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Estimation of the Endogenous Pancreatic/Biliary Zinc Pool and the Effect of Phytate and Calcium on Zinc Homeostasis

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Abstract

The pancreas is an important organ in the maintenance of zinc homeostasis. Endogenous zinc is continuously secreted via pancreatic exocrine fluid or to a lesser extent in bile. Much of the endogenous secretion must be reabsorbed to sustain zinc homeostasis. The objective of this study was to estimate the relative size of the pancreatic/biliary zinc pool in comparision to the dietary zinc intake, and to study the effect of the phytate and calcium on the zinc homeostasis using a rat model. At the termination of the experiment, pancreatic/biliary fluid was collected from the rats. Both radioactivity and total zinc were measured and the relative size of the pancreatic/biliary zinc pool was estimated. To determine the effect of phytate and calcium on zinc homeostasis, dietary zinc intake, the amount of zinc in pancreatic/biliary fluid and fecal zinc excretion were measured. The flow rate of pancreatic/biliary fluid, as corrected for tubing constriction, gives the corrected zinc concentration in the pancreatic/biliary fluid was 2.2 times higher than dietary zinc intake. To maintain zinc homeostasis, zinc absorption/reabsorption was very efficient in the current model; 76%, 88% of absorption/reabsorption for low calcium group and high calcium group, and 81%, 85% for phytate group and non-phytate group, respectively.

Key words: zinc homeostasis, pancreatic/biliary zinc pool, calcium, phytate

INTRODUCTION

The endogenous zinc is mainly derived from pancreatic/biliary secretions and the zinc that reenters the intestinal lumen transmucosally. The endogenous zinc sources are important for the maintenance of zinc homeostasis. The pancreas is the most important organ for the source of the endogenous zinc and thus, for zinc homeostasis(1-6). Large amounts of endogenous zinc are recycled via the pancreatic fluid secretion(7,8). The rat has no gallbladder, therefore, the pancreatic/biliary secretion has been considered as a major factor in zinc homeostasis in rat model(9,10). The amount of zinc secreted into the duodenum through the pancreatic duct may be two or more times the dietary zinc intake(11-13). Pekas(12) reported that there was more ^{65}Zn secreted in pure pancreatic fluid than in bile or duodenal secretions of pigs. Pancreatic duct ligation abolished ^{65}Zn from the duodenal aspirate in the dog(8). Matsesheet al.(14), using a perfusion technique, reported an elevated zinc concentration in the duodenum.

For many years, the dietary phytate has been shown to reduce the bioavailability of zinc in laboratory animals and in humans(15-19). The elevation of calcium in the diet containing phytate accentuated the phytate effect (17,20,21). Zinc in the pancreatic fluid at pH 8.0~8.3 is secreted into the upper duodenum with a pH about 6.0. In the gastrointestinal tract, secreted zinc appeared to be ionized and thus vulnerable to phytate complexation.

Oberleas(18) reported the presence of two pancreatic zinc pools. In the first pool, zinc is contained in stable complexes with zinc-dependent enzymes and other large molecular weight proteins. In the second pool, zinc is loosely bound to small molecular weight compounds and is dissociated in the duodenum and thus subject to complexation by phytate and other complexing agents. The latter labile pancreatic zinc pool must be largely reabsorbed to sustain zinc homeostasis.

The objective of this study was 1) to estimate the size of the pancreatic/biliary zinc pool and 2) to study the effect of calcium and phytate on zinc absorption/reabsorption and zinc homeostasis.

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MATERIALS AND METHODS

Experimental design and diets

The experimental design was a randomized complete block design with a 2×2 factorial arrangement of treatments. The rats were fed casein-based semi-purified diets with added phytate and calcium. The two variables were two levels of phtyate, 0 and 4.7g/kg as Na phytate(Sigma Chemical Co., St Louis, MO) and two levels of calcium, 8 and 16g/kg created by adding appropriate levels of calcium carbonate(Fisher Scientific, Pittsburgh, PA). The diets were supplied in aluminum feed cups and the distilled water was supplied ad libitum from the polyethylene water bottles with butyl tubber(neoprene) stoppers.

