Difructose Anhydride III Enhances Zinc Absorption in vivo: Zinc Gluconate is More Suitable for the Effects of Difructose Anhydride III than Zinc Yeast

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Summary We examined the effect of difructose anhydride III (DFAIII) on zinc absorption in rats in two experiments. First, we compared the absorption of zinc from zinc gluconate and zinc yeast with or without DFAIII by measuring concentrations in plasma collected from the portal vein after a single oral administration of zinc gluconate or zinc yeast with or without DFAIII. The peak plasma zinc concentration and the incremental area under the curve (IAUC) of plasma zinc 6 h after administration were highest in the zinc gluconate with DFAIII group. Second, we examined the effect of DFAIII on the absorption of zinc with phytic acid. Plasma zinc levels in groups both with and without DFAIII were nearly the same as those at the baseline. Our results showed that DFAIII was better with zinc gluconate than zinc yeast for zinc absorption, and that DFAIII does not alter the inhibitory effects of phytic acid on zinc absorption.

Key Words: DFAIII, zinc absorption, zinc gluconate, zinc yeast, phytic acid

Introduction Zinc is an essential trace element for plants, animals and humans. It plays an important role in numerous biochemical mechanisms such as immunity actions on several hormones, and more than 200 enzymes are zinc dependent [1]. Humans and animals experiencing zinc deficiency exhibit a wide variety of symptoms, including impaired growth, anorexia, impaired sexual development, geophagia, dwarfism, anemia, and dermatitis [2]. Human zinc deficiency has been reported worldwide [3–5]. Zinc supplementation is increasingly being used in important fields of research, such as in the prevention or improvement of the above symptoms [6, 7]. Although the symptoms of severe zinc deficiency are obvious, assessment of marginal zinc deficiency is difficult due to the lack of clinical signs and reliable laboratory indicators.

In recent years, difructose anhydride III (DFAIII) has been suggested to have a positive effect on calcium, magnesium and zinc absorption in vitro [8]. As its safety has been demonstrated by acute and sub-acute toxicity test, DFAIII is a candidate as a food supplement to improve calcium insufficiency. A small amount of DFAIII exists in chicory tubers; with the natural occurrence in caramel and roasted chicory root has been measured at about 2%. This amount is not sufficient to meet the high demands for both scientific and industrial purposes. DFAIII can be produced in large quantities with high purity from inulin using inulase II from Arthrobacter sp. H65-7 [9].

DFAIII has been found to stimulate calcium absorption and increase bone mineral density, and to stimulate iron absorption and prevent anemia in rats [10, 11]. The two proposed mechanisms for the promotion of calcium absorption are the following: 1) intact DFAIII stimulates paracellular mineral absorption by increasing the passage of tight junctions in the small and large intestine [12, 13]; and 2) ingested...
DFAIII is fermented in the large intestine, producing short-chain fatty acids that promote calcium absorption [10, 14]. The promoting effect of DFAIII on calcium absorption has been demonstrated by in vivo balance studies and human urinary calcium excretion [15] and balance studies. Compared to the information on calcium absorption, there is no information about DFAIII’s effect on zinc absorption from in vivo and human studies. Further, there is little information about zinc absorption from zinc gluconate or zinc yeast, which has been approved as food ingredients by the Health, Labour and Welfare Ministry in Japan.

Some food components as known to have adverse effects on zinc absorption. Phytic acid is a P storage substance found in foods such as beans, tubers and cereal grains. Ingestion of phytic acid substantially reduces zinc bioavailability due to the formation of insoluble salts [16]. Phytic acid serves as an excellent chelator of mineral ions such as calcium, zinc, iron. We hypothesized that DFAIII might suppress the inhibitory effects of phytic acid on mineral absorption because some food components, namely, highly fermentable types of insoluble dietary fiber and casein phosphopeptides (CPP), are known to suppress this effect of phytic acid [17].

In this study, we examined the effect of DFAIII ingestion on zinc absorption in rats by measuring zinc concentration in blood collected from the portal vein over a 6-h period after a single administration of zinc with or without DFAIII and compared the effects of two zinc sources, zinc gluconate and zinc yeast. In addition, we also investigated the effect of DFAIII on zinc absorption after a single oral administration of zinc with phytic acid.

**Materials and Methods**

**Experimental animals**

Six-week-old male Sprague-Dawley rats weighing 220–240 g (Kudo Ltd., Kumamoto, Japan) were housed in plastic cages. Temperature and humidity were maintained at 20–24°C and 50–60%, respectively. The lighting was controlled for a 12-h light-dark cycle (8:00–20:00 of light).

**Chemicals**

We used DFAIII (>97%) manufactured by Nippon Beet Sugar Mfg. Co., Ltd. (Tokyo, Japan). DFAIII has two glyco-side linkages between two fructose moieties (Fig. 1). Zinc gluconate and phytic acid were purchased from Wako Pure Chemical Industries (Osaka, Japan). Zinc yeast was purchased from Miwa Seiyaku Co., Ltd. Zinc gluconate has a bound structure of gluconic acid, which is a kind of organic acid, and zinc, is easily dissolvable and ionizes easily in aqueous solution. Zinc yeast has loosely bound structure of yeast protein and zinc, and is practically insoluble in water.

