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Enhanced Absorption of Calcium after Oral Administration of Maltitol in the Rat Intestine

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Abstract

The enhancing effects of maltitol (α-D-glucopyranosyl-1,4-sorbitol) on absorption of calcium by the rat intestine have been studied by use of [^{45}Ca]$CaCl_2$ in-vivo.

After intragastric administration of [^{45}Ca]$CaCl_2$ solution with maltitol, plasma ^{45}Ca concentration remained at the maximum level for more than 80 min, whereas for animals given [^{45}Ca]$CaCl_2$ solution without maltitol, plasma ^{45}Ca concentration declined sharply after the peak. Determination of ^{45}Ca radioactivity remaining in the various segments of the gastrointestinal tract revealed that administration of maltitol elicited slower gastric emptying and slower intestinal transit, resulting in extensive ^{45}Ca distribution along the small intestine throughout the experimental period. The luminal contents of the small intestine were significantly higher in rats given maltitol than in the control group.

These results suggest that the enhancing action of maltitol on intestinal calcium absorption could be attributed to reduced gastrointestinal calcium transit and increased luminal fluid content, presumably because of the osmotic activity of maltitol; this would not only accelerate the dissolution of calcium into the increased luminal contents, but also enable a larger area of the small intestine to absorb calcium for a longer period of time.

Maltitol (α-D-glucopyranosyl-1,4-sorbitol), a sugar alcohol produced by the hydrogenation of maltose, has been used as a filler in solid pharmaceuticals and as a sweetener in many foods. The enhancing effects of maltitol on intestinal calcium absorption were first reported by Goda et al (1992), who demonstrated that the consumption of a 10% maltitol diet by rats resulted in elevated calcium absorption. In-vitro experiments using everted ileal segments of rats suggested that maltitol accelerated passive diffusion of calcium in the lower part of small intestine (Goda et al 1993; Kishi et al 1996). Another disaccharide sugar alcohol, lactitol (Ammann et al 1988) and the monosaccharide sugar alcohols sorbitol (Vaughan & Filer 1960; Suzuki et al 1985), mannitol (Armbrecht & Wasserman 1976) and xylitol (Hämäläinen et al 1985) were reported to increase intestinal calcium absorption. Although it has been assumed that the enhancement of intestinal calcium absorption by maltitol and other sugar alcohols results from enhancement of passive diffusion (Bronner 1987; Goda et al 1993; Kishi et al 1996), which might be triggered by interactions of the sugar alcohols with the brush-border membranes (Suzuki et al 1985; Kishi et al 1996), the exact mode of action is unknown. Although intake of large quantities (approximately 20–70 g day^{-1}) of non-digestible saccharides and sugar alcohols causes diarrhoea (Ellis & Krantz 1941; Patil et al 1987), the maximum non-effective dose of maltitol is relatively high, approximately twice that of sorbitol (Koizumi et al 1983a, b). Maltitol is thus a potentially useful agent as an enhancer of the intestinal calcium absorption.

In this study we have investigated the relationship between the gastrointestinal transit and the plasma concentration of orally administered ^{45}Ca using various segments of the gastrointestinal tract and the plasma to clarify the putative factors involved in the maltitol-induced enhancement of calcium absorption in the gastrointestinal tract in-vivo.

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Materials and Methods

Materials

Maltitol was of commercial grade (Towa Chemical, Tokyo, Japan). The specific activity of [^{45}Ca]$CaCl_2$ (Du Pont, Boston, MA) was 485 MBq mg^{-1}, and non-radioactive $CaCl_2$ and HCl were of analytical reagent grade (Kokusan, Tokyo, Japan). Other reagents and solvents were also of analytical reagent grade.

Animals

Male Wistar rats, 190–210 g, 7 weeks (Charles River Japan, Yokohama, Japan), were used. The animals had free access to water and solid laboratory diet (CRF-1, Oriental Yeast, Tokyo, Japan) which contains 1·22 g calcium/100 g diet. The animals were housed in an animal room at 23 ± 2°C and 55 ± 10% relative humidity, and were acclimatized for more than 1 week before use.