Animal preparation

Forty eight male Sprague-Dawley rats(Harlan Sprague-Dawley Inc., Indianapolis, IN) were utilized in this experiment. Initial weight was 97~105g. The rats were individually housed throughout the experiment. All animals were fed the low calcium, non-phytate diet for a 3 week growth period to increase body weight. Following the growth period was a 4 week depletion period. The twenty four rats in the low calcium group were fed the casein-based low calcium, phytate containing diet, whereas the twenty four rats in the high calcium group were fed with the casein-based high calcium, phytate containing diet during the depletion period. The deficiency at the end of this period was regarded as mild.

After the 7 week growth and depletion periods, the animals were reassigned by weight to each of four treatment groups for 3 weeks of fecal collection. They were injected with 10μCi ^{65}Zn and placed in individual metabolic cages. The collection period consisted of 2 weeks of initial collection period followed by 1 week after dietary crossover. The groups represented here are as defined in the initial collection period.(Table 1 shows the experimental design and diet plan).

Food consumption and fecal collection

Food consumption was recorded daily for the combined 3 week collection period. Feces were collected at nearly the same time daily throughout the collection period. The feces from each rat for each day was placed in a tube, was counted for radioactivity and stored for determination of the total zinc concentration. The average body weight of the rat at the terminous of the collection periods was 341±7g.

Pancreatic/biliary fluid collection

At the end of experimental period, rats were anesthetized with a mixture of Ketaset(Ketamine HCl, Aveco Co. Inc. Fort Dodge, IA) and Xylazine(1ml/kg body weight), or Na pentobarbital(40mg/kg body weight). Pancreatic/biliary fluid was collected by the modified method of Rolf et al.(22). Intramedic non-radiopaque polyethylene tubing(i.d. 0.28mm, o.d. 0.61mm) was used for pancreatic/biliary duct cannulation.

Fig. 1 shows the rat surgery for the collection of the pancreatic/biliary fluid. The distal segment of the common bile duct was hemisected and the tapered end of the tubing was introduced into the common pancreatic/biliary duct incision until the complete circumference was covered by duct tissue. The external end of the tubing was inserted into the polyethylene tube placed in an ice bath so to promote gravity flow of the pancreatic/biliary fluid. Five ml of 5% bovine serum

Table 1. Experimental design and diet plan

Experimental period	Week	Low Ca groups		High Ca groups	
Growth period	3 weeks	Low Ca and non-phytate (48 rats)			
Depletion period	4 weeks	Low Ca and phytate (24 rats)		High Ca and phytate (24 rats)	
Initial collection period	2 weeks	Low Ca and phytate (12 rats)	Low Ca and non-phytate (12 rats)	High Ca and phytate (12 rats)	High Ca and non-phytate (12 rats)
Crossover collection period	1 week	Low Ca and non-phytate (12 rats)	Low Ca and phytate (12 rats)	High Ca and non-phytate (12 rats)	High Ca and phytate (12 rats)

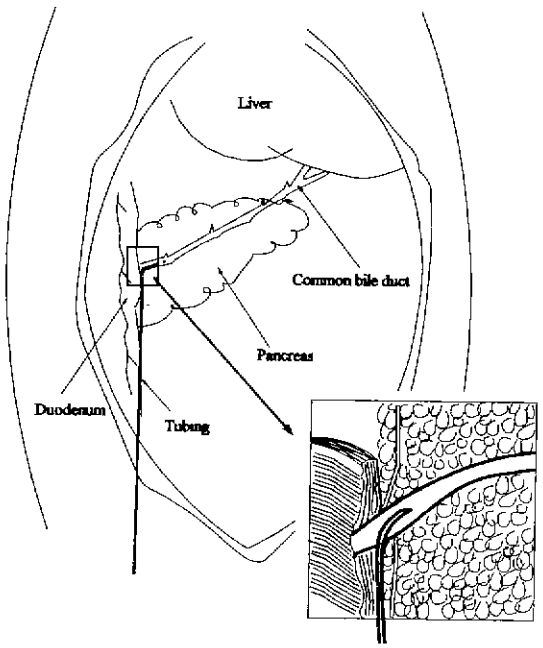

Fig. 1. Illustration of surgery to collect the pancreatic/biliary fluid in the rat.
The tapered end portion of the polyetylene tubing(i.d. 0.28mm, o.d. 0.61mm) was introduced into the common pancreatic/biliary duct incision approximately 1mm above the ampulla, which allowed collection of mixed pancreatic/biliary secretions.

albumin in 0.1M $NaHCO_3$ was infused into the duodenum above the Sphincter of Oddi to stimulate the flow of pancreatic/biliary fluid. Pancreatic fluid was collected until the rat was exhausted.

