Journal of Trace Elements in Medicine and Biology 30 (2015) 43–48

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

journal homepage: www.elsevier.com/locate/jtemb

NUTRITION

Resistant starch does not affect zinc homeostasis in rural Malawian children☆,☆☆

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ARTICLE INFO

Article history:
Received 5 December 2014
Accepted 14 January 2015

Keywords:
Zinc
Endogenous zinc
Zinc homeostasis
Malawi
Resistant starch

ABSTRACT

Objective: This study tested the hypothesis that Malawian children at risk for zinc deficiency will have reduced endogenous fecal zinc (EFZ) and increased net absorbed zinc (NAZ) following the addition of high amylose maize resistant starch (RS) to their diet.
Methods: This was a small controlled clinical trial to determine the effects of added dietary RS on zinc homeostasis among 17 stunted children, aged 3–5 years consuming a plant-based diet and at risk for perturbed zinc homeostasis. Dual zinc stable isotope studies were performed before and after 28 d of intervention with RS, so that each child served as their own control. The RS was incorporated into fried wheat flour dough and given under direct observation twice daily for 28 d. Changes in zinc homeostatic measures were compared using paired Student's *t*-tests and linear regression analysis.
Results: Children had a mean height-for-age *Z*-score of −3.3, and consumed animal source foods ≤twice per month. Their habitual diet contained a phytate:zinc molar ratio of 34:1. Children avidly consumed the RS without complaints. EFZ was 0.8 ± 0.4 mg/d (mean ± SD) both before and after the intervention. Fractional absorption of zinc was 0.38 ± 0.08 and 0.35 ± 0.06 before and after the RS intervention respectively. NAZ was 1.1 ± 0.5 and 0.6 ± 0.7 before and after the RS intervention. This reduction of NAZ corresponded with diminished dietary zinc intake on the study day following intervention with RS. Regression analysis indicated no change in zinc absorption relative to dietary intake as a result of the RS intervention.
Conclusion: Consumption of RS did not improve zinc homeostasis in rural African children without zinc deficiency. RS was well tolerated in this setting.

Background

Zinc deficiency affects tens of millions of children throughout the developing world [1], and has been implicated in the pathogenesis of stunting [2], impaired cognitive development [3], and infectious morbidity [4,5]. Previous studies of zinc homeostasis among children in rural Malawi have demonstrated that perturbations of zinc homeostasis are related to the abundance of phytate in the diet, which is a primary inhibitor of zinc absorption [6–9], and impaired conservation of zinc secreted into the intestinal lumen, but not reabsorbed [10–14], known as endogenous fecal zinc (EFZ).

Resistant starch (RS) is present in cereals, tubers, legumes and some fruits. RS resists digestion in the small bowel resulting in increased colonic starch which serves as a substrate for the fermentative production of short chain fatty acids, which in animal models exert effects on colonic luminal pH and colonic microbiota profiles. In humans, RS and enhanced colonic short chain fatty acids have been reported to confer health benefits in patients with metabolic syndrome and inflammatory bowel disease [15–19]. It has also been

☆ This study was supported by the Bill & Melinda Gates Foundation, Child Health Research Internship, Children's Hospital Colorado Research Institute, and NIH K24 DK083772. The sponsors played no role in the study design, data collection or data analyses.
☆☆ The trial was registered with ClinicalTrials.gov under the protocol number NCT01811836.
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http://dx.doi.org/10.1016/j.jtemb.2015.01.005
0946-672X/

speculated that RS may improve absorption of cationic minerals such as zinc, because of increases in particular bacterial enzymes, including acidic phytase [8,20–22]. High amylose maize starch contains larger quantities of RS than other cereal varieties [23], and is a common additive to many commercial food products [24]. In this study we tested the hypothesis that 3–5 year old rural Malawian children at risk for perturbed zinc homeostasis will have reduced EFZ and increased net absorbed zinc (NAZ) following addition of high amylose maize starch to the diet for 28 d.

Methods

Subjects

Eligible subjects were stunted children, aged 3–5 years, consuming animal source foods ≤twice/month who resided in Masika, Malawi. Masika is an isolated village of subsistence farmers growing primarily maize, cassava, legumes, and rice. Residents live in mud huts with grass roofs, collect water from shallow wells, and travel over 50 km to reach the nearest paved road. The screening process consisted of an anthropometric assessment and a food frequency questionnaire. Stunting and infrequent consumption of animal source foods were chosen as eligibility criteria in order to select children at greater risk for perturbed zinc homeostasis [25,26].

The study was approved by the College of Medicine Research and Ethics Committee at the University of Malawi, the Institutional Review Board from Washington University School of Medicine in St. Louis, and the University of Colorado Denver. Written and oral informed consent was obtained through a three step community-based process; (1) the entire community was informed about the study, (2) caretakers of potential participants attended a presentation about the study, and finally, (3) each caretaker individually provided consent for their child to participate.