Administration of [^{45}Ca]$CaCl_2$ solution

[^{45}Ca]$CaCl_2$ was dissolved in distilled water and diluted with non-radioactive $CaCl_2$ solution to prepare 175 mM $CaCl_2$ solution containing 5 kBq mL^{-1} of ^{45}Ca (control). The experimental solution contained 175 mM $CaCl_2$, 5 kBq mL^{-1} ^{45}Ca and 20% (w/v) maltitol. After a 24-h fast, rats were administered 1 mL of the [^{45}Ca]$CaCl_2$ solutions (7 mg calcium equivalent) into the stomach via a gastric tube.

Determination of ^{45}Ca radioactivity in the plasma and the luminal contents of gastrointestinal segments

Rats were anaesthetized with ether 20, 40, 60, 90 and 120 min after administration of the [^{45}Ca]$CaCl_2$ solution, a separate group of five rats being used at each time-point. An abdominal incision was made, a blood sample (approx. 8–12 mL) was withdrawn from the aorta by means of a heparinized syringe, and the sample was centrifuged at 1600 *g* for 15 min. Scintillator (Pico Fluor 40, Packard, Chicago; 10 mL) was added to the supernatant from the blood sample (plasma; 2 mL) and ^{45}Ca radioactivity was determined by means of a liquid scintillation spectrophotometer (Tri-Carb 2000CA, Packard). The fraction of the exogenously administered calcium recovered in the plasma was computed from the ^{45}Ca radioactivity in the plasma, and expressed as μg calcium equivalent (mL plasma)$^{-1}$.

After collection of the blood sample the gastrointestinal tract was separated into stomach, 5 cm duodenum, four small intestinal segments of equal lengths (upper jejunum, lower jejunum, upper ileum and lower ileum), caecum and colon. The luminal contents of each segment were collected by gentle manual squeezing into a plastic centrifuge tube and then weighed. The collected luminal contents were mixed with HCl (0·1 M; 20 mL), vigorously shaken, and then centrifuged at 1600 *g* for 15 min. ^{45}Ca radioactivity in the supernatant was determined and the amounts of exogenously administered calcium remaining in the luminal contents were computed in the same manner as described above for ^{45}Ca in the plasma.

Determination of total calcium concentration in the plasma

Total calcium concentration in the plasma was determined by the methylxylenol blue method (Calcium E-Test; Wako, Osaka, Japan) 20, 40, 60, 90 and 120 min after administration of the [^{45}Ca]$CaCl_2$ solution, a separate group of five rats being used at each time-point.

Results

Concentration of ^{45}Ca in plasma after administration of the solutions

Figure 1 shows the time-courses of the changes in the concentration of ^{45}Ca radioactivity in plasma, expressed as μg calcium equivalent mL^{-1}, after intragastric administration of the [^{45}Ca]$CaCl_2$ solutions. The radioactivity in the plasma of the maltitol group increased at a similar rate as that of the control group until 40 min after administration.

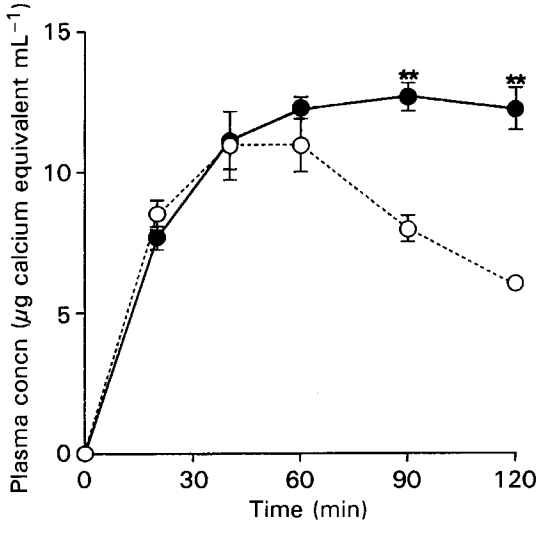

Figure 1. Time-courses of the concentration of radioactivity in plasma after intragastric administration of [^{45}Ca]$CaCl_2$ solution without (○) and with (●) maltitol. The concentration of exogenously administered calcium in the plasma was computed from ^{45}Ca radioactivity, and expressed as μg calcium equivalent mL^{-1}. Each point represents the mean ± s.e.m. (n = 5). $^{**}P < 0.01$, significantly different from the result for the control group given [^{45}Ca]$CaCl_2$ without maltitol.