For the determination of the effect of calcium and phytate on the zinc homeostasis, the flow rate, total zinc concentration in the pancreatic/biliary fluid were measured. ^{65}Zn radioactivity, protein concentration and serum ^{65}Zn radioactivity were also measured; for the weak radioactivity, no difference between dietary groups were shown.

Analysis for zinc

Glass and plastic ware were routinely monitored to avoid contamination. All glassware used in the extraction were cleaned by soaking for 24h in 10% nitric acid, followed by rinsing with a large volume of deionized water. For analysis, samples of diets and feces were dried in ceramic dishes for 24 hours in a convection oven at 105°C. They were then ashed in a muffle furnace with no exposed metal heating elements. The furnace was initially set at 100°C and the temperature raised 50°C every hour to 300°C. The temperature was then raised to 450°C and the samples were ashed for 24 hours. The ashed samples were cooled in a desiccator. To each cooled tube was added 50μl each of 50% hydrogen peroxide and concentrated nitric acid to destroy any organic matter left in the sample(23). Vessels were dried in a dry bath at 100°C for 3 hours and samples were then ashed at 400°C for an additional 12 hours. The drying and ashing procedure was repeated until only white ash remained. After ashing, samples were diluted with 10% nitric acid and analyzed using a Perkin-Elmer Model 5000 atomic absorption spectrophotometer(Perkin Elmer, Inc., Norwalk, CT).

The accuracy of the atomic absorption analysis and the completeness of digestion were verified against the National Institute of Standards Technology(NIST) peach leaves standard(standard reference material 1547). The zinc value found for the standard peach leaves was 17.9μg Zn/g sample compared with 18.3μg Zn/g sample reported by NIST.

Statistical analysis

ANOVA was used for group differences and Scheffe's test were used for the difference between individual groups.

RESULTS AND DISCUSSION

Dietary Zn content and food intake

Dietary zinc content was 6.8μg/g diet for low Ca/phytate group, 6.2μg/g diet for low Ca/non-phytate group, 6.6μg/g diet for high Ca/phytate group, and 6.8 μg/g diet for high Ca/non-phytate group, respectively, which gives that the average dietary zinc intake of four groups is 6.61μg Zn/g diet per day.

During the initial collection period, the food intake for low Ca/phytate group, low Ca/non-phytate group, high Ca/phytate group and high Ca/non-phytate group were 13.9±0.3g, 15.5±0.2g, 15.9±0.5g, and 19.9±0.5g diet/day/rat, respectively($p<0.05$). The average food intake of four dietary group during the initial collection period was 16.33±0.5g diet/day/rat.

Fecal Zn excretion

Daily fecal Zn excretion, during the initial collection period, for low Ca/phytate group, low Ca/non-phytate group, high Ca/phytate group and high Ca/non-phytate group were 85.81±1.23μg/total feces, 52.61±1.36μg/

Table 2. Effect of dietary calcium and phytate on pancreatic/biliary fluid and serum ^{65}Zn radioactivity in four diets[1,2]

Measure	Treatment (Initial collection period / Crossover collection period)			
	Low Ca/phytate	Low Ca/non-phytate	High Ca/phytate	High Ca/non-phytate
	Low Ca/non-phytate (4)	Low Ca/phytate (7)	High Ca/non-phytate (10)	High Ca/phytate (10)
Flow rate[3], ml/h	0.46±0.11^{a}	0.64±0.08^{a}	0.73± 0.04^{a}	0.69±0.06^{a}
Protein[4], mg/ml	23.42±4.89^{a}	24.27±1.57^{a}	27.63± 2.81^{a}	25.33±1.55^{a}
Zinc[5], μg/ml	4.55±1.48^{ab}	3.38±0.87^{ab}	6.40± 1.75^{a}	1.98±0.47^{b}
^{65}Zn radioactivity[6], (pancreatic/biliary fluid) cpm/ml	75.5 ±9.3^{a}	49.3 ±3.2^{ab}	42.0 ±12.5^{ab}	16.6 ±5.3^{b}
^{65}Zn radioactivity[7], (serum), cpm/ml	20.2 ±5.4^{a}	9.1 ±2.2^{b}	8.1 ± 1.7^{b}	3.1 ±1.3^{b}