**Experimental procedure**

The animals were fed a commercial stock diet (MF, oriental Yeast, Tokyo, Japan) ad libitum for a 1-week adaptation period and then deprived of the stock diet for 18 h before portal cannulation. A portal cannula (polyethylene tube 0.28 mm i.d., 0.61 mm o.d.) filled with heparinized saline solution except at sampling times was directly inserted into the portal vein at day 8. This operation and the experiments were performed under anesthesia with diethyl ether.

**Effect of difference in zinc sources on the promotive effect of zinc absorption by DFAIII (Experiment 1)**

Rats were divided into 5 groups. The rats of one group were orally administered distilled water only as a control. Rats of the zinc yeast and zinc yeast with DFAIII groups were orally administered a distilled water suspension of zinc yeast (10 mg Zn/kg body weight) with or without DFAIII (1 g DFAIII/kg body weight), respectively. Rats of the zinc gluconate and zinc gluconate with DFAIII groups were administered a distilled water solution of zinc gluconate (10 mg Zn/kg body weight) with or without DFAIII (1 g DFAIII/kg body weight).

An adequate volume of heparin in saline was injected through the portal cannula before portal blood sampling; then, 200 µl of blood was collected at 0, 0.5, 1, 2, 4, and 6 h from the portal vein of each animal under anesthesia with diethyl ether. Blood samples were promptly collected into a polypropylene tube containing heparin-Na and centrifuged at 5000 × g for 10 minutes. The supernatant was separated as a plasma sample and kept frozen at –80°C until analysis.

**Effect of phytic acid on the promotive effect of zinc absorption by DFAIII (Experiment 2)**

Rats were divided into 3 groups. One group was administered distilled water as a control. The other groups were administered zinc gluconate and phytic acid with or without DFAIII (zinc: 10 mg/kg body, DFAIII 1 g/kg body, phytic acid; 1 g/kg body). Procedures were the same as in experiment 1.

**Analysis**

Plasma zinc concentrations were measured by a commer-
cially available kit from Wako Pure Chemical (Zinc-Test).

Statistical analysis

Data are shown as means ± SE. The incremental area under the zinc curve (IAUC) was calculated according to the method described by Wolever and Jenkins [18]. In experiment 1, the plasma zinc data was analyzed by three-way ANOVA to determine the main effects of DFAIII, the type of zinc, the time course of changes and the interactions with respect to plasma zinc concentration. IAUC was analyzed by two-way ANOVA, and Turkey’s test was used for comparison of means within a factor. In experiment 2, the plasma zinc data were analyzed by two-way repeated measures ANOVA. Differences were considered significant at \( P < 0.05 \). Statistical analysis was performed using by Stat View 5.0 (SAS Institute, Inc., Cary, NC, USA).

Results

Effect of difference in zinc sources on the promotive effect of zinc absorption by DFAIII (Experiment 1)

Time courses of plasma zinc concentrations after oral administration of zinc gluconate or zinc yeast with or without DFAIII are shown in Fig. 2. The basis values of the plasma zinc concentrations in all groups were not significantly different. In the zinc gluconate with DFAIII group, the plasma zinc concentration at 0.5–4 h after administration was significantly higher than those of the other groups.

Three-way ANOVA showed that the three main effects (DFAIII, zinc and time) and the interaction (DFAIII and zinc) were significant (Table 1).

The IAUCs of plasma zinc during the period 0–6 h after oral administration are shown in Fig. 3. The IAUC of plasma zinc in the zinc gluconate group was almost the same as that of zinc yeast. However, the IAUC of plasma zinc in the zinc gluconate with DFAIII group was more than 2 times that of the zinc yeast with DFAIII group. The IAUC of plasma zinc in zinc gluconate with DFAIII was significantly higher than those of all other groups (\( P < 0.05 \)).

Table 1. Three-way ANOVA of plasma zinc in the portal vein in rats administered zinc gluconate or zinc yeast with or without DFAIII.

<table>
<thead>
<tr>
<th>Main effects</th>
<th>Interactions</th>
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<tbody>
<tr>
<td>DFAIII (D)</td>
<td>D × Z</td>
</tr>
<tr>
<td>Zinc (Z)</td>
<td>D × T</td>
</tr>
<tr>
<td>Time (T)</td>
<td>Z × T</td>
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<td></td>
<td>D × Z × T</td>
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<tr>
<td>Plasma zinc</td>
<td></td>
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<tr>
<td>( P &lt; 0.0001 )</td>
<td>( P = 0.0205 )</td>
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To test the main effects and interaction of DFAIII, the type of zinc and the time course of changes in plasma zinc, three-way ANOVA was used.

