J Nutr Sci Vitaminol, 1998, **44**, 869–875

Effect of L-Lactic Acid on the Absorption of Calcium in Gastrectomized Rats

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

Yakult Central Institute for Microbiological Research, Kunitachi, Tokyo 186–8650, Japan

(Received August 24, 1998)

Summary The effect of dietary L-lactic acid (LA) (0.5, 1.0, or 2.5 g/100 g of diet) on the absorption of calcium in gastrectomized rats was evaluated for 28 d. Calcium phosphate was used as a source of calcium. The apparent calcium absorption ratio and the calcium contents of the femur and tibia in gastrectomized rats fed the control diet were significantly less than those in sham-operated rats. In the gastrectomized rats, the apparent calcium absorption ratio and the calcium contents of bone in the rats fed the lower doses of LA diets (LA 0.5 or 1.0 g/100 g of diet) were not affected; however, the apparent calcium absorption ratio and the calcium contents of bone in the rats fed the highest doses of LA diet (LA 2.5 g/100 g of diet) were greater than those in gastrectomized rats fed the control diet. Dietary LA (2.5 g/100 g of diet) also enhanced the phosphorus absorption and bone phosphorus content in the gastrectomized rats. We speculated that the highest dose of dietary LA might be associated with the dissolving of a water-insoluble form of calcium salt in the diet, thereby facilitating the calcium absorption and resulting in increased bone calcium content in gastrectomized rats.

Key Words lactic acid, calcium, absorption, gastrectomy

It is well documented that gastrectomy has developed osteopenia (*1–3*), suggesting that the stomach is important for calcium homeostasis. Some theories have been advanced to explain the effects of gastrectomy on calcium homeostasis. First, gastric acid is thought to dissolve insoluble calcium in the diet, thereby facilitating the absorption of calcium from the small intestine (*4–7*). Second, hypocalcinocemic gastric peptide hormone, released from the stomach in response to gastrin, is thought to enhance the uptake of calcium into bone (*2, 8*). Third, the rate of delivery of calcium to the small intestine from the stomach is considered a potentially important determinant of calcium absorption (*9, 10*).

* To whom correspondence should be addressed.

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

	Control	LA 0.5%	LA 1.0%	LA 2.5%
Casein	20	20	20	20
DL-Methionine	0.3	0.3	0.3	0.3
Corn oil	5	5	5	5
Mineral mixture[1]	3.5	3.5	3.5	3.5
Vitamin mixture[1]	1	1	1	1
Choline bitartrate	0.2	0.2	0.2	0.2
Cornstarch	55	51.2	47.5	36.2
Sucrose	10	10	10	10
Cellulose powder	5	5	5	5
Lactic acid preparation[2]	0	3.8	7.5	18.8

[1] Mineral mixture and vitamin mixture were based on the AIN-76 formulation.
[2] Made up in cornstarch, supplying lactic acid 0.5, 1.0, and 2.5 (g/100 g of diet).

We previously demonstrated that the inhibition of gastric acid secretion by dietary omeprazole (OM), a proton pump inhibitor, decreased the apparent calcium absorption and that dietary L-lactic acid (LA), which is present in fermented products such as yogurt, prevented the inhibition of calcium absorption in rats fed OM (*11*).

In the present study, we reexamined the effect of dietary LA on calcium absorption to identify the mechanism of the enhanced calcium absorption observed in rats fed LA. The possibility that the dietary acidity induced by dietary LA might prevent calcium malabsorption was tested in gastrectomized rats.

MATERIALS AND METHODS

Materials. We prepared LA in a powder form as previously reported (*11*). LA solution (Wako Pure Chemicals, Osaka, Japan) and cornstarch (Oriental Yeast, Tokyo, Japan) were mixed in the ratio of 1 to 2 (wt/wt), and a 9th volume of distilled water was added. The mixture was then lyophilized and assayed for LA content with a Determina LA kit (Kyowa Medix, Tokyo). The LA content in this preparation was 13.3 g/100 g of LA preparation.

