Contribution of calcium and other dietary components to global variations in bone mineral density in young adults

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Abstract

A research project on comparative international studies of osteoporosis using isotope techniques was organized by the International Atomic Energy Agency (IAEA) with the participation of 12 countries (Brazil, Canada, Chile, China, Croatia, Hungary, Philippines, Russia, Singapore, South Africa, Turkey, and the United Kingdom). Participating centers in 11 countries (all but the UK) made measurements and collected data on men and women aged 15 to 49 years. In addition to studies of bone mineral density (BMD) at the femoral neck and lumbar spine using DEXA, anthropometric, lifestyle, and nutritional data were also collected. The results of the nutritional studies are reviewed in this paper. Overall, about 8% of the observed variability in spine BMD could be attributed to nutritional factors in men and women; in men, no such relationship could be determined. No single nutritional component (not even calcium) stood out as being of particular importance across all participating centers.

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Key words: osteoporosis, bone mineral density, women, calcium intake

Introduction

Osteoporosis is a crippling bone disease characterized by loss of bone tissue from the skeleton, which in turn leads to an increase in bone fragility and propensity to fracture under minimal trauma. More than 200 million people (mainly, but not only, post-menopausal women) are thought to be affected worldwide. International comparisons are usually made not on the basis of osteoporosis incidence *per se* but rather by using hip-fracture rates and/or measurements of bone mineral density (BMD) as proxies. Approximately 1.7 million hip fractures occur worldwide each year, and this incidence is expected to increase fourfold by 2050 because of the increasing numbers of older people [1].

It is now widely recognized that the causes of osteoporosis are multifactorial in nature and that there are wide variations in incidence across different populations. However, much work still remains to be done to quantify the differences in incidence and to "unravel" some of the contributing factors.

In 1994 the IAEA started a coordinated research project on this subject with the participation of the 12 countries—Brazil, Canada, Chile, China, Croatia, Hungary, Philippines, Russia, Singapore, South Africa, Turkey, and the United Kingdom. The UK participant served as a central reference laboratory with responsibilities for quality control and data evaluation.

The main objectives of the project were:

» To make comparative measurements of bone mineral density (BMD) of selected human subjects (young adults) in different parts of the world (having different geographical, cultural, and ethnic backgrounds),

» To determine the age range over which peak BMD is achieved and sustained,

» To collate and evaluate the BMD data in relation to gender, nutrition, and lifestyle factors,

» To conduct supplementary studies of trace and other elements in bone (mainly autopsy and biopsy samples of iliac crest).

This paper focuses on the nutritional aspects of the project.

Methods

Subject recruitment

The study protocol specified a target of enrolling 350 subjects at each center, with each cohort stratified equally by sex and age into seven 5-year age bands. Only a few centers had access to local population-based registers to select a population-based random sample so that the selection of participants varied between centers. Most used local university or hospital staff, and friends or relatives of hospital attendees. All centers excluded subjects with a longer than three month history of chronic disease affecting bone metabolism such as renal failure, hepatic failure, gastrointestinal disease, primary hyperparathyroidism, Paget's disease of bone, or thyroid disease. Other exclusion criteria included a history of hormone supplementation (such as estrogen or corticosteroids), pregnancy or lactation, previous low energy fracture, prolonged immobilization (more than 1 month), and over-exposure to toxic metals or irradiation.

Subject characterization

Each center interviewed subjects for approximately one hour using a modified version of the World Health Organization (WHO) osteoporosis questionnaire. Information sought included age, socio-demographic status, ethnicity, fracture history (both in the subject and their family), tobacco use, reproductive history (females only), physical activity, diet, and hormone use (especially estrogen).

Assessment of bone mineral density

Each center measured BMD using either Hologic (Bedford, Mass., USA) or Lunar (Madison, Wisc., USA) densitometry machines. Cape Town, Santiago, Sao Paulo, Shanghai, and Toronto used Hologic scanners while Ankara, Beijing, Debrecen, Manila, Moscow, Obninsk, Singapore and Zagreb used Lunar machines. Known systematic differences between the scanner types were taken into account in the study design with cross-calibration and standardization of BMD derived from all the equipment using a so-called "European Spine Phantom" (ESP). Calculations were performed using regression analysis of the ESP measurements based on an exponential model. The values of standardized BMD thus generated are referred to here as *sBMD*. Anteroposterior sBMD of the lumbar spine (L2-4) and the femoral neck were the primary values used for comparison between centers as these sites were assessed by both makes of scanning equipment. This methodology and the results obtained will be described in more detail in a subsequent publication.

Dietary evaluation

As part of the "subject evaluation" mentioned above, the subjects were also questioned regarding daily consumption of meat, fish, vegetables, and dairy products. Since this was not a mandatory part of the project, only five centers (Beijing, Debrecen, Manila, Shanghai, and Singapore) were able to provide sufficient information for further detailed evaluation. From the information provided, and using locally applicable food composition tables, average daily intakes of protein (g), carbohydrate (g), fat (g), energy (kcal), and calcium (mg) were calculated for all except Singapore, which recorded calcium intake only. Most of the nutritional evaluation presented in this paper is based on these data. However, for the discussion on calcium, additional literature was drawn upon.

Bone composition studies

A supplementary project conducted with bone (iliac crest, femoral neck, and rib) samples collected from four countries (Brazil, China, Russia, and Turkey) was concerned with trace and other nutritional elements that may play a role in bone health (including calcium, fluorine, iron, potassium, magnesium, manganese, sodium, phosphorus, strontium and zinc). A standardized protocol was devised for the separation of the samples into cortical and trabecular components and their preparation for analysis (mainly by neutron activation).

Results

Bone mineral density (BMD)

A total of 5,950 subjects were enrolled in the study (2,073 men and 3,877 women, M:F = 1:1.9); China and Russia were both represented by two different cities. Sample sizes ranged from 137 in Obninsk to 1,323 in Cape Town. Extreme statistical outliers for anthropometry, bone mineral density, and diet were identified using a box and whisker plot and these subjects (77 in all; 1.3% of the sample) were excluded from further analysis.

The details of the BMD results will be described in a subsequent publication. However, figures 1 and 2 are illustrative of the results obtained. There was no consistent pattern of behavior of sBMD with respect

to age (fig.1). Across the whole study population, highly significant ($p < .001$) differences in sBMD were observed between the sexes at both skeletal sites, and also between many of the centers (fig. 2). In regression models, approximately 12% to 20% of the global variation in sBMD was found to be explained by anthropomorphic differences while a further 4% to10% was accounted for by the country of origin.

Multivariate nutritional evaluation

Four of the centers (Beijing, Debrecen, Manila, and Shanghai) provided sufficient information for multivariate statistical evaluation of the sBMD data using anthropometry (age, height, weight) and proximate nutrients (energy, protein, fat, carbohydrate, and calcium) as independent variables. As in the overall regression analyses, anthropomorphic indices and the center of origin accounted for about one-fourth of the variability in spine sBMD (29.8% in men and 22.6% in women). Table 1 illustrates the results obtained when the nutrition variables were entered into the model (nutritional components are shaded). Men and women behaved differently in the sense that nutrient intake contributes at least in part to the variability of BMD across centers in men but not in women. In men, some of the dietary components had small but statistically independent influences on spine sBMD and determined a further 8% of its variability (R^2 for the model is 30%). Similar effects were seen at the femoral neck where nutritional components increased the model R^2 by 1% in men, but had no impact on the R^2 in women.

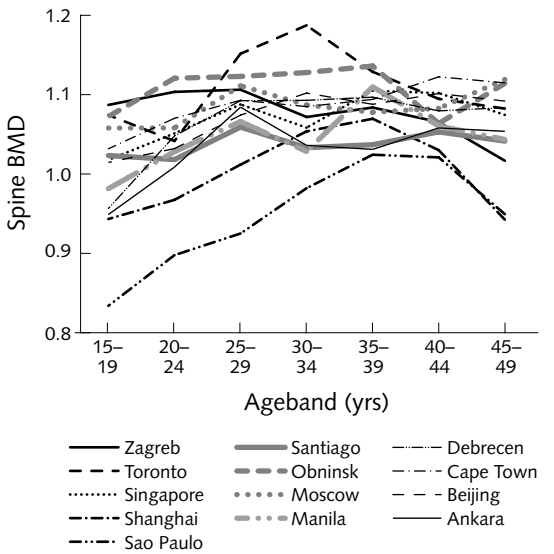

FIG. 1. Spine sBMD (g/cm^2) for women at 13 centers. These subjects have been categorized into 5-year agebands

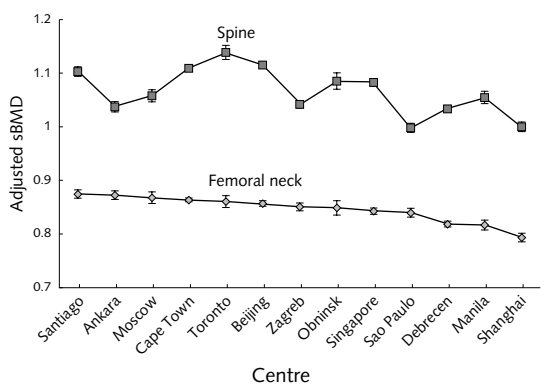

FIG. 2. Mean (± SEM) adjusted sBMD following linear regression of age, height, and weight to 35 years, 160 cm and 60 kg in women. The centers are ranked in order of descending femoral neck sBMD.

This kind of evaluation emphasizes differences between centers. Within individual centers, most of these differences disappear. One exception is the case of calcium in Shanghai men (fig. 3). This was the only set of results that showed a significant correlation between sBMD and calcium intake.

Single nutrient evaluation (with emphasis on calcium)

Five of the centers (Beijing, Debrecen, Manila, Shanghai, and Singapore) provided sufficient information for evaluation of calcium intakes in individual subjects included in the study of BMD. Figure 4 illustrates the results obtained. It is apparent that the single European country in this group (Hungary) has much higher intakes than the four Asian countries.

To permit an evaluation of the relationship between calcium intakes and sBMDs for all of the centers included in this project, typical calcium intake data

TABLE 1. Effect of age, height, weight, diet, and center on spine sBMD (g/cm^2)

	Men		Women	
	B	*p*-value	B	*p*-value
Age	–0.003	< .001	0.002	.0002
Height	0.035	NS	0.003	.001
Weight	0.003	< .001	0.001	.013
Energy	0.0001	< .001	10^{-5}	NS
Protein	–0.0004	NS	–0.0007	NS
Fat	–0.0015	< .001	–0.0004	NS
Carbohydrate	–0.0006	< .001	10^{-5}	NS
Calcium	10^{-5}	NS	0.0001	NS
Beijing	Reference center			
Manila	–0.074	.003	–0.078	< .001
Shanghai	–0.137	< .001	–0.095	< .001
Debrecen	–0.152	< .001	–0.132	< .001

Beijing is the reference center as it has the highest mean values for spine sBMD (1.092 ± 0.149 g/cm^2 and 1.093 ± 0.108 g/cm^2 for men and women, respectively).
B, slope; NS, not significant.

were drawn from the literature; i.e., for the five centers mentioned above, actual mean values were used; otherwise, literature values were used [2]. Figure 5 illustrates the results obtained. The regression lines do not show significant correlations.

None of the other major nutritional components (protein, fat, carbohydrate, energy) when treated as single independent variables showed any significant relationships with sBMD. Similarly, none of the other nutritional components reported by some centers (e.g., sodium, magnesium, copper, zinc) revealed any interesting relationships.

Bone analysis data

Four countries (Brazil, China, Russia, and Turkey) conducted studies on bone samples (mainly iliac crest) collected from apparently healthy victims of sudden death (mostly traffic accidents). Results were reported for more than 30 elements including most of the trace and other nutritional elements that are thought to play a role in bone health (including calcium, fluorine, iron, potassium, magnesium, manganese, sodium, phosphorus, strontium, and zinc).

This work will be reported in detail in a subsequent publication. Suffice it to say here that the values

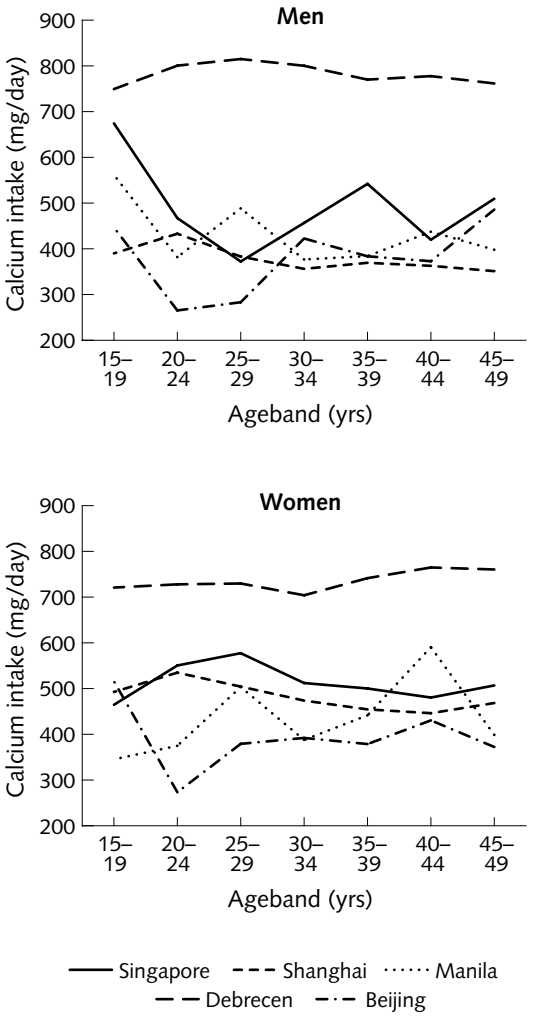

FIG. 4. Mean daily calcium intake (mg/day) in men and women studied in five centers

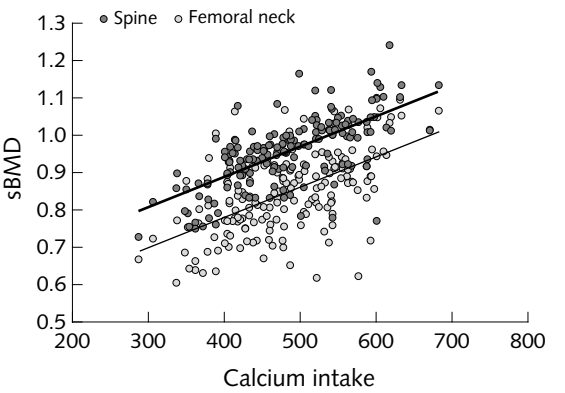

FIG. 3. Mean sBMD (g/cm^2) at the spine and femoral neck against daily calcium intake (mg) in Shanghai men

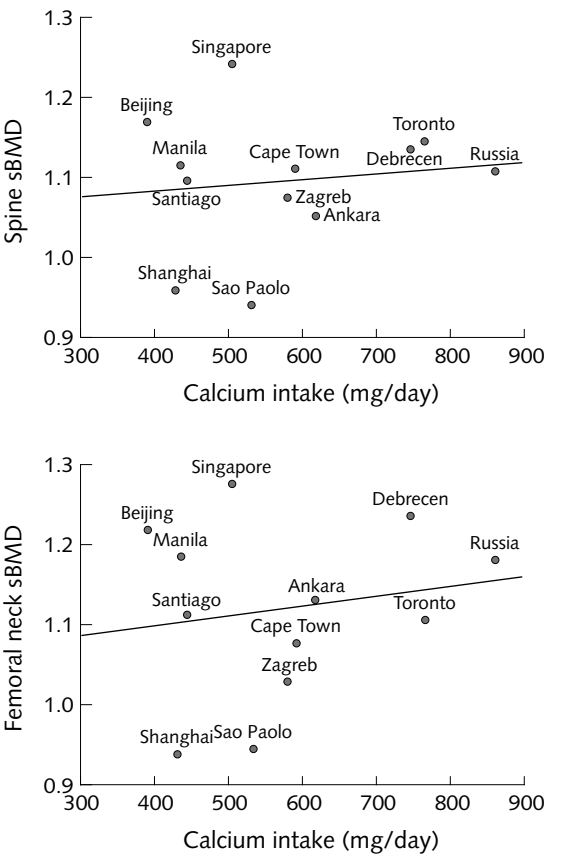

FIG. 5. Mean sBMD (g/cm^2) for spine and femoral neck against daily calcium intake (mg)

obtained were consistent with what has already been reported elsewhere [3]. Unfortunately, nothing of significance was found that throws any new light on the possible role of these elements in relation to osteoporosis.

Discussion

Because calcium is a major bone-forming mineral, it has long been assumed that primary or secondary calcium deficiency must, in some way, underlie osteoporosis and fracture risk. It is also well known that normal dietary intakes of calcium vary significantly from one population to another. Relevant data for adult population groups taken from a global IAEA database have a range from 210 to 1,650 mg/day [2, 4]. Putting these facts together one might suppose that differences in calcium intake should be the major reason for the differences in osteoporosis risk between different countries, and for the significant differences in sBMD observed in this IAEA project.

Unfortunately, things are not so simple. This study now adds to the growing body of evidence (e.g., [5]) that there is no clear relationship between calcium intake and bone strength. As suggested recently by Nordin [6] it is beginning to appear that calcium requirements must be understood on a sliding scale. There is no single, universal calcium requirement, only a requirement linked to the intake of other nutrients.

This conclusion strengthens the understanding that osteoporosis is a multifactorial disease. At the time that this IAEA project started in 1994, a list of risk factors was drawn up by the participants and consultants (table 2). The simple fact that so many different factors are involved makes it exceedingly difficult to design a study in free-living populations that can identify the effects of any one of them treated as a single independent variable.

Table 2. Risk factors for osteoporosis

Well established
Age: elderly
Sex: female
Race: Caucasian or Asian
Gonadal deficiency
Post-menopausal women
Early menopause
Hypogonadal men
Probable
Low body mass
Excessive smoking
Excessive alcohol
Sedentary life style
Malabsorption of calcium in the elderly
Possible
Low calcium intake
High caffeine intake
High protein intake (industrialized countries)
Low protein intake (developing countries)
Vitamin K deficiency
Minor and trace element deficiency: boron, calcium, copper, magnesium, manganese, strontium, and zinc
Minor and trace element excess: aluminum, cadmium, fluorine, heavy metals, and sodium

However, this should not be interpreted as saying that nutrition is if no significance for osteoporosis. Obviously, nutritional recommendations for the delivery and maintenance of good bone health must include attention to all of the nutritional factors that, on the basis of evidence from clinical, biochemical, and animal studies, are known to play an important role. There is no reason yet to downplay the significance of any of the nutritional factors listed in table 2.

References

1. United Nations Sub-Committee on Nutrition ACC/SCN. Calcium: an emerging issue for developing countries. In: Third report on the world nutrition situation.Geneva: World Health Organization, 1997: 44–6.
2. Parr RM, Crawley H, Abdulla M, Iyengar GV, Kumpulainen J. Human dietary intakes of trace elements: A global literature survey mainly for the period 1970–1991: I. Data listings and sources of information. Report NAHRES-12. Vienna: IAEA, 1992.
3. Iyengar GV, Tandon L. Minor and trace elements in human bones and teeth. Report NAHRES-39. Vienna: IAEA, 1999.
4. Parr RM, Iyengar GV. The role of minerals and trace elements in osteoporosis. In: Abdulla M, Bost M, Gamon S, Arnaud P, Chazot G., eds. New aspects of trace element research. London: Smith-Gordon, 1999:248–52.
5. Kanis JA. The use of calcium in the management of osteoporosis. Bone 2001;24(4):279–90.
6. Nordin C. Calcium requirement is a sliding scale. Am J Clin Nutr 2000;71:1381–3.

Validating the analytical methodologies for determining some important trace elements in food consumed in India

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Abstract

This paper reports on the development, standardization, and application of instrumental as well as radiochemical neutron activation analysis (INAA and RNAA) techniques for determining the concentrations of iron, zinc, cobalt, cesium, strontium, selenium, thorium, and calcium in food consumed in India. Based on the analysis of 20 diet samples, prepared as per the data on dietary intake patterns of an adult in four provinces of India and that of an average adult Indian, the geometric mean (GM) intake of various elements for the reference Indian man was estimated to be 15.9 (10.7–34.4) mg for iron, 8.6 (5.1–15.6) mg for zinc, 17.0 (8.3–31.4) μg for cobalt, 4.76 (2.8–11.8) μg for cesium, 1.46 (0.79–2.96) mg for strontium, 52.4 (35.0-130.8) μg for selenium, 0.75 (0.44–1.75) μg for thorium, and 0.35 (0.17–0.67) mg for calcium. A comparison of the daily dietary intakes of these trace elements by the reference Indian man was made with that of the International Commission on Radiological Protection (ICRP) reference man and also with the world average compiled by the International Atomic Energy Agency (IAEA). When compared with the ICRP reference man data, the daily dietary intakes of all the eight elements by the reference Indian man were considerably lower by factors ranging from 1.4 for strontium to as much as 18.0 for cobalt. However, when compared with the world average, daily dietary intakes by the reference Indian man were comparable for iron and lower by factors 1.2 to 1.9 for zinc, selenium, and calcium.

Key words: analytical methodologies, trace elements, neutron activation analysis (NAA), reference man, diet samples

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Mention of the names of firms and commercial products does not imply endorsement by the United Nations University.

Introduction

The significance of important essential trace elements such as iron, zinc, cobalt, selenium, and calcium for human health and nutrition, as well as their use for diagnostic and therapeutic purposes has been well understood and documented [1, 2].There is another category of trace elements such as cesium, strontium, thorium, etc. whose importance has been recently recognized because of their similarity in behavior to their radioactive counterparts, ^{137}Cs, ^{90}Sr, and ^{232}Th which are encountered in operations of the nuclear fuel cycle and contribute to the internal radiation dose to the occupational workers. The data on such trace elements in the diet along with the information on their concentrations in human tissues could also be useful in ascertaining the biokinetic behavior of the radioisotopes of some of these elements that may get accidentally incorporated into the human body [3, 4]. The measurement of the concentrations of the mentioned elements in food was, therefore, undertaken by the Internal Dosimetry Division, of the Bhabha Atomic Research Centre (BARC). This work was part of an IAEA coordinated research program on the reference Asian man (Phase II) to establish their database on daily dietary intake and organ content of different trace elements for the adult Indian population (reference Indian man).

This paper describes instrumental neutron activation analysis (INAA) as well as radiochemical neutron activation analysis (RNAA) techniques employed for the determining iron, zinc, cobalt, cesium, strontium, selenium, thorium, and calcium concentrations in food consumed in India. The analytical methodologies were validated by analyzing four standard reference materials (National Institute of Science and Technology, Gaithersberg, Md.,USA) namely: citrus leaves, orchard leaves, bovine liver, and total diet samples. The daily dietary intakes of the mentioned elements by the reference Indian man are also reported. A comparison of the daily intake of trace elements by the Indian adult population has been made with the limited data available for the

International Commission on Radiological Protection (ICRP) reference man and with the world average, i.e., IAEA data on dietary intake obtained from 11 countries including the United States, China, and Brazil.

Materials and methods

Sampling and sample preparation

The commonly employed methods for studying the daily dietary intake of trace elements are based on the analysis of duplicate diet samples, the market basket, or the cooked total diet. The method employed for obtaining representative samples for analysis depends mainly on whether the population in the country is homogeneous and whether national statistics on the dietary consumption pattern of the country's entire population are available. The Indian population is not homogeneous in nature, yet in terms of their diet consumption patterns, the task of obtaining the representative samples was made simple because of the extensive surveys conducted by the National Nutrition Monitoring Board (NNMB) of India [5–7], which provided dietary intake data on various food materials consumed by different Indian population groups, living both in rural and urban areas of the country. On the basis of these extensive studies, Dang et al. [8], proposed the average daily intake of various food materials which form part of the daily diet of the average adult Indian male and female.

The consumption data on various food materials as proposed by Dang et al. [8], for reference Indian man were used to prepare cooked market basket diet samples representing the national diet consumed by the adult Indian. The food was cooked in the main ethnic style prevailing in the country. A preliminary study showed that the average of the individual dietary intakes by the population groups from West Bengal, Kerala, Punjab, and Maharashtra provinces could represent the national consumption. In order to capture the variability in the consumption of various trace elements across the cross-section of India, the diets were prepared using data on consumption of individual food ingredients by the adult population in these provinces (table 1). The main ingredients, such as rice, wheat, millet, pulses, jaggery etc., were procured from those provinces, but the vegetables, fruits, milk, meat, etc., although typical of those regions, were procured from Mumbai's local market.