[1]Values are means±SEM. Each mean is the average of the rats in each group(rat numbers in each groups are shown in the parenthesis)
[2]The mean difference among treatment means were analyzed by Scheffe's test. Values with different superscript within the same row are significantly different at $p<0.05$
[3]Ca effect($p>0.058$), phytate effect($p>0.626$), Ca and phytate interaction($p>0.149$)
[4]Ca effect($p>0.353$), phytate effect($p>0.612$), Ca and phytate interaction($p>0.560$)
[5]Ca effect($p>0.832$), phytate effect($p>0.014$), Ca and phytate interaction($p>0.246$)
[6]Ca effect($p>0.004$), phytate effect($p>0.010$), Ca and phytate interaction($p>0.965$)
[7]Ca effect($p>0.006$), phytate effect($p>0.003$), Ca and phytate interaction($p>0.220$)

total feces, 60.89±0.90μg/total feces, and 32.27±0.92 μg/total feces, respectively($p<0.05$). The average fecal zinc excretion of phytate group and non-phytate group was 73.4±1.07μg/total feces/day and 42.4±1.14μg/total feces/day. The average fecal zinc excretion of low Ca group and high Ca group was 69.2±1.29μg/total feces/day and 46.6±0.91μg/total feces/day.

Analysis of pancreatic/biliary fluid

Flow rate:

Analytical data of pancreatic/biliary fluid are shown in Table 2. Flow rate of pancreatic/biliary fluid among four dietary groups ranged from 0.46±0.11ml to 0.73± 0.04ml per hour with an average flow rate of 0.63± 0.07ml among the four dietary groups. There were no significant differences among the groups.

The flow rate data was similar to that reported by Berger and Schneeman(24). They reported flow rates without protein stimulation of 1.34±0.05ml/h. Stimulation with three different proteins(casein, soyprotein isolate and egg white) produced flow rates of 1.71±0.08ml/h, 1.86±0.09ml/h, 1.62±0.09ml/h, respectively. The tubing size(i.d. 0.68mm, o.d. 1.18mm) which they used was larger than that used in the present study. Finley and Johnson(25) showed a similar flow rate of 1.10±0.05ml/h, under unstimulated conditions and 1.30±0.02ml/h with protein stimulation using PE 10 tubing. Considering all factors, the flow rates of the above and the present study were similar.

Zn concentration:

Average total zinc concentration in the pancreatic/biliary fluid was 3.97±0.76μg/ml for the low Ca groups and 4.19±0.98μg/ml for the high Ca groups. This zinc concentration is similar to that reported by Berger and Schneeman(24). In their study, the zinc concentration in the pancreatic/biliary fluid was 2.69μg/ml without protein stimulation. The zinc conentration after stimulation was casein 4.35μg/ml, soy protein 5.27μg/ml, and egg albumin 4.93μg/ml.

Zn homeostasis and the size of the pancreatic/biliary Zn pool

Pancreatic flow may be restricted by the tubing wall. The tubing size must be considered to estimate the flow rate of pancreatic/biliary fluid in the intact animal. Correction of pancreatic/biliary secretion for tubing size is shown in Fig. 2. The pancreatic/biliary fluid must flow through the smaller inside cross- sectional area($0.14mm^2 \times \pi = 0.0616mm^2$), and is constricted by the tubing wall which can be estimated from the outside area as($0.305mm^2 \times \pi = 0.2922mm^2$). The outside area($0.2922mm^2 - 0.0616mm^2 = 0.2306mm^2$) is 3.74 times the inside area. This restriction difference must be

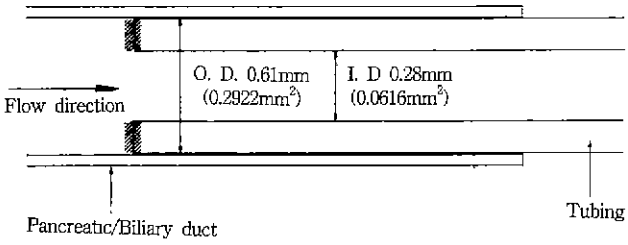

Fig. 2. Model of tubing and pancreatic/biliary duct size on the collection of pancreatic/biliary fluid.
The tubing size was i.d. 0.28mm and o.d. 0.61mm. Only the cross-sectional area of the lumen of the tubing allowed flow of pancreatic/biliary fluid.