Diets. The ingredients of the diets are given in Table 1. We prepared four types. The control diet was based on the AIN-76 formulation (*12*). An LA 0.5% diet, LA 1.0% diet, and LA 2.5% diet were also prepared. The LA preparation was added to the control diet at levels of 3.8 g/100 g, 7.5 g/100 g, and 18.8 g/100 g of the diet as a source of LA (0.5 g/100 g, 1.0 g/100 g, and 2.5 g/100 g of diet, respectively), and the LA contained in the preparation was replaced by an equal amount of sucrose.

Animals and surgical procedures. Five-week-old male Sprague-Dawley rats (Clea Japan, Tokyo) were housed in individual stainless-steel cages in a temperature-controlled (24±1°C) room with 60±5% humidity and a 12 h light-dark

cycle. After a 3-d adaptation period in which all rats were fed the control diet, they were separated into two groups of 7 and 28 rats. In each rat of the 28-rat group, a gastrectomy was performed by resection of the stomach, followed by the joining of the esophagus and duodenum end to end. In each rat of the 7-rat group, a sham operation was performed. After these operations, the rats were deprived of food for 24 h, then allowed free access to homogenized, pasteurized cow's milk (Snow Brand, Tokyo) for 48 h (*3*). All rats were then fed the control diet at 12 g/d for the next 7 d. Dietary treatments began on day 10 of postoperation, at which time the 28 gastrectomized rats were separated into 4 groups of seven animals each with similar mean body weights. Each group was fed an experimental diet, and the sham group was fed the control diet. All rats were fed 15 g of diet per day and allowed free access to deionized water ad libitum for 28 d. All rats also received an intramuscular injection of vitamin B-12 (0.5 mg/kg; Wako Pure Chemical) every second week (*3*), starting 10 d after the operations.

Sample collection and analyses. Fecal samples collected from days 5, 12, 19, and 26 for 3 d during each period were cleaned of foreign adhering matter, dried by lyophilization, and ground to a fine powder. After the 28-d feeding period, all rats were anesthetized by an intraperitoneal injection of sodium pentobarbital (40 mg/kg body weight), and the femora and tibiae from the right leg were excised and cleaned of adhering matter and dried to a constant weight.

The femora, tibiae, and fecal samples were examined for calcium and phosphorus after wet ashing, as described previously (*13*), with the use of an inductive-coupled plasma emission spectrometer (ICPS-2000; Shimadzu, Kyoto, Japan). The apparent absorption ratios of calcium and phosphorus were calculated as the calcium and phosphorus intake minus the fecal excretion and are expressed as the percentages of calcium and phosphorus intake.

These studies were approved by the ethical committee for animal experiments of the 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 (*14*) was used to reveal significant differences among groups. A difference between means was considered significant at $p < 0.05$. All statistical analyses were conducted with a statistical software program (STATISTICA, Statsoft, Tulsa, OK, USA).

RESULTS

Body weights and food intakes

The body weights and food intakes are shown in Table 2. The initial and final body weights of the gastrectomized rats were significantly lower than those of the sham-operated rats. The food intake was not significantly different among groups.

Table 2. Body weights and food intake in rats fed experimental diets for 28 d.[1]

	Sham	Control	LA 0.5%	LA 1.0%	LA 2.5%
Initial body weight (g)	154 ± 4^{a}	145 ± 6^{b}	144 ± 5^{b}	145 ± 5^{b}	143 ± 5^{b}
Final body weight (g)	275 ± 8^{a}	242 ± 7^{b}	241 ± 12^{b}	241 ± 13^{b}	232 ± 13^{b}
Food intake (g)	419 ± 1^{ns}	414 ± 12	405 ± 13	395 ± 38	392 ± 23

[1] Values are means and standard deviations for seven rats. Means in the same row not sharing common superscript letters are significantly different by Tukey's test ($p < 0.05$).