To the cooked meal, 2.2 L of drinking water evaporated to about one-forth volume, was added and the total material was homogenized in a blender fitted with a titanium blade to avoid cross-contamination. The samples were freeze dried and powdered, and three aliquots were taken for the trace element analysis.

Development of analytical methods

A number of analytical methods such as atomic absorption spectrophotometry (AAS), spectropho-

TABLE 1. A comparison of daily dietary intake (g) of various foods by the adult male population in a few selected provinces of India and the all India average

Food Item	Daily intake (g)				
	Maharastra	Kerala	West Bengal	Panjab	All India average
Cereals					
Rice	241	392	502	102	280
Wheat	—	—	60	301	80
Other cereals	196 (Jawari)	—	—	195 (Maize and Bajra)	65 (Jawari)
Pulses	33	15	76	62	32
Milk	49	12	77	230	90
Vegetables					
Green	—	2	40	39	20
Others (including roots and tubers)	62	122	67	137	75
Sugar and jaggery	31	58	39	88	26
Spices	13	13	12	13	12
Meat	27	30	58	5	15
Fruits	9	20	—	14	18
Oil	15	12	19	26	15
Nuts	—	8	—	—	13

tometry, inductively coupled plasma-mass spectrometry (ICP-MS), fluorimetry, etc. have been used by various workers for the analysis of trace elements in biological samples [1, 2]. However, the advantage of neutron activation analysis (NAA) over other analytical methods lies in the fact that it is a blank-free and matrix-independent method which is also adequately sensitive for the measuring elemental concentrations at sub-nanogram levels. Therefore, the analytical methods employing neutron activation (both instrumental neutron activation and radiochemical neutron activation) were developed for determining the nutritionally as well as radiologically important trace elements. Thorium and strontium were determined using radiochemical neutron activation analysis (RNAA) and iron, zinc, cobalt, cesium, selenium, and calcium were determined using instrumental neutron activation analysis (INAA). The details of these analytical techniques are given below. The important nuclear parameters such as radioisotopes employed for the determining these elements along with their half-lives, the characteristic gamma energies and the minimum detection limit (MDL) achieved, are given in table 2.

Determination of thorium by RNAA

The concentration of thorium present in the biological samples was determined using the radiochemical neutron activation analysis (RNAA). The samples were first irradiated along with the thorium standard in the APSARA reactor (swimming pool type) in a thermal neutron flux of $\approx 10^{13}$ n cm^{-2} s^{-1} for 14 hours. The nuclear reaction taking place during the neutron irradiation is shown below:

$$^{232}\mathrm{Th}\ (\mathrm{n}, \gamma)\ ^{233}\mathrm{Th} \underset{\beta^-}{\rightarrow}\ ^{233}\mathrm{Pa}$$

After cooling for 8 to 10 days, allowing the decay of short lived activities of ^{24}Na, ^{42}K, ^{38}Cl, etc., the irradiated samples were digested in concentrated HNO_3 until a clear solution was obtained. ^{233}Pa produced during irradiation of thorium present in the sample was separated, first by co-precipitating with manganese dioxide, and then with barium sulphate. The radioactive ^{233}Pa was quantitatively carried along with barium sulphate precipitate, which was filtered, dried, and sealed in a polyethylene bag for counting. ^{233}Pa, which emits characteristic gamma rays of 311.8 keV, was measured using a 54 cc HPGe detector (M.S. Eurisys, Mesures, France) coupled to a 4K pulse height analyzer. Thorium present in the sample was quantified by comparing the ^{233}Pa activity formed in the sample and the standard. Further details of the radiochemical separation procedure have been described elsewhere [9].

Determination of strontium by RNAA

The concentration of strontium present in the biological samples was also determined using the radiochemical neutron activation analysis (RNAA). The samples were irradiated along with a strontium standard in the APSARA reactor in an irradiation position with a thermal neutron flux of $\approx 10^{12}$ n cm^{-2} s^{-1} for 1 hour. The nuclear reaction that takes place during the neutron irradiation is shown below:

$$^{87}\mathrm{Sr}\ (\mathrm{n},\gamma)\ ^{87\mathrm{m}}\mathrm{Sr}$$

After irradiation, the sample was digested along with 100 mg of strontium and 50 mg of calcium carriers in concentrated HNO_3. About 1 to 2 ml of 30% hydrogen peroxide was added to aid the digestion process, until a clear solution was obtained. It was then evaporated to dryness and dissolved in 1N HCl and 5ml of saturated solution of oxalic acid was added and pH was raised to 3 to 4 with ammonium hydroxide to precipitate calcium oxalate. The calcium oxalate precipitate which carries ^{87m}Sr quantitatively, was filtered and counted for its characteristic gamma line of 389 keV. The stron-

TABLE 2. Radioisotopes, technique, half-lives, gamma energies used and minimum detection limit (MDL) for the analysis of iron, zinc, cobalt, selenium, cesium, calcium, strontium, and thorium using NAA

Element	Isotope	Technique	Half-Life (days)	Gamma-ray measured (keV)	MDL
Iron	^{59}Fe	INAA	45	1,098.6 and 1,291.5	1 µg
Zinc	^{65}Zn	INAA	245	1,115.4	0.15 µg
Cobalt	^{60}Co	INAA	1,924	1,173 and 1,332	2 ng
Selenium	^{75}Se	INAA	127	136 and 265	10 ng
Cesium	^{134}Cs	INAA	754	795.8	1 ng
Calcium	^{47}Sc	INAA	3.6	160	40 µg
Strontium	^{87m}Sr	RNAA	0.117	389	50 ng
Thorium	^{233}Pa	RNAA	27	311.8	0.05 ng

RNAA, Radiochemical neutron activation analysis.
INAA, Instrumental neutron activation analysis

tium standard, irradiated along with the sample, was also subjected to the same radiochemical separation and counted in the same geometry using 54 cc HPGe detector coupled to a 4K analyzer.

Determination of iron, zinc, cobalt, cesium, selenium, and calcium by INAA

The other trace elements, iron, zinc, cobalt, cesium, selenium, and calcium were determined by instrumental neutron activation analysis (INAA). The samples along with the known quantities of the standards were irradiated in a neutron flux of 10^{13}n cm^{-2} s^{-1} in the APSARA for 14 hours. After irradiation, the samples were allowed to cool for 3 to 4 weeks for the decay of short lived activities of ^{24}N, ^{42}K, ^{38}Cl, etc. The irradiated samples and standards were then counted for 16 hours using a 54 cc HPGe detector coupled to a 4K analyzer. The iron, zinc, cobalt, cesium, selenium, and calcium present in the sample were quantified by comparison of the induced activities formed in the sample and the respective standards. The details of the nuclear parameters for the radioisotopes produced on neutron irradiation of these elements, their half-lives, and minimum detection limits achieved by using INAA can be found in table 2.

Results and discussion

The development and subsequent validation of the analytical methodologies forms an important feature of the study as the trace elements studied were present in extremely small concentrations (micro and sub-micro levels) in the diet samples. After the development of the analytical methods for iron, zinc, cobalt, cesium, selenium, calcium, thorium, and strontium, these methods were put to test by analyzing the standard reference materials obtained from the National Institute of Science and Technology (NIST) (Gaithersburg, Md., USA). The agreement between the concentrations of these elements in the reference materials obtained in the present work with the certified concentrations was quite good as is seen in the table 3.

Table 4 shows the mean, geometric mean, and the range of daily dietary intakes of the eight elements by the reference Indian man (adult male). Daily dietary intakes of iron, zinc, cobalt, selenium, cesium, strontium, thorium, and calcium were obtained by multiplying their measured concentrations with the total weight of the diet. The minimum and maximum intake values were for the groups from Kerala (southern India) and Punjab (northern India), respectively. It is important to mention here that the staple food in Punjab is wheat and in Kerala it is rice. Wheat had higher concentra-

TABLE 3: Comparison of the concentrations of iron, zinc, cobalt, selenium, thorium, cesium, strontium, and calcium in selected standard reference materials obtained with certified values (literature values given in parentheses)

Standard reference material	Elemental concentration							
	Iron (μg/g)	Zinc (μg/g)	Cobalt (μg/g)	Selenium (μg/g)	Thorium (ng/g)	Cesium (ng/g)	Strontium (μg/g)	Calcium (mg/g)
Citrus leaves (SRM 1572)	87.5 ± 2.8 (90)	29.5 ± 1.6 (29)	0.023 ± 0.002 (0.02)	0.04 ± 0.01 (0.03)	13 ± 2 (15)	100 ± 7 (98)	95 ± 4 (100)	30.9 ± 1.7 (31.5)
Orchard leaves (SRM 1571)	287 ± 15 (300)	23 ± 2 (25)	0.18 ± 0.02 (0.2)	0.07 ± 0.01 (0.08)	60 ± 2.8 (64)	44.7 ± 5.8 (40)	41.0 ± 2.5 (37)	20.1 ± 1.2 (20.9)
Bovine liver (SRM 1577)	276 ± 20 (268)	138 ± 8 (130)	0.19 ± 0.0 (0.18)	0.98 ± 0.1 (1.1)	3.8 ± 0.2 (3.2)	20 ± 4 (17)	0.14 ± 0.01 (0.14)	0.12 ± 0.01 (0.12)
Total diet (SRM 1548)	33.5 ± 1.7 (32.6)	29.7 ± 2.1 (30.8)	0.032 ± 0.005 (—)	0.23 ± 0.02 (0.245)	1.4 ± 0.3 (1.6)	12 ± 4 (14)	2.9 ± 0.3 (4.1)	1.78 ± 0.07 (1.74)

TABLE 4. Daily dietary intake of some important trace elements by the reference Indian man

	Daily intake of elements							
	Iron (mg)	Zinc (mg)	Cobalt (μg)	Selenium (μg)	Thorium (μg)	Cesium (μg)	Strontium (mg)	Calcium (g)
Mean ± SD	17.0 ± 6.9	9.1 ± 3.5	17.9 ± 7.4	57.4 ± 29.3	0.82 ± 0.39	5.2 ± 2.6	1.59 ± 0.68	0.37 ± 0.14
Range	10.2–34.4	5.3–16.7	8.3–31.4	35.0–130.8	0.44–1.75	2.6–11.8	0.79–2.96	0.17–0.67
Geometric mean	15.9	8.6	17.0	52.4	0.75	4.76	1.46	0.35
(Standard geometrical deviation)	(1.43)	(1.43)	(1.50)	(1.51)	(1.53)	(1.52)	(1.52)	(1.49)
No. of sample	20	20	19	17	20	20	20	20

tions of most of the elements studied [10].

The comparison of the daily dietary elemental intake by the reference Indian man with that of the ICRP reference man [11] and also with the limited data compiled by the IAEA on the average intake[12] by an adult in various countries of the world, are shown in table 5. While dietary intake data are available for the ICRP reference man for the eight elements in this study, the IAEA only has data available on four elements for the world average. The daily dietary intakes of the eight elements by reference Indian man are considerably lower by factors ranging from 1.4 for strontium to 18.0 for cobalt as compared with the ICRP reference man data. On the other hand, the Indian intakes for four elements are lower in comparison to the world average (recent data) by small factors ranging from 1.0 to 1.9. This range of variation between the Indian and the IAEA data is understandable, since the body weight ratio of the adult male representing the world population and reference Indian man is also about 1.4, and perhaps the intake requirement is also lower by the same proportion. The consistently higher intake values for the ICRP reference man, even in comparison to the world average, underscore the urgent need to review and revise the ICRP reference man data of the 1970s.

TABLE 5. A comparison of the daily dietary intake of trace elements by the reference Indian man, with that for the ICRP reference man and the world average compiled by the IAEA

Element	Daily dietary intake		
	Indian reference man	ICRP reference man	World average[a]
Iron (mg)	15.9	27	15.6
Zinc (mg	8.6	17	10.7
Cobalt (μg)	17.0	300	—
Selenium (μg)	52.4	150	72.0
Thorium (μg)	0.75	3	—
Cesium (μg)	4.76	10	—
Strontium (mg)	1.46	2	—
Calcium (g)	0.35	1	0.67

a. IAEA data is based on the preliminary results of intake received from 11 countries including the United States, China, and Japan (TEMA-7, 1990).

Conclusions

The analytical methods developed by using both instrumental neutron activation analysis (INAA) and radiochemical neutron activation analysis (RNAA) were found to be adequately sensitive to permit the determination of the daily dietary intakes of eight elements (iron, zinc, cobalt, cesium, strontium, thorium, and calcium) for the reference Indian man. The techniques were validated by analyzing NIST standard reference materials containing trace elements in concentrations similar to those present in these samples. The results for the reference materials showed a good agreement with their certified values and thus, assured the reliability of the data generated for the concentration of trace elements in food consumed in India.

The daily dietary intakes of the eight elements by the reference Indian man were considerably lower by factors ranging from 1.4 for strontium to 18.0 for cobalt as compared to the ICRP reference man data. However, the Indian intakes for four elements are lower as compared to the world average by small factors ranging from 1.0 to 1.9. The consistently higher intake values for the ICRP reference man, underscores the urgent need to review and revise the ICRP reference man data of the1970s.

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References

1. Underwood EJ. Trace elements in human and animal nutrition. 4th ed. New York: Academic Press, 1977.
2. Prasad AS. Trace elements in human health and disease. Vol. 1. Zinc and copper. New York: Academic Press, 1976.
3. Dang HS, Jaiswal DD, Sharma RC, Krishnamony S. Studies on the biological half-lives of three important radionuclides released in nuclear power reactor operations. Health Physics. 1995; 69(3):400–02.
4. Dang HS, Pullat VR, Pillai KC. Gastrointestinal absorption factor for uranium incorporated in diet. Radiation Protection Dosimetry 1992;40(3):195–7.
5. National Nutrition Monitoring Board (NNMB). Report on the urban population: Nutritional and anthropometric survey. Hyderabad: National Institute of Nutrition, 1984.
6. National Nutrition Monitoring Board (NNMB). Report on the rural population: Nutritional and anthropometric survey. Hyderabad: National Institute of Nutrition, 1980.
7. National Nutrition Monitoring Board (NNMB). Report on the repeat survey of rural population for nutritional and anthropometric status. Hyderabad: National Institute of Nutrition, 1988–1990.
8. Dang HS, Jaiswal DD, Parameswaran M, Krishnamony S. Physical, anatomical, physiological and metabolic data for reference Indian man—A proposal. BARC Report No. BARC/1994/E-043. Mumbai: Bhabha Atomic Research Centre,1994.

9. Dang HS, Jaiswal DD, Sunta CM, Soman SD. A sensitive method for the determination of Th in body fluids. Health Physics 1989;57(3):393–6.
10. Dang HS, Jaiswal DD, Suma Nair. Daily dietary intake of trace elements of radiological nutritional importance by the adult Indian population. J Radioanal Nuclear Chem 2001;249(1):95–101.
11. International Commission on Radiological Protection. Report of the task group on reference man. Oxford: Pergamon Press, 1975.
12. Parr RM, Abdulla M. Dietary intakes of trace elements and related nutrients in eleven countries: Preliminary results from an IAEA CRP. TEMA-7. Dubrovnik, Yugoslavia: IAEA, 1990; 22–25.

Instrumental neutron activation analysis of minor and trace elements in food in the Russian region that suffered from the Chernobyl disaster

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Abstract

The control of food quality, using the analysis of essential and toxic element contents, assumes an urgent importance within the regions that suffered from the Chernobyl disaster. Instrumental neutron activation analysis was used to study contents of 17 chemical elements (calcium, chlorine, cobalt, chromium, cesium, iron, mercury, potassium, magnesium, manganese, sodium, rubidium, antimony, scandium, selenium, strontium, and zinc) in foods within the south and southwest territories of the Kaluga Region that was exposed to radionuclide contamination. The radionuclide contamination ranges up to 15 Ci/km^2 there. Flesh and meat products, dairy products, bread, vegetables, legumes, roots, fruits, and mushrooms were analyzed. The concentration of essential and toxic elements in the different foods were in the normal ranges.

Key words: Food, minor and trace elements, instrumental neutron activation analysis, Chernobyl disaster

Introduction

The main etiologic factor of various diseases, syndromes, and pathologic conditions is an excess, deficiency, or imbalance of trace element intake into the human body [1]. Children, pregnant women, elderly, and weakened people, including those recovering from surgery and diseases, are the most sensitive to each change in the homeostasis of trace elements. An inadequate essential trace element intake may result in undesirable consequences that can multiply against a background of additional unfavorable environmental influences such as high levels of radiation and organic and inorganic toxicants. Therefore, in regions contaminated with radionuclides by the Chernobyl disaster, controlling the minor and trace elements in food is a current problem.

We used instrumental neutron activation analysis (INAA) to estimate the essential and toxic elements in different foods within the south and southwest territories of the Kaluga Region with radionuclide contamination ranges up to 15 Ci/km^2 [2].

Materials and methods

The meat and meat products, dairy products, bread, vegetables, legumes, roots, fruits, and mushrooms were bought in local shops. The products were washed and cleaned, placed into sealed, plastic containers and transported in a special car to the laboratory for analysis where they were weighed and homogenized within one day after collection. Portions of the homogenates weighing 50 g were put into polyethylene vessels, frozen, and lyophilized. The plastic containers and utensils used for food collection and storage were carefully washed with acetone and alcohol beforehand. A titanium knife was used to clean the vegetables, roots, and fruits. The IAEA reference material H-9 (mixed human diet) [3] was used to determine the accuracy of the method.

A VVR-C-type research nuclear reactor (water-water reactor (special)) was used to irradiate the samples. For the short-lived radionuclide INAA, we used a reactor horizontal channel equipped with a pneumatic transfer system. The neutron flux density was 1.7×10^{13} n.cm^{-2} s^{-1}. Ampoules with samples and standards were put into polyethylene containers and irradiated for 30 seconds. For the long-lived radionuclide INAA, a vertical reactor channel with the neutron flux density of 1.2×10^{13} n.cm^{-2} s^{-1} was used for irradiation. Samples and standards were wrapped in aluminum foil and put in a quartz ampoule that was pre-washed with acetone and alcohol. The ampoule was sealed, placed into an aluminum container and irradiated for 120 hours. A coaxial Ge(Li) detector of the 98 cm^3 active volume

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and spectrometric unit including a multichannel analyzer coupled to a personal computer (NUC 8100, Hungary) were used for measurement. Measurements were conducted 1 minute and 1.5 hours after irradiation for the short-lived radionuclide analysis. The first measurement lasted for 10 minutes and the second one for 20 minutes. To analyze the long-lived radionuclides, measurements were started 15 days after irradiation. The time of measurements was one hour for the standards and three hours for the samples and the IAEA reference material.

TABLE 1. Some characteristics and conditions of radionuclides used for INAA of minor and trace element contents in foods

Element	Radionuclide	Half-life	Used ϒ-ray energy MeV	Conditions of analysis[a]
Calcium	^{49}Ca	8.75 min	3085.0	A
Chlorine	^{38}Cl	37.29 min	1642.0; 2167.0	A
Cobalt	^{60}Co	5.26 yrs	1332.5	C
Chromium	^{51}Cr	27.8 days	320.1	C
Cesium	^{134}Cs	2.05 yrs	795.8	C
Iron	^{59}Fe	45.6 days	1291.6	C
Mercury	^{203}Hg	46.91 days	279.8	C
Potassium	^{42}K	12.4 hrs	1524.2	B
Magnesium	^{27}Mg	9.46 min	844.0	A
Manganese	^{56}Mn	2.58 hrs	846.8	B
Sodium	^{24}Na	14.96 hrs	1369.5; 2754.0	B
Rubidium	^{86}Rb	18.66 days	1078.7	C
Antimony	^{124}Sb	60.9 days	602.7	C
Scandium	^{46}Sc	83.89 days	889.2	C
Selenium	^{75}Se	120.4 days	264.6	C
Strontium	^{78m}Sr	2.83 hrs	388.5	B
Zinc	^{65}Zn	245.7 days	1115.5	C

a. Irradiation time, decay, and measurement:
A. 30 seconds, 60 seconds, 600 seconds; sample-detector distance: 10 cm; shielding: 5 cm lead.
B. 30 seconds, 90 minutes, 20 minutes; sample-detector distance: 0 cm; shielding: 5 cm lead.
C. 120 hours, 15 days, 1 or 3 hours; sample-detector distance: 3 cm; shielding: 5 cm lead.

The conditions of analysis for each element are given in table 1. Time of irradiation, decay, and measurement, and a sample-detector distance were chosen for optimal estimation of the largest number of chemical elements within a minimum statistical error. The conditions of analysis were calculated in advance using a specially developed computer program [4].

Results and discussion

The results and certified values of IAEA H-9 for each element were within the certified 95% confidence interval. Calcium is the only exception (table 2). The mean concentration of calcium was 21% higher than the certified mean value for IAEA H-9.

The short- and long-lived radionuclide INAA data of the minor and trace elements foods are presented in table 3. Comparison with published data [5, 6] showed that the essential and toxic element concentrations in different foods within the radionuclide-contaminated territories of the Kaluga Region were in the normal range.

TABLE 2. INAA of the IAEA reference material, H-9 (human mixed diet), as compared with certified values

	Element concentration, μg/g dry mass		
	Our results	Certified values	
Element	Mean ± SE	Mean	95% confidence intervals
Calcium	2,800 ± 350	2,310	2,150–2,470
Chlorine	12,300 ± 300	12,500	11,000–14,000
Cobalt	0.046 ± 0.002	0.043	0.038–0.048
Chromium	0.164 ± 0.016	0.15	0.11–0.19
Cesium	0.029 ± 0.002	0.025	—
Iron	35.7 ± 3.9	33.5	31–36
Mercury	< 0.01	0.0048	0.0034–0.0062
Potassium	8,100 ± 500	8,300	7,600–9,000
Magnesium	760 ± 200	785	730–840
Manganese	11.2 ± 1.3	11.8	11.0–12.6
Sodium	8,400 ± 200	8,100	7,400–8,800
Rubidium	8.6 ± 0.2	8.0	7.4–8.6
Antimony	0.016 ± 0.002	—	—
Scandium	0.0022 ± 0.0003	—	—
Selenium	0.12 ± 0.02	0.11	0.10–0.12
Strontium	< 30	3.0	2.6–3.4
Zinc	28.8 ± 1.0	27.5	25.7–29.3

TABLE 3. INAA of minor and trace elements in foods in regions contaminated by the Chernobyl disaster (Mean of element content in 1g of wet mass)

	INAA with short-lived radionuclides							INAA with long-lived radionuclides									
Food	Calcium Mg	Chlorine mg	Potassium mg	Magnesium µg	Manganese µg	Sodium µg	Phosphorus mg	Cobalt Ng	Chromium ng	Cesium Ng	Iron µg	Mercury ng	Rubidum µg	Antimony ng	Scandium ng	Selenium Ng	Zinc µg
Milk	2.53	1.11	1.74	193	0.4	317	7.12	1.1	50	3.2	4.1	2.2	4.8	2.5	2.4	<4	6.2
Cheese	35.8	33.9	0.90	842	2.3	8,390	19.9	2.7	189	3.7	17	2.5	1.1	0.6	2.0	350	114
Pork	0.39	1.08	3.06	587	0.2	440	3.38	5.6	<15	6.2	40	9.2	5.6	0.7	1.6	356	126
Beef	0.54	2.41	1.62	404	0.4	900	7.26	28	61	12	52	6.7	1.9	1.2	0.3	55	140
Sausage	1.50	41.3	3.06	500	4.4	11,900	33.2	75	175	37	188	19	14	1.4	2.0	387	216
White bread	0.64	9.83	1.74	1,170	17	2,680	90.5	55	<14	6.0	20	1.1	1.1	0.3	1.4	69	9.0
Rye bread	0.57	11.8	1.19	995	9.0	2,530	10.5	35	42	3.2	35	0.8	3.5	3.7	1.4	133	15
Potato	0.10	1.97	5.30	340	3.1	21	11.3	3.0	20	0.1	5.2	11	1.3	0.7	0.2	2	1.3
Tomato	0.31	0.58	1.55	161	0.3	15	1.70	8.2	12	0.8	4.3	<0.2	0.8	0.1	0.1	8	1.2
Onion	0.33	0.34	3.35	224	1.8	48	4.17	6.1	<110	2.5	9.5	1.1	0.4	0.7	0.3	19	3.6
Parsley	13.1	16.8	18.4	1,610	13	320	54.1	111	140	9.8	104	5.3	2.7	1.5	2.8	22	85
Beet	0.30	0.61	5.61	284	3.4	224	7.13	3.1	18	0.3	4.1	8.7	3.5	2.5	2.4	6	2.6
Carrot	0.93	0.50	5.73	282	12	300	30.8	5.4	50	0.3	4.3	10	2.2	2.5	0.2	10	3.1
Turnip	0.55	1.62	6.37	366	4.1	1,140	45.9	6.7	50	0.2	4.5	5.2	1.8	5.4	2.4	9	9.3
Peas	0.75	0.67	9.75	2,030	12	19	15.4	228	<16	16	59	15	18	0.9	0.3	89	47
Kidney bean	5.34	0.22	13.1	1,970	26	16	23.5	286	9	1.9	78	1.8	3.1	0.4	0.1	9	43
Fruits (dry)	0.83	0.43	8.40	1,360	3.0	136	79.4	—	—	—	—	—	—	—	—	—	—
Mushrooms (dry)	1.12	0.75	8.25	1,450	22	2,600	23.5	707	48	143	63	3.4	83	4.5	<1.7	883	167

References

1. Avtsin AP, Zhavoronkov AA, Rish MA, Strochkova LS. Human trace elements. Moscow: Medetsina, 1991.
2. Radiation and hazard. Moscow: Obninsk, 1993;(3) App.1.
3. Parr RM. On the role of neutron activation analysis in the certification of new reference material for trace element studies, mixed human diet, H-9. J Radioanal Nucl Chem 1988;123:259–71.
4. Korelo AM, Zaichick V. Software to optimise the multi-element INAA of medical and environmental samples. In: Activation analysis in environmental protection. Dubna: Joint Institute of Nuclear Research,1993: 326–32.
5. Gabovich RD, Priputina LS. Hygienic bases of nutritional protection from hazardous chemical agents. Kiev: Zdorovja, 1987.
6. WHO. Trace elements for human nutrition. WHO Report of Expert Committee No 532. Geneva: WHO, 1975.