NS: Not significant, \( P > 0.05 \).

Fig. 2. Time courses of zinc concentrations in portal vein blood after oral administration of zinc gluconate or yeast zinc with or without DFAIII.

×, distilled water (\( n = 6 \)); ○, zinc gluconate, (\( n = 9 \)); ●, yeast zinc, (\( n = 9 \)); △, zinc gluconate with DFAIII, (\( n = 12 \)), ▲; yeast zinc with DFAIII, (\( n = 8 \)). Each symbol represents the mean ± SE.

Effect of phytic acid on the promotive effect of zinc absorption by DFAIII (Experiment 2)

Time courses of plasma zinc concentrations after oral administration of zinc gluconate and phytic acid with or without DFAIII are shown in Fig. 4. There was no change in plasma zinc after administration in any group.

Discussion

We examined the effect of DFAIII on zinc absorption of rats after a single oral administration of zinc with or without DFAIII by measuring zinc concentrations in portal venous plasma. The plasma zinc concentration in portal blood should be a reflection of the degree of absorption. Therefore, we attempted two experiments: one to elucidate the effect of DFAIII on zinc absorption from zinc gluconate or zinc yeast (Experiment 1) and another to elucidate the effect of DFAIII on the zinc absorption from zinc gluconate with phytic acid (Experiment 2).

In Experiment 1, we followed the time courses of plasma zinc concentrations after a single oral administration of zinc.
Zinc Absorption Increases by DFAIII

Vol. 39, No. 2, 2006

The total increment of the plasma zinc level, IAUC, was determined. The increase in the IAUC of zinc induced by coadministration of DFAIII was significantly greater in the zinc gluconate group than in the zinc yeast group. This result showed that DFAIII had more impact on zinc absorption from zinc gluconate than from zinc yeast. We thought that the difference in zinc absorption between zinc gluconate and zinc yeast with coadministration of DFAIII was due to differences in their ionization characteristics. It has been reported that DFAIII directly affects epithelial tissue by opening the tight junctions located on the luminal side adjacent to epithelial cells, and that increases in calcium, magnesium and zinc absorption occur by the entrance of these ions through the paracellular route regulated by the tight junctions [12, 8]. It is noted that zinc yeast is not very soluble in water, and is difficult to ionize. On the other hand, zinc gluconate exists almost entirely as zinc ions in the body.

Stimulation of zinc absorption by DFAIII has been demonstrated by in vitro experiments [8], but zinc sulfate, which was used in that study, is not approved as a food ingredient or food additive. The two sources of zinc used in this study, zinc gluconate and zinc yeast, are approved as foods in Japan. The results of this study thus provide valuable information for application to functional food ingredients in human diets. Mild zinc deficiency is observed often in children and pregnant women [19, 20]. The supplemental intake of zinc with DFAIII may be more useful for preventing and improving zinc deficiency when zinc gluconate rather than zinc yeast is used.

The issue of zinc transport is complex; mechanisms of intestinal zinc absorption have been partially elucidated [21], but are not yet fully understood. In the small intestine in rats, zinc absorption has been shown to occur in the duodenum, jejunum, and ileum [22]. It has been reported that zinc absorption occurs through the epithelial tissue in the intestine [8]. Zinc absorption is also involved in the zinc transporters, ZnT-1, 2, 3 and 4, which are expressed only in the duodenum and upper jejunum and not in the ileum or colon. From these facts, it is thought that the mechanism by which DFAIII increases zinc absorption may be associated with these transporters as well as the paracellular pathway. We need to carry out further research about the mechanism by which DFAIII increases zinc absorption.

Some food components are reported to reverse the suppression of zinc absorption by phytic acid. CPP keeps zinc in the soluble form at a neutral pH, which is considered important for mineral absorption and stimulation of zinc absorption [17]. In experiment 2, we examined the effect of DFAIII on zinc absorption after a single oral administration of zinc gluconate with phytic acid with or without DFAIII. Ingestion of phytic acid abolished the increase of zinc absorption by coadministration of DFAIII. DFAIII in a single oral administration did not reverse the inhibitory effect of phytic acid on zinc absorption; it might not keep zinc in the soluble form as does CPP.

The large intestine is involved in mineral absorption [23, 24]. DFAIII is known to be involved in promoting calcium absorption in the large intestine [10, 12–14]. Therefore, further research needs to be done in the large intestine on this topic.

DFAIII is especially beneficial for promoting mineral absorption because it has effects in both the small and large intestine [25]. At present, CPP is known to promote mineral absorption only in the small intestine, and insoluble fibers and resistant starch are involved in promoting mineral absorption only in the large intestine. The supplemental
intake of zinc with DFAIII may be more useful for zinc absorption than these components.

In conclusion, it has been demonstrated that a single oral administration of zinc gluconate with DFAIII causes a significant increase in zinc absorption from the small intestine in vivo. Zinc gluconate is more suitable for DFAIII promotion of zinc absorption than zinc yeast.

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References