pH compared with the rats fed the OM 0.03% diet (study 2).

Calcium absorption. The absorption of calcium is shown in Table 3. The levels

Table 2. Body weight, food intake, and gastric pH of rats fed experimental diets for 7 d.[1]

	Study 1					Study 2					Study 3		
	Stan-dard	FA 0.03%	OM 0.005%	OM 0.01%	OM 0.03%	Stan-dard	OM 0.03%	LA 0.5%	LA 1.0%	LA 2.5%	Stan-dard	OM 0.03%	SMB
Initial body weight (g)	106 $\pm 4^{ns}$	106 ± 3	106 ± 4	106 ± 3	106 ± 4	108 $\pm 7^{ns}$	108 ± 4	108 ± 4	108 ± 5	108 ± 4	106 $\pm 6^{ns}$	106 ± 5	106 ± 3
Final body weight (g)	142 $\pm 8^{ns}$	142 ± 5	143 ± 7	138 ± 5	141 ± 6	146 $\pm 12^{ns}$	139 ± 6	140 ± 5	143 ± 3	145 ± 8	144 $\pm 10^{ns}$	136 ± 8	140 ± 5
Food intake (g)	94.2 $\pm 8.4^{ns}$	91.3 ± 5.8	96.1 ± 6.3	92.2 ± 4.6	89.6 ± 5.6	99.8 $\pm 9.0^{ns}$	90.9 ± 7.7	87.6 ± 3.7	95.1 ± 4.7	96.0 ± 7.9	107.0 $\pm 6.9^{ns}$	98.2 ± 7.1	95.8 ± 8.9
Gastric pH	3.1 $\pm 0.8^{a}$	4.0 $\pm 0.4^{ab}$	3.5 $\pm 0.4^{a}$	3.6 $\pm 0.6^{ab}$	4.6 $\pm 0.1^{b}$	3.8 $\pm 0.2^{a}$	4.6 $\pm 0.3^{b}$	4.5 $\pm 0.1^{bc}$	4.0 $\pm 0.3^{ac}$	3.8 $\pm 0.4^{a}$	3.6 $\pm 0.9^{a}$	4.5 $\pm 0.2^{b}$	4.4 $\pm 0.4^{ab}$

[1] Values are means $\pm$ SD. Means in the same row not sharing a common superscript letter are significantly different by Tukey's test ($p < 0.05$).

of fecal calcium excretion were highest in the rats fed the OM 0.03% diet among the groups of rats fed an experimental diet (studies 1, 2, and 3). The apparent calcium absorption ratios were significantly decreased in the rats fed the OM 0.03% diet compared with the rats fed the standard diet (studies 1, 2, and 3). The dietary FA and lower doses (0.01 and 0.005%) of OM did not affect the calcium absorption (study 1). An increase in the LA content of the diet resulted in the increase of the apparent calcium absorption ratio (study 2). The rats fed the SMB diet significantly increased the apparent calcium absorption ratio compared with the rats fed the OM 0.03% diet (study 3).

Discussion

It is well documented that gastrectomy and achlorhydric patients have reduced bone mineral content (*11–13*), possibly as a result of a calcium deficiency because of an impaired bioavailability of dietary calcium. Some studies have attributed the effects of partial gastrectomy and the use of antiulcer medications to calcium malabsorption, but other studies have failed to confirm this (*4*, *14*, *15*). The results of studies of intestinal calcium absorption in achlorhydric patients and experimental animals are conflicting (*16*, *17*). We hypothesized that in these studies, achlorhydria had not been induced completely in the patients and experimental animals; we therefore first tested the effects of different kinds of antiulcer medications (OM, a proton pump inhibitor, and FA, an H_2-receptor antagonist) on dietary calcium absorption from the rat intestine. As shown in Table 3, OM inhibited calcium absorption dose dependently with a potency greater than that of FA, and OM at the highest dose increased the gastric pH. These results indicated that a strong inhibition of gastric acid secretion increases the pH in the stomach and affects the intestinal calcium absorption.