Zinc and iron interactions evaluated between different mineral sources in different nutritional matrixes

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Abstract

We compared the absorption of BioZn, SFE-171, SO_4Fe (reference standard) and SO_4Zn (reference standard) alone or in combination in water and in an infant dessert. When mineral interactions were evaluated, zinc and iron were administered in a 1:1 molar relation. There 160 rats divided in 16 groups of 10 animals each which received: $SO_4{}^{65}Zn$, $Bio^{65}Zn$, $SO_4{}^{65}Zn + SO_4Fe$, $Bio^{65}Zn + SFE\text{-}171$, $SO_4{}^{59}Fe$, $^{59}SFE\text{-}171$, $SO_4{}^{59}Fe + SO_4Zn$ and $^{59}SFE\text{-}171 + BioZn$ either in water or an infant dessert. The results showed that BioZn has bioavailability similar to SO_4Zn both in water (23.36 ± 3.14% vs. 21.48 ± 6.03%. respectively) and in an infant dessert (19.89 ± 3.27% vs. 18.31 ± 4.76%, respectively). When these zinc compounds were administered with iron no statistical difference of zinc absorption was found ($Bio^{65}Zn + SFE\text{-}171$ in water 22.70 ± 6.30%, $Bio^{65}Zn + SFE\text{-}171$ in the infant dessert 18.07 ± 5.89%, $SO_4{}^{65}Zn + SO_4Fe$ in water 24.67 ± 5.70% and $SO_4{}^{65}Zn + SO_4Fe$ in the infant dessert 20.56 ± 5.20%). For iron, the absorption of $^{59}SFE\text{-}171$ in water was higher ($p < .01$) than $SO_4{}^{59}Fe$ in water and $^{59}SFE\text{-}171 + BioZn$ in water (32.35 ± 8.32% vs. 26.27 ± 8.83% vs. 23.69 ± 8.37%, respectively). Iron absorption from $SO_4{}^{59}Fe$ in water was higher ($p < .01$) than $SO_4{}^{59}Fe + SO_4Zn$ in water (26.27 ± 8.83% vs. 20.21 ± 8.72%, respectively). Iron absorption in the infant dessert was higher ($p < .01$) for $^{59}SFE\text{-}171 + BioZn$ than $SO_4{}^{59}Fe$, $^{59}SFE\text{-}171$ and $SO_4{}^{59}Fe + SO_4Zn$ (22.81 ± 6.97% vs. 16.12 ± 6.14% vs. 16.90 ± 6.23% vs. 15.04 ± 6.25%, respectively). Statistical differences ($p < .01$) were found between iron absorption from $^{59}SFE\text{-}171$ in water and the infant dessert (32.35 ± 8.32% vs. 16.90 ± 6.23%, respectively) and for $SO_4{}^{59}Fe$ (26.27 ± 8.83% vs. 16.12 ± 6.14% respectively). Zinc and iron interactions evaluated in a 1:1 molar relation of the minerals were observed only for iron absorption in water but not in infant dessert. No negative effect was found for zinc absorption neither in water nor in infant dessert.

Key words: iron, zinc, absorption, interaction, micronutrients, minerals

Introduction

There is a high incidence of iron and zinc deficiencies in the the world [1–5]. The interactions between trace elements are primarily antagonistic. Thus, it is expected that when two chemically similar ions are present in the intestinal lumen the one having molar excess will tend to exclude the other [6, 7]. In terms of dietary intake, recommendations for iron and zinc are on the same order of magnitude. However, preparations of nutrient supplements generally provide iron in much higher amounts than zinc. Therefore, the interference of iron on zinc absorption is predominantly observed [8, 9]. Many studies showed that high concentrations of iron can have a negative effect on zinc absorption when zinc and iron are given in a water solution. However, when they are given in a meal such an effect is not observed, possibly because in this case zinc can be absorbed via an alternative pathway with the aid of ligands formed during digestion [10, 11]. On the other hand, studies should be carried out to define the amounts to be used for fortifying foods with iron and zinc and to find the optimal ratio to avoid antagonistic interactions between them. Many authors recommend the addition of iron and zinc in a 1:1 molar ratio [6, 12]. We determined the zinc and iron interactions of different mineral sources when they were given in water or in an infant dessert.

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Materials and methods

Animals

We used 160 female inbred 3-month old Sprague-Dawley rats. The rats were distributed in 16 groups. Each group of animals was maintained in stainless steel cages of 315 mm by 445 mm and 240 mm high with a stainless steel grated floor and collection trays of the same material, thus preventing the feces from coming in contact with the animals. They had free access to water and were nourished with a standard diet (Nutrimentos Diet N° 3, Nutrimentos, Buenos Aires, Argentina). The animals were maintained with cycles of 12 hours of light and 12 hours of darkness throughout the experiment.

Administration of the products

Each iron and zinc compound was intrinsically labeled with ^{59}Fe and ^{65}Zn, respectively (NEN, Du Pont, Nemours and Co., Wilmington, Del., USA, catalogue NEZ-037 and NEZ-109, respectively). BioZn is zinc gluconate stabilized with glycine [13] and SFE-171 is ferrous sulfate microencapsulated with phospholipids [14]. The animals were food-deprived for 12 hours before the administration of the preparations. Each group of animals received: $SO_4{}^{65}Zn$, $Bio^{65}Zn$, $SO_4{}^{65}Zn + SO_4Fe$, $Bio^{65}Zn$ + SFE-171, $SO_4{}^{59}Fe$, ^{59}SFE-171, $SO_4{}^{59}Fe + SO_4Zn$ and ^{59}SFE-171 + BioZn either in water or in an infant dessert. The products were administered through a syringe coupled to a plastic gastric catheter, which allowed the standardization of the intake volume to 1 ml. The animals were again given food four hours after the product intake. The iron and zinc doses were 73 and 85 μg/kg, respectively (1:1 molar ratio). The infant dessert contained carbohydrates 16.4 g / 100 g, lipids 2.3 g / 100 g, proteins 3.4 g / 100 g, calcium 124 mg / 100 g, vitamin A 359 IU / 100 g, vitamin D 62 IU/ 100 g, folic acid 15.6 μg / 100 g, vitamin B12 0.36 μg / 100 g, and additives.

Absorption studies

We measured the activity retained by each rat as a function of time by a gamma spectrometer with a 5 cm by 5 cm NaI (Tl) well crystal in optimal electronic conditions (Alfanuclear, model ZX, Buenos Aires, Argentina). All the animals were measured every day for 10 days. To determine zinc or iron absorption, the ^{65}Zn or ^{59}Fe radioactivity retained by each rat was measured using a whole-body geometry introducing the animal in a covered lucite box, adapted to the size of the animal and to the detector geometry. We determined the zinc or iron retention percentage for each rat as a function of time. The zinc or iron absorption values were determined by the extrapolation of the final portion of the retention curve to the initial time (t = 0) by means of a linear regression analysis of the experimental data in order to correct the data for physical radioisotopic decay and physiological elimination.

Statistical analysis

The data are presented as mean ± SD. The results were evaluated by a two-way analysis of variance (ANOVA). To test the differences among the means we used the Tokey method considering statistically significant the probability levels < .01 [15].

Results

Zinc from BioZn had a similar absorption to SO_4Zn both in water (23.36 ± 3.14% vs. 21.48 ± 6.03%, respectively) and in the infant dessert (19.89 ± 3.27% vs. 18.31 ± 4.76%, respectively) (fig. 1). When these zinc compounds were administered with iron, no statistical difference of zinc absorption was found (BioZn + SFE-171 in water 22.70 ± 6.30%, BioZn + SFE-171 in the infant dessert 18.07 ± 5.89%, $SO_4Zn + SO_4Fe$ in water 24.67 ± 5.70% and $SO_4Zn + SO_4Fe$ in the infant dessert 20.56 ± 5.20%).

Figure 2 shows that iron absorption from SFE-171

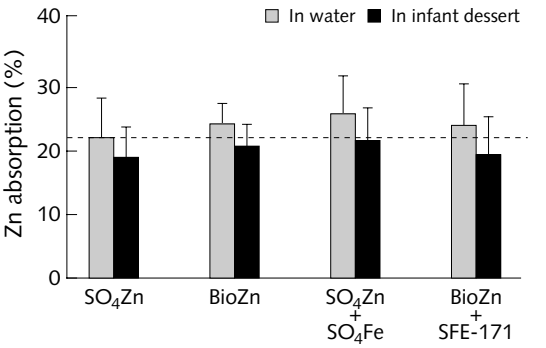

FIG.1. Zinc absorption and interaction in water and in an infant dessert

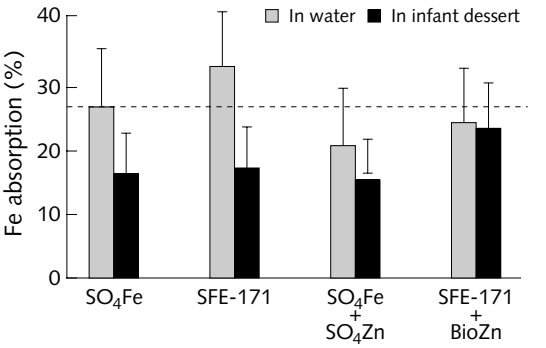

FIG. 2. Iron absorption and interaction in water and infant dessert

in water was higher ($p < .01$) than SO_4Fe in water and SFE-171 + BioZn in water (32.35 ± 8.32% vs. 26.27 ± 8.83% vs. 23.69 ± 8.37%, respectively). Iron absorption from SO_4Fe in water was higher ($p < .01$) than SO_4Fe + SO_4Zn in water (26.27 ± 8.83% vs. 20.21 ± 8.72%, respectively), but was similar to iron absorption of SFE-171 + BioZn in water (26.27 ± 8.83% vs. 23.69±8.37%, respectively). Nevertheless iron absorption from SFE-171 was higher than SFE-171 + BioZn (32.35 ± 8.32% vs. 23.69 ± 8.37%, respectively). Iron absorption in the infant dessert was higher ($p < .01$) for SFE-171 + BioZn than SO_4Fe, SFE-171 and SO_4Fe + SO_4Zn (22.81 ± 6.97% vs. 16.12 ± 6.14% vs. 16.90 ± 6.23% vs. 15.04 ± 6.25%, respectively). Statistical differences ($p < .01$) were found between iron absorption from water and the infant dessert for SFE-171 (32.35 ± 8.32% vs. 16.90 ± 6.23%, respectively) and for SO_4Fe (26.27 ± 8.83% vs. 16.12 ± 6.14%, respectively).

Discussion

Fortification and supplementation of foods with zinc and iron are effective strategies, but when using iron and zinc together one of the ions may inhibit the absorption of the other [6].

Two principally different types of trace element interactions can occur in a biological system [6]. When trace elements share the same absorption pathway, a high concentration of one element may interfere with the absorption of the others [16, 17]. In the second type of interaction, the deficiency of one element can affect the metabolism of another element [18]. In the case of food fortification or supplementation the first type of interaction is the most important to be considered; nevertheless, the second type of interaction should also to be taken into account.

It is known that iron and zinc do not form similar coordination complexes in aqueous solutions, and it is believed that they do not compete directly for the same absorptive sites; however, in specific circumstances this interaction takes place. Therefore, we evaluated the iron-zinc interactions when they were administered in water or in an infant dessert.

In figure 1, we see the values of zinc absorption and the effect of iron on zinc absorption when they were administered in water or in an infant dessert. Zinc from BioZn has similar absorption to SO_4Zn both in water and the infant dessert, demonstrating that the nutritional matrix does not affect the zinc absorption for both compounds. When SO_4Zn and BioZn were administered with iron as SO_4Fe and SFE-171 in the infant dessert or in water, no statistical differences on zinc absorption were found, demonstrating that in these experimental conditions no negative interaction on zinc absorption was detected. Nevertheless, the principal advantage between both zinc compounds is that BioZn does not produce any modification of the sensorial properties of the fortified food.

In figure 2, we see the results of iron absorption and the effect of zinc on it. In water, the iron absorption from SO_4Fe was higher than from SO_4Fe + SO_4Zn and the iron absorption from SFE-171 was also higher than from SFE-171 + BioZn. These results show that in our experimental conditions zinc interfered with iron absorption in both cases. Nevertheless iron absorption from SFE-171 + BioZn did not differ significantly from the absorption of the reference standard (SO_4Fe).

For the infant dessert, iron absorption was lower for each iron compound in water demonstrating that the nutritional matrix produces a negative effect on iron absorption. Nevertheless, iron absorption from SFE-171 + BioZn was significantly higher than SO_4Fe, SO_4Fe + SO_4Zn and SFE-171. This result can be explained if we consider that BioZn contains gluconate and glycine in a molar ratio 2:2:1 with regard to iron. In gastric conditions these compounds can interact with iron through a weak ligand effect, protecting it from the inhibitory effect of the nutritional matrix and improving iron absorption [19, 20]. Iron absorption from SO_4Fe + SO_4Zn was similar to that from the reference standard (SO_4Fe), demonstrating that in infant dessert zinc has no negative interaction on iron absorption.

Under our experimental conditions, iron had no negative effect on zinc absorption either in water or in an infant dessert. We observed a negative interaction of zinc on iron absorption in water, however, had no negative effect on iron absorption in the infant dessert.

References

1. WHO Commission on Health and Environment. Report of the panel on food and agriculture. Geneva: World Health Organization, 1992.
2. Pennington JAT. Intake of minerals from diets and foods: is there a need for concern? J Nutr 1996;126:2304s–8s.
3. Ruz M. Trace element intake and nutriture in Latin America. Adelaide, Australia: Proceedings of the International Congress of Nutrition;1994:296–300.
4. Sandstead HH, Smith Jr J. Deliberations and evaluations of approaches, endpoints and paradigms for determining zinc dietary recommendations. J Nutr 1996;126: 2410s–8s.
5. Stephenson L, Latham M, Ottesen E. Global malnutrition. Parasitology 2000;121:S5–22.
6. Lonnerdal B. Iron-zinc-copper interactions. In: Micronutrient interactions. Impact on child health and nutrition. Washington D.C.: USAID, 1996:3–10.
7. O'Dell B. Mineral interactions relevant to nutrient requirements. J Nutr 1989;19:1832–8.
8. Solomons N. Competitive interaction of iron and zinc in

the diet: consequences for human nutrition. J Nut. 1986; 116:927–35.
9. Solomons NW, Ruz M. Zinc and iron interactions: concepts and perspectives in the developing world. Nutr Res 1996;17:177–85.
10. Whittaker P. Iron and zinc interactions in humans. Am J Clin Nutr 1998;68:442–6.
11. Rossander-Hulten L, Brune M, Sandstrom B, Lonnerdal B, Hallberg L. Competitive inhibition of iron absorption by manganese and zinc in humans. Am J Clin Nut. 1991; 54:152–6.
12. Davisson L, Almgren A, Sandstrom B, Hurrell R. Zinc absorption in adult humans: the effect of iron fortification. Br J Nutr 1995;74:417–25.
13. Salgueiro MJ, Zubillaga MB, Lysionek AE, Sarabia MI, Caro RA, De Paoli T, Hager A, Ettlin E, Weill R, Boccio J. Bioavailability, biodistribution and toxicity of BioZn-AAS™: a new zinc source. Comparative studies in rats. Nutrition 2000;16:762–6.
14. Boccio J, Zubillaga M, Caro R, Gotelli M, Weill R. New procedure to fortify fluid milk and dairy products with high-bioavailable ferrous sulfate. Nutr Rev 1997;55:240–6.
15. Dawson-Saunders B, Trapp R. Basic and clinical biostatistics. Washington, D.C.: Appleton and Lange, 1994.
16. Lonnerdal B, Keen C, Ohtake M, Tamura T. Iron, zinc, copper and manganese in infant formulas. Am J Dis Child 1983;137:433–7.
17. Hill C, Matrone G. Chemical parameters of the study in vivo and in vitro interactions of transition elements. Fed Proc 1970;29:1474–81.
18. Cohen N, Keen C, Lonnerdal B, Hurley L. Effects of varying dietary iron on the expression of copper deficiency in the growing rats: anemia, ferroxidase I and II, tissue trace elements, ascorbic acid, and xanthine deshydrogenase. J Nutr 1985;115:633–49.
19. Whitehead M, Thompson R, Powell J. Regulation of metal absorption in the gastrointestinal tract. Gut 1996; 39:625–8.
20. Powell J, Whitehead M, Ainley C, Kendall M, Nicholson J, Thomson R. Dietary minerals in the gastrointestinal tract: Hydroxypolymerisation of aluminium is regulated by luminal mucins. J Inorg Biochem 1999;75:167–80.

Iron fortification of wheat flour: bioavailability studies

Rakhshanda Bilal, Samina Roohi, Tanvir Ahmad, and Trinidad P. Trinidad

Abstract

Bioavailability refers to that fraction of nutrients which is utilized by the body out of the total indigested amount. Various direct and indirect methods for the determination of bioavailability are available. We determined the bioavailability of iron from fortified wheat flour using both in vitro and in vivo methods. The bioavailability data will be used to make the recommendation for a fortification strategy in Pakistan. The in vitro bioavailability of iron from fortified wheat flour was determined using in vitro enzymatic digestion and fermentation by simulating the condition of the small intestine and colon in the laboratory. Different products were prepared from the fortified ferrous sulfate ($FeSO_4$) and unfortified wheat flour. The total iron of the samples was measured by the wet-digestion method and analyzed on an atomic absorption spectrometer (AAS). To obtain the percentage of iron released, the samples were subjected to pepsin digestion and dialysis. The dialysate was collected at 3, 6, 9, and 12 hours and read on an AAS. The retentates from the above were subjected to the fermentation condition of the colon by inoculating it with human fecal inoculum and incubating it for 24 hours at 37°C under anerobic conditions. The dialysate was collected at 3- and 6-hour intervals and read on an AAS. More iron was released from fortified wheat flour (4.6%), leavened chapati (6.8%), and Nan (15.1%) than from the unfortified control samples. Fermentation and leavening resulted in a better release, which was evident from in vitro digestion results.

Key words: Iron fortification, *in vitro*, bioavailability, wheat flour

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Introduction

Iron is an essential nutrient and iron deficiency (ID) and iron-deficiency anemia (IDA) are widely prevalent in developing countries, especially in the low socioeconomic strata of the population. The incidence of iron-deficiency anemia is high in Pakistan and it is a major public health problem with many adverse consequences, especially in young children and women of reproductive age [1, 2].

Among many strategies to control IDA, fortification of appropriate foods with suitable a type of iron is considered most promising because of its long-lasting effects and minimum consumer involvement. Iron supplementation is considered to be a short-term solution, whereas, food diversification requires maximum input on the part of the consumer [2].

The Government of Pakistan is planning to fortify wheat flour with iron in order to combat iron deficiency and its consequent anemia. There are various choices for the fortificants (forms of iron to be used as premix) and the vehicle (most commonly consumed food by the population). Prior to launching a nationwide fortification program, a feasibility study has begun to determine the best form of iron fortificant and its level.

This paper summarizes the preliminary data, on *in vitro* bioavailability studies done on various products prepared from wheat flour fortified with ferrous sulfate ($FeSO_4$) in order to compare their relative bioavailability. Further studies are ongoing with elemental iron-fortified wheat flour using the *in vitro* method. In the final phase of study, the *in vivo* bioavailability studies will be undertaken using stable isotopes of iron and tissues retention methodologies [3].

Materials and methods

Well-milled grade No. 1 (80% extraction) wheat flour was used for the fortification purposes.

Ferrous sulfate was used as the fortificant (USP 24,

Dr. Paul Lohmann, GmbH KG, Emmerthal, Germany). The wheat flour was fortified with $FeSO_4$ (at 30 PPM) by using both a large-scale mechanical mixer and a small-scale mixer for the homogeneity study.

Preparation of products

Nan is a product made from wheat flour. The dough is prepared using a starter for fermentation long with milk. The round flattened dough is cooked in an earthen furnace, rather than on a hot plate (chapati), leavened and unleavened chapatis were prepared from the fortified and unfortified wheat flour. The wheat flours (fortified and unfortified) were kneaded with the deionized water, separately. The chapatis were prepared on a non-stick hot plate (Tawa) using a gas burner. The leavened chapatis were prepared from wheat flour after leavening the dough. Nan from fortified and unfortified wheat flour was prepared by Nan house (Ghausia Nan House, Islamabad). The leavening process at the Nan house involved the addition of milk and yeast for the preparation of the dough.

The samples were freeze-dried (Edwards Company, Modulyo, Manor Royal, Crawley, West Sussex, UK) and ground with a mortar and pestle first, then with an electric grinder (Moulinex, Paris, France). The powdered samples were stored in labeled jars at –15°C.

Total iron determination

The wet-digestion method was used to determine the homogeneity of iron in the wheat flour [4]. The homogeneity of mixing was better with the small-scale method than with the large-scale method (fig. 1), therefore the small-scale method was adopted for the fortification of wheat flour.

The total iron of the products, prepared from the fortified and unfortified wheat flour, was also determined by the wet digestion method. Samples were digested using concentrated H_2SO_4 and 30% H_2O_2 in a Kjeldahl flask at 150°C for 10 to 15 minutes. The digested samples were then measured using an AAS [4].

In vitro enzymatic digestion

The *in vitro* release of iron was done with the pepsin digestion and dialysis method by simulating the conditions of the stomach and small intestine in the laboratory. Duplicate samples (20 g freeze-dried) were homogenized with 80 g of deionized water. The pH of the mixture was adjusted to 2.0 with 6N HCl and enzymatically digested using 3.2 ml of pepsin-HCl solution [8 g pepsin (Sigma Chemical Company, St. Louis, Mo., USA, 600 units/mg solid from pig stomach) in 50 ml of 0.1M HCl] for three hours at 37°C in a metabolic shaker water bath. Aliquots (20g) of the digested samples were placed in dialysis bags (spectrapor 1, width 23mm, 6000–8000 MW cutoff) where 5 ml pancreatin-bile solution [1g pancreatin (porcine pancreas Grade VI, Sigma Chemicals Company) plus 6.25 g bile extract (porcine, Sigma chemicals Company) in 100 ml of 0.5 M sodium bicarbonate Solution (pH 7.5)] was added and incubated for 12 hours at 37°C in a water bath to determine iron release potentially available for absorption in the small intestine. The dialysate was collected at 3, 6, 9, and 12 hours and were read on the AAS [5].

