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Bioavailability

Enhanced intestinal absorption of micronutrients in streptozotocin-induced diabetic rats maintained on zinc supplementation

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ABSTRACT

In view of the deficiency of zinc concomitant with other minerals in diabetic condition, it is desirable to increase the absorption capability of the same by improving the intestinal health. In continuation of our previous report on the virtue of zinc supplementation on diabetic complications, and a significant favourable consequence in the restoration of the compromised structural integrity of small intestines in diabetic situation, it would be relevant to examine the permeability characteristics of the intestines. Groups of hyperglycemic rats were treated for six weeks with supplemental zinc (5-times and 10-times of normal level) to examine its possible influence on intestinal absorption of trace elements zinc, iron and calcium. Everted segments of isolated duodenum, jejunum and ileum portions of small intestine excised from these rats were evaluated for *ex vivo* uptake of iron, zinc and calcium from the incubation medium containing mineral fortified digesta of finger millet as a provider of these trace minerals. The extent of *ex vivo* uptake of zinc, iron, and calcium was severely compromised in the intestinal segments isolated from diabetic rats suggesting the loss of functional integrity concomitant with diminished ultra-structural integrity. This was more prominent in the case of iron uptake followed by that of calcium and zinc. Treatment with supplemental zinc improved the mineral uptake *ex vivo* by the isolated intestinal segments, which was maximum for iron followed by zinc and calcium. This favourable influence was seen more in the jejunal segment probably as a result of improving the expression of applicable transporter protein (s) as observed previously. Thus, dietary zinc supplementation was evinced here to have a promoting stimulus on the intestinal absorption of zinc, iron and calcium, which could encourage a dietary approach to counter the dyshomeostatic state of these trace elements prevalent in diabetes.

1. Introduction

Diabetes is a hyperglycaemic condition consequential of lack of insulin secretion and/ or action and is associated with several secondary complications. This progressive, devastating disorder is due to loss of glycemic control and metabolic homeostasis through erosion of pancreatic β-cell function and insulin resistance [1]. After crossing the reversible pre-diabetes stage, the body develops certain changes to endure serious long-term complications including neuropathy, cardiomyopathy, retinopathy, and nephropathy due to instability in the microvessels and nerves [2]. Among the proposed mechanisms for the commencement of diabetic induced complications, one might be the anomalous homeostasis of trace elements like zinc, iron and calcium.

A connotation between zinc and pancreas proficiency in diabetes is known for a long time. Numerous clinical and epidemiological studies propose that diminished zinc status is allied with diabetes. Diabetes induces zinc dyshomeostasis; inversely, zinc deficiency also was evinced to prompt the risk of diabetes and its complications [3]. Diabetes exhibits undue zinc excretion due to an association of elevated zincuria with the urine volume, polyuria as of osmotic diuresis being one indication of diabetes mellitus which also is evidenced in our previous study [4]. In addition to hyperzincuria, other anticipated mechanisms behind Zn loss comprise dominant intestinal excretion of zinc, which could be due to inept intestinal absorption of this mineral or excretion from the pancreas or could be the both. This well-documented knowledge contradicts an old report that STZ-induced diabetic rats exhibited a higher amount of zinc deposition in hepatic and renal tissue that could be due to a higher daily absorption of dietary zinc [5]. The apparent daily absorption of dietary Zn and Cu per 100 g body wt was three-fold higher in STZ-diabetic rats, while dietary Fe absorption per day was not altered.

Our previous study documented that dietary zinc supplementation hold significant favourable protective influence to maintain diabetic intestinal structural integrity by affording them improved fluidity [Our

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unpublished data] and maintained the zinc efflux by controlling the diabetes induced zinc dyshomeostasis through modulation of the respective zinc transporters in the intestine [6]. In continuation of this study, we extended to understand the virtue of zinc supplementation as to whether it could improve the efficiency of micronutrient absorption in the intestines of diabetic animal.

2. Material and methods

2.1. Chemicals and materials

All the chemicals used in this experimentation were acquired from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA) or SISCO Research Laboratories (Mumbai, India) and were of analytical grade.

2.2. Experimental animals

All the experimental processes in this animal study were legitimated by the Institutional Animal Ethics Committee (CSIR-CFTRI, Mysore, India) and deterrents were taken to lessen pain and distress to the animals. Adult 12 weeks old Wistar female albino rats (140–150 g) were acquired from the Experimental Animal Production Facility of this Institute. Rats were separately housed in stainless steel cages under controlled temperature (25 ± 2 °C) and humidity (40–70%) with 12/12 h light-dark cycle. Rats were fed with *ad libitum* diet and water. Diabetes was persuaded by a single *i.p*. injection of streptozotocin (STZ) (40 mg/kg body weight dissolved in freshly prepared 0.1 M; pH 4.5, citrate buffer). Rats having fasting blood glucose levels > 15 mmol/L, formerly determined by Huggett and Nixon [7], were considered as diabetic for further observations.