In the second and third studies, we tested the effect of dietary LA and SMB on calcium absorption in rats fed OM, which appeared in study 1 to have great potential as an agent for producing achlorhydria in an animal. When the rats were treated with higher doses of dietary LA (1.0 and 2.5 g/100 g of diet), the gastric pH was decreased and the intestinal calcium absorption was increased. However, when the rats were treated with the lowest dose of dietary LA (0.5 g/100 g of diet) or SMB, the apparent calcium absorption ratio was increased without decreasing gastric pH. These findings indicate that gastric pH is not the only factor to explain the stimulatory effect of LA on calcium absorption.

A correlation between the increased acid load of organic acid and a concomitant slowing of gastric emptying has been reported in human and animal studies (*18*, *19*). The rate of gastric emptying is also considered a potentially important determinant of nutrient absorption by controlling the delivery of nutrients to the small intestinal epithelium (*18–21*). In our present studies we did not examine the emptying rate, but the rate of gastric emptying and the solubilization of calcium because of lactic acid feeding were speculated to be involved in the calcium absorption.

Table 3. Calcium absorption in rats fed experimental diets for 7 d.[1]

	Study 1					Study 2					Study 3		
	Standard	FA 0.03%	OM 0.005%	OM 0.01%	OM 0.03%	Standard	OM 0.03%	LA 0.5%	LA 1.0%	LA 2.5%	Standard	OM 0.03%	SMB
Intake (mg/3 d)	203 $\pm 17^{ns}$	197 ± 18	219 ± 13	202 ± 12	196 ± 14	237 $\pm 25^{ns}$	207 ± 23	205 ± 12	218 ± 12	223 ± 21	255 $\pm 13^{a}$	204 $\pm 18^{b}$	228 $\pm 26^{ab}$
Fecal excretion (mg/3 d)	62 $\pm 11^{a}$	70 $\pm 16^{a}$	68 $\pm 10^{a}$	66 $\pm 10^{a}$	109 $\pm 15^{b}$	79 $\pm 12^{a}$	113 $\pm 19^{b}$	84 $\pm 12^{a}$	84 $\pm 14^{a}$	72 $\pm 15^{a}$	94 $\pm 6^{a}$	126 $\pm 16^{b}$	112 $\pm 21^{ab}$
Absorption (mg/3 d)	141 $\pm 14^{ab}$	127 $\pm 9^{a}$	150 $\pm 10^{b}$	136 $\pm 9^{ab}$	86 $\pm 17^{c}$	158 $\pm 15^{a}$	94 $\pm 9^{b}$	121 $\pm 15^{c}$	134 $\pm 14^{cd}$	152 $\pm 9^{ad}$	161 $\pm 16^{a}$	78 $\pm 14^{b}$	116 $\pm 29^{c}$
Apparent absorption ratio (%)	70 $\pm 5^{a}$	65 $\pm 6^{a}$	69 $\pm 4^{a}$	67 $\pm 4^{a}$	44 $\pm 8^{b}$	67 $\pm 2^{a}$	46 $\pm 4^{b}$	59 $\pm 6^{a}$	61 $\pm 6^{a}$	68 $\pm 4^{a}$	63 $\pm 4^{a}$	38 $\pm 6^{b}$	50 $\pm 10^{c}$

[1] Values are means $\pm$ SD. Means in the same row not sharing a common superscript letter are significantly different by Tukey's test ($p < 0.05$).

Reynolds (*22*) also reported that some medications bind to the divalent cations, resulting in poorly absorbed complexes. If OM has the ability to bind calcium and make unabsorbed complexes under the experimental conditions used in the present study, OM intake may stimulate calcium excretion in the feces and decrease the calculated apparent calcium absorption ratios. We therefore tested the calcium binding ability of OM in vitro; we observed no calcium binding ability of OM (data not shown).

In the present study, dietary LA and the SMB containing LA increased the calcium absorption in a rat achlorhydric model. Although the significance of gastric acid in terms of overall calcium absorption is not known, under the present experimental conditions the stimulatory effect of LA and SMB on calcium absorption may be partly associated with the decreased gastric pH and rate of gastric emptying.

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