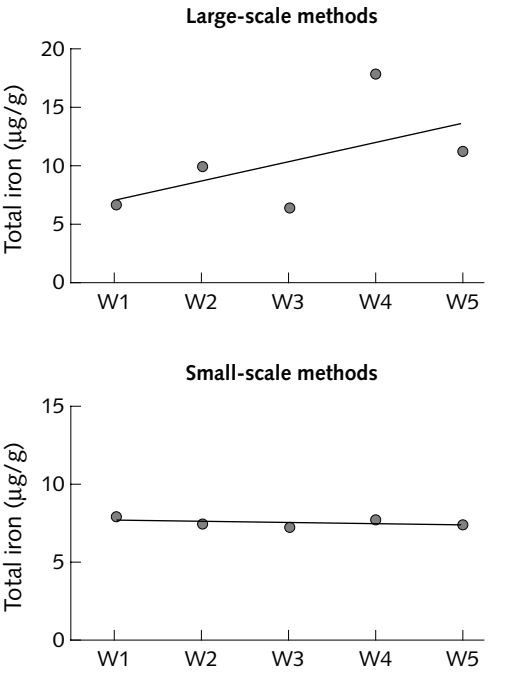

FIG.1. Total iron contents of the fortified wheat flour determined by wet digestion in large-scale and small-scale methods

In vitro fermentation

The retentate from the *in vitro* enzymatic digestion was freeze-dried and 0.5 g was placed in a serum bottle and 40 ml fermentation media [a mixture of 2L deionized water, 1L 0.5 M sodium bicarbonate buffer solution, 1L macro mineral solution (0.04 M $NaHPO_4$ and 0.5M KH_2PO_4), 5 ml reducing solution (mixture of 1.25 g cysteine-HCl, 50g KOH pellets and 1.25 g Na_2S in 100 ml double deionized (DDI) water)]. The mixture was flushed with carbon dioxide until it was colorless. The bottles were sealed with rubber stoppers and aluminum seals using a hand crimper and stored overnight at 4°C.

The next day, the bottles were placed in a 37°C water bath for 1 to 2 hours before inoculation. A 10ml fecal inoculum (prepared from 1:15 dilution of fresh feces from a human volunteer) was put into each bottle and incubated for 24 hours at 37°C. After 24 hours, the bottles were opened and 1 ml of 0.6% merthiolate solution (Sigma Chemical Company) was added. The contents were transferred to dialysis bags that were previously soaked in double deionized water. The tubes were then immersed in 100 ml double deionized water for six hours. The dialysate was collected at 3 and 6 hours and read as before using an AAS [5].

Results

The total iron and the percentage of iron released after *in vitro* digestion from the unfortified and fortified leavened and unleavened chapatis and Nan are given in table 1. The percentage of iron released in the fortified Nan (14.3%), leavened chapati (6.4%) unleavened chapati (2.6%) after *in vitro* digestion was higher than the control (4.6%, 2.9% and 0.5 %, respectively).

The percentage of iron released after fermentation in all the wheat flour products was greater than the controls (fig. 2), which can be attributed to the large amount of fiber present. The fiber complexes with the iron, thus inhibiting its absorption in the small intestine. However, in the actual system, the complexes are broken down during the fermentation process in the large intestine due to the action of phytases [6], which results in better absorption/uptake in the intestine.

For Nan, the fermentation took place while in the dough stage, therefore the percentage of iron released was greater after pepsin digestion (14.3%) with only 0.8% released after *in vitro* fermentation (table 1). The same is true for the leavened chapatis.

Conclusion

Fermentation process increases the percent of iron released because of the action of phytases.

The fortified Nan showed a maximum iron release, which confirmed the earlier observation that leavening and fermentation have a positive effect on bioavailability of iron in high phytate flour.

The maximum benefit of fortified wheat flour will be derived if it is eaten as fermented products, i.e., leavened chapati or Nan.

Acknowledgements

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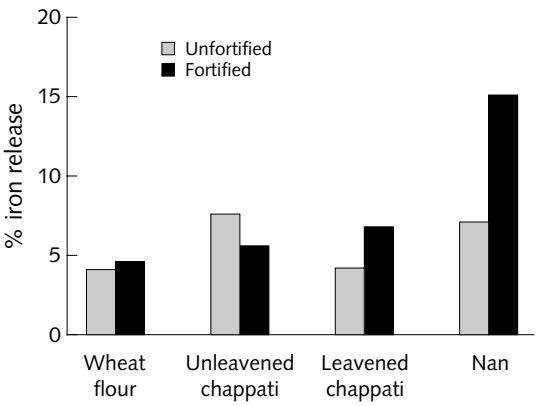

FIG. 2. Total percentage of iron released after *in vitro* digestion and fermentation in products prepared from unfortified and fortified wheat flour.

TABLE 1. Total iron and the percentage of iron released after *in vitro* digestion and fermentation of products prepared from unfortified and fortified wheat flour

Sample no.	Samples	Total (µg/g)	% release of iron		
			After *in vitro* digestion	After *in vitro* fermentation	Total % release
1	Wheat flour (no addition)	21.25	1.7	2.4	4.1
2	Wheat flour[a]	65.19	0.9	3.7	4.6
3	Unleavened chappati (no addition)	73.72	0.5	7.1	7.6
4	Unleavened chappati[a]	77.00	2.6	3.1	5.6
5	Leavened chappati (no addition)	61.89	2.9	1.3	4.2
6	Leavened chappati[a]	62.66	6.4	0.4	6.8
7	Nan (no addition)	76.76	4.6	2.5	7.1
8	Nan[a]	74.05	14.3	0.8	15.1

a. Fortified with $FeSO_4$ at 30 ppm.

References

1. Andersen PP, Lorch RP, Rosegrant MW. Food policy report. Washington, D.C.: International Food Policy Research Institute, 1999.
2. Viteri FE. Prevention of iron deficiency. In: Howson CP, Kennedy ET, Horwitz A. Prevention of micronutrient deficiencies: A toolkit for policy makers and public health workers. Washington D.C.: Institute of Medicine, National Academy of Sciences, 1998; 45–83.
3. Brune M, Rossander-Hulten L, Hallberg L, Gleerup A, Sandberg AS. Iron absorption from bread in humans: Inhibiting effects of cereal fiber, phytate and inositol phosphates with different numbers of phosphate groups. J Nutr 1992;122:442–9.
4. Association of Official Analytical Chemists. Official methods of analysis. 10th ed., Washington D.C.: AOAC, 1965:192.
5. Trinidad T P, Wolves TMS, Thompson LU. Availability of calcium for absorption in the small intestine and colon from diets containing available and unavailable carbohydrate: an in vitro assessment. Int J Food Sci Nutr 1996;47:83–8.
6. Mellon F, Sandstrom B. Stable isotopes in human nutrition. London: Academic press, Harcourt Brace & Company, 1996:34–9.

The effect of different iron fortificants on iron absorption from iron-fortified rice

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Abstract

Iron absorption from rice fortified with different iron fortificants, e.g., ferrous sulfate ($FeS0_4$), sodium iron EDTA (NaFeEDTA), ferrous fumarate (FeFum), and ferrous bisglycinate (FeBis) was determined using an in vitro enzymatic digestion method simulating conditions in the small intestine and an in vivo method using radioisotope techniques. The in vitro method showed that the percentage of dialyzable iron from NaFeEDTA (15.7 ± 0.9) and $FeS0_4$-fortified rice (13.2 ± 1.5) was significantly greater than that from FeFum (6.4 ± 0.6; $p < .05$*) and FeBis fortified rice (3.3 ± 0.8;* $p < .05$*). Iron absorption in vivo was investigated from $FeS0_4$ and NaFeEDTA fortified rice with and without fish and vegetables in 10 borderline iron-deficient subjects. Iron absorption (mg) from NaFeEDTA fortified rice (0.44 ± 0.11) was significantly greater than from $FeS0_4$-fortified rice (0.22 ± 0.05;* $p < .05$*) and the unfortified rice (0.17 ± 0.02;* $p < .05$*). Iron absorption (mg) from a meal consisting of iron-fortified rice, fish, and vegetables was significantly greater from NaFeEDTA (0.88 ± 0.24) and $FeS0_4$ (0.67 ± 0.10) -fortified rice than from the unfortified rice (0.41 ± 0.08;* $p < .05$*). This study concluded that both NaFeEDTA and FeS04 are effective iron fortificants for rice. The binder used in the study may have a significant role in the release of iron from iron-fortified rice for absorption. Further studies on the use of other binders to maximize iron release and minimize iron loss during cooking should be conducted to improve iron absorption from the fortified rice/rice-fish-vegetable meals. Results from this study can be used as a basis for food iron fortification programs as well as in the establishment of recommended dietary allowances for iron among Filipinos.*

Key words: iron absorption, iron fortificants, iron-fortified rice

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Introduction

Iron-deficiency anemia is prevalent in the Philippines especially among infants and preschool children and pregnant and lactating women. Thirty-one out of every 100 Filipinos are anemic [1]. Given this result, an effective nationwide intervention is necessary to combat this disease. One nutrition strategy to solve this problem is the food fortification intervention program. Rice, the staple food of most Filipinos, is a good vehicle to fortify with iron. Previous studies have shown that iron absorption from ferrous sulfate-fortified rice, fried fish, and jackfruit with coconut milk was significantly greater than that from unfortified rice with the same meal [2]. However, the study did not investigate the effect of other iron fortificants such as sodium iron EDTA (NaFeEDTA), ferrous fumarate (FeFum), or ferrous bisglycinate (FeBis). In addition, other iron fortificants may be as or more effective than ferrous sulfate ($FeS0_4$) for fortifying rice. We determined the effect of the different iron fortificants on iron absorption from iron-fortified rice with and without fish and vegetables using *in vitro* and *in vivo* methods. The Results of this study can be used as a basis for an effective iron fortification program as well as for establishing recommended dietary allowances for iron for Filipinos.

Materials and methods

Preparation of the iron-fortified rice

The method used in this study was a modification of the method of Peil et al. [3]. Milled premium grade rice (95% head rice) was used in the preparation of the premix iron-fortified rice and milled grade No.1 (80% head rice) was used in the preparation of the enriched rice samples (National Food Authority Circular No. AO-97-08-001). A mixture of carboxy ethyl cellulose (CEC), carboxy methyl cellulose (CMC) (food grade; Dow Chemicals and Amchem Marketing Inc., Midland, Mich., USA) and organic solvents such as

isopropyl alcohol (food grade, J.T.Baker, Pillipsburg, New Jersey, USA), ethanol (food grade; Ajax Laboratory Chemicals, NSW, Australia) and absolute alcohol (food grade; Ajax Laboratory Chemicals, NSW, Australia) was used as the coating solution. Ferrous sulfate (Alyson's Chemical Enterprises, Inc., Quezon City, Philippines, USP Grade), sodium iron EDTA (food grade, Dr. Paul Lohman, Emmerthal, Germany), ferrous fumarate (food grade, Dr. Paul Lohmann, Emmerthal, Germany) and ferrous bisglycinate (food grade, Dr. Paul Lohmann, Emmerthal, Germany) were used as iron fortificants. The iron fortificant was dispersed in the coating solution before pouring onto rice grains tumbling in a rotary mixer. The coated iron-fortified rice samples were air dried for 24 hours to eliminate residual alcohol and moisture that was absorbed during processing. The premix iron-fortified rice contained 1,200 mg iron per 100 g rice and was packed in polyethylene bags and labeled. Enriched rice samples were prepared by dispersing one part of iron-fortified premix rice to 200 parts of ordinary rice. The enriched rice was estimated to contain 6 mg iron per 100 g rice. The amount of iron-fortified rice used in this study provided 3 mg of iron per 100 g or one-third of the recommended dietary allowances for Filipino men.

The binder solution was tested alcohol toxicity and was found to be negative. The alcohol evaporated during the drying of the iron-fortified rice.

In vitro enzymatic digestion

Duplicate 20 g samples of freeze-dried cooked rice fortified with $FeS0_4$, NaFeEDTA, FeFum, and FeBis were homogenized with 80 g deionized water. The pH of the mixture was adjusted to pH 2.0 with 6N HCl and enzymatically digested *in vitro* using 3.2 ml of pepsin-HCL solution (8 g pepsin, 600 units/mg solid from hog stomach, Sigma Chemical Company, St. Louis, Mo., USA) in 50 ml of 0.1M HCl) for three hours. Aliquots (20 g) of the digested samples were placed in a dialysis bag (Spectrapor 1, width 23 mm, 6000–8000 MW cutoff; molecular porous membrane, Spectrum Laboratories, Inc., Rancho Dominguez, Calif., USA) where 5 ml pancreatin-bile solution (1 g pancreatin (porcine pancreas Grade VI, Sigma Chemical Company, St. Louis, Mo., USA) plus 6.25 g bile extract (porcine, Sigma Chemical Company) in 250 ml of 0.1M sodium bicarbonate solution (pH 7.5)) was added and incubated for nine hours to determine the dialyzable iron potentially available for absorption in the small intestine [4]. The dialysates were collected every three hours and replaced with 100 ml double distilled water. Dialysates were read in the atomic absorption spectrometer for iron content. The percentage of dialyzable iron was calculated as follows:

$$\% \text{ dialyzable iron} = \frac{\text{mg dialyzable iron } (\sum t_1 \ldots t_3)}{\text{mg total iron}} \times 100$$

In vivo iron measurements

Subjects

Ten healthy adults, 7 males and 3 females, 30 ± 2 years of age, 100 ± 2% of ideal weight were selected as subjects after receiving a physical examination. Blood samples were drawn from the subjects for hemoglobin [5] and hematocrit [5] to assess their iron status and to determine background radioactivity. The protocol for this study was approved by the Human Ethics Committee, Philippine Council for Health Research and Development, Department of Science and Technology. The subjects signed an informed consent form.

Preparation of the radio-iron labeled fortified rice [2]

The iron-fortified rice was labeled with ^{55}Fe (1.0 uCi/subject for rice, fish, and vegetables; Amersham Pharmacia, Buckinghamshire, England) or ^{59}Fe (0.5 uCi/subject for rice alone; Amersham Pharmacia, Buckinghamshire, England). The isotope together with the iron fortificant was dispersed uniformly in the coating solution it was poured onto the rice grains. The coated iron-labeled fortified rice was air dried for 24 hours. The rice was cooked and served to the subjects with or without fish and vegetables. It was assumed that there is an isotopic exchange between the native iron and isotopic iron in the coating solution. However, complete isotopic exchange takes place in the small intestine [6].

Test meals

Food samples were prepared in the Nutrient Availability Section of the Nutritional Science and Technology Division at the Food and Nutrition Research Institute (FNRI) from raw materials bought from the Bicutan Market. The test meal consisted of rice (100 g uncooked), fish (hasa-hasa, 40 g uncooked) and vegetables (kangkong, 44 g uncooked). The rice was washed once and boiled with water in a rice cooker. Leafy vegetables were washed, the stem separated and cooked with water and salt and served with pure kalamansi juice (Philippine lemon, *Citrus Microcarpa*, 1 g). Salt was added to the fish after it was cleaned and the meat separated from the bones and then fried in a stainless steel pan. The meal was homogenized, freeze-dried, and analyzed for total iron [7], non-heme iron [8], ascorbic acid [9], phytic acid [10], and tannic acid [11].

Feeding regimen

All foods and meals were eaten between 7:00 to 9:00 AM after an overnight fast for four consecutive days as follows: rice (A); rice, fish, vegetables (B); rice, fish, vegetables (B); rice (A). No food or drink was allowed for three hours after the meal was consumed. After 14

days a blood sample was drawn from the subjects for radioactivity measurements. This feeding regimen was done for unfortified rice and rice fortified with sodium iron EDTA and ferrous sulfate (fig. 1). After feeding all of the above unfortified and iron-fortified foods and meals, a reference dose containing 3 mg iron as ferrous sulfate and 30 mg ascorbic acid labeled with ^{59}Fe was given as a drink to all subjects after an overnight fast. After 14 days, another blood sample was drawn from the subjects for radioactivity measurements. The reference dose measures the absorptive capacity of each individual subject [12]. The total amount of radio-iron labeled food or reference dose given to each subject was 3.5 uCi ^{59}Fe and 6.0 uCi ^{55}Fe.

Iron absorption measurements

Duplicate 5 ml samples of whole blood and standard whole blood samples (Philippine General Hospital Blood Bank) labeled with ^{55}Fe and ^{59}Fe (same amount of ^{55}Fe and ^{59}Fe used to label the meal) were digested [13]. The radioactivity of the digested blood was read in a liquid scintillation counter (Beckman LS 6500, Beckman, Fullerton, Calif., USA). The percentage of iron absorption from the test foods, meals, and reference dose were calculated based on the estimation of blood volume of subject from sex, height, and weight using the Tulane table [14] with the assumption that 100% of the radioactivity was incorporated into the red blood cells [15].

Statistical analysis

The results were expressed as means ± SEM. Differences between treatments were examined by two-way repeated measures analysis of variance and Tukey's studentized range test using the Statistical Analysis System program (SAS Institute, Inc., Cary, NC, USA).

Day	Activities
0	Hematological characteristics and background data
1	Feeding of NaFeEDTA-fortified rice labeled with ^{55}Fe
2	Feeding of NaFeEDTA-fortified rice labeled with ^{59}Fe Fish and vegetables
3	day off
4	Feeding of NaFeEDTA-fortified rice labeled with ^{55}Fe
18	Blood extraction Feeding of unfortified rice labeled with ^{55}Fe
19	Reference dose labeled with ^{59}Fe
20	— day off —
21	Feeding of unfortified rice labeled with ^{55}Fe
35	Blood extraction Feeding of $FeS0_4$-fortified rice labeled with ^{55}Fe
36	Feeding of $FeS0_4$-fortified rice labeled with ^{59}Fe Fish and vegetables
37	— day off —
38	Feeding of $FeS0_4$-fortified rice labeled with ^{55}Fe
52	Blood extraction

FIG. 1. Protocol for the *in vivo* study

Results

The total iron in the uncooked and cooked enriched rice was used to determine the homogeneity of the iron-fortified rice and the iron loss during cooking (table 1).

In vitro iron availability

The percentage of dialyzable iron from unfortified and iron-fortified rice with NaFeEDTA, $FeS0_4$, FeFum, and FeBis is shown in table 2. The percentage of dialyzable iron from NaFeEDTA- and $FeS0_4$-fortified rice was significantly greater than that from FeFum- and FeBis-fortified rice and the unfortified rice (table 2; $p < .05$). No significant differences were observed on the percentages of dialyzable iron between NaFeEDTA- and $FeS0_4$-fortified rice. On the other hand, the percentage of dialyzable iron from FeFum-fortified rice was significantly greater than that from FeBis-fortified rice and the unfortified rice, while FeBis-fortified rice and the unfortified rice did not differ significantly (table 2; $p < .05$). Based on the above results, it was decided to determine iron absorption in humans from

TABLE 1. Iron content of unfortified and fortified enriched rice, cooked and uncooked

Enriched rice	Uncooked, mg/100g	Cooked, mg/100g	Iron loss (%)
Unfortified	1.4 ± 0.4	1.0 ± 0.5	28.6 ± 0.5^{d}
+ NaFeEDTA	4.8 ± 0.7	2.1 ± 0.2	56.2 ± 0.4^{a}
+ FeS04	3.5 ± 0.6	2.6 ± 0.1	25.7 ± 0.4^{e}
+ FeFum	4.1 ± 0.3	2.2 ± 0.2	46.3 ± 0.3^{b}
+ FeBi,s	3.5 ± 0.8	2.4 ± 0.8	31.4 ± 0.8^{c}

Letters *a, b, c, d,* and *e* denote significant differences between treatments at $p < .05$, $n = 6$.

TABLE 2. Dialyzable iron (%) from unfortified and iron-fortified rice, *in vitro* (Mean ± SEM; $n = 4$)

Test food	Dialyzable iron (%) Uncooked	Dialyzable iron (%) Cooked
Unfortified rice	4.0 ± 0.8^{bx}	4.4 ± 0.8^{cx}
+ NaFeEDTA	26.9 ± 1.5^{ax}	15.7 ± 0.9^{ay}
+ Fe sulfate	4.7 ± 0.3^{bx}	13.2 ± 1.5^{ay}
+ Fe fumarate	2.1 ± 0.2^{ax}	6.4 ± 0.6^{by}
+ Fe bisglycinate	2.5 ± 0.2^{ax}	3.3 ± 0.8^{cy}

Letters *a, b,* and *c* denote significant differences between treatments ($p < .05$).
Letters *x* and *y* denote significant differences between uncooked and cooked rice ($p < .05$).

NaFeEDTA- and FeS04-fortified rice with and without fish and vegetables.

In vivo iron absorption study

The physical and hematological characteristics of the subjects are shown in table 3. The composition of the meal (cooked) is shown in table 4. The iron content of all the test foods and meals ranged from 1.0 to 4.7 mg of iron per 100 g sample and 1.0 to 3.5 mg per 100 g sample for non-heme iron (table 4). Ascorbic acid was only present in the meal containing rice, hasa-hasa, and kangkong served with kalamansi juice. The phytic acid content of all test foods and meals was 96 mg/100g, rice was the main source of phytic acid. Tannic acid was greater in the meal than in rice alone, vegetables were the main source of tannic acid (table 4).

Table 5 shows the iron absorbed (mg) from unfortified rice, NaFeEDTA-, and $FeS0_4$-fortified rice with and without fish and vegetables. Iron absorbed from NaFeEDTA-fortified rice was significantly greater than that from $FeS0_4$-fortified rice and unfortified rice (table 5; $p < .05$). However, iron absorbed from NaFeEDTA- and $FeS0_4$-fortified rice with fish and vegetables did not differ significantly and was significantly greater than that absorbed from unfortified rice with fish and vegetables (table 5; $p < .05$). For all treatments, iron absorbed from rice with and without NaFeEDTA and $FeS0_4$ plus fish and vegetables was significantly greater than that absorbed from rice alone, fortified and unfortified (table 5; $p < .05$). The absorption values presented were adjusted to 40% of the reference dose absorption representing subjects with border-line iron stores [16].