utilized to correct the secretion rate to non-cannulated conditions. The average flow rate, 0.63±0.07ml/hr adjusted for tubing then extended for 24 hours gives approximately 56.55ml/day(0.63ml/hr×3.74×24 hrs) of pancreatic/biliary secretion. With average zinc concentration of 4.13μg/ml, the corrected zinc concentration in the pancreatic/biliary fluid for one day would be 233.55μgZn/day(56.55ml/day×4.13μgZn/ml), which is 2.2 times greater than the 107.94μgZn/day dietary zinc intake(16.33g dietary intake/day×6.61μgZn/g diet). These data support the fact that the pancreatic/biliary secretion of zinc is two or more times greater than the zinc consumed each day(8,12-13) and thus the reabsorption of this pancreatic/biliary secretion is important to maintain zinc homeostasis in the body. In the present study, zinc in urine, hair and nails were not estimated.

Effect of dietary Ca on Zn homeostasis

Mean zinc intake for one day was 95.3±1μgZn/day in the low Ca dietary groups and 120.1±3μgZn/day in the high Ca dietary groups(Fig. 3). The flow rate of the pancreatic/biliary fluid with tubing correction was 49.4±6.3ml/day(0.55ml/h×24h×3.74) in the low Ca groups and was 63.7±2.7ml/day(0.71ml/h×24h×3.74) in the high Ca groups. The zinc concentration in the pancreatic/biliary secretion corrected for flow constriction was 196±7.6μgZn/day in the low Ca groups and was 267±3.0μgZn/day in the high Ca groups. Thus, the duodenal zinc pool for one day considered as the sum of the dietary zinc intake and pancreatic/biliary zinc secretion was 291.3±9μgZn/day in the low Ca groups and was 387.1±6μgZn/day in the high Ca groups. The fecal zinc excretion was 69.2±1μgZn/day or 24% of the duodenal zinc pool in the low Ca groups and 46.6±1μgZn/day or 12% of the duodenal zinc pool in the high Ca groups. Absorbed zinc fraction from

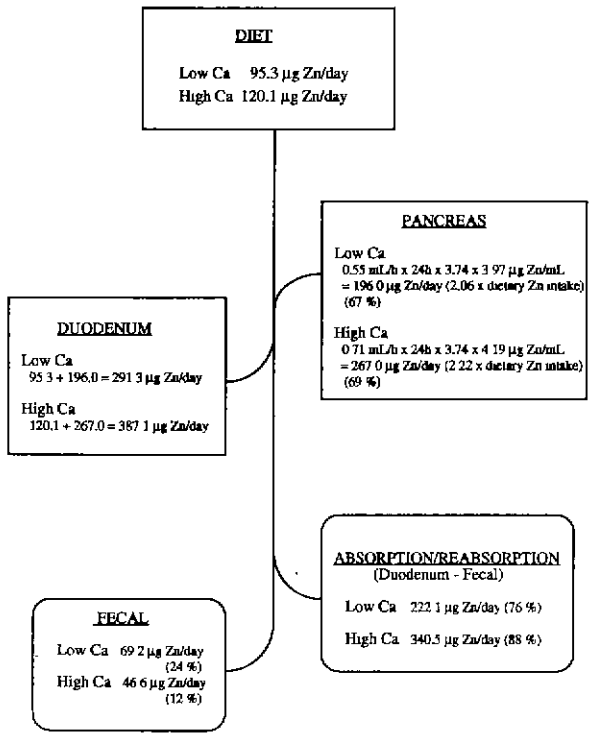

Fig. 3. Mathematics of calcium effect on zinc homeostasis.
Calculation of fecal zinc excretion, true absorption and pancreatic/biliary zinc pool during the initial collection period. Zinc in urine, hair and nails were not estimated.

the duodenal zinc pool was 222.1±8μgZn/day which was 76% of duodenal zinc pool in the low Ca groups and 340.5±5μgZn/day which was 88% of the duodenal zinc pool in the high Ca groups.

The data show that, in both low Ca groups and high Ca groups, the estimates of absorption were 76% absorption/reabsorption in low Ca groups and 88% absorption/reabsorption in high Ca groups.