Table 3. Apparent absorption ratio of calcium and phosphorus in rats fed experimental diets for 28 d.[1]

	Sham	Control	LA 0.5%	LA 1.0%	LA 2.5%
Apparent absorption ratio (%)					
Calcium					
4–7 d	57.3 ± 3.4^{a}	13.5 ± 10.3^{b}	13.2 ± 7.7^{b}	19.5 ± 4.8^{bc}	28.5 ± 6.9^{c}
11–14 d	60.3 ± 2.8^{a}	15.5 ± 6.8^{b}	17.3 ± 3.5^{b}	17.2 ± 5.8^{b}	29.9 ± 8.5^{c}
18–21 d	54.4 ± 4.3^{a}	10.5 ± 6.5^{b}	18.8 ± 6.0^{b}	16.0 ± 10.8^{b}	36.6 ± 9.0^{c}
25–28 d	48.6 ± 4.8^{a}	9.9 ± 10.9^{b}	17.9 ± 12.5^{bc}	20.9 ± 11.2^{bc}	26.1 ± 8.5^{c}
Phosphorus					
4–7 d	70.3 ± 2.3^{a}	33.8 ± 8.0^{b}	31.5 ± 6.2^{b}	37.1 ± 3.4^{bc}	44.3 ± 5.5^{c}
11–14 d	68.5 ± 8.9^{a}	29.9 ± 5.4^{b}	31.8 ± 3.3^{b}	31.8 ± 4.8^{b}	42.8 ± 6.4^{c}
18–21 d	71.8 ± 2.8^{a}	40.0 ± 4.0^{b}	40.1 ± 6.0^{bc}	33.7 ± 10.7^{b}	50.3 ± 7.0^{c}
25–28 d	66.1 ± 2.3^{a}	34.4 ± 8.3^{b}	39.6 ± 8.6^{b}	40.6 ± 8.2^{b}	43.0 ± 7.1^{b}

[1] Values are means and standard deviations for seven rats. Means in the same row not sharing common superscript letters are significantly different by Tukey's test ($p < 0.05$).

Apparent absorption ratios of calcium and phosphorus

The absorption ratios of calcium and phosphorus are shown in Table 3. The apparent absorption ratios of calcium and phosphorus in the gastrectomized rats fed each experimental diet were significantly lower than those of the sham-operated rats throughout the four absorption study periods. In the gastrectomized rats, the apparent absorption of calcium in the rats fed the LA 2.5% diet was significantly higher than in the rats fed the control diet throughout the four absorption study periods. Among the gastrectomized rats, the apparent absorption of phosphorus in the rats fed LA 2.5% diet was also higher than in the rats fed the control diet throughout the four absorption study periods, but no significant differences were found at the final absorption study period among the 4 gastrectomized rat groups. The apparent absorption ratios of calcium and phosphorus were not significantly different throughout the four absorption study periods among the gastrectomized rats fed the lower doses of LA (LA 0.5% and 1.0% diet) and the rats fed the control diet.

Table 4. Bone mineral content in rats fed experimental diets for 28 d.[1]

	Sham	Control	LA 0.5%	LA 1.0%	LA 2.5%
Femur					
Dry weight (g)	0.40 ± 0.02^{a}	0.24 ± 0.02^{b}	0.23 ± 0.01^{b}	0.24 ± 0.03^{bc}	0.27 ± 0.01^{c}
Ca (mg/g dry)	210.1 ± 3.2^{a}	147.7 ± 6.8^{b}	146.8 ± 7.6^{b}	149.3 ± 7.7^{b}	161.1 ± 7.0^{c}
P (mg/g dry)	110.7 ± 1.7^{a}	83.0 ± 3.6^{b}	81.9 ± 4.5^{b}	83.3 ± 3.9^{b}	90.1 ± 3.3^{c}
Tibia					
Dry weight (g)	0.32 ± 0.01^{a}	0.20 ± 0.01^{b}	0.20 ± 0.02^{bc}	0.20 ± 0.02^{bc}	0.22 ± 0.01^{c}
Ca (mg/g dry)	208.5 ± 1.7^{a}	162.1 ± 7.5^{b}	163.3 ± 6.9^{b}	169.4 ± 9.4^{b}	180.5 ± 5.4^{c}
P (mg/g dry)	111.4 ± 1.2^{a}	88.5 ± 4.9^{b}	90.6 ± 3.2^{b}	93.2 ± 5.4^{b}	99.8 ± 2.6^{c}

[1] Values are means and standard deviations for seven rats. Means in the same row not sharing common superscript letters are significantly different by Tukey's test ($p < 0.05$).