TABLE 3. Physical and hematological characteristics of subjects

Subject	Age (yr)	Sex	Body mass index (BMI) (kg/m^2)	Hemo-globin (g/dl)	Hemat-ocrit (%)
RM	21	M	22	12.3	41
JL	27	M	22	13.0	43.5
RS	28	M	22	12.9	43
AA	25	M	22	13.5	45
RM	40	M	25	13.2	44
RR	39	M	25	13.2	43
HS	28	M	23	13.2	44
JJ	27	F	25	12.9	43
ER	28	F	23	12.4	41.5
RA	40	F	23	9.0	31
Mean ± SEM	30.3 ± 2.1		23.2 ± 0.4	12.6 ± 0.4	41.9 ± 1.3

Discussion

The *in vitro* study showed that NaFeEDTA and $FeS0_4$ were more effective iron fortificants than FeFum and FeBis (table 2). This result may be due in part to the binder that was used in the study. Comparisons of the iron content of cooked and uncooked fortified rice resulted in iron losses ranging from 25.7 to 56.2% with those from NaFeEDTA greater than those from FeFum which were greater than from FeBis which were greater than from FeS04 (table 1; $p < .05$). Cooking rice included washing the rice. The binding property of the coating solution containing the above fortificants in rice may be weaker than the one containing ferrous sulfate. On the other hand, all iron fortificants gave a significantly greater percent of dialyzable iron when cooked than when uncooked except for NaFeEDTA (table 2; $p < .05$). Around 41.6% of dialyzable iron from the rice fortified with NaFeEDTA was lost during cooking. Nevertheless, the iron released from rice fortified with NaFeEDTA was not significantly different from that of rice fortified with $FeS0_4$. Other binders can be used to improve the binding property of the other fortificants to rice. This

Table 4. Composition of the test food or meal (cooked; mg/100g)

Test food or meal	Total iron	Non-heme Iron	Ascorbic acid	Phytic acid	Tannic acid
Rice					
Unfortified	1.0 ± 0.5	1.0 ± 0.5	0	96 ± 1.2	15.2 ± 0.5
+ NaFeEDTA	2.1 ± 0.2	2.1 ± 0.2	0	97 ± 0.9	15.1 ± 0.2
+ Fe sulfate	2.6 ± 0.1	2.6 ± 0.1	0	96 ± 0.2	15.3 ± 0.7
+ Fe fumarate	2.2 ± 0.2	2.2 ± 0.2	0	95 ± 0.5	15.0 ± 1.0
+ Fe bisglycinate	2.4 ± 0.8	2.4 ± 0.8	0	96 ± 0.4	15.2 ± 0.4
Rice + fish + vegetables					
Unfortified	3.2 ± 0.6	2.0 ± 0.4	69.6 ± 0.2	95 ± 1.0	151.6 ± 1.1
+ NaFeEDTA	4.7 ± 0.4	3.5 ± 0.2	69.7 ± 0.5	98 ± 0.5	151.4 ± 0.9
+ Fe sulfate	4.1 ± 0.1	3.5 ± 0.3	69.5 ± 0.8	97 ± 0.8	151.7 ± 0.2

TABLE 5. Iron absorption from unfortified and iron-fortified rice with and without fish and vegetables (mean ± SEM; $n = 10$)

Test food	Intake (g) (cooked)	Non-heme iron per intake (mg)	Iron absorption (%)	Iron absorbed (mg)
Unfortified				
Rice	245 ± 0^{az}	2.45 ± 0.00^{az}	6.9 ± 0.7^{ax}	0.17 ± 0.02^{ay}
Rice + fish + vegetables	337 ± 4^{by}	5.51 ± 0.08^{bz}	7.3 ± 1.3^{ax}	0.41 ± 0.08^{by}
NaFeEDTA				
Rice	260 ± 5^{ay}	5.51 ± 0.11^{ay}	8.0 ± 2.0^{ax}	0.44 ± 0.11^{ax}
Rice + fish + vegetables	347 ± 4^{by}	12.10 ± 0.19^{by}	7.3 ± 1.9^{ax}	0.88 ± 0.24^{bx}
Ferrous sulfate				
Rice	280 ± 5^{ax}	7.35 ± 0.13^{ax}	3.0 ± 0.7^{ay}	0.22 ± 0.05^{ay}
Rice + fish + vegetables	365 ± 1^{bx}	12.69 ± 0.01^{bx}	5.3 ± 0.8^{bx}	0.67 ± 0.10^{bx}

Letters *a* and *b* denote significant differences between rice and rice, fish, and vegetables ($p < .05$).
Letters *x, y,* and *z* denote significant differences between unfortified rice or rice, fish ,and vegetables and iron-fortified rice or rice, fish, and vegetables ($p < .05$).

may decrease the iron loss during cooking and increase the percentage of iron released. NaFeEDTA can be more effective than $FeS0_4$ as an iron fortificant for rice. Studies have shown a significant increase in iron absorption from meals fortified with NaFeEDTA as compared with $FeS0_4$ from a variety of low iron availability foods, such as cereals, legumes, and milk [17–20]. Therefore, NaFeEDTA may have the potential to be a better fortificant than FeS04 for rice. Similarly, FeBis and FeFum may also be better fortificants with a different binder. However, the cost of both the binder and the fortificant should always be considered.

A significantly lower percentage of dialyzable iron was observed from rice fortified with FeFum and FeBis as compared to that fortified with NaFeEDTA and $FeS0_4$ ($p < .05$). Cellulose, a component of the binder, has been shown to inhibit mineral absorption [21]. Cellulose may entrap or form insoluble complexes with FeFum and FeBis and inhibit the release of iron for potential absorption. NaFeEDTA and $FeS0_4$ may not have been affected by the presence of cellulose in the binder solution in the present study.

The absorption of iron from NaFeEDTA- and $FeS0_4$-fortified rice with and without fish and vegetables was determined in humans. They were both effective iron fortificants for rice in the presence of fish and vegetables. For rice alone, NaFeEDTA was more effective than $FeS0_4$ (table 5; $p < .05$). Iron absorbed from NaFeEDTA-fortified rice with fish and vegetables was significantly greater than for NaFeEDTA-fortified rice alone (table 5; $p < .05$). This suggested that the presence of 40 g of fish and 70 mg of ascorbic acid per 100g sample enhanced the absorption of iron from the meal (table 4). Similar results were observed with FeS04-fortified rice and unfortified rice, with and without fish and vegetables. The enhancing effect of ascorbic acid and meat, fish, and poultry on iron absorption is well-known [22–27]. The phytic and tannic acid present in the rice and meals in this study did not show an inhibitory effect on percentage of iron absorbed. A previous *in vitro* study showed no effect of either phytic acid or tannic acid from Filipino regional meals [27]. A tannic acid content less than 3000 mg per meal did not affect iron absorption [27]. The meal used in this study contained 151.6 mg per 100 g of tannic acid (table 4).

The percentage of dialyzable iron, *in vitro* from $FeS0_4$-fortified rice did not differ significantly from that for NaFeEDTA-fortified rice while *in vivo* results gave contradictory results ($p < .05$; tables 3 and 5). Physiological factors should be considered when investigating the availability of iron from foods. The *in vitro* method estimates iron released from the food and meals for its potential absorption and is used for screening several foods or meals. It is also an alternative for the *in vivo* methods as they are expensive and recruiting human subjects is difficult. However, *in vitro* results have to be validated *in vivo.*

Conclusion and recommendation

In conclusion, both NaFeEDTA and $FeS0_4$ are effective fortificants of iron in rice. The binder used in the study may have a significant role in the release of iron from iron-fortified rice for absorption. NaFeEDTA may have the potential to be a more effective iron fortificant for rice than $FeS0_4$ with another binder. Similarly, FeBis and FeFum may also be better fortificants with a different binder. Further studies on the use of other binders to maximize iron absorption and minimize iron loss during cooking should be conducted to improve iron absorption from the fortified rice and rice-fish-vegetable meal. Results from this study can be used as a basis for a food iron fortification program and for

establishing recommended dietary allowances for iron for Filipinos.

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References

1. FNRI. Fifth national nutrition survey. Manila: Food and Nutrition Research Institute, Department of Science and Technology, 1998.
2. Valdez, DH, Mallillin AC, Cruz EM, Kuizon MD, Cabrera IZ. Iron absorption from FVRice-iron fortified. Phil J Sci 1996;125(4):291–9.
3. Peil A, Barrett, Rha C, Langer R. Retention of micronutrients by polymer coatings used to fortify Rice. J Food Sci 1981;47:260–2.
4. Trinidad TP, Wolever TMS, Thompson LU. Availability of calcium for absorption in the small intestine and colon from diets containing available and unavailable carbohydrates: an in vitro assessment. Int J Food Sci Nutr 1996;47:83–8.
5. ICHS, International Committee on Standardization in Hematology. Recommendation for reference methods for hemoglobinometry in human blood (ICHS Standard EP 6/3, 1977). J Clin Path 1978;31:139–43.
6. Bjorn-Rasmussen E, Hallberg L, Walker RB. Food iron absorption in man. II. Isotopic exchange of iron between labeled foods and between a food and an iron salt. Am J Clin Nutr 1973;26:1311–9.
7. Association of Official Agriculture Chemists (AOAC). Official methods of analysis. 10th ed. Washington D.C.: AOAC, 1965.
8. Torrance JD, Bothwell TH. A simple technique for measuring storage iron concentration in formalized liver samples. S Afr J Med 1968;33:9–11.
9. Roe JH, Kuether CA. Determination of total ascorbic acid. J Biol Chem 1943;147:399.
10. Association of Official Analytical Chemists. Phytate in foods: anion exchange method. J Assoc Off Anal Chem 1986;69:2.
11. Earp CF, Ring SH, Rooney LW. Evaluation of several methods to determine tannins in sorghum with varying kernel characteristics. Cereal Chem 1981;58:134–8.
12. Magnusson B, Bjorn-Rasmussen E, Hallberg L, Rossander L. Iron absorption in relation to iron status. Model proposed to express results of food iron absorption measurements. Scand J Hematol 1981;27:201–8.
13. Eakins JD, Brown DA. An improved method for the simultaneous determination of iron-55 and iron-59 in blood by liquid scintillation counting. Int J Appl Radiat Isot 1966;17:391–7.
14. Nadler SB, Hidalgo JU, Block T. Prediction of blood volume in normal human adults. Surgery 1962;51: 224–32.
15. Hosain F, Marsaglia G, Finch CA. Blood ferrokinetics in normal man. J Clin Invest 1967;46:1–9.
16. Magnusson B, Bjorn-Rasmussen E, Hallberg L, Rossander L. Iron absorption in relation to iron status. Model proposed to express results of food iron absorption measurements. Scand J Hematol 1981;27:201–8.
17. MacPhail AP, Bothwell TH, Torrance JD, Derman DP, Bezwoda WR, Charlton RW. Factors affecting the absorption of iron from Fe(III)EDTA. Br J Nutr 1981;45: 215–27.
18. Viteri FE, Garcia-Ibanez R, Torun B. Sodium iron NaFeEDTA as an iron fortification compound in Central America. Absorption studies. Am J Clin Nutr 1978;31: 961–71.
19. Layrisse M. Martinez-Torres C. Fe(III)EDTA complex as iron fortificant. Am J Clin Nutr 1977;30:1166–74.
20. Martinez-Torres C, Romano EL, Renzi M, Layrisse M. Fe(III)EDTA complex as iron fortificant: further studies. Am J Clin Nutr 1979;32:809–16.
21. Slavin JL, Marlett JA. Influence of refined cellulose on human bowel function and Ca and Mg balance. Am J Clin Nutr 1980;33:1932–39.
22. Layrisse M, Martinez-Torres C, Roche M. Effect of interaction of various foods on iron absorption. Am J Clin Nutr 1968;21:1175–83.
23. Layrisse M, Martinez-Torres C, Roche M. Effect of histidine, cysteine, glutathione or beef on iron absorption in humans. J Nutr 114:217–23.
24. Taylor PG, Martinez-Torres C, Romano EL, Layrisse M . The effect of cysteine-containing peptides released during meat digestion on iron absorption in humans. Am J Clin Nutr 1986;43:68–71.
25. Ballot D. The effects of fruit juices and fruits on the absorption of iron from a rice meal. Br J Nutr 1987;57:331–43.
26. Cook JD, Monsen ER. Vitamin C, the common cold and iron absorption. Am J Clin Nutr 1977;30:235–41.
27. Trinidad TP, Madriaga JR, Valdez DV, Cruz EM, Mallillin AC, Sison CC, Kuizon MD. Iron absorption from adequate Filipino meals. The Nucleus 1989;27:39–50.

The use of zinc stable isotopes in the study of iron-zinc interactions in Chilean women

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Abstract

The objective of this study was to compare the fractional zinc absorption (FAZ) and the size of the rapidly exchangeable zinc pool (EZP) after three months of iron supplementation in women consuming ferrous sulfate between meals. Twenty-one non-anemic apparently healthy women received on average 55.1 ± 18.5 mg elemental iron per day as ferrous sulfate, and five received no supplemental iron. Fractional absorption of zinc was determined before and three days after finishing the third month of iron supplementation by using an extrinsic labeling with zinc stable isotopes and a dual isotope enrichment method in urine. EZP was determined from urine enrichment following intravenous administration of ^{70}Zn. Results of selected zinc-related variables in the iron supplemented women were (before vs. after iron supplementation): FAZ with meal 0.22 vs. 0.24, p = *.23; FAZ in fasting state 0.58 vs. 0.69,* p = *.005; EZP 177 mg vs. 160 mg,* p = *.058; plasma zinc 90.6 vs. 86.1 μg/dl,* p =*.065. The control group remained unchanged. The capacity to absorb zinc was increased three days after terminating a period of iron supplementation as compared with the pre-iron period. This may be attributable to impairment of zinc status by the iron supplements as evidenced by a trend for lower plasma zinc and EZP.*

Key words: zinc, iron supplementation, zinc absorption, mineral interactions

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Introduction

Iron deficiency is a common nutritional disorder worldwide, and Chile is not an exemption to this situation [1]. Zinc deficiency has also been identified in selected groups of the Chilean population, such as infants [2], preschool children [3], and short-stature schoolchildren [4].

Iron and zinc interaction has been described in animal and human models [5]. A number of studies have reported decreased zinc absorption in the presence of high amounts of iron, whereas others have failed to observe this effect [5, 6]. The extent of the potential effects of increased iron consumption through iron fortification or iron supplementation on zinc absorption and metabolism remains to be elucidated [6]. The use of zinc stable isotopes as tracers may constitute a useful tool to explore the nature of this mineral-mineral interaction.

We evaluated the effects of iron supplements administered between meals during a period of three months in a group of apparently healthy non-anemic non-pregnant women by using a zinc stable isotope-based methodology. Fractional zinc absorption (FAZ) and the size of the rapidly exchangeable zinc pool (EZP) were determined before and after the supplementation.

Material and methods

Subjects

Twenty-six apparently healthy non-anemic non-pregnant women (age range 18 to 41 years) volunteered to take part in the study. Their average BMI was 25.8 ± 3.1 kg/m^2. Twenty-one individuals were instructed to take between meals, (i.e., between 10:00–11:00 hours and/or 16:00–17:00 hours), 1 to 2 tablets of ferrous sulfate per day, which provided 40 to 80 mg of elemental iron per day. Five subjects received a placebo. The number of tablets consumed was monitored every other week by a member of the team (JC).

At the beginning of the study and after three months of supplementation a series of dietary, anthropometric, and biochemical determinations were conducted. FAZ and EZP were determined.

Determinations

Anthropometric evaluation was carried out using standardized procedures [7]. A dietary interview was performed by the team nutritionists (AR and MV). Energy and nutrient intakes were calculated with the FP2 software (ESHA Research, Salem, Ore., USA) by using a database which contained locally generated nutrient composition data as well as information from the literature [8].

Fasting blood (7 ml) was obtained by antecubital puncture and kept in trace-element free containers until analysis. Whole blood was used to determine hematocrit and the concentration of hemoglobin by coulter counter. Serum and plasma were separated within two hours of collecting the samples. Serum was used to determine ferritin concentration by enzyme-linked immunosorbent assay (ELISA) ([9]. Plasma was used to determine zinc concentration by atomic absorption spectrophotometry according to the method of Smith et al. [10]. Hair was collected from the proximal centimeter of the occipital scalp. It was washed with non-ionic detergent, dried, ashed, and analyzed by atomic absorption spectrophotometry.

Fractional zinc absorption and the size of the rapidly exchangeable zinc pool were determined according to the dual isotope ratio technique using extrinsic labeling and urine enrichment method of Friel et al. [11].

These procedures were carried out before the start of the iron supplementation and three days after completion of the three month intervention. All subjects signed an informed consent form.

On day 1 the subjects received an oral dose of ^{68}Zn in water (accurately weighed quantity of approximately 2 mg) and an IV dose of ^{70}Zn (accurately weighed quantity of approximately 0.5 mg). On day 2, they were given an oral dose of ^{67}Zn in a standard meal (accurately weighed quantity of approximately 1.0 mg). The standard meal consisted of 230 ml of whole fat milk labeled with ^{67}Zn plus white bread and a slice of cheese.

Spot timed urine samples were collected from day 4 to 9 after the stable isotope administration. Urine samples were digested and extracted according to the method of Veillon et al. [12]. The percentages of ^{67}Zn, ^{68}Zn, and ^{70}Zn enrichments were determined by inductively coupled plasma-mass spectrometry (ICP-MS) in the Pediatric Section Laboratory, University of Colorado, Denver. With the exception of the final ICP-MS determination, all procedures were conducted at the Department of Nutrition, Faculty of Medicine, University of Chile.

Statistical analyses (descriptives, paired comparisons, and correlations) were performed using the STATA 6.0 statistical package (Stata Statistical Software, College Station, Texas, USA, 1999).

Results

The average energy and selected nutrient intakes at the time of the initial assessment is shown in table 1. Observed zinc intakes represented, on average, 84% and 100% of adequacy of the recommended intakes [13] in the iron-supplemented and control groups, respectively. Consumption of supplemental iron averaged 55.1 ±18.5 mg per day, which corresponded to a total amount of 4,961 ± 1,668 mg during the 90 days of the experiment. None of the subjects reported major discomfort attributed to the use of the supplement.

The iron nutrition-related indices before and after the supplementation are described in table 2. The only significant change observed was the increased ferritin concentration in the group receiving supplemental iron.

There was a significant increase of FAZ from a water solution given on an empty stomach, but not from a standard meal (table 3). Plasma zinc and the size of the EZP decreased, but not significantly, in the iron supplemented group.

Discussion

Zinc has five stable isotopes, three of which are in naturally low abundance that make them suitable to be used as "tracers." These are ^{67}Zn (natural abundance 4.1%), ^{68}Zn (18.8), and ^{70}Zn (0.6%). The use of these stable isotopes along with accurate and precise determination techniques already available will contribute to the understanding of zinc homeostasis under a variety of conditions. Thus, intestinal absorption of zinc, excretion of endogenous zinc, and zinc metabolism through compartmental analysis are among the most relevant applications [14].

Most of the iron-zinc interaction studies have been conducted by evaluating the results of the simultaneous administration of both minerals [5, 6]. Witthaker

TABLE 1. Energy and nutrient intakes at the beginning of the study

	Iron supplemented (N = 21)	Control (N = 5)	*p*
Energy (kcal)	1,560 ± 368	1,657 ± 182	.58
Protein (g)	53.5 ± 14.4	63.7 ± 10.5	.15
Calcium (mg)	506 ± 277	621 ± 188	.39
Iron (mg)	10.6 ± 2.33	10.7 ± 2.62	.94
Zinc (mg)	6.7 ± 1.84	8.0 ± 1.32	.14

TABLE 2. Iron nutrition indices of the groups before and after three months of iron supplementation

		Before iron supplementation	After 3 months of iron supplementation	*p*
Hematocrit (%)	Iron supplemented	41.5 ± 2.5	41.9 ± 2.5	.53
	Control	41.2 ± 1.6	41.0 ± 2.6	.80
Hemoglobin (g/dl)	Iron supplemented	14.0 ± 0.9	14.1 ± 1.0	.31
	Control	14.1 ± 0.6	14.0 ± 0.8	.71
Serum ferritin (μg/L)	Iron supplemented	28.5[a]	35.0	.01
	Control	24.4	25.2	.87

a. Geometric mean

TABLE 3. Fractional zinc absorption (FAZ), size of the rapidly exchangeable zinc pool (EZP), hair zinc, and plasma zinc concentrations in the experimental groups before and after three months of iron supplementation

		Before iron supplementation	After 3 months of iron supplementation	*p*
FAZ 68 Zn (fasting)	Iron supplemented	0.58 ± 0.20	0.69 ± 0.21	.005
	Control	0.61 ± 0.14	0.60 ± 0.13	.76
FAZ 67 Zn (test meal)	Iron supplemented	0.22 ± 0.07	0.24 ± 0.06	.23
	Control	0.24 ± 0.09	0.23 ± 0.07	.86
EZP (mg)	Iron supplemented	176.6 ± 38.3	160.3 ± 42.4	.058
	Control	167.1 ± 32.1	171.8 ± 20.5	.64
Hair zinc (μg/g)	Iron supplemented	143.7 ± 28.2	139.3 ± 29.2	.23
	Control	141.4 ± 15.2	138.5 ± 13.1	.67
Plasma zinc (μg/dl)	Iron supplemented	90.6 ± 12.0	86.1 ± 9.4	.065
	Control	92.6 ± 5.7	93.9 ± 4.3	.74

[15] reviewed 29 studies on the effects of iron on zinc absorption. Fourteen of the studies showed negatives effects, whereas 15 failed to find such effects. The main conclusions from that review were that iron, in an iron: zinc ratio 2:1 or higher, strongly interferes with zinc absorption provided the minerals are given in water or simple beverages. In more complex food matrices the effect of iron is, in most cases, absent. It is also evident that only non-heme iron is capable of interfering with zinc absorption.

In interventions where the supplements are consumed between meals the cumulated evidence does not allow for firm conclusions on the potential negative effects of iron on zinc. Hambidge et al. [16] reported that pregnant women receiving 150 mg or more per day of iron had reduced plasma zinc levels in the third trimester. O'Brien [17] found similar results in Peruvian women receiving prenatal iron and folate supplements (60 mg iron/day). In contrast, Sheldon et al. [18] did not find any adverse effects of 240 mg of ferrous fumarate (2 times daily) in pregnant women. Yip et al. [19] did not observe reduced plasma zinc in children receiving 30 mg per day of ferrous sulphate. Sandstrom et al. [20] failed to detect a reduction of zinc absorption in individuals receiving 50 mg of iron during two weeks.

The use of zinc stable isotope-based methodologies can help determine the occurrence and magnitude of the effects of iron supplementation on zinc absorption and metabolism. There are two published papers relevant to this issue. Fung et al. [21] studied zinc absorption in 13 women at preconception, late pregnancy, and lactation. The iron supplements were administered with the midday meal. They noted a significant increase of zinc absorption during lactation in the group as a whole. Interestingly, four subjects who received iron supplements did not show an increase, which may suggest some degree of interference in zinc absorption. Unfortunately, the small sample size precludes reaching any firm conclusions in this regard. O'Brien et al. [22] studied Peruvian pregnant women who received either iron-folate supplements with or without zinc, or no supplements (controls). Zinc absorption approached 20% in both iron (with and without zinc) supplemented groups, as compared with 47% in the controls. The results are hard to interpret because the subjects consumed their supplements at the same time that the zinc absorption tests were performed.

In our study, zinc absorption determinations were carried out three days after suspending the iron supplement. Thus, we evaluated the effects excluding any

luminal mineral-mineral interaction or the participation of recently absorbed iron. When iron supplements were taken between meals there was a significant increase in zinc absorption (19%) as determined on fasting conditions, and a trend (non-significant) to decreased plasma zinc levels (5%) and a reduced size of the EZP (9%). These findings suggest the occurrence of a downward change in zinc status, which in turn signaled the enterocyte to increase zinc absorption capacity. Increased zinc absorption was only observed in the fasting state because the results are not diminished by the other food constituents that affect zinc absorption from meals. Ongoing studies conducted by our group with extended periods of supplementation, and with the incorporation of additional zinc status indices may help to clarify the nature and extent of the iron-zinc interaction in individuals receiving iron supplements.