Effect of dietary phytate on Zn homeostasis

Mean daily zinc intake was 99.7±2μgZn/day in the phytate dietary groups and 115.7±1μgZn/day in the non-phytate dietary groups(Fig. 4). Thus zinc concentration in the pancreatic/biliary secretion corrected for flow rate was 295.1±4.3μgZn/day in the phytate groups and 161.2±11.7μgZn/day in the non-phytate groups. The duodenal zinc pool for one day, the sum of the dietary zinc intake and pancreatic/biliary zinc secretion, was 394.8±6μgZn/day in the phytate groups and 276.9±13μgZn/day in the non-phytate groups. The fecal zinc excretion was 73.4±1μgZn/day or 19% of the duo-

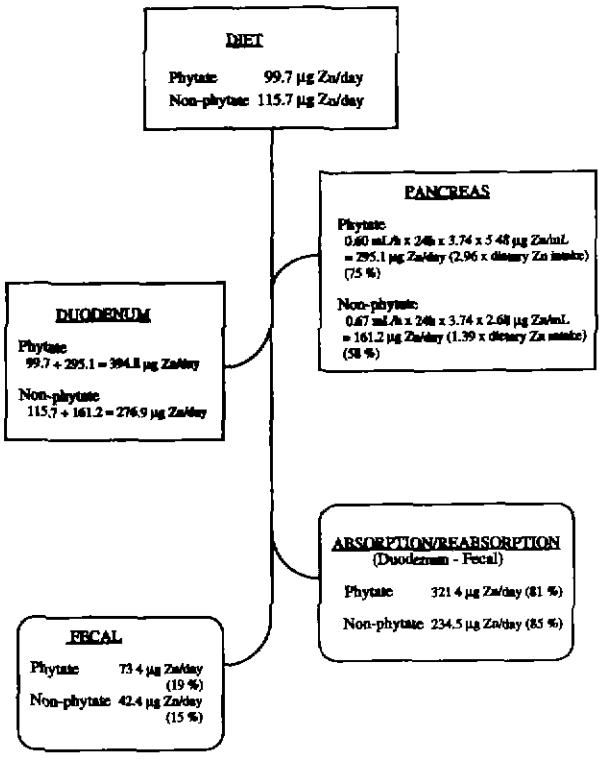

Fig. 4. Mathematics of phytate effect on zinc homeostasis.
Calculation of fecal zinc excretion, true absorption and pancreatic/biliary zinc pool during the initial collection period. Zinc in urine, hair and nails were not estimated.

denal zinc pool in the phytate groups and 42.4±1μg Zn/day or 15% of the duodenal zinc pool in the non-phytate groups. The absorbed zinc fraction was 321.4 ±5μgZn/day or 81% of the duodenal zinc pool in the phytate groups and 234.5±2μgZn/day or 85% of the duodenal zinc pool in the non-phytate groups.

These data were not corrected for urinary zinc excretion or other unsensible zinc losses. These results support the mechanism of zinc homeostasis. The minimum requirement for the zinc homeostasis to sustain the pancreatic/biliary zinc pool for one day is by reabsorption of 295.1±4.3μgZn/day in the phytate groups and 161.2±11.7μgZn/day in the non-phytate groups. It accounts for about 75% in the phytate groups and 58% in the non-phytate groups of the total duodenal zinc pool. The minimum requirement for the zinc homeostasis to sustain the pancreatic/biliary zinc pool for one day is by reabsorption of 196±7.6μgZn/day in the low Ca groups and 267±3.0μgZn/day in the high Ca groups. It accounts for about 67% in the low Ca groups and 69% in the high Ca groups of the total duodenal zinc pool. All animals of four dietary groups exhibited symptoms of mild zinc deficiency. Under the conditions of the current study, all animals were able to sustain the homeostasis at the level permitted by the current diet which showed mild deficiency, but were not able to improve their homeostatic upto the normal zinc nutriture.

In conclusion, the results of the present study indicate: (1) that across all groups for the corrected flow rate, pancreatic/biliary fluid had about 2.2 times higher zinc concentration in the pancreatic/biliary secretion for one day(233.55μgZn/day) compared to the dietary zinc intake(107.94μgZn/day) and (2) that both of the dietary phytate groups and the dietary calcium groups showed sufficient rates of absorption/reabsorption to sustain zinc homeostasis at the mild zinc deficient level, but not for the optimum zinc status level.

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