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Original Research Article

Dietary iron intakes based on food composition data may underestimate the contribution of potentially exchangeable contaminant iron from soil

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ABSTRACT

Iron intakes calculated from one-day weighed records were compared with those from same day analyzed duplicate diet composites collected from 120 Malawian women living in two rural districts with contrasting soil mineralogy and where threshing may contaminate cereals with soil iron. Soils and diet composites from the two districts were then subjected to a simulated gastrointestinal digestion and iron availability in the digests measured using a Caco-2 cell model. Median analyzed iron intakes (mg/d) were higher ($p < 0.001$) than calculated intakes in both Zombwe (16.6 vs. 10.1 mg/d) and Mikalango (29.6 vs. 19.1 mg/d), attributed to some soil contaminant iron based on high Al and Ti concentrations in diet composites. A small portion of iron in acidic soil from Zombwe, but not Mikalango calcareous soil, was bioavailable, as it induced ferritin expression in the cells, and may have contributed to higher plasma ferritin and total body iron for the Zombwe women reported earlier, despite lower iron intakes. In conclusion, iron intakes calculated from food composition data were underestimated, highlighting the importance of analyzing duplicate diet composites where extraneous contaminant iron from soil is likely. Acidic contaminant soil may make a small but useful contribution to iron nutrition.

1. Introduction

The assessment of iron intakes in low-income countries is most frequently calculated from 24-h recalls or records using food composition data. However this procedure does not consider iron sources extrinsic to the food. The importance of these extrinsic sources of iron in low-income countries has been emphasized by Harvey et al. (2000) because some of this iron may be available for absorption. Sources include contamination of foods from soil, dust, and water; metal fragments from milling; leaching of iron into foods through the use of iron cooking pots (Prinsen Geerligs et al., 2004), and the practice of geophagia.

A recent cross-sectional study of women living in two rural districts in Malawi with contrasting soil mineralogy revealed a low risk of iron deficiency among the women, notwithstanding diets based on unrefined cereals with high concentrations of phytate and negligible intakes of heme iron from cellular animal foods. This suggested that perhaps some contaminant iron joined the common non-heme iron pool (i.e. was exchangeable) and thus was available for absorption.

Therefore we have compared iron intakes of the Malawian women in these two districts, calculated from weighed food records and Malawian food composition data, with the results from the analysis of duplicate diet composites collected on the same day. We hypothesized that the iron intakes based on the analyzed

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duplicate diet composites (Siyame et al., 2014) would be higher than those based on the calculated iron intakes presented here which do not account for contaminant iron or any contribution of iron from drinking water. We also examined the potential bioavailability of iron from the composite diets and soils from the two districts using a Caco-2 cell model system (Wawer et al., 2012).

2. Materials and methods

2.1. Study site and participants

Details of the study site, recruitment of participants, and their socio-demographic, anthropometric, and biochemical iron status have been published earlier (Hurst et al., 2013; Siyame et al., 2014). Briefly, a convenience sample of 120 apparently healthy Malawian women aged 18–50 years participated in the study. The women were living in six rural villages in Zombwe Extension Planning Area (EPA) ($n = 60$), characterized by acid soils with a low pH (median 5.2), and six rural villages in Mikalango EPA ($n = 60$), with calcareous Eutric Vertisols (median pH = 7.8). Verbal informed consent was obtained from the traditional authorities in the villages and from the participants, and the study protocol was approved by the National Health Sciences Research Committee of Malawi.

2.2. Assessment of iron intakes from weighed food intakes

One-day weighed food records were collected from the women in their homes by trained research assistants at the same time as weighed duplicate diet composites (including drinking water). Women were instructed not to change their normal dietary pattern during the diet-composite day. Money was given to the women to reimburse them for the cost of the food.

Intakes and major food sources of iron were calculated from the weighed food records using a Malawian food composition database compiled by the investigators (Yeudall et al., 2005). The values for the iron of the major plant-based staples in this food composition database were based on chemical analyses in our laboratory; details have been published earlier (Ferguson et al., 1990). The source of the other values was the WorldFood Dietary Assessment System (Bunch and Murphy, 1997). For composite dishes, recipe data were used and the iron values for raw foods adjusted for changes in retention and yield after cooking by using retention (USDA, 2007) and yield factors (Matthews and Garrison, 1987), where appropriate. Adjustments were made to all added iron values for any differences between the moisture content of the food stated in the Malawian Food Composition Database and the external source value.