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References

1. Ruz M. Trace element intake and nutriture in Latin America. In: Wahlqvist ML, Truswell AS, Smith R, Nestel PJ, ed. Nutrition in a sustainable environment. London: Smith-Gordon and Co., 1994:296–300.
2. Castillo-Durán C, Rodríguez A, Venegas GV, Alvarez P, Icaza G . Zinc supplementation and growth of infants born small for gestational age. J Pediatr 1995;127: 206–11.
3. Ruz M, Castillo C, Lara X, Rebolledo A, Atalah E. A 14-month zinc supplementation trial in apparently healthy Chilean preschool children. Am J Clin Nutr 1997; 66: 1406–13.
4. Castillo-Durán C, García HR, Venegas P, Panteon E, Torrealba I, Concha N, Pérez P. Zinc supplementation increases growth velocity of male children and adolescents with short stature. Acta Paediatr 1994;83:833–7.
5. Solomons NW. Competitive interaction of iron and zinc in the diet: consequences for human nutrition. J Nutr 1986; 116:927–35.
6. Solomons NW, Ruz M. Zinc and iron interaction: concepts and perspectives in the developing world. Nutr Res 1997;17:177–85.
7. Gibson RS, ed. Principles of nutrition assessment. Oxford: Oxford University Press, 1990.
8. Schmidt-Hebbel H, Pennacchiotti I. Tabla de composición química de alimentos chilenos. Santiago: Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, 1985.
9. International Nutritional Anemia Consultative Group (INACG). Measurements of iron status. Washington, D.C.: Nutrition Foundation, 1985.
10. Smith JC Jr, Butrimovitz GP, Purdy WC. Direct measurement of zinc in plasma by atomic absorption spectroscopy. Clin Chem 1979;25:1487–91.
11. Friel JK, Naake VL, Miller JV, Fennessey PV, Hambidge KM. The analysis of stable isotopes in urine to determine the fractional absorption of zinc. Am J Clin Nutr 1992;55: 473–7.
12. Veillon C, Patterson K, Moser-Veillon P. Digestion and extraction of biological materials for zinc stable isotope determinations by inductively coupled plasma mass spectrometry. J Anal At Spectrom 1996;11:727–30.
13. Food and Nutrition Board, Institute of Medicine. Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Washington, D.C.; National Academy Press, 2001.
14. Krebs NF, Miller LV, Naake VL, Lei S, Westcott JE, Fennessey PV, Hambidge KM. The use of stable isotope techniques to assess zinc metabolism. Nutr Biochem 1995;6:292–301.
15. Whittaker P. Iron and zinc interactions in humans. Am J Clin Nutr 1998;68(suppl):442S–6S.
16. Hambidge KM, Krebs NF, Jacobs MA, Favier A, Guyette L, Ickle DN. Zinc nutritional status during pregnancy: a longitudinal study. Am J Clin Nutr 1983;37:429–42.
17. O'Brien KO, Zavaleta N, Caufield LE, Yang DX, Abrams SA. Influence of prenatal iron and zinc supplements on supplemental iron absorption, red blood cell iron incorporation, and iron status in pregnant Peruvian women. Am J Clin Nutr 1999;69:509–15.
18. Sheldon WL, Aspillaga MO, Smith PA, Lind T. The effect of oral iron supplementation on zinc and magnesium levels during pregnancy. Br J Obstet Gynaecol 1985;92:892–8.
19. Yip R, Reeves JD, Lonnerdal B, Keen CL, Dallman PR. Does iron supplementation compromise zinc nutrition in healthy infants? Am J clin Nutr 1985;42:683–7.
20. Sandstrom B, Davidsson L, Cederblad A, Lonnerdal B. Oral iron, dietary ligands and zinc absorption. J Nutr 1985;115:411–4.
21. Fung EB, Ritchie LD, Woodhouse LR, Roehl R, King JC. Zinc absorption in women during pregnancy and lactation: a longitudinal study. Am J Clin Nutr 1997;66:80–8.
22. O'Brien KO, Zavaleta N, Caufield LE, Wen J, Abrams SA. Prenatal iron supplements impair zinc absorption in pergnant Peruvian women. J Nutr 2000;130:2251–5.

Domestic drinking water—an effective way to prevent anemia among low socioeconomic families in Brazil

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Abstract

Iron deficiency and iron-deficiency anemia are common in the developing world. We evaluated the feasibility of iron fortification of domestic drinking water to prevent and control iron deficiency and iron-deficiency anemia. Twenty-one families representing 88 persons, including children, were selected to participate in this study. Twelve families added an iron solution plus ascorbic acid to their domestic drinking water over a four months period and nine families added a placebo. Blood samples were collected, before and after the four months, for hemoglobin and serum ferritin measurements. Iron-fortified drinking water increased hemoglobin (children 10.9 ±1.1 g/dl to 11.7 ± 1.1 g/dl $p < .01$, *adults 12.9 ± 1.7 g/dl to 13.7 ± 1.7 g/dl* $p < .01$*) and ferritin (children 27.6 ± 21.6 ng/dl to 33.8 ± 22.1 ng/dl, adults 74.8 ± 41.3 ng/dl to 106.2 ± 93.9 ng/dl* $p < .05$*). No significant changes in hemoglobin and ferritin were found in the placebo group after 4 months. Preparation, distribution, and consumption of the solutions were successful. Iron fortification of household drinking water can be a simple and effective alternative to deal with iron deficiency and iron-deficiency anemia in less developed areas.*

Key words: drinking water, anemia

Introduction

Iron-deficiency anemia is estimated to effect two billion people worldwide. Its consequences justify various strategies and interventions to combat it, especially groups at risk, such as children six months to six years of age and women of reproductive age [1]. The functional consequences of iron-deficiency anemia are related to a reduction of hemoglobin and tissue iron, that affects the ability of a person to perform normal physical and mental activities. Studies in different countries have demonstrated impaired productivity as a result of iron deficiency [2]. Iron deficiency and iron-deficiency anemia can affect children's neural development and mental retardation [3, 4]. During pregnancy, iron-deficiency anemia is associated with an increased risk of maternal and fetal morbidity and mortality [5]. Iron deficiency in infancy and childhood is associated with a significant loss of cognitive abilities, apathy, inactivity, and decreased resistance to infections [5, 6].

Fortification of widely consumed and centrally processed staple food with iron is considered to be the backbone of improving iron intake in industrialized and developing countries [7, 8]. The problem is often the choice of the most appropriate food vehicle as well as the iron compound to be used as a fortificant. These may vary from one country to another depending on the sociocultural, economic, and technological conditions. Several foods such as wheat flour, sugar, salt, and monosodium glutamate have been used as iron carriers but the success of iron fortification will depend on the one most suitable and acceptable to the target population. The problem with conventional iron fortification of foods such as wheat flour, sugar, and salt has also been the bioavailability of the added iron salts as well as the taste and discoloration of the food vehicle. In addition, an industrial technological infrastructure is required for the production of the iron-fortified foods and an available network is needed for their distribution. They are not always available in developing areas of the world.

Iron fortification in developing countries requires new innovative approaches adapted to their socioeconomic and cultural environment. They must be simple and sustainable. One such approach is the use of drinking water as a well-suited and appropriate vehicle to carry iron to the low socioeconomic population of Brazil [9, 10]. Drinking water has previously and successfully been fortified with iodine [11] and fluoride

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[5, 11]. Water is universally consumed. Safe and clean water is essential for drinking, cooking, and preparing infant formulas and weaning foods. Preliminary studies with the fortification of water with different iron compounds indicated that taste, color, and turbidity of water enriched with ferrous sulfate can be overcome for its use by preschool children. In previous studies in our laboratory rats that consumed iron-fortified drinking water did not become anemic. In this study ferrous sulfate was the most effective form of iron [9]. Clinical studies with institutionalized preschool children in a day care center showed that consumption of iron-fortified drinking water for eight months reduced drastically the prevalence of their anemia [10].

The study was conducted to test the feasibility and logistics of iron fortification of drinking water at home to guarantee a daily iron intake for the whole family.

Materials and methods

The subjects were low socioeconomic families attending the Health Care Center of the University of São Paulo Medical School in Ribeirão Preto, an agricultural town located in the interior of the State of São Paulo, Brazil. Families with children between one and six years old and the adults living in the house were invited to participate in the study. Some of the children had borderline normal hemoglobin and ferritin levels. The adults were not anemic and their ferritin levels were higher than those of the children (table 1). Twenty-one families consisting of 88 members who agreed to participate in the study were randomly divided into two groups. A group of 12 families (44 members), the experimental group, received iron-fortified drinking water at their home. Another group of nine families (44 members), the placebo group, received their usual drinking water without iron. The experimental period lasted for four months. Written informed consent was obtained from the head of each family, and the Human Studies Committee of the University of São Paulo Medical School Health Center approved the project. Each family had an earthen pot for storing about 10 L of drinking water. This is traditionally available in most homes in this area. A concentrated solution of ferrous iron (we used ferrous sulfate 7 hydrated) and ascorbic acid was prepared in our laboratories and dispensed in 10 ml bottles for daily use by the families. Bottled 10-ml placebo water solutions were prepared in the same way. The addition of a 10-ml bottle of the iron stock solution to the 10 L pot of drinking water of the experimental group was calculated to have a final concentration of 10 mg of elemental iron and 60 mg of ascorbic acid per liter of drinking water. This was kept for the four month study period. The control (placebo) group added the 10 ml water solution without iron to their drinking water in the same way for the same four month period. The families did not know which one was receiving iron and they were trained to add the bottled solutions to the drinking water pot every morning. Each week the families were provided a fresh stock of iron or iron-free solutions, sufficient to last for seven days. Samples from the domestic drinking pots were checked for their iron concentration and the amount of water consumed by both groups was recorded. A questionnaire was designed and used throughout the study to interview all members of the families about the use, consumption, reactions, side effects related to the drinking water. Hematological data were obtained through blood samples collected from all family members at the beginning and at the end of the four-month study. Hemoglobin was determined on fresh blood using the cyanomethemoglobin method. Serum samples were stored at –70°C and thawed for ferritin analysis using a ferritin radioimmunoassay kit (Ciba-Corning Diagnostic, Irvine, Calif., USA). Statistical analyses of the data were carried out using nonparametric rank tests.

Results

Eighty-nine percent of the placebo group and all those in the experimental group regularly added the respec-

TABLE 1. Hemoglobin and serum ferritin levels of children and adults before and after the introduction of a placebo or iron-fortified drinking water for a period of four months

	Before treatment		After treatment	
	Placebo group (mean ± SD)	Iron group (mean ± SD)	Placebo group (mean ± SD)	Iron group (mean ± SD)
Children				
Hemoglobin (g/dl)	11.3 ± 1.3	10.9 ± 1.1	10.9 ± 1.2	11.7 ± 1.1*
Serum ferritin (ng/dl)	38.4 ± 27.7	27.6 ± 21.6	35.0 ± 28.8	33.8 ± 22.1
Adults				
Hemoglobin (g/dl)	13.2 ± 1.1	12.9 ± 1.7	12.5 ± 1.3	13.7 ± 1.7*
Serum ferritin (ng/dl)	58.3 ± 34.2	74.8 ± 41.3	51.1 ± 30.7	106.2 ± 93.9**

Values significantly different after treatment * $p < .01$, ** $p < .05$

tive solutions to their household drinking water pots. Both groups found this activity to be simple and easy to manage. About 75% to 78% of the family members of both groups consumed water daily from the drinking pots. No significant difference was observed in the amount of water consumed by the same age members of both groups, before and after the introduction of the iron enrichment program. The families were similar with respect to their socioeconomic background and their dietary habits. Their diet was based on rice and beans as the staple foods and they occasionally used animal products, such as meat and poultry. Limited amounts of fruits and green leafy vegetables were included in their daily meals.

The mean hemoglobin and serum ferritin levels of children and adults before and after the introduction of the iron-fortified drinking water or a placebo for a period of four months are shown in table 1. The adult mean hemoglobin levels were higher than those of children in both groups. The mean hemoglobin levels of the children and adults who received iron-fortified drinking water for four months increased significantly ($p < .01$) as compared to their baseline levels. The hemoglobin values of the placebo group were similar at the beginning and end of the study.

Serum ferritin levels in both groups were higher in adults than in children. The mean serum ferritin levels in children and adults increased in those who received the iron-fortified drinking water ($p < .01$) and stayed the same in the placebo group. The ferritin increase in the children in the iron-fortified water group was not significantly different, but the increase was statistically significant ($p < .05$) for adults. Both hemoglobin and ferritin levels in the placebo group stayed the same from the beginning to the end of the experiment.

Discussion

Water is an essential component of the human diet that is consumed daily by everyone, but it has not been well explored as a vehicle for mineral micronutrients. In Ribeirão Preto, where this study was carried out, water is treated and distributed by the City. It is a common practice in Southern Brazil to store drinking water in earthen pots. This offered us an opportunity to consider adding iron to household drinking water as a way to regularly supply this important micronutrient directly to low socioeconomic families. Using drinking water as an iron carrier is a simple, inexpensive, and practical way to reach all age groups [9, 10]. It is an easier carrier than other traditional carriers, such as wheat flour, sugar, and salt.

The initial difficulties in adding iron to drinking water were the solubility and stability of ferrous sulfate for a long period. After some time the solution increases its color and turbidity. This problem was solved by adding a solution of ascorbic acid and ferrous sulfate to the water each week. Ascorbic acid increases the absorption of iron and thereby its bioavailability [12–14]. Other water-soluble compounds such as iron EDTA or iron chelates can also be added to drinking water. They do not change the taste and color of the water but they are more expensive. We have had a positive experience with them as water fortificants.

Another aspect of this study was the development of a simpler mechanism to supply iron and ascorbic acid to the families in the community. Appropriate concentrated solutions with known amounts of ferrous sulfate and ascorbic acid were prepared in our laboratory and supplied in 10 ml glass bottles with caps, however, local pharmacies can easily prepare them. The solutions were delivered to the families weekly. The families added the 10 ml solution of iron and ascorbic acid to the 10 L of water in their earthen drinking water pots. The iron-fortified water was acceptable to all members of the family, although it has a residual iron taste detected by some adolescents. Our results showed that domestic drinking water has a great potential as an effective and acceptable carrier of iron in developing countries. The industrial and technological infrastructure is not always available for fortifying other food products with iron. Hemoglobin enriched cookies [15] and iron fortification of wheat flour and other food products, although technologically feasible, have not been successful in many developing countries. They have no industrial infrastructure and no means of distributing the enriched product to those most in need.

The amount of iron chosen was 10 mg in the form of elemental iron plus 60 mg of ascorbic per liter of drinking water. This amount is half of that used in an earlier study [10], but the previous study was carried out in 2 to 6 year old children who had a higher degree of anemia (58%).

Compliance by the families was monitored by weekly visits, which confirmed that fortifying the drinking water used by the household can control iron deficiency and iron-deficiency anemia. The effectiveness of the program is evidenced by the adherence, as determined by the questionnaire and by the improvement of hematological indices after the four months period (table 1).

The findings of this study agree with those of a previous study [10], where a group of 2 to 6 year old children had a higher percentage anemia and lower levels of ferritin. These children were followed for eight months and their hemoglobin and ferritin serum levels increased significantly. Fifty-eight percent of them were anemic initially (< 11.0 g/dl), decreasing to 3% at the end of the study. Their mean initial serum ferritin values (13.7 ± 8.9 μg/L) increased to 22.6 ± 11.8 μg/L in four months and to 25.6 ± 10.5 μg/L after the eight month intervention with iron-fortified drinking water.

We conclude that fortification or enrichment of household drinking water is feasible and can be adapted easily to reach low socioeconomic families at the community level. Iron fortification of water is a simple, inexpensive, and appropriate strategy for the prevention of iron-deficiency anemia in Brazil and other countries.

References

1. WHO. National strategies for overcoming micronutrient malnutrition. 86th Session, Provisional Agenda item 10.2. Geneva: World Health Organization, 1991.
2. Fairbanks VF, Beutler E. Iron. In: M Shils, Young VR, eds. Modern nutrition in health and disease. Philadelphia: Lea & Febiger, 1988:193–225.
3. Beard JL. Iron deficiency and neural development. Arch Latinoam Nutr 1999;49(Suppl 21):34–9.
4. Hurtado EK, Claussen AH, Scott K. Early childhood anemia and mild or moderate mental retardation. Am J Clin Nutr 1999;69:115–9.
5. FAO/WHO. International conference on nutrition. Major issues for nutrition strategies. Geneva and Rome: Food and Agricultural Organization and World Health Organization, 1992.
6. Helyar L, Sherman AR. Moderate and severe iron deficiency lowers numbers of spleen T-lymphocyte and B-lymphocyte subsets in the C57113 16 mouse. Nutr Res 1992;12: 1113–22.
7. International Nutritional Anemia Consultative Group (INACG). Combating iron deficiency anemia through food fortification technology. Washington: US Agency for International Development, 1990.
8. Hurrell RF. Preventing iron deficiency through food fortification. Nutr Rev 1997;55:210–22.
9. Ferreira JF, Aranda RA, Bianchi MLP, Desai ID, Dutra-de-Oliveira JE. Utilização da agua potável como veículo de nutrientes: estudos experimentais com ferro. Arch Latinoamer Nutr 1991;41:400–7.
10. Dutra-de-Oliveira JE, Ferreira JF, Vasconcellos VP, Marchini JS. Drinking water as an iron carrier to control anemia in preschool children in a day-care center. J Am Coll Nutr 1994;13:198–202.
11. Penelope N. Food fortification in developing countries. Washington: US Agency for International Development, 1993.
12. Disler PB, Lynch SR, Chariton RW, Bothwell TH, Walker RB. Studies on the fortification of cane sugar with iron and ascorbic acid. Br J Nutr 1975;34:141–52.
13. Cook JD, Reusser MD. Iron fortification: an update. Am J Clin Nutr 1983;38:648–59.
14. Gillooly M, Torrance JD, Bothwell TH, MacPhail AP, Derman D, Mills W, Mayet F. The relative effect of ascorbic acid in iron absorption from soy-based and milk-based infant formulas. Am J Clin Nutr 1984;40:522–7.
15. Walter T, Hetirampf E, Pizarro F, Olivares M, Llaguno S, Letelier A, Vega V, Stekel A. Effect of hemoglobin-fortified cookies on iron status of school children: a nationwide program in Chile. Am J Clin Nutr 1993;57: 190–4.

Study in China on ingestion and organ content of trace elements of importance in radiological protection

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Abstract

To improve knowledge of the ingestion and organ content of calcium, cesium, iodine, potassium, strontium, thorium, and uranium that have a high priority in radiological protection, a total diet and organ content studies was conducted. The total diet study used the market basket sample method with representative sampling. For the organ content specimens of muscle, skeleton, liver, kidney, lung, and thyroid were collected during autopsies of normal, healthy adult accidental death victims. The concentration of above stated elements in the diet and organs were analyzed by nuclear and nuclear-related techniques with strict quality assurance measures. The results are presented in the paper and compared with previous national and international data and the parameters of current International Commission on Radiological Protection (ICRP) Reference Man. The study provided a new and reliable database for setting the parameters for the Chinese reference man and the reference Asian man. It also facilitated the study on biokinetics behavior of their radiation counterparts in human body and helped in the realistic assessment of their internal radiation doses.

Key words: trace elements, radiological protection, ingestion, organ content

Introduction

In order to estimate the internal dose and derive the annual limits of intake for radionuclides in the field of radiation protection, there is a need to have accurate knowledge of the corresponding stable element intakes and their concentrations in organs, especially for some trace elements of importance in radiological protection such as cesium, potassium, calcium, strontium, iodine, thorium, and uranium because their radiation counterparts ^{137}Cs, ^{90}Sr, ^{131}I, ^{232}Th and ^{238}U are the sources of radiation exposure to the nuclear industry workers engaged in power production and to the general public at the time of any nuclear accident (e.g., Chernobyl, 1986). This study was conducted under a coordinated research project (CRP) entitled Dietary intake and organ content of trace elements of importance in radiological protection, reference Asian man phase II. [1] This CRP was organized by the International Atomic Energy Agency (IAEA) to strengthen the construction of radiation protection in the Asian region [2].

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Methods

Sampling and sample collection

In the diet study, diet samples were collected according to the sampling strategy devised by the Institute of Nutrition and Food Hygiene, Chinese Academy of Prevention Medicine for the first total diet study in China in 1990 [3]. Based on geographic, socioeconomic, and dietary habits, China was divided into four regions. For each region three provinces were selected for the survey. In each of these provinces three survey points (one in town and two in the countryside) were selected. In each point 30 families were randomly selected to weigh and record their food for three successive days. The sampling strategy took into account all relevant geographic, socioeconomic, and ethnic variables to ensure regionally and nationally representative sampling.

Foods were classified into 12 categories. Various foods were collected from nearby markets and prepared and cooked separately according to local dietary habits. In this way 12 food categories were formed for each region (A total of 48 samples for the four regions). The samples were dried and powdered for further analysis to estimate their contribution to the total daily intake.

Also 12 samples from each region were combined into four regional total diet samples and the 48 samples were combined into a national total diet sample.

For the organ content study, suitable organ specimens were obtained from accident victims or other cases of sudden violent death. The autopsies were carried out within 24 hours of death. The tissue and organ specimens collected included bone (rib), kidney, liver, lung, muscle, and thyroid. Samples were obtained from 31 autopsies. The samples were dried and ground to a powder before analysis.

Analytical methods

Inductively coupled plasma-mass spectrometry (ICP-MS) was used to determine cesium, thorium, and uranium; ICP-atomic emission spectrometry (ICP-AES) was used for calcium, and strontium; epithermal neutron activation analysis (ENAA) was used for iodine and atomic absorption spectrophotometry (AAS) used for potassium.

Quality assurance and quality control (QA/QC)

To obtain high quality data, quality assurance and quality control measures were conducted during the study. Standard operating procedures were developed covering all aspects of the study. These included: representative sampling, avoid external contamination in sampling, sample preparation, and chemical analysis, including checking the purity of standards and reagents.

The analytical methods must be validated using the National Institute of Standards and Technology standard reference materials (NIST SRMs) supported by IAEA [4]. The results showed a good agreement between the measured values and certified values. Most of the discrepancies were within 20%. Even though the discrepancies in NIST-SRM 1548 for thorium and in NIST-SRM 8414 for strontium more than 20%, but they were within 1 SD for thorium and within 2 SD for strontium.

During the element analysis phase internal quality control measures were adopted by using some suitable reference materials, and duplicates sample aliquots were kept for reanalysis, if necessary.

Samples (10%) were sent to the Central Reference Laboratory (CRL) for cross-checking analysis, as the external quality control measures [5]. Z scores were calculated for each sample that was analyzed in both laboratories. Normally, the Z score should have values between –2 and +2.

When the target value of the relative SD was assumed as 10%, the Z scores numerical values were less than 2 for 60% of the samples analyzed for cesium, iodine, and strontium. If we assume the target value of the relative SD is 20%, then 95% of the samples have numerical value of Z scores less than 2 for cesium, iodine, and strontium analysis, but this would be true for only 70% of the samples analyzed for thorium and uranium.

Results

Dietary intakes

The national average concentrations and SD for seven elements in 12 food categories are listed in table 1. The corresponding average daily dietary intakes for the seven elements were estimated by the following formula:

$$I_i = \sum_j C_{ij} \cdot D_j$$

Here, I_i is the daily intake of the element i; C_{ij} is the concentration of the food category j; D_j is the daily consumption of the food category j. The diet composition and food consumption was quoted from the data of the second total diet study in China, 1992 [6]. Table 2 shows a comparison of the results with the recommended values, the national data in 1990, and the available international data.

The intakes of calcium, iodine, and potassium increased in present study as compared to the data in 1992 [6]. As the intake of these elements used to be insufficient for the Chinese [3, 6, 7], this means that the Chinese diet has improved. Comparison of the present data with the data of ICRP [8] and the renewal value of Iyengar [9] shows that intakes of the elements are quite close to each other except for uranium.

Element contents in organs and tissues

The concentrations of calcium, cesium, potassium, strontium, thorium, and uranium in muscle, rib, kidney, liver, and lung and iodine in thyroid for 31 adult men were obtained (table 3). Some elements were concentrated in certain organs and tissues. If assuming the element concentration in muscle is 1, the relative concentrations of calcium, strontium, thorium, and uranium are 1,937, 406, 10, and 3 in skeleton, respectively, and thorium and uranium are 42 and 3 in the lung, respectively.

Based on the concentration detected the elemental contents of the organs and tissues were calculated by multiplying the masses of relevant organ and tissue of the Chinese reference man [10].