Calcium and phosphorus contents of the femur and tibia

The dry weights of the femur and tibia and the calcium and phosphorus contents per g dry weight of femur and tibia are shown in Table 4. The dry weights and the contents of calcium and phosphorus of the femur and tibia in the gastrectomized rats fed each experimental diet were significantly lower than in the sham-operated rats. Among the gastrectomized rats, the dry weights and the contents of calcium and phosphorus of both bones in the rats fed the LA 2.5% diet were significantly higher than those in rats fed the control diet. The dry weights and contents of calcium and phosphorus of the femur and tibia were not significantly different among the gastrectomized rats fed the lower doses of LA (LA 0.5% and 1.0% diet) and the rats fed the control diet.

DISCUSSION

Gastric acid has been suggested to play an important role in the intestinal absorption of calcium from ingested food because calcium solubility is thought to be a prerequisite for calcium absorption and the solubility of calcium is highly pH-dependent (*5*, *15*). However, other study has demonstrated a lack of effect of a high intragastric pH on dietary calcium absorption (*16*). The significance of gastric acid secretion regarding dietary calcium absorption is thus controversial. Our previous findings also suggested that the gastric acidity induced by dietary LA is not the only factor in the stimulatory effect of LA on calcium absorption (*11*). In the present study, we reexamined the effect of dietary LA on calcium absorption to identify the mechanism of LA-enhanced calcium absorption. The purpose of the present study was to examine the possibility that the dietary acidity induced by dietary LA might prevent calcium malabsorption in gastrectomized rats.

The gastrectomy decreased the apparent calcium absorption ratio, as previously reported by Ohta et al (*3*), but in the present study the apparent phosphorus

absorption ratio was also decreased by gastrectomy. We used water-insoluble calcium phosphate as a source of calcium and phosphorus, and Ohta et al used water-insoluble calcium carbonate and water-soluble potassium dihydrogen-phosphate as the sources of calcium and phosphorus, respectively (*3*). These differences suggest that the calcium phosphate in the diets we used here might not be sufficiently dissolved and that the bioavailability not only of calcium but also of phosphorus was thus impaired in the gastrectomized rats.

We previously demonstrated that the inhibition of gastric acid secretion by dietary OM decreased the apparent calcium absorption and that dietary LA prevented the inhibition of calcium absorption in rats fed OM (*11*). Given the presence of LA throughout the gastric tract, two theories have been advanced to explain the stimulatory effects of dietary LA on calcium absorption in rats fed OM. First, the LA may enable an insoluble calcium source to change into a soluble or ionized form, resulting in the increased bioavailability of dietary calcium (*17*). Second, the LA may control the delivery of calcium to the small intestine by delaying the emptying of the stomach (*18*, *19*), which is considered a potentially important determinant of calcium absorption (*9*, *10*). Dietary LA (2.5 g/100 g of diet) increased the intestinal absorption of calcium and phosphorus even in the gastrectomized rats (Table 3). We have speculated that the stimulatory effect of LA on calcium absorption in gastrectomized rats may be associated with a change in the water-insoluble form of calcium salt to a soluble and/or ionized form (*5*, *15*).

As shown in Table 3, dietary LA (2.5 g/100 g of diet) increased the intestinal calcium absorption in the gastrectomized rats, but the lower doses of LA (0.5 and 1.0 g/100 g of diet) did not affect the apparent absorption ratios of calcium. When we previously tested the effect of dietary LA on calcium absorption in rats fed OM, the intestinal calcium absorption was increased even when the LA concentration was 0.5 g/100 g of diet (*11*). We do not know the reason for this difference in results, but it may reflect an impaired capacity in the stomach to convert insoluble dietary calcium into soluble calcium that can be absorbed in the small intestine. Thus the degree of the effect of LA on calcium absorption in the gastrectomized rats was less than in OM-induced achlorhydric rats.

In the present study, dietary LA enhanced calcium absorption and bone calcium content even in the gastrectomized rats. We speculated that the dietary acidity induced by dietary LA might be associated with a dissolving of a water-insoluble form of calcium salt in the diet, thereby facilitating the calcium absorption and resulting in an increased bone calcium content in gastrectomized rats.