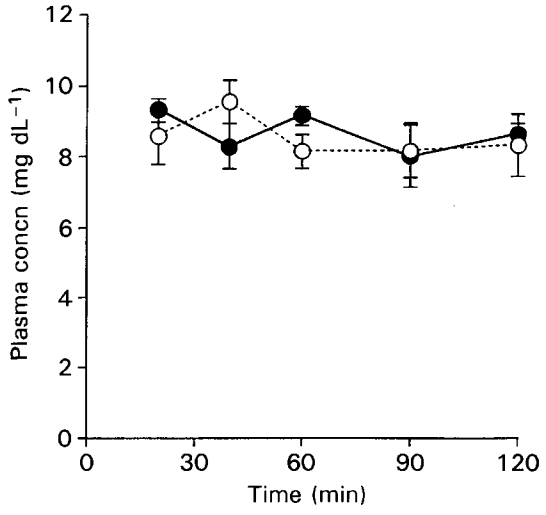

Figure 2. Total concentration of calcium in plasma after intragastric administration of [^{45}Ca]$CaCl_2$ solution without (○) and with (●) maltitol. Each point represents the mean ± s.e.m. (n = 5).

The radioactivity in the plasma of the control group reached a maximum at 40 min and then gradually decreased towards a low level (approx. 50% of the maximum) at 120 min whereas the radioactivity in the plasma of the maltitol group reached a plateau approximately 60 min after administration and remained high until 120 min, the longest period examined; the values for the maltitol group were significantly ($P < 0.01$) greater than those for the control group 90 min and 120 min after administration. As shown in Figure 2, there was no difference between the total concentration of plasma calcium in the control and maltitol groups throughout the experimental periods after intragastric administration of the [^{45}Ca]$CaCl_2$ solutions.

Luminal ^{45}Ca remaining after administration

The amounts of ^{45}Ca radioactivity remaining in the various segments of the gastrointestinal tract were determined at various times after intragastric administration of the [^{45}Ca]$CaCl_2$ solution. As shown in Figure 3, in both groups of animals distribution of the luminal ^{45}Ca was shifted to the lower parts of the intestine with increasing time after administration. In animals given [^{45}Ca]$CaCl_2$ without maltitol a major fraction of the ^{45}Ca was evacuated from the stomach within 20 min of the administration of [^{45}Ca]$CaCl_2$; 60 min after administration more than 90% of the remaining ^{45}Ca was recovered from the ileal segments. After 90 min and 120 min large amounts of ^{45}Ca were present in the lower ileum. In contrast, for animals given [^{45}Ca]$CaCl_2$ solution with maltitol at all the time-points examined a significantly greater