The estimated contents in organs and tissues are listed in table 4 and compared with the previous data. The content of cesium, iodine, thorium and uranium

TABLE 1. The national average concentration of the 7 elements in various food categories of China

Food category		Calcium mg/kg	Cesium μg/kg	Iodine μg/kg	Potassium g/kg	Strontium mg/kg	Thorium μg/kg	Uranium μg/kg
Grain	Mean	466	7.98	122	1.145	1.74	6.22	5.47
	SD	210	3.40	28	0.840	0.42	9.63	5.24
Beans	Mean	1,456	25.0	511	2.715	5.68	6.82	6.95
	SD	950	18.2	353	0.929	2.33	4.43	2.74
Yam	Mean	217	16.3	598	3.044	1.21	3.25	4.46
	SD	66	14.1	475	0.472	0.76	2.22	6.58
Meat	Mean	148	23.4	786	2.112	1.08	3.08	2.59
	SD	71	18.3	632	0.243	0.60	5.18	2.92
Eggs	Mean	671	15.2	661	1.193	1.64	0.30	0.65
	SD	376	20.9	566	0.218	1.11	0.19	0.47
Aquatic product	Mean	2,271	13.8	730	3.298	2.88	2.67	3.08
	SD	3,285	4.4	640	0.897	2.28	3.40	4.58
Milk	Mean	1,210	4.6	89	1.243	0.54	0.23	0.46
	SD	316	2.5	59	0.227	0.38	0.22	0.35
Vegetables	Mean	724	11.0	527	2.672	3.55	3.02	2.52
	SD	244	2.8	435	0.607	1.34	1.84	3.18
Fruits	Mean	123	6.86	11	1.219	0.74	0.30	0.20
	SD	54	4.72	2	0.044	0.55	0.05	0.08
Sugar	Mean	294	12.1	79	0.499	0.45	1.88	0.73
	SD	176	17.4	126	0.704	0.26	2.40	0.64
Beverages	Mean	29.6	0.88	10	0.022	0.19	0.14	1.89
	SD	13.6	1.31	8	0.032	0.07	0.10	1.96
Alcohol	Mean	31.5	0.41	9	0.056	0.13	0.22	0.61
	SD	13.5	0.20	7	0.054	0.10	0.10	0.31

TABLE 2. Daily intakes of elements compared with recommended values for the Chinese adult man

	Calcium mg	Cesium μg	Iodine μg	Potassium g	Strontium mg	Thorium μg	Uranium μg
Daily intake (a)	723	13.0	364	2.11	2.86	5.35	6.75
Recommended value (b)	800 (RDA)		150 (RDA)	1.88–5.63 (ESADDI)		950.7 (ALI)	1509.6 (ALI)
Ratio a/b	0.90		2.42	1.12		0.0056	0.0045
1990 value	582	—	—	1.12	1.5	3.62	1.08
ICRP 23 value [8]	1100	10	200	3.3	1.9	3	1.9
Iyengar 1998 [9]	—	10	200	—	1.5	1.15	1.27

RDA, recommended daily allowance.
ESADDI, estimated safe and adequate daily dietary intake.
ADI, acceptable daily intake.
ALI, Annual limit of intake.

TABLE 3. Element concentration in main tissues and organs of the Chinese adult man (g-1 Fresh), $n = 31$

	Calcium Mg		Cesium Ng		Potassium mg		Strontium μg		Thorium ng		Uranium ng		Iodine μg	
Tissues	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Muscle	0.07	0.03	24.9	15.9	1.76	0.29	0.14	0.06	0.26	0.57	0.93	1.16	—	—
Skeleton	120	26	11.9	10.2	1.03	0.21	56.9	32.6	2.46	2.97	2.31	1.83	—	—
Liver	0.07	0.04	14.0	5.7	1.87	0.52	0.12	0.06	0.42	0.87	0.74	1.15	—	—
Kidney	0.10	0.04	14.1	6.0	1.62	0.32	0.13	0.10	0.28	0.57	1.04	0.86	—	—
Lung	0.12	0.13	19.4	16.1	1.34	0.34	0.15	0.07	11.0	11.2	2.61	2.37	—	—
Thyroid	—	—	—	—	—	—	—	—	—	—	—	—	678	200

in the Chinese adult man are first reported in this study. Calcium in organs and tissues is higher than before. This might reflect an increased intake of calcium from the diet. The amount of strontium in muscle, liver, kidney, and lung, of uranium in muscle, liver, and lung are much higher than that of ICRP reference man. Iodine was much higher in the thyroid of residents living near the sea (20.4 mg) than in those who lived far from the sea (15.0 mg).

Conclusion

Some new sensitive and reliable analytical methods such as ICP-MS, ICP-AES, ENAA, and some proper QA/QC measures were developed and successfully applied for trace element analysis in biological samples. The data generated by the study provided a new and reliable database for setting the parameters for the Chinese reference man and the reference Asian man, and facilitated the study on biokinetics behavior of their radiation counterparts in the human body and helped in the realistic assessment of their internal radiation doses. The study also provided the national basic data for evaluating the present status of the Chinese diet, against which the deficiency or excess of one or more of these elements could be identified. Some discrepancies in the dietary intakes and organ contents of elements between the Chinese adult man and the ICRP reference man may be attributed to variation in diet composition, natural environment, and progress in analytical techniques including quality assurance measures.

Acknowledgements

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TABLE 4. Content of element in main tissues and organs of the Chinese adult man

Elements	Tissue or organ	Average contents (a)	Previous value	ICRP 23 value [8] (b)	Ratio a/b
Calcium, g	Muscle	1.725	0.428	0.87	1.98
	Skeleton	960.0	504.0	1,010	0.95
	Liver	0.102	0.026	0.090	1.13
	Kidney	0.028	0.006	0.029	0.97
	Lung	0.146	0.034	0.087	1.68
Cesium, μg	Muscle	620	—	570	1.09
	Skeleton	95.2	—	160	0.60
	Liver	19.7	—	20	0.99
	Kidney	4.1	—	2.3	1.78
	Lung	24.2	—	6.2	3.90
Potassium, g	Muscle	44.0	57.0	84	0.52
	Skeleton	8.24	12.2	15	0.55
	Liver	2.64	2.9	4.5	0.59
	Kidney	0.470	0.550	0.59	0.80
	Lung	1.68	2.30	1.9	0.88
Strontium, mg	Muscle	3.50	1.50	0.42	8.33
	Skeleton	455	360	320	1.42
	Liver	0.169	0.130	0.032	5.29
	Kidney	0.038	0.038	0.018	2.09
	Lung	0.188	0.300	0.057	3.29
Thorium, μg	Muscle	6.50	—	—	—
	Skeleton	19.70	—	—	—
	Liver	0.592	—	—	—
	Kidney	0.081	—	—	—
	Lung	13.80	—	—	—
Uranium, μg	Muscle	23.2	—	5.3	4.39
	Skeleton	18.5	—	59	0.31
	Liver	1.04	—	0.45	2.32
	Kidney	0.302	—	7	0.04
	Lung	3.26	—	1.0	3.26
Iodine, mg	Thyroid	17.6	—	12	1.47

cesium, thorium, and uranium detection in a part of diet and tissues samples. We appreciate the advice and comments of Drs. Parr and Iyengar from the IAEA, and Kawamura from the central research laboratory, National Institute of Radiological Sciences, Japan, and we thank all of the participants in the study.

References

1. International Atomic Energy Agency. Report of the project formulation meeting for phase 2 of the reference Asian man project: CRP on ingestion and organ content of trace elements of important radiological protection. Report IAEA/ RCA-95-03 IAEA. Vienna: IAEA, 1995.
2. IAEA. Compilation of anatomical, physiological and metabolic characteristics for a reference Asian man in 1998. Vol. 1. Data summary and conclusions. IAEA-TECDOC-1005. Vienna: IAEA, 1998.
3. Chen J, Gao J. Study on Chinese diet in 1990. Hygiene Research 1993;22 (Suppl)1–66 (in Chinese).
4. Kawamura H, Parr RM, Dang HS, Tian W, Barnes RM, Iyengar GV. Analytical quality assurance procedures developed for the IAEA s reference Asian man project (Phase 2). J Radioanal Nuclear Chem 2000;245(1) 123–6.
5. Kawamura H, Parr RM, Iyengar GV, Dang HS. Analysis of food and tissue samples for IAEA s reference Asian man project (phase 2): Quality assurance aspects. J Radioanal Nuclear Chem 2001;249(1):5–10.
6. Zhu HD, Gao JQ, Yin XY, Wang SL, Liu GD, Hou XL, Wang DP, Liu QF, Wang JX, Chen RS. Chinese dietary composition and intakes of elements and radionuclides by ingestion. Chin. Med Sci J 1997;12(4):195–201.
7. Ge KY. The dietary and nutritional status of Chinese population (1992 national nutrition survey). Vol. 2. Beijing: Peoples Medical Publishing House, 1996: 133–218.
8. International Commission on Radiological Protection. Report of the task group on reference man. ICRP publication 23. Oxford: Paragon Press, 1975.
9. Iyengar GV. Revaluation of the trace element in reference man. Radiat Phys Chem 1998;51:545–60.
10. Wang JX, Chen RS, Zhu HD. Data of anatomical physiological and metabolic characteristics for Chinese reference man. Beijing: Atomic Energy Press, 1999:21–8 (in Chinese).

Estimation of daily micronutrient intake of Filipinos

Erlinda Natera, Trinidad Trinidad, Divina Valdez, Hisao Kawamura, Lorna Palad, and Kunio Shiraishi

Abstract

The Fourth National Nutrition Survey of the Food and Nutrition Research Institute conducted in 1993 showed an increasing prevalence of micronutrient-related diseases in various age groups. Hence, the daily diet consumed by the average Filipino was examined for its nutrient content.

A total of 19 regional diet samples were collected and analyzed for phosphorous, iron, zinc, magnesium, manganese, calcium, potassium, and sodium by using inductively coupled plasma atomic emission spectrometry (ICP-AES). Iodine was determined by inductively coupled plasma mass spectrometry (ICP-MS). Benchmark data for the abovementioned micronutrients showed decreased intake values as compared to the recommended dietary allowance established in 1989. The information will be useful in assessing the existing nutritional status so that appropriate nutrient interventions can possibly be put in place.

Key words: micronutrients, food consumption, inductively coupled plasma atomic emission spectrometry (ICP-AES), inductively coupled plasma spectrometry (ICP-MS), Philippines

Introduction

New evidence from nutritional studies demonstrates the important role of micronutrients in the prevention of various diseases. Hence the inflow of new information relating to dietary characteristics from different population groups in developing countries are continuously being published. One of these is the Philippines where malnutrition exists among inhabitants of the different islands.

While considerable progress has been made by local authorities in addressing the problem by the use of food supplements, baseline information on the dietary composition and daily micronutrient intake is inadequate.

In the Philippines, the Food and Nutrition Research Institute (FNRI) is responsible for monitoring the nutritional status of the population. A nationwide nutrition survey has been conducted every five years since 1978. The daily food consumption (mean one day per capita intake) has been decreasing since the first survey [1]. Subsequent increases in the incidence of micronutrient-related diseases, such as anemia and goiter, are included in the surveys. Likewise the magnitude and extent of underweight and stunted growth among children in different age groups have increased with population growth.

This work hopes to contribute information needed to address the malnutrition problem. The data may be useful in redefining strategies and national nutrition policies. It may serve as a basis for assessing dietary requirements.

Background

Food basket of the Filipino

The food basket of the average Filipino adult is normally a combination of rice, fish, vegetables, chicken and fruit (table 1). The average consumption of cereals, mostly rice and its products, is 340 g representing 42.3% of the total food intake; fish is approximately 99 g (12.3% of the total food consumed); and vegetables is 106 g representing 13.2% of the total amount. The quantity of chicken consumed per day is 48 g and that of fruit is 83 g. Meat and poultry share 5.9% of the total 18.2% for fish, meat, and poultry.

The rest of the food basket contains dried beans,

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TABLE 1. Mean one day per capita food consumption (g)

FNRI 1993 [1]	
Cereals	340
Starchy roots	17
Sugar and syrups	19
Fish, meat, and poultry	147
Eggs	12
Milk and milk products	44
Dried beans	10
Vegetables	106
Fruits	77
Fats and oils	12
Miscellaneous	20

nuts, and seeds (10 g); starchy roots and tubers (17 g); sugar (19 g), and beverages, condiments and other miscellaneous food add 20 g to the total weight of approximately 804 g. Milk and its products are rarely consumed by the average Filipino, only 56 g. The consumption of fats and oils amounts to 12 g [1].

The urban population consumes twice as much meat, poultry, milk, and milk products as those in rural areas.

Factors relating to food consumption

Geographic differences

The Philippines is composed of 7,150 islands with a population of 75.2 million distributed in three main groups namely Luzon (42.52 million), Visayas (15.14 million), and Mindanao (17.66 million). These islands are located in the southeastern part of Asia and are divided into 79 provinces located in 16 regions including the National Capital Region [2].

Those who reside in Regions 1 and 2 in the northern part of the Philippines consume the most rice, 344 g per capita per day. Visayas (Regions 6 to 8), which is centrally located and Mindanao (Regions 10 to 12) located in the southern part, are corn eating regions [1]. The National Capital Region consume the least amount of rice, 252 g per capita per day.

All regions of the central and southern parts of the country consume an average of 100 g per day of fish and its products which is more than that consumed by those living in the northern part. In addition to fish, the inhabitants of the northern regions consume some meat, poultry, eggs, milk, and vitamin-rich foods and beverages.

Household size and economic status

The average Filipino household consists of six members. However, the consumption amounts reported here are taken from households of seven or more. The mean one-day per capita food consumption decreases with an increase in the household size, i.e., cereals and its products reduce from 452 g for a household of 1 to 2 people to 319 g for a household of 9 and above [1]. Therefore large households are nutritionally at risk.

The occupational groups, such as professionals, overseas contract workers, administrators, executives, managers and entrepreneurs, have more diverse and better diets than those who earn less. The disadvantaged households, such as fishermen, farmers, janitors, carpenters, blue collar workers, and the unemployed, have a limited choice and provision for other types of food.

There is a wide gap in consumption between the lowest and highest per capita income groups. The lowest per capita income group normally has a large household and they buy cheap food like corn, green and yellow vegetables, and starchy roots and tubers. Other factors that affect food consumption are meal patterns, culture, religion, food supply, and other dietary practices, i.e., use of leftovers.

Materials and methods

Sampling

Samples of a one-day diet consumed by an average Filipino adult were prepared from commonly eaten foods as documented by the FNRI [3]. The total diet (test meals) from the nine regions consisted of breakfast, lunch, dinner, and one snack. The selection was based on the low cost, availability of food items, and the ease of preparation. This included food eaten raw, processed foods, and beverages. The meals were cooked from edible portions of the raw market sampling using the regional recipes prepared by FNRI.

The other type of sampling is the purchase of regional cooked meals (duplicate diet) in the quantity and selection recommended by FNRI. The total freeze-dried sample weight ranged from 131 to 401 g.

Sample preparation

Each meal was weighed, homogenized, and freeze-dried. The dried samples were pulverized to achieve a powdery texture, mixed thoroughly, and stored at –20°. Approximately 0.9 g of the powdered samples was put into a pressure vessel (similar to Teflon), weighed, and placed in a vacuum oven for one to one and a half hours. After cooling, the sample was checked for moisture content.

Analysis

Digestion was performed using a stepwise microwave digestion system. First, approximately 5 ml of nitric acid was added to 0.9 g aliquots of the freeze-dried

samples and digested in pressure vessels. The samples were further digested with the addition of nitric acid plus perchloric acid, and finally treated with nitric acid plus hydrofluoric acid. All acids used were of 'TamaPure' grade (Tokyo, Japan). After cooling for one hour, the solutions were transferred into other Teflon beakers.

Two ml of nitric acid (68%) was added to the samples and the resultant solutions were dried on a ceramic-top hotplate at 100 to 120°C. White precipitates were obtained at this stage which were dissolved with nitric acid and refluxed in Teflon beakers covered with Teflon watch glasses. After cooling, the solutions were quantitatively transferred to 25 ml volumetric flasks and purified water was added to the mark and mixed thoroughly.

Each sample was analyzed for phosphorous (at the wavelength of 213.6 nm), calcium (317.9 nm), magnesium (285.2 nm), manganese (257.6 nm), zinc (206.2 nm), copper (224.7 nm), sodium (588.9 nm), and potassium (766.4 nm) using inductively coupled plasma atomic emission spectrometry (ICP-AES) [4].

Iodine was determined by using Schnetger and Muramatsu's method of sample combustion and recovery of iodine in an absorbing solution [5]. Finally each sample was analyzed using inductively coupled plasma mass spectrometry (ICP-MS).

One blank and one reference material were run using the same procedures with each batch of samples. Standard reference materials (SRM) used were: SRM 1566a oyster tissue, SRM 1548a typical diet, SRM 1575 pine needles and NIES/NIRS (National Institute of Enviromental Studies, Ibaraki, Japane and National Institute of Radiological Science. Chiba. Japan) typical Japanese diet. Both samples and SRMs were run in duplicates.

Results and discussion

Assessment of the concentration and the nutrient intake values

The concentration and the corresponding intake values for phosphorous, manganese, magnesium, iron, calcium, copper, sodium, potassium, zinc, and iodine based on the regional meals analyzed are given in table 2. Regional variation (large standard deviations) in the concentration and intake values of nutrients were observed in the duplicate and total diet samples due to the seasonal availability of ingredients and the wide selection of seasoning and condiments applied in the preparation of test meals. The sampling period was conducted for a period of two years with a sample size of 19 "one day diets" representing 13 regions including the National Capital Region.

Contributing factors such as those listed enhanced the divergence of values obtained. The same observations were documented in the previous research conducted in 1992 in the National Capital Region (NCR) [6].

TABLE 2. Mean concentration (μg/g dry) and daily intake (mg) of micronutrients from the Filipino diet

Nutrient	Concentration μg/g dry	SD	Daily intake mg	SD
Phosphorus (P)	1,804	464	564	143
Manganese (Mn)	8.75	3.88	2.83	1.54
Magnesium (Mg)	435	121	136	37
Iron (Fe)	25.8	29	8.35	9.79
Calcium (Ca)	788	363	251	139
Potassium (K)	3,367	1,216	1,041	330
Sodium (Na)	4,012	1,521	1,247	496
Copper (Cu)	3.69	1.64	1.19	0.68
Zinc (Zn)	16.3	4.7	5.16	1.73
Iodine (I)	0.38	0.55	0.12	0.48

Based on the above observations, it is safe to present the data as typical intake values. Hence, values may be presented either by mean (table 1) or by minimum, maximum, and median values (fig. 1).

Observed intake versus recommended dietary allowance

Segments of the population in the Philippines vary in their daily nutrient intake because the habitual food consumed varies from one region to another. Hence establishing the safe range of nutrient intake for the Filipino may be simple, while establishing a nutrient requirement to prevent detectable signs of impaired function may be complicated. However, both may require baseline data for the average dietary nutrient intake.

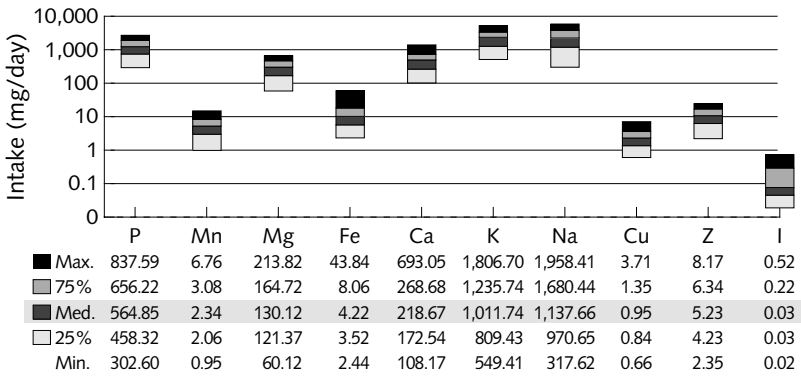
Phosphorous and calcium

The average phosphorus intake of Filipinos, estimated to be 976 mg per day, comes mostly from green leafy vegetables, shrimps, seaweed, rice, corn, banana, beans, eggs, and fish, while the calcium intake is approximately 450 mg per day [7]. Therefore the calcium:phosphorus ratio is 0.46.

In this study the calcium:phosphorus ratio is 0.45 computed from mean intake values of 251 mg for calcium and 564 mg for phosphorus (table 2). Earlier local data showed intake values of 460 mg and 284 mg for phosphorus and calcium, respectively (table 3)[6].

Manganese and magnesium

The estimated intake for manganese is 1.65 mg and for magnesium it is 123 mg [6]. A slight increase in the value for magnesium was observed in this study while



	P	Mn	Mg	Fe	Ca	K	Na	Cu	Z	I
Max.	837.59	6.76	213.82	43.84	693.05	1,806.70	1,958.41	3.71	8.17	0.52
75%	656.22	3.08	164.72	8.06	268.68	1,235.74	1,680.44	1.35	6.34	0.22
Med.	564.85	2.34	130.12	4.22	218.67	1,011.74	1,137.66	0.95	5.23	0.03
25%	458.32	2.06	121.37	3.52	172.54	809.43	970.65	0.84	4.23	0.03
Min.	302.60	0.95	60.12	2.44	108.17	549.41	317.62	0.66	2.35	0.02

FIG. 1. Daily micronutrient intake from the Filipino diet

that for manganese increased from 1.65 mg in 1992 to 2.8 mg in 2000 (table 3). The sources of these two nutrients in the Filipino diet are cereals, leafy vegetables, and nuts. To date there is no information correlating these nutrients to the existing problem of stunted growth and underweight among Filipino children.

Sodium and potassium

Sodium regulates the volume of extracellular fluids and maintains acid-base balance. The common use of salt (NaCl) provides an adequate intake of 6 g or less per day as suggested by the Food and Nutrition Committee [7]. Sodium deficiency rarely occurs because, in addition to salt, fish sauce or shrimp sauce is often added to foods during cooking.

Most of the sodium loss from the body is attributed to sweat especially in tropical climates, hence the sodium requirement may increase during the hot season. A wide variation in individual values was obtained for sodium since sampling was performed during both cold and hot months of the year.

Potassium is the main cation in tissue and blood cells. Like sodium it plays a major role in the regulation of the acid-base balance [7]. The major food sources of potassium are bananas, vegetables, and other local fruits.

One calculation of the sodium and potassium content in the average one day per capita daily food consumption gave estimated values of 4,600 and 1,300 mg, respectively, with a sodium:potassium ratio of 3.5. However, the mean sodium and potassium contents of institutional diets reported in 1953 gave a ratio of 1.5.

The sodium:potassium ratio in this study is calculated to be 1.2 with sodium and potassium contents of 4,012 mg and 3,367 mg, respectively. In 1992 the ratio was computed to be 2.1 (table 3) [6, 7]. The decreasing values observed may not reflect the real picture for there are signs of increasing intake of sodium in the diet because of the excessive use of fish sauce and shrimp sauce [1].

An increasing incidence of hypertension due to excessive salt intake was reported in the Fourth Nationwide Nutrition Survey [1]. Processed foods, such as eggs, fruits, and vegetables preserved with salt, also contribute to the increased sodium intake.

Iron

Rice contributes 29% of the iron in the diet; followed by fish (19.6%), and dark green vegetables, such as malunggay, ampaliya, and mungbeans (7.6%). The rest comes from fruits, such as banana, lemon (calamansi), and meat and poultry (4.3%) [7]. A typical breakfast of rice, mungbeans, and banana is estimated to provide 2.6 mg of iron. Reported values for iron, for 39 regional meals, range from 7.33 to 21.33 mg with a mean of 12.96 mg [7].

This study reports a mean intake of 8.35 mg of iron while in 1992 it was 5.8 mg (table 2), which coincides with the problem of iron-deficiency anemia present in 37.2% of the Filipinos in 1993 [6]. It is expected that with this increase in iron intake that the next nationwide survey will report a corresponding decrease in the incidence of iron-deficiency anemia, especially in infants and lactating mothers.

TABLE 3. Comparison of daily intake with recommended dietary allowance (mean, mg)

Nutrient	1989 RDA [7]	1992 Data [6]	2000 Data
Phosphorus	976	460	564
Magnesium	170	123	136
Manganese	2.9	1.65	2.83
Iron	10.7	5.85	8.35
Calcium	450	284	251
Potassium	1,300	757	1,041
Sodium	4,600	1,596	1,247
Copper		0.82	1.19
Zinc	11	5.02	5.16
Iodine	0.065		0.12

Iodine

The iodine content of the Filipino diet was reported to be approximately 65 μg [7], however, in this study, the mean concentration in the diet is 0.38 μg (table 2), which is significantly smaller than the previous study. Despite the fact that fish and seafood were included in most of the test meals prepared and sampled in some regions, the decreased values obtained maybe due to a loss of iodine in the cooking process. The meals tested included mostly fried fish where 20% of the iodine is lost and boiled fish where 58% of the iodine is destroyed [7]. However, three test meals from the southern region had high iodine values ranging from 0.3 mg to 0.5 mg because the test meals contained raw fish and seafood.

Endemic goiter is prevalent in the Philippines. Thirty-six (36) out of 100 Filipino children have moderate to severe iodine-deficiency disorders and those in the south (Mindanao) face the highest risk of iodine-deficiency disorders [1].

Copper

Seafoods, organ meats, legumes, and nuts are dietary sources of copper. The adult dietary intake of copper ranged from 1 to 1.5 mg per day [7]. Drinking water maybe one source of copper, coming from the pipes.

