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Note

Difructose Anhydrides III and IV Equally Promote Calcium Absorption from the Luminally Perfused Rat Small Intestine

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We examined the effects of di-D-fructose anhydride (DFA) III and IV on Ca absorption in luminally perfused segments of the small intestine in anesthetized rats. The calcium absorption rate with perfusion of 10 mmol/l $CaCl_2$ was similarly increased by addition of 100 mmol/l DFAIII or IV, and these promotive effects of both DFAs were pronounced at perfusion rate of 0.15 ml/min than at 0.3 ml/min. The promotive effects were higher in the duodenojejunum than in the ileum.

Key words: calcium absorption; di-D-fructose anhydride III; di-D-fructose anhydride IV; small intestine

Lower calcium intakes increase the risk of osteoporosis. Calcium deficiency can efficiently be restored by enhancing Ca absorption because the absorption rate of Ca is usually low. It has been reported that highly fermentable nondigestible saccharides, including dietary fiber and oligosaccharides, promote Ca absorption in the large intestine with increasing intestinal organic acid production.[1–3] Previously, we showed that a nondigestible saccharide, di-D-fructofranosyl 1, 2′: 2, 3′ anhydride (DFAIII), increased Ca absorption in rats.[4,5] Di-D-fructofranosyl 2, 6′: 2′, 6 anhydride (DFAIV), an isomer of DFAIII, enhanced Ca transport equally with DFAIII in a rat balance test.[6] But, the promotive effect of DFAIV was much greater than that of DFAIII in an *in vitro* study using the isolated mucosa of the small intestine.[7] The reason for the contradictory results between *in vivo* and *in vitro* studies has not been evident. Also, there has not been direct evidence that DFAs increase Ca transport in the small intestine with *in situ* experiments.

The present study was conducted to compare the promotive effects of DFAIII and IV on Ca absorption in the luminally perfused duodenojejunum and ileum in anesthetized rats.

This study was approved by the Hokkaido University Animal Committee, and the animals were maintained in accordance with the Hokkaido University guidelines for the care and use of laboratory animals.

Male Sprague-Dawley rats, weighing 250 g, were acclimated with a stock diet (CE-2, Clea Japan, Tokyo) for 3 d, and fasted for 1 d before the experiments. A perfusion segment was made in the jejunum or the ileum in each rat with an abdominal midline incision (3 to 4 cm) under pentobarbital anesthesia (sodium pentobarbital, 40 mg/kg). Briefly, a polyethylene catheter (SP 28; I.D. 0.4 mm, O.D. 0.8 mm; Natsume Seisakusyo, Tokyo) was inserted into the bile-pancreatic duct to divert bile-pancreatic juice (BPJ) from the small intestine, because BPJ contains calcium and possibly several factors affecting calcium absorption. After ligation of the pylorus, two small cuts in the small intestine were made just distal of the pylorus and about 10 cm distal from the ligament of Treitz, the lumen was washed out, and two silicone tubes (O.D. 1.0 mm and I.D. 0.5 mm in the proximal cut, O.D. 3.5 mm and I.D. 2.0 mm in the distal cut) were inserted and fixed (a 20-cm segment of the duodenojejunum). The 20-cm ileal segment was similarly made in the just proximal part of the ileum from the ileocecal junction. The rats with catheters were placed in a Bollman-type restraining cage during the perfusion experiment in a temperature controlled room at 25 °C.

A solution containing 10 mmol $CaCl_2$, 30 mmol MOPS (pH 6.5), 0.25 mmol [^{3}H] 1,2-polyethylene glycol 4000 (18.5 MBq/g, New England Nuclear Research Products, Boston, MA) per l with 100 mmol DFAIII or DFAIV, or 50 mmol NaCl (control) was perfused from the catheter of the proximal side at 0.15 ml/min or 0.30 ml/min using a syringe pump (Model sp220i, WPI, Sarasota, FL). The perfusate was collected from the catheter on the distal side every 20 min (0.15 ml/min) or 10 min (0.30 ml/min). We used only a lower perfusion speed, 1.5 ml/min, in the ileum loop because the *in vivo* ileal transit rate is much lower than that of the jejunum.[8] DFAIII and IV were kindly provided by Nippon Beet Sugar MFG Co., Ltd. (Obihiro, Japan).

In a preliminary study to characterize and set up the experimental conditions, we observed the effects of D-

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Abbreviations: DFAIII, di-D-fructofranosyl 1, 2′: 2, 3′ anhydride; DFAIV, di-D-fructofranosyl 2, 6′: 2′, 6 anhydride; ANOVA, analysis of variance

glucose and L-glutamine as energy sources and the dose-dependency of DFAIII on Ca absorption using ligated jejunal loops in rats under the same conditions as in the perfusion experiments. The two sets of experiments were done in 15-cm ligated segments just distal from the Treitz ligament by an instillation of test solution for 20 min. There were no effects from the addition of D-glucose (10 mmol/l) or L-glutamine (6 mol/l) on Ca absorption with or without DFAIII. The means of Ca absorption were: 7.17% without DFAIII, 11.8% with DFAIII, n = 18, $P = 0.007$ for DFAIII (D), $P = 0.609$ for energy source (E) and $P = 0.534$ for D × E. Calcium absorption increased in a dose-dependent and nonsaturable manner up to 150 mmol DFAIII/l in the jejunal loops (Fig. 1).