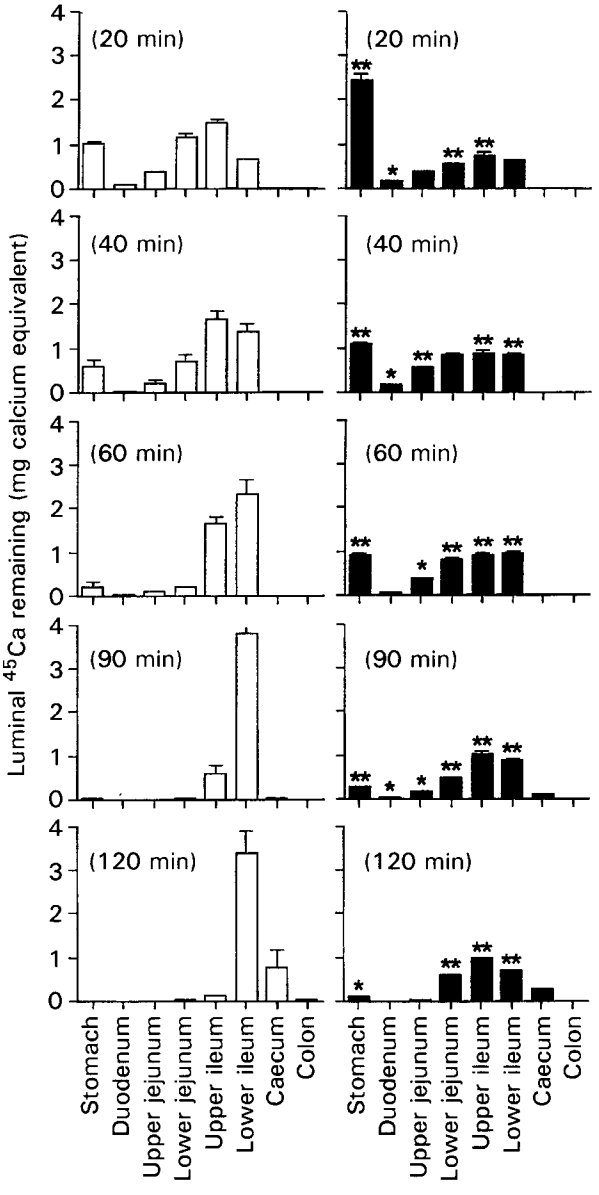

Figure 3. Distribution of luminal ^{45}Ca remaining at various times after intragastric administration of [^{45}Ca]$CaCl_2$ solution without (□) and with (■) maltitol. Each column represents the mean and s.e.m. (n = 5). *$P < 0.05$, **$P < 0.01$, significantly different from the value for the corresponding segment of the control group given [^{45}Ca]$CaCl_2$ without maltitol.

amount of ^{45}Ca radioactivity remained in the stomachs than in those of the control group, indicating slowed gastric emptying in animals given maltitol. At 40 min and thereafter dispersed distribution of ^{45}Ca in the small intestine was observed for the maltitol group; no apparent accumulation of ^{45}Ca radioactivity was observed in any specific segment. For both groups of animals a negligible amount of ^{45}Ca radioactivity was found in the caecum and the colon until 120 min after the administration of [^{45}Ca]$CaCl_2$. Figure 4 shows the changes in the

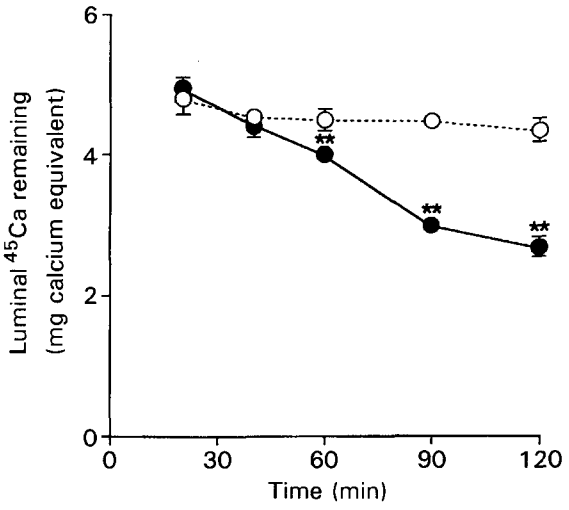

Figure 4. Exogenous calcium remaining in the whole gastrointestinal tract after intragastric administration of [^{45}Ca]$CaCl_2$ solution without (○) and with (●) maltitol. Each point represents the mean ± s.e.m. (n = 5). **$P < 0.01$, significantly different from the result for the control group given [^{45}Ca]$CaCl_2$ without maltitol.

amounts of ^{45}Ca remaining in the whole gastrointestinal tract. The amount of ^{45}Ca remaining in the whole gastrointestinal tract 20 min and 40 min after administration of [^{45}Ca]$CaCl_2$ did not differ significantly between the two groups. Between 40 min and 120 min after administration the level of ^{45}Ca in the lumen of the whole gastrointestinal tract of the control group was unchanged, whereas that of the maltitol group decreased linearly with time; after 120 min the amount of ^{45}Ca in the whole gastrointestinal tract of the maltitol group was approximately 40% less than in that of the control group.

Effect of maltitol administration on the luminal contents of the gastrointestinal tract

Figure 5 shows the changes in the luminal contents of the various segments of the gastrointestinal tract after intragastric administration of control and maltitol solutions. The luminal contents of the stomach and small intestine contained an abundance of water and fluid, whereas those in caecum were of a similar consistency to mud. Throughout the experiment the stomach contents of the maltitol group were significantly ($P < 0.01$) higher than those of the control group. At 40 min and 60 min after administration the luminal contents of the upper and lower jejunum were significantly higher (approx. 100%; $P < 0.01$) in animals given the solution with maltitol than in the control group. At 90 min and 120 min after administration the luminal contents both of the jejunal and ileal segments were significantly ($P < 0.05$) higher for animals given maltitol–$CaCl_2$ solution than for animals of the control group. The luminal contents of caecum and colon of the maltitol group were similar to those of the control group throughout the experiment. Figure 6 shows the changes in the luminal contents of the whole gastrointestinal tract after intragastric administration of the [^{45}Ca]$CaCl_2$ solution. The amount obtained from the control group gradually decreased with time after administration whereas that from the maltitol group was unchanged with time and remained high throughout the experiment. As a consequence the total contents were significantly ($P < 0.01$) higher in the maltitol group than in the control group.