We are grateful to Dr. H. Hara of the Department of Bioscience and Chemistry, Faculty of Agriculture, Hokkaido University, for his helpful advice regarding the surgical procedures for gastrectomy.

REFERENCES

1) Morgan DP, Paterson CG, Woods CG, Pulvertaft CN, Fourman P. 1965. Search for osteomalacia in 1228 patients after gastrectomy and other operations on the stomach. *Lancet* **ii**: 1085–1088.
2) Persson P, Gagnemo-Persson R, Chen D, Axelson J, Nylander AG, Johnell O, Håkanson R. 1993. Gastrectomy causes bone loss in the rat: Is lack of gastric acid responsible? *Scand J Gastroenterol* **28**: 301–306.
3) Ohta A, Ohtsuki M, Hosono A, Adachi T, Hara H, Sakata T. 1998. Dietary fructooligosaccharides prevent osteopenia after gastrectomy in rats. *J Nutr* **128**: 106–110.
4) Mahoney AW, Holbrook RS, Hendricks DG. 1975. Effects of calcium solubility on absorption by rats with induced achlorhydria. *Nutr Metab* **18**: 310–317.
5) Allen LH. 1982. Calcium bioavailability and absorption. *Am J Clin Nutr* **35**: 783–808.
6) Eastell R. 1985. Calcium absorption and achlorhydria. *N Engl J Med* **313**: 1481–1482.
7) Sheikh MS, Santa Ana CA, Nicar MJ, Schiller LR, Fordtran JS. 1987. Gastrointestinal absorption of calcium from milk and calcium salts. *N Engl J Med* **317**: 532–536.
8) Persson P, Håkanson R, Axelson J, Sundler F. 1989. Gastrin releases a blood calcium-lowering peptide from the acid-producing part of the rat stomach. *Proc Natl Acad Sci USA* **86**: 2834–2838.
9) Heany RP, Smith KT, Recker RR, Hinders SM. 1989. Meal effects on calcium absorption. *Am J Clin Nutr* **49**: 372–376.
10) Skeikh MS, Santa Ana CA, Nicar MJ, Schiller LR, Fordtran JS. 1988. Calcium absorption: effect of meal and glucose polymer. *Am J Clin Nutr* **48**: 312–315.
11) Chonan O, Takahashi R, Yasui H, Watanuki M. 1998. Effect of L-lactic acid on calcium absorption in rats fed omeprazole. *J Nutr Sci Vitaminol* **44**: 473–481.
12) Ad Hoc Committee on Standards for Nutrition Studies. 1977. Report of the American Institute of Nutrition Ad Hoc Committee on Standards for Nutritional Studies. *J Nutr* **107**: 1340–1348.
13) Chonan O, Watanuki M. 1995. Effect of galactooligosaccharides on calcium absorption in rats. *J Nutr Sci Vitaminol* **41**: 95–104.
14) Zur JH. 1989. Biostatistical Analysis, 2nd ed. Prentice-Hall, Englewood Cliffs, NJ.
15) Recker RR. 1985. Calcium absorption and achlorhydria. *N Engl J Med* **313**: 70–73.
16) Bo-linn GW, Davis GR, Buddrus DJ, Morawski SG, Ana CS, Fordtran JS. 1984. An evaluation of the importance of gastric acid secretion in the absorption of dietary calcium. *J Clin Invest* **73**: 640–647.
17) Ohta A, Osakabe N, Yamada K, Saito Y, Hidaka H. 1993. Effects of fructooligosaccharides and other saccharides on Ca, Mg and P absorption in rats. *J Jpn Soc Nutr Food Sci* **46**: 123–129.
18) Ebihara K. 1996. Effect of lactic acid on postprandial plasma-glucose and -insulin responses in rats administered glucose solution. *Nutr Res* **16**: 1575–1585.
19) Liljeberg HGM, Björck IME. 1996. Delayed gastric emptying rate as a potential mechanism for lowered glycemia after eating sourdough bread: studies in humans and rats using test products with added organic acids or organic salt. *Am J Clin Nutr* **64**: 886–893.