Study design

This was a small controlled clinical trial to determine the effects of dietary RS on zinc homeostasis. Subjects participated in a dual zinc stable isotope study before and after the intervention, so that each child served as his/her own control. The sample size of 20 children was chosen assuming that 18 children would successfully complete the dose administration and sample collection. The sample size was chosen to detect a 20% change in EFZ with 95% specificity and 80% power, assuming that variances would be similar to those of a previous village-based study in rural Malawi [26]. The primary outcomes were changes in EFZ and NAZ before and after intervention with RS, as measured by an isotope dilution method [27]. Secondary outcomes were changes in fractional absorption of zinc (FAZ), and total absorbed zinc (TAZ), before and after intervention with RS, measured by dual isotope tracer ratio method [28]. Neither subjects nor clinical research workers were blinded to the intervention. Laboratory personnel were blinded during analyses. The trial was registered with ClinicalTrials.gov under the protocol number NCT01811836.

Participation

On the first day of participation, caretakers completed a questionnaire detailing demographic and social characteristics of the child as well as sanitation and hygiene practices. Anthropometric measurements were made at this time. The zinc stable isotope study was started on day 1 and required 8 d to complete collection of specimens. Upon completion of the first stable isotope test the RS intervention was initiated. After 28 d of intervention with RS, an additional zinc stable isotope study was undertaken. The RS intervention was continued over the 8 d required for this second test. Anthropometric measurements were repeated on day 38. Total participation time for each child was 45 d.

Study intervention

On study d 9–45, participants were given 8.5 g/d of RS which was added to a locally produced fried cake, known as a mandasi, comprised of white wheat flour, whole fluid milk, whole eggs, baking powder, salt, sugar, and soybean oil. The average weight of each mandasi was 40 g, and provided approximately 150 kcal. RS, in the form of the ingredient Hylon VII, (Ingredion, Bridgewater, NJ, USA), was added prior to cooking. The mandasi was prepared daily for delivery to each subject's home every morning and evening. Children consumed the mandasi under the direct observation of study staff. Caretakers of subjects were encouraged to not alter the child's intake of habitually consumed foods during the intervention.

Measurement of habitual dietary intake

A weighed food record protocol was developed based on 24-h dietary recall protocols and a previous weighed food record performed in Malawi. Two local enumerators with a high school education were hired to perform the in-home weighed food record with each subject two times, ideally on a weekday and a weekend day. The enumerators recorded the ingredients and final weight of each recipe that was prepared, and then weighed the amount each child consumed at meals. The enumerators arrived at the subject's home before sunrise and stayed until after the last meal of the day was finished. Records were kept for everything the child consumed including water and the study's intervention mandasi.

The enumerators recorded data in their native language, Chichewa, and nurses fluent in Chichewa and English translated the data. The weighed food records and recipe ingredients were entered in Microsoft Excel. Previous analyses of common dishes consumed in central and southern Malawi was used to establish nutrient content for each recipe (Jaimie Hemsworth, personal communication, iLiNS Project [29]). Microsoft Access was used to calculate combined nutrient values for the summed portions of each recipe consumed by subjects every day. Consumption of common sauces was accounted for by application of a correction factor based on previous data from a 24-h dietary survey by this research team, showing that the median ratio of solid:liquid was 1.37 (25th percentile 0.83, 75th percentile 2.27) [30].

Dual zinc stable isotope test

Clinical. This procedure required 8 d to complete and was conducted twice during the study. On the first day fasted subjects arrived at the Chipalonga Health Post with their caretakers at 0600. Baseline stool and urine specimens were collected prior to isotope dosing. To measure zinc absorption, carefully weighed doses of oral zinc stable isotopes were administered with each of the three main meals of the day, and each subject received an exactly weighed amount of ~125 μg ^{70}Zn, (first administration) or ^{67}Zn (second administration). Additionally, each child received ~50 μg of the same oral isotope with a morning snack of mandasi. No isotope was given with an afternoon snack of banana or mandasi. The diet given with the stable isotopes was chosen to correspond to the habitual amount of zinc, iron, and phytate consumed in the local area, (i.e. maize porridge, beans, boiled vegetable leaves, mandasi, and bananas). Halfway through the meal or snack small drops of the isotope were delivered by a syringe into the child's mouth, alternating with bites of food. The dose vial and the oral syringe used for isotope delivery were rinsed three times with distilled zinc-free water to ensure complete administration of the isotope dose. All losses from drooling or spit were collected on ashless filter paper for later isotope analysis, and this quantity was subtracted from the measured