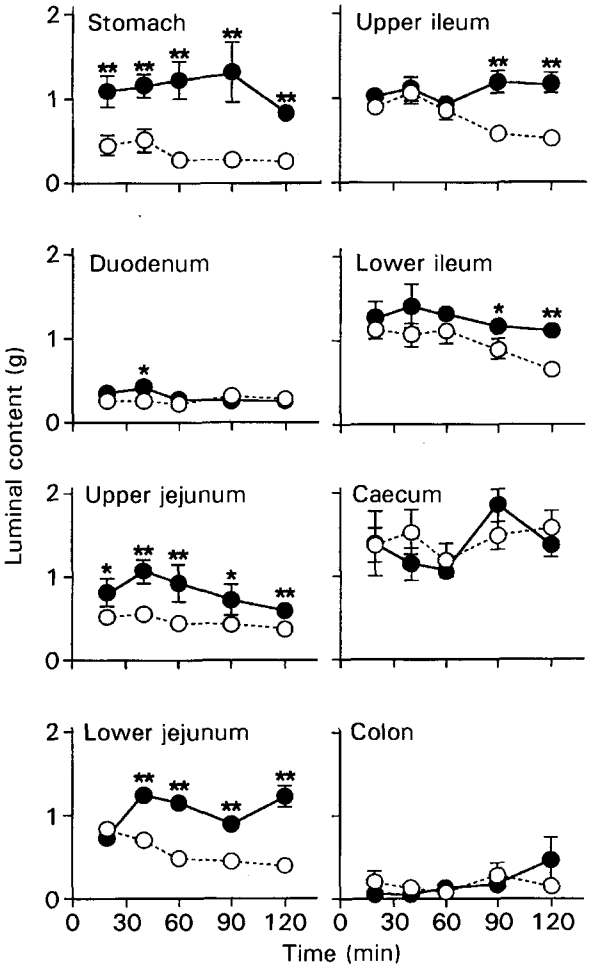

Figure 5. Luminal contents of different segments of the gastrointestinal tract of rats after intragastric administration of [^{45}Ca]$CaCl_2$ solution without (○) and with (●) maltitol. Each point represents the mean ± s.e.m. (n = 5). *$P < 0.05$, **$P < 0.01$, significantly different from the value for the corresponding segment of the control group given [^{45}Ca]$CaCl_2$ without maltitol.

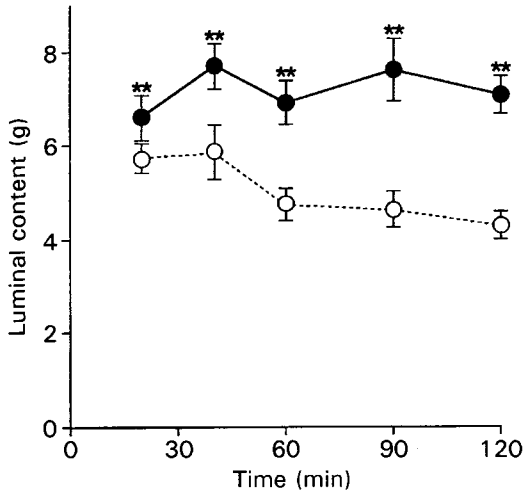

Figure 6. Luminal contents of the whole gastrointestinal tract after intragastric administration of [^{45}Ca]$CaCl_2$ solution without (○) and with (●) maltitol. Each point represents the mean ± s.e.m. (n = 5). $^{**}P < 0.01$, significantly different from the result for the control group given [^{45}Ca]$CaCl_2$ without maltitol.