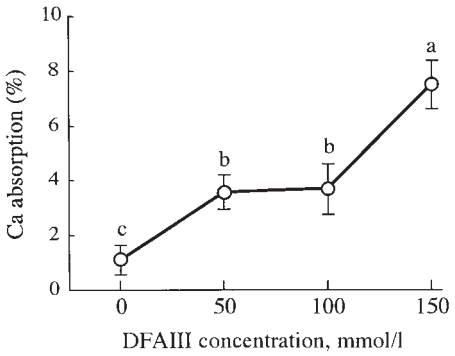

Fig. 1. Calcium Absorption Rate in the Jejunal Segment Instilled Solution with Increasing Concentrations of DFAIII.

Values are means ± SEM (n = 6). The P value for ANOVA was < 0.001. Values not sharing a common letter are significantly different ($P < 0.05$).

Calcium concentration in the perfusate was measured by atomic absorption spectrophotometry (Z-5310, Hitachi, Tokyo) after treatment with perchrolic acid (final 0.5 mol/l). The radioactivity of [^{3}H] 1,2-polyethylene glycol was measured with a liquid scintillation system (LSC-5100, Aloka, Tokyo) with a scintillation cocktail containing 32 mmol DPO and 1 mmol dimethyl-POPOP/l of toluene:ethylene glycol monoethyl ether (1:1).

Calcium absorption rates were calculated using the following equation:

$$\text{Absorption rate (\%)} = 100 \times (\text{1,2-polyethylene glycol/calcium in collected perfusate}) / (\text{1,2-polyethylene glycol/calcium in infused solution})$$

Each value represents the mean ± SEM. The results were analyzed by one-way analysis of variance (ANOVA). Duncan's multiple-range test[9] was used to determine whether mean values were significantly different between groups ($P < 0.05$).

The addition of both DFAs to the perfusion medium clearly increased Ca absorption in the jejunal segment (Fig. 2). These results agree with the findings of the *in vitro* experiments with isolated mucosa of the small intestinal,[7,10] and are concrete and direct evidence for enhancement of Ca absorption by DFAs in the small intestine.

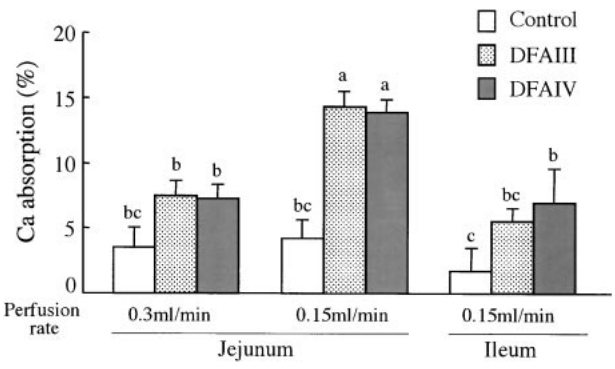

Fig. 2. Calcium Absorption Rate in the Jejunum and Ileum with Perfusion of Test Solution Containing 100 mmol DFAIII or DFAIV/l or 50 mmol NaCl/l at 0.15 or 0.3 ml/min.

Values are means ± SEM (n = 6). The P value for ANOVA was < 0.001. Values not sharing a common letter are significantly different among all the groups ($P < 0.05$).

The increased absorption rates with DFAIV perfusion were very similar to those with DFAIII at both 0.15 ml/min and 0.3 ml/min in the jejunal segments of anesthetized rats, which agrees with the results of *in vivo* balance studies,[4,6] but does not agree with the results using the isolated intestinal mucosa. The calcium absorption rate stimulated by DFAIV was much higher than that by DFAIII in the experiment with stripped mucosa of the jejunum and ileum.[7] The reason for the contradiction between the results of *in situ* and *in vitro* experiments is not known. The rate of mucus secretion or blood flow in the isolated intestinal mucosa might different from the intestine in the *in vivo* or *in situ* condition. DFAIII or IV promotes paracellular transport by direct action to the mucosal epithelial cells dependent on their chemical structure, and this effect might closely reflect the absorption rate in the isolated mucosa independent of blood flow. The blood flow rate might be a rate-limiting step for absorption through the simple diffusion pathway promoted by DFAIII or IV in *in vivo* or *in situ* experiments.

The present results indicated that lowering perfusion speed considerably increased calcium absorption. At a perfusion rate of 0.3 ml/min, Ca absorption with both DFAIII and IV was approximately doubled compared with that in the control perfusion, and absorption with both DFAs were 3 times higher at the low perfusion rate. Previously, we found that DFAIII promotes Ca absorption by activating the paracellular transport pathway

through tight junction.[10] It has been reported that transit speed in the small intestine is an important factor for determination of diffusional Ca transport *via* the paracellular pathway, especially at high Ca levels in the diet.[11] We used a perfusion medium with a high but physiological Ca concentration, 10 mmol/l,[12] much higher than the *K*t of active Ca transport, 0.32 mmol/l, in the duodenum.[13] Also, we found a nonsaturable increase in Ca absorption up to 150 mmol DFAIII/l. This confirms that diffusional transport through tight junction is responsible for the promotion of Ca absorption by DFA.

Calcium absorption in the ileum loops was lower than those in the jejunum loops in all perfusions. The rates of calcium absorption with both DFAIII and IV in the ileum were less than half those in the jejunum. It has been reported that the paracellular transport of Ca is done mainly in the ileum,[14] but the transit speed was much slower in the ileum than in the jejunum.[8] Perhaps our results do not disagree with the previous results because the much slower transit speed might restore the lower absorptive rate in the ileum.

In conclusion, the nondigestible saccharides DFAIII and DFAIV enhance calcium absorption to the same degree in the jejunum of anesthetized rats. The calcium absorption rate for the ileum was much lower than that for the duodenojejunum in the present condition.

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