2.3. Diets and animal treatment

Normal basal diet (AIN-76) contained were revised with the addition of zinc carbonate as Zn salt and conformed to 5 times (0.32 g/kg diet) and 10 times (0.64 g/kg diet) of RDA. Rats were disseminated into six groups,of which three groups were diabetic (12 rats in each group) and three groups were normal (eight rats in each group) and were maintained on corresponding diets for 6 weeks. The 6 animal groups were as follows: (1) Normal control (N), (2) Normal + Zn-dose 1 (N + Zn-1), (3) Normal + Zn-dose 2 (N + Zn-2), (4) Diabetic control (D), (5) Diabetic + Zn-dose 1 (D + Zn-1), (6) Diabetic + Zn-dose 2 (D + Zn-2).

2.4. In vitro intestinal absorption studies

After sacrificing the experimental animals the small intestine was quickly excised. After thoroughly rinsing both inside and outside with 0.9% saline, it was everted and cut uniformly into 10 cm segments. Uptake of micronutrients – Fe, Zn, Ca, by these segmented intestine isolated from experimental diabetic animals were assessed.

2.4.1. Preparation of finger millet digesta

The finger millet digesta was prepared following the standard procedure established in our lab [8]. In brief, finger millet subjected to replicated gastrointestinal digestion employing pepsin, pancreatin and bile salts with respective temperature and pH. Finger millet powder (10 g) fortified with calcium, iron and zinc salts (calcium phosphate dibasic anhydrous, ferric stearate and zinc sulfate) (to provide calcium, iron or zinc at 5 mg 100 g^{-1} flour) was dissolved in 80 g water in a 250 mL Erlenmeyer flasks. The pH was adjusted to 2.0 by adding 6 M HCl followed by freshly prepared pepsin solution (3 g) and the sample mixture was made up to 100 g with water. The mixing sample was then incubated at 37 °C in a shaking water bath for 2 h at 100–120 strokes/min. The pH of this homogenised gastric digest was adjusted to 5.0 by adding NaOH, 25 g of the pancreatic mixture was added and incubated in a shaking water bath for 2 h at 37 °C. At the end of the incubation period, the pH was measured and set to 7.0. The Erlenmeyer flasks were closed with parafilm in order to reduce CO_2 losses. Portions (20 g) of this digesta were taken as intestinal incubation medium in *in vitro* intestinal absorption studies.

2.4.2. Processing of mineral absorption by everted gut sacs

Segments of 10 cm length were cut from each section of the intestine. Duodenum segments from the first 12 cm posterior to the common bile duct, jejunal segments from 15 cm beyond 10 cm of the pyloric end and ileal segments were taken from the region proximately anterior to the ileo-cecal junction. The intestinal sections were everted over a thin glass rod. Respective everted intestinal sac was filled with Krebs–Ringer phosphate buffer of pH 7.4 comprising 10 mM glucose. Absorption of the specific mineral was observed by incubating aerobically, the everted rat intestinal segments positioned in 50 mL conical flask comprising a known volume (10 mL) of the mineral source, *viz.*, finger millet digesta (finger millet subjected to imitated gastrointestinal digestion procedure defined above). The flasks were aerated with 95% oxygen and 5% carbon dioxide mixture under incubation at 37 °C in a shaking water bath (Julabo) for exactly 30 min at 110 strokes/min. A pilot study was done for optimization of required incubation time for the intestinal segments in these assessments. In this study, range of different time interval for the incubation of the intestinal segments was carried out correspondingly, 15, 30, 45 and 60 min. Among them 30 min incubation period showed most differentiated result among groups; thus, 30 min was fixed for all subsequent experiments.

At the completion of incubation, the sacs were removed and the fluid adhering to the mucosal surface was rinsed with the medium. The mucosal medium, serosal fluid, and the intestinal tissue were collected distinctly. The intestinal sacs were dehydrated at 65 °C in an oven to a constant weight. Weighed samples were subjected to cold acid digestion in a mixture of concentrated nitric, sulphuric and perchloric acids (3:3.1:1, v/v) for overnight. The digested samples were then boiled for about 4–5 h for evaporating acid fumes and the volume was made up to a known volume by deionised water. The mucosal and serosal fluid samples were acidified with 5% nitric acid.

2.4.3. Mineral analysis

Zinc, iron, and calcium contents in the mucosal medium, serosal fluid, and intestinal tissue samples were determined by Atomic Absorption Spectrometry (Shimadzu AAF-6701, Kyoto, Japan). Calibration of the mineral measurements was implemented using zinc, iron, and calcium standards and appropriate acid blanks. All measurements were carried out with standard flame operating conditions as endorsed by the manufacturer. The amount of mineral absorbed was computed by the combined values of mineral present in the serosal fluid and the intestinal epithelial tissue, as against the unabsorbed portion remaining in the mucosal fluid at the end of incubation.

2.5. Statistical analysis

Statistical analysis was done by using Prism 6.0; Graph-Pad Software (San Diego, CA, USA). To evaluate differences between normal (nondiabetic) and diabetic groups over zinc dose (normal, dose level-1, and dose level-2), data were analyzed by two-way analysis of variance (ANOVA). For this analysis, the two dependent variables were zinc dose and group (normal *vs*. diabetic). Results were evaluated using the Tukey multiple comparison test, and considered significantly different at $P < 0.05$.

3. Results and discussion

Effect of dietary zinc supplementation on the uptake of zinc by segments of duodenum, jejunum and ileum portions of the small intestine when incubated with the digesta of finger millet flour is