Discussion

This study supported and expanded the results of a previous study (Goda et al 1992) which showed, by means of calcium-balance study, that intestinal absorption of calcium was enhanced by administration of maltitol. By using [^{45}Ca]$CaCl_2$ as an indicator of exogenous calcium we found that the maximum concentration of exogenous calcium in the plasma derived from oral administration of calcium solution accounted for approximately 13% (control group) or 15% (maltitol group) of total plasma calcium, suggesting a remarkable contribution of absorbed calcium to the total plasma calcium pool. Nevertheless plasma calcium concentrations were unchanged after oral administration of calcium solution in both control and maltitol groups (Figure 2), and were within the normal range reported in the literature (Tajima 1989). It was thus confirmed that the plasma calcium level was strictly controlled by homoeostasis. An increase in intestinal calcium absorption might, however, contribute to an increase in the calcium content of bones and an increase in their breaking force (Goda et al 1995).

According to the results of experiments showing that greater amounts of ^{45}Ca remained in the stomachs of the maltitol group than in those of the control group at all time-points examined (Figure 3) maltitol seemed to slow down gastric emptying of calcium solution. This behaviour seems to be attributable to the osmotic activity of maltitol, which might have caused exudation and inhibited absorption of water by maltitol (Niwa et al 1980). Despite the difference in gastric emptying of calcium, the same rate of increase in plasma ^{45}Ca radioactivity was observed for the control and maltitol groups until 40 min after administration (Figure 1). This result indicates that the calcium transferred to the small intestine of rats given maltitol was more efficiently absorbed than it was by control rats. It is possible that delay in gastric calcium emptying might have enabled the upper part of small intestine to absorb calcium for longer. Active calcium transport, a saturable transcellular process, occurs predominantly in the upper part of small intestine, whereas passive calcium diffusion, a non-saturable paracellular process, takes place throughout the whole length of the intestine (Pansu et al 1983). The maltitol-induced increase in calcium absorption was more prominent at time-points after 60 min, when a large portion of the ^{45}Ca was already transferred to the lower part of the small intestine. By use of the intestinal sac technique of everted rat ileum it has been shown that sugar alcohols including maltitol elicited 1·6–2·6 times greater absorption of calcium, and this increase was completely inhibited by a calmodulin antagonist which presumably involved an inhibitor of paracellular path (Kishi et al 1996). Thus both active transport in the upper small intestine and passive diffusion of calcium in the lower part seem to be enhanced by administration of maltitol.

Intake of large quantities of non-digestible saccharides and sugar alcohols cause diarrhoea as a result of osmotic activity and bacterial fermentation (Itoh & Matsuo 1992; Kishi et al 1996). Maltitol is known to be only slightly hydrolysed (approximately 10%; Oku 1996), and absorbed (approximately 8%; Oku et al 1971) in the small intestine. When the gastrointestinal tracts were removed and the luminal contents collected, the contents in the dilated small intestine of rats given maltitol were rich in water. The greater contents of the gastrointestinal tracts of the maltitol groups must be attributed to the osmotic activity of maltitol resulting from its non-digestibility and non-absorbability. Intestinal calcium might be precipitated by secreted HCO_3^- ion owing to the formation of sparingly soluble $CaCO_3$. A large amount of exuded water might be advantageous for the dissolution of calcium in the luminal contents. The absorbable saccharides might not elicit the dissolution of calcium in the small intestine, because they are easily removed from the lumen by intestinal absorption.

A continuous decline of ^{45}Ca radioactivity remaining in the whole gastrointestinal tract was measured in rats given maltitol (Figure 4), and long-lasting maximum levels of ^{45}Ca in the plasma

were observed for this group (Figure 1). Remarkable accumulation of ^{45}Ca was observed in the lower part of the small intestine of the control group, whereas there was no accumulation of ^{45}Ca in the same part by the maltitol group and the distribution of ^{45}Ca in the small intestine was very extensive (Figure 3). This extensive distribution of ^{45}Ca in the small intestine of the maltitol group, presumably because of delayed gastric emptying and slow intestinal transit, will probably result in an increase in the absorption area for calcium in the upper and lower small intestine. In the lower small intestine, maltitol-induced elevation of fluid content (Figure 5) would accelerate the dissolution of calcium and the enlargement of the distribution area, thereby enhancing passive diffusion of calcium. This elevation of the fluid content of the small intestine might be common to non-digestible saccharides and sugar alcohols, which enhance calcium absorption (Brommage et al 1993).

In conclusion, the enhancing effects of maltitol on calcium absorption were at least partly attributable to an increase in passive diffusion through the lower part of intestine, primarily as a result of dissolution of calcium into the increased luminal contents, owing to osmotic activity and secondarily as a result of an increase in the distribution area of calcium.

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