2.3. Elemental analyses of duplicate diet composites and soil samples

Calculated iron intakes were compared with iron intakes based on chemical analysis of the duplicate diet composites collected on the same day. Concentrations of aluminum and titanium in the diet composites and iron in the soil samples collected from the two EPAs were also analyzed by inductively coupled plasma spectrometry (ICP-MS). For the analyses, aliquots of the homogenized freeze-dried duplicate diet composites or finely ground soil samples were microwave digested in 3.0 mL of 70% trace analysis grade HNO_3 (Fisher Scientific, UK), 2.0 mL H_20_2 and 3.0 mL milli-Q water (18.2 MΩ cm; Fisher Scientific UK Ltd., Loughborough, UK). The acid digests were then analyzed by inductively coupled plasma mass spectrometry (ICP-MS) (X-Series^{II}, Thermo Fisher Scientific Inc., Waltham, MA, USA). Iron and aluminum were quantified using an external multi-element calibration standard (Claritas-PPT grade CLMS-2 from SPEX Certiprep Inc., Metuchen, NJ, USA); titanium was determined semi-quantitatively. Internal standards were introduced to the sample stream on a separate line and included Sc (20 μg/L), Rh (10 μg/L), Ge (10 μg/L) and Ir (5 μg/L) in 2% trace analysis grade HNO_3 (Fisher Scientific, UK). Two standard reference materials (SRMs) from National Institute of Standards and Technology (NIST; Gaithersburg, MD, USA) were included to check on the accuracy and precision of the ICP-MS procedures for iron and aluminum – NIST 1573a (tomato leaves) and NIST 1577c (bovine liver); no certified values were available for titanium. Values for the iron and aluminum content of tomato leaves (NIST 1573a) were: Fe, 328.3 mg/kg (89% recovery); Al 476.1 mg/kg (79% recovery) compared to certified values of Fe, 368 mg/kg and Al, 598 mg/kg. Corresponding values for iron for bovine liver (NIST 1577c) were 185.6 mg/kg (93% recovery) compared to a certified value for Fe of 197.9 mg/kg. Operational sample blanks ($n = 10$) were run to determine limit of detection (LOD; 3*standard deviation, SD) and limit of quantification (LOQ, 10*SD) values in diet composite samples.

2.4. Phytate analysis of duplicate diet composites

Inositol penta-(IP5) and hexa-(IP6) phosphates were determined by a modified method of Lehrfeld (1989). Briefly, inositol phosphates were extracted from aliquots of the freeze-dried powdered diet composites (0.5 g) with 5 mL of 0.67 M HCl (BDH, Aristar). Dried extracts were then reconstituted with 1 mL of distilled deionized water and the inositol phosphates concentrated using a Hypersil column (H30DS-250A, HICHROM, Berkshire, UK). Inositol phosphates were then separated and analyzed in duplicate by high-performance liquid chromatography using a Waters 2690 Separation Module (Waters, Milford, MA, USA) and a differential refractometer (410 Differential Refractometer, Waters, MA, USA). Phytate as IP5 and IP6 concentrations were calculated from regression equations derived from different concentrations of standard solutions and peak areas of the sample. The inter-run coefficient of variation (CV) for the HPLC method was 7.2%. Concentration of combined IP4, IP5 and IP6 in maize flour (72% extraction rate) was 755 mg phytate/100 g (CV = 3.4%) compared to a generated laboratory mean value from other previous studies of 796 mg phytate/100 g of maize flour.

2.5. Cell culture

Unless otherwise stated all chemicals and enzymes were purchased from Sigma–Aldrich (UK). Caco-2 cells (HTB-37; ATCC, USA) were grown in collagen-coated 6-well plates (Greiner, UK) at a density of 4.75×10^4 in 2 mL of Dulbecco's modified Eagle's medium (DMEM media; LGC, UK) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 5 mL of 5000 u/mL Penicillin/Streptomycillin solution (Gibco, UK) and 5 mL of 100× non-essential amino acids. Medium was replaced every 2 days. Cells between passages 28 and 30 were used for experiments at 13 days post seeding, and 24 h prior to experimentation, cells were switched to serum-free medium (MEM, Invitrogen) supplemented as above with the exception of fetal bovine serum.

2.6. In vitro digestion and preparation of cell monolayers

Composite diet and soil samples were subjected to a simulated gastrointestinal digestion (with the addition of ascorbic acid (AA) at 1:10 or 1:30 iron:AA molar ratio to improve the sensitivity of the model by facilitating uptake of iron into the cells) according to the method of Glahn et al. (1996). Briefly, for the gastric phase of digestion, the samples were exposed to pepsin at pH 2 at 37 °C for 1 h after which the pH was raised