A nutritional copper deficiency has not been established in the Philippines. The symptoms may include neutropenia, hypo-pigmentation of skin and hair, osteoporosis, and vascular abnormalities [7]. The mean dietary intake for copper in this study was 1.19 mg, while in 1992 it was 0.82 mg per day (table 3).

Zinc

Meat, eggs, and oysters are good sources of zinc. The average Filipino diet is estimated to contain 11 mg. Clinical deficiency symptoms include growth retardation, poor appetite, and mental lethargy. In the Philippines zinc intake is inadequate in pregnant and lactating mothers [7]. In the 1992 and 2000 studies, the zinc intakes reported were 5.02 mg and 5.16 mg, respectively (table 3).

Conclusions and recommendations

In this study the estimates of daily intakes by an average Filipino for micronutrients, i.e., manganese, iron, copper, zinc, and iodine, and for macronutrients, i.e., phosphorus, magnesium, calcium, sodium, and potassium were generally larger than those reported for the national capital region in 1992, that lacked iodine data.

However, the average Filipino diet may not meet the estimated recommended dietary allowances for iron, zinc, iodine, calcium, and phosphorous (table3). Although this is not conclusive due to the small population covered by this work, the aforementioned statement maybe substantiated by reported incidence of micronutrient-related diseases.

There is a need to expand the study to complete the information on the micronutrient composition of the Filipino diet. Further, seasonal sampling of the daily diet must be included, covering all 16 regions, different age groups, and both sexes. The Filipino diet is diverse and is dependent on geographical location, culture, and religion as well as on economic status.

The results of this study strongly indicate that studies on iodine, iron, zinc, phosphorous, and calcium may be needed in order to address the existing micronutrient-related diseases.

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References

1. Fourth National Survey, Philippines. Manila: Food Consumption Food and Nutrition Research Institute, Department of Science and Technology, 1993.
2. National Mapping Resource Information Authority (NAMIRIA). Political map of the Philippines. Compilation based from the National Statistics Coordination Board (NSCB), the National Statistics Office (NSO), and the Department of Interior and Local Government (DILG). Quezon City: Prostyle Grafix,1999.
3. Food and Nutrition Research Institute. Food composition tables, Philippines, Manila: FNRI, 1990.
4. Shiraishi K, Kawamura H, Ouchi Y, Tanaka G. Determi-

nation of 15 mineral elements in total diet samples by ICP-AES. Nippon Eiyo Shokuryo, Gakkaishi 1986;39(3): 209–15.
5. Schnetger B, Muramatsu Y. Determination of iodine. Analyst 1996;121:1627–31.
6. Natera ES, Cuevas GD, Azanon EM, Palattao MB, Espiritu RT, Cobar MC, Palad LH, Torres B, Shiraishi K. Compilation of anatomical, physiological and dietary characteristics for a Filipino reference man. Philippine Tech J 1995;20(3):71–89.
7. RDA Committee. Recommended dietary allowances for Filipinos. Manila: Food and Nutrition Research Institute, Department of Science and Technology, 1989.

High prevalence of subclinical vitamin A deficiency in Jordan: a forgotten risk

Ibrahim Mahmud Dib Khatib

Abstract

This longitudinal study assessed growth and vitamin A status of schoolchildren after earlier surveys had linked stunting among Jordanian children to dietary zinc and iron inadequacies. A group of 1,023 subjects ages 5.5 to 9.9 years were randomly recruited for study from seven disadvantaged semirural districts. Baseline assessment included anthropometric and laboratory data with the relevant dietary information. Over nine months of study, the subjects received a daily snack meal. Immediately before the final assessment, each student received one 100,000 IU vitamin A capsule. At baseline there was a 19.9% prevalence of stunting, 18.8% for anemia, and 21.8% for subclinical vitamin A deficiency. Mean and median serum retinol concentrations were 248 (sd ± 66) and 242 μg/L, respectively. In 98% of the cases, vitamin A-rich vegetables were consumed three or more days per week. About 60% of subjects had serum retinol levels in the range 200 to 300 μg/L. Only vitamin A foods from animal sources showed an influence ($p < .05$*) on mean serum vitamin A values and growth score. Dietary and capsule supplementation had a significant positive impact only on serum retinol levels (*$p < .01$*) and on the anemia (*$p < .05$*) indicators. The conclusion underlines vitamin A deficiency among schoolchildren as a public health problem, and that the situation is anticipated to be more profound among preschool children, who are usually at greater risk of becoming deficient. Launching another, but controlled, intervention study in other sites, preferably with use of a tracer to rule out malabsorption in young children, is highly indicated.*

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Key words: vitamin A (VA), serum retinol concentration μg/L (SRC), linear growth score (height-for-age Z score), relative ponderal growth score (weight-for-height Z score), hemoglobin (Hb)

Introduction

Overview

The causality link between vitamin A deficiency (VAD) and xerophthalmia, the preventable nutritional blindness that hits young children in many developing communities, has been recognized for many decades. Besides, VAD is well documented as a significant contributor to severe infections, including diarrhea, and to increased vulnerability to other nutritional disorders, such as iron-deficiency anemia [1]. Over the second half of the twentieth century the study of VAD increased among health researchers and planners in the majority of developing countries. Unfortunately, this concern about VAD was not observed in Jordan for over three decades, ending only in the mid 1990s.

National research

During the early 1960s, two important health surveys on VAD were carried out in Jordan. The first, sponsored by the WHO, was a clinic-based intervention study on a group of young children who were admitted to hospitals with ocular signs of xerophthalmia [2]. This study estimated that there were more than 300 cases of clinical VAD in a one year period. The mean serum retinol concentration (SRC) in this trial was about 55 μg/L. The second survey, which was known as the Jordan Pediatric Study [3], reported that about 37% of the children under five years old had a mean serum vitamin A falling below the cutoff point (200 μg/L). The community-based nutrition research continued, with an almost total disregard of VAD. Exclusively, the focus encompassed areas of infant feeding, the epidemiology of anemia, and child growth deficits which reflected poor diet and general malnutrition [4–7]. The appar-

ent reason for this evasion of VAD was the absence of a specialized laboratory capable of testing for vitamin A; the expertise was lacking and the equipment was not affordable.

With the global concern for child undernutrition in the early 1990s [8, 9] and the increased evidence of the importance of the roles dietary micronutrients play in health and disease [10, 11], the local nutrition researchers began to look at the child-growth deficiency in the country as a consequences of a hidden type of hunger. Two consecutive surveys for the study of growth of infants and children [12, 13] were launched. With the finding that growth stunting begins only during late infancy [12–14] and continues afterward at a prevalence rate that exceeds 15%, assessment of weaning diets for old infants and feeds for weanlings pointed to the possibility of dietary inadequacies of iron and zinc intakes [12]. The dietary information collected failed to indicate the likelihood of a concomitant inadequacy of dietary vitamin A (VA) (table 1) However, when rusks, fortified with both iron and zinc, were introduced at mid-infancy, only limited, though significant ($p < .05$), improvements in the growth curves of older infants were achieved [13]. This finding was a sufficient indication for considering the repetition of the study with one essential modification. Clearly there was a justification for incorporating some other micronutrients, including vitamin A, in any supplement mixture to be used in future nutritional interventional trials. This conclusion on the need for bringing VAD to light again became justifiable more than ever after the risk of iodine deficiency become more under control with the legislation for the iodination of food grade salt that was issued in 1995 [15].

In the summer of 1997, A VAD-pilot study in three moderately advantaged rural areas near Amman was carried out with support from the WHO and UNICEF. Serum retinol of 400 children under five years old was measured [16]. High performance liquid chromatography (HPLC) was used to assess vitamin A status [17]. About 35% the study subjects had serum retinol concentration (SRC) below 300 μg/L (table 1). Such a finding was salient since blood sampling took place in July, the peak season of vegetables and fruits in Jordan, and the study sample was selected from a relatively well-to-do population. From a nutritional standpoint, this was alarming, and warranted a replication of the trial in other population sectors, and at different times and locations. Based on the success of the VA-pilot trial, a laboratory able to handle analysis for larger scale surveys on VAD was set up at the campus of the Jordan University of Science and Technolgy in 1998.

Table 1. Background summary

Nutrient intakes of infants during the 10th month of age		
Intake	(units)	% of RDA
Energy	(kcal)	88
Protein	(g)	96
Vitamin A	(μg RE)	79
Zinc	(mg)	37
Iron	(mg)	38
N = 30 infants. Source: ref. 12.		

The 1997 pilot survey of vitamin A status of the children under 5 years old	
Serum retinol (N = 400)	
Mean (SD)	339 (± 89) μg/L
Median	328 μg/L
Range	141–624 μg/L
Distribution of children by serum retinol levels (N = 400)	
Vitamin A (μg/L)	Frequency %
< 200	4
200–299	31
≥ 300	65

Distribution of children by growth centile levels (N = 445)		
Growth centile	Sample size (n)	Frequency %
Height-for-age		
< 3rd	68	15.3
> 3rd–25th	206	46.3
> 25th	171	38.4
Weight-for-height		
< 3rd	9	2.0
> 3rd–25th	141	31.7
> 25th	295	66.3

Source: ref. 16.

The Ministry of Education initiative

Influenced by the spreading awareness of importance of micronutrients in child nutrition, Jordan's Ministry of Education (MOE), with support from the Social Security Package Program at the Ministry of Planning, initiated a 'school snack service' (SSS) in September 1999. Immediately before beginning the snack distribution, the MOE-steering committee of the SSS advocated a scientific evaluation of the impact of the service to be carried out along with the snack distribution activity. Upon short notice from the MOE, this uncontrolled longitudinal study was launched the first week that snack distribution began. The study design was tailored to accomplish the objectives set in advance by the MOE.

Study objectives

The study evaluated the impact of serving a mid-morning snack on growth and nutritional status of primary schoolchildren. According to the SSS plans, the population targetted to benefit from the snack service and to be studied included only primary schoolchildren in the first three elementary grades, who lived in villages and hamlets of seven of the less furtunate Jordanian districts.

Methods

Study design

As the MOE initiated the SSS to be an uncontrolled, food-based supplementation project, this study had to follow the longitudinal approach that entails carrying out 'before' and 'after' assessments. The survey investigations were carried out in two phases, at the start and at the end of the nine month period of snack distribution. Baseline (before) data were collected during the first week of September 1999 . This 'phase 1' assessment included the collection of blood and serum samples for laboratory testing, measurement of major anthropometric indicators, and the recording of basic dietary information for each of the study subjects. Phase 2, the final (after) assessment included the collection of laboratory and anthropmetric data and the field work was carried out near the end of May 2000, a few days before the cessation of the SSS project activities.

Research assistance

A team of 22 research assistants, all university graduates and representing the disciplines of nutrition, nursing, and biotechnology, assisted in the field work. An intensive two-day workshop was held for all the team members just before departure to the field. The workshop was meant to ensure quality performance in the field work, with a special emphasis on the standardized application of anthropometry. The exercise evoked scientific stimulation and enthusiasm among the field workers, and thus their readiness for coping with the anticipated harsh conditions of the distant survey locations.

Subjects

The districts which the government records refer to as under-advantaged represent the south, east, and west regions of the country. Seven of these districts were targeted for snack distribution under the SSS tentative program. The were approximately 10,000 primary schoolchildren, all registered in the first three elementary grades. Families of the snack recipients were semi-rural, scattered in 218 villages. The pre-determined sample size was 10% of the study population. At the onset of phase1 Jordan's General Department of Statistics helped in the random selection of the sample by cluster sampling. Primary schools in 38 villages were selected as representative sites. At each of the schools, students were randomly selected. Each school subsample included only the apparently healthy subjects. On the day of the school visit, each student who showed no obvious signs of illness was considered eligible for participation in the study. The purpose of applying such a strict condition was the elimination of the probable effect of an on-going illness on the biochemical and hematological readings. Of 1,048 children selected from the 38 schools, 1,023 were studied in both phases of the survey. All were between 5.5 to 11.0 years old. These subjects came from villages that represent the regions of Aqaba (n = 112), Karak (n = 32), Ma'an (n = 79), Tafeelah (n = 234), Dair-Alla (n = 130), South Shoenah (n = 180), and North Badia (n = 256). However, to avert the confounding effect of puberty on the growth scores of study children, statistical analysis of anthropometric data was confined to those children who were in the 5 to 9.9 year-old age group. There were 604 girls and 419 males. For the dietary assessment by direct interview and the 24-hour dietary record, only subsamples of children and mothers were recruited.

Supplements

Each subject received the snack five times a week during the nine months of the study, It consisted of a 50g pack of iron fortified biscuits, 100 ml of sterilized fresh cow's milk, and one small sized piece of fruit; the snack fruit-alternatives that changed every week were apples, oranges, and bananas. The biscuits were enriched with 3.5 mg ferrous sulphate. In late April 2000, and upon the preliminary findings of the phase1 assessment which revealed an unforeseen shortcoming of the SSS, the Ministry of Education distributed an additional supplement in the form of a vitamin A oil capsule to all the subjects. Each subject received only a single dose of 100,000 IU retinyl palmitate three weeks before the final assessment was carried out.

Data collection

Anthropometric, basic dietary, and laboratory data were collected. All the anthropometric data were collected following the standard methodology recommended by the WHO expert groups [18]. Standardization of growth measurements was applied in the field at all stages. Height measurements were to the nearest 1 millimeter, and only averages of duplicate readings were recorded. An adult-type Seca beam balance scale (Vogel and Halke GmbH-Hamburg, Germany), reading up to 200 kg was used to measure weight. The children, wearing only underwear, were weighed with measurement precision of 100g. Mid-upper arm circumference (MUAC) and arm triceps skinfold thickness (SFT) were also taken. However, these were found to add little extra weight to the analysis of major anthropometric indicators.

At baseline, a dietary questionnaire highlighting the child's consumption of various food categories was administered. At a later stage, a 24-hour dietary record technique was used to obtain information to calculate the child's nutritional intake of major nutrients.

Immediately after blood samples were obtained, simple hematological tests, hemoglobin (Hb) and hematocrit (PCV), were done in the field. Serum was separated from the blood samples on the spot. The sera were primarily needed for the assessment of serum retinol and ferritin concentrations. For the hemoglobin measurement the Drabkin's spectrophotometric method was applied.

A 3 ml venous blood sample was obtained from subjects using a syringe with 21 gauge needle, and transferred to a 5 ml plain tube. These were rapidly wrapped with carbon paper. Immediately before centrifugation for serum separation, capillary pipettes were used to withdraw the required blood volume for hemoglobin and hematocrit testing. Serum was kept in polyethylene vials and immediately placed in a cooler. The vials were frozen in an ordinary refrigerator until transported to the Jordan University of Science and Technology laboratories and stored at –20°C. Direct exposure to natural light was avoided throughout the whole collection process. The sera collected were tested within the first week of storage; laboratory testing included the determination of serum retinol and ferritin concentrations.

Data analysis

Derivation of anthropometric indices and the prevalences of stunting and wasting, were computed using 'Anthro.' the CDC/WHO package [19]. The Statistical Package for Social Studies (SPSS), Version 4 was used for other statistical analyses. The cutoff point used to identify malnutrition was a Z score less than –2 SD.

Results

The recipient children's socioeconomic status (SES) was apparently very low, as labelled by the SSS project. On the other hand, the results of the statistical data analyses indicated a wide spectrum of information that revealed unknown aspects of child nutrition in the study area.

A cutoff point at the level of two-thirds of the RDA was set to estimate nutritional inadequacies. At baseline, dietary assessment by the 24-hour diary method showed that habitual daily dietary intakes of the schoolchildren were not far below the RDA reference cutoff values (table 2). The nutrients estimated were: energy 92%, protein 82%, vitamin A 67%, calcium 76%, and iron 62%. However, with the snack contributing about 25% of the RDA, the total intake during the study period was deemed fairly adequate (table 1). Obviously, most of the daily protein intake came from cereal foods (bread, rice, crackers, and biscuits) and powdered cow's milk.

The phase 1 results showed high figures for the prevalence of stunting (low Z score values for height-for-age). On the other hand, the prevalence of wasting (low Z scores for weight-for-height) was minimal. The Prevalence of stunting was 19.6% with no apparent sex differences (table 3). There were no observable changes in anthropometric indicators during the course of snack supplementation (table 4). The occurrence of such significant stunting was not unexpected in the light of previous reports of the 1990s [12–14].

At baseline there was a high prevalence of vitamin A deficiency (VAD) in most of the study districts (table 5). The mean and median serum retinol concentrations for the population under study were 248 ± 66 μg/L and 242 μg/L, respectively. The overall prevalence of VAD averaged 21.8 %. Except for the district of Tafeelah, all districts have a VAD prevalence of 20% or higher, with North Badia at 33.8%. These findings were somewhat surprising since more than 98% of subjects reported that they consumed vitamin A-containing foods three or more days per week. There was an overall decline in these prevalence rates after the snack supplementation and the vitamin A capsule (table 6). When the high prevalence rates of VAD and stunting were analyzed on the basis of diet the consumption of meats, but not vegetables, had a crucial role. Consumption of meat had a significantly positive impact on vitamin A status ($p < .05$) and a higher impact ($p < .01$) on linear growth achievements (weight-for height) (table 7). The serum retinol concentration of non-stunted children (250 ± 69 μg/L) was significantly greater than that of the stunted children (235 ± 63 μg/L) ($p < .005$). No significant sex-linked differences were detected in the

TABLE 2. Baseline assessment of mean daily dietary intakes of schoolchildren

Nutrient supply	% RDS (± SD)
Usual dietary consumption	
Energy (kcal)	92 (± 13)
Protein (g)	82 (± 11)
Vitamin A (μg RE)	67 (± 16)
Calcium (mg)	76 (± 16)
Iron (mg)	62 (± 18)
Extra nutrients from snack	
Energy (kcal)	25
Protein (g)	25
Vitamin A (μg RE)	25
Calcium (mg)	25
Iron (mg)	25

Statistics were assumed valid throughout the study period.Only one100,000 IU retinyl palmitate capsule was given as a supplement near the end of the period.

baseline vitamin A values.

Mild to moderate anemia was common in young schoolchildren. The mean hemoglobin concentration of th schoolchildren was found to be 11.9 g/dl (table 8), with prevalence of anemia (Hb < 11.0 g/dl) of about 18.8% (table 9). The prevalence of severe anemia was less than 0.3 , indicating the presence of iron-deficiency anemia. The baseline mean serum ferritin concentration was 15.3 ng/ml. After the snack supplementation ($p < .01$) the mean serum ferritin value increased to 15.9 ng/ml ($p < .01$) (table 8). As a result, the proprtion of children with suspected iron deficiency dropped from 15.2% to 10.1% level (table 9).

Although there were no sex-related differences observed at baseline, after the snack-supplementation hemoglobin levels of the girls increased significantly (Hb = 12.2 g/dl) ($p < .005$) as compared to the boys (mean Hb = 12.0 g/dl). No reason for this inter-gender difference in hemoglobin values could be identified. A further inter-gender difference was related to weight-for-height (table 10). The explanation for this finding could be that male children were leaner than the females, particularly in those whose puberty onset was imminent.

The results underlined three common nutritional disorders among schoolchildren in the study; VAD, anemia, and stunting (table 11). With the exception of Tafeelah, children in all the study districts suffered from these disorders. Tafeelah is a hilly area with relatively fertile soil; olive trees are grown in this district, and the consumption of olive oil is common.

At baseline linear growth levels did not correlate with hemoglobin or with serum retinol levels. This indicated a possibility of a past or a chronic type of nutritional deprivation. In fact, this agreed with earlier studies, which micronutrient-deficiency states were implicated in the evolution of stunting starting from mid-infancy [12, 13]. On the other hand, there has been a long-standing awareness of anemia among Jordanian physicians and researchers. Accordingly, the focus on anemia and the interest in taking action to alleviate it would be expected to continue. Thus, only the figures on the prevalence of subclinical VAD in schoolchildren were found stunning (table 11). Such findings indicate failing health standards, and therefore merit recognition by nutrition and health researchers as a reminder of the early 1960s surveys [2, 3].

TABLE 3. Prevalence of malnutrition among study children (Z scores < –2)

	Chronic malnutrition		Acute malnutrition	
	Stunting		Wasting	
Age group (yr)	No. affected (n)	%	No. affected (n)	%
Combined sexes				
5–5.9	14 (71)	19.72	0 (71)	0.00
6–6.9	64 (318)	20.13	7 (318)	2.20
7–7.9	67 (324)	20.68	2 (324)	0.62
8–8.9	39 (247)	15.79	5 (247)	2.02
9–9.9	11 (35)	31.43	0 (35)	0.00
All 5–9.9	195 (995)	19.60	14 (995)	1.41
Males				
5–5.9	4 (29)	13.79	0 (29)	0.00
6–6.9	25 (132)	18.94	3 (132)	2.27
7–7.9	27 (139)	19.42	2 (139)	1.44
8–8.9	21 (86)	24.42	2 (86)	2.33
9–9.9	1 (5)	20.00	0 (5)	0.00
All 5–9.9	78 (391)	19.95	7 (391)	1.79
Females				
5–5.9	10 (42)	23.81	0 (42)	0.00
6–6.9	39 (186)	20.97	4 (186)	2.15
7–7.9	40 (185)	21.62	0 (185)	0.00
8–8.9	18 (161)	11.18	3 (161)	1.86
9–9.9	10 (30)	33.33	0 (30)	0.00
All 5–9.9	117 (604)	19.37	7 (604)	1.16

Discussion

Early studies affirmed that stunting evolves gradually after mid-infancy, and that the enrichment of infant weaning foods with selected micronutrients may alleviate the problem [12, 13]. Apart from zinc and iron, vitamin A may be the key component in the selection of micronutrients to be considered for all supplementary preparations aiming at the prevention of childhood stunting in the country.

The socioeconomic constraints in the study area seemed to influence nutritional status in general, and the development of anemia, in particular. According to the prevalent prices and the cost of living in Jordan, expenditures for food were limited which ultimately has had an impact on the availability of quality food in the household. Under normal circumstances, along with the abundance of cereal foods in all meals in

TABLE 4. Frequency distribution of study children according to prevalence of malnutrition before and after supplementation

		Stunting		Wasting	
Gender	Phase	No. affected (*n*)	%	No. affected (*n*)	%
Combined	Before	195 (995)	19.6	14 (995)	1.4
	After	170 (917)	18.5	6 (916)	0.7
Males	Before	78 (391)	20.0	7 (391)	1.8
	After	72 (366)	19.7	2 (366)	0.6
Females	Before	117 (604)	19.4	7 (604)	1.2
	After	98 (551)	17.8	4 (550)	0.7

Jordanian households, appreciable amounts of items from the other food groups are generally served. With increasing economic constraints, the consumption of cereal foods becomes proportionally larger and meats less. Deviances from an average balanced diet, that is based on the four major food groups, are more common today than in earlier years. This is most likely to be attributed to continuing deterioration of the purchasing power of the local currency, the Jordanian Dinar (JD).

Dietary deviances from the norm seem to affect the vitamin A status of young schoolchildren. Based on recent WHO-criteria [20], the finding that subclinical VAD in the under-developed sectors of Jordan's population exceeded the 20% prevalence cutoff point, has put the country on the of list countries imperiled by VAD as a public health problem. Moreover, this scientific opinion has been underlined further with a general trend of low serum retinol values found in in the studied subjects. At baseline, the percentage of children with subclinical VAD (SRC < 200 μg/L), or being at risk of becoming deficient (SRC 200 to < 300 μg/l) was 82.2% (table 5). From another standpoint, this study has highlighted a more alarming situation in the country, since the epidemiology of VAD is known to be more common in the preschool years. The exceptionally high prevalence of subclinical VAD has affected the villages and small hamlets in the North Badia, followed by Aqaba and Ma'an, more than other the sites. These three areas are the furthest from the capital, Amman,

TABLE 5. Baseline frequency distribution of study children according to level of serum vitamin A concentration (μg/L) [Study population mean SRC= 248 ± 66 μg/L; median SRC = 242 μg/L]

Region	N	Serum vitamin A concentration μg/L	Frequency	Valid %	Cumulative %
Population	869	< 200 (deficient)	211	21.8	21.8[a]
		200–299	576	59.4	81.2
		300–399	158	16.3	97.5
		400–499	21	2.2	99.7
		500–599	3	0.3	100.0
Aqaba	109	< 200 (deficient)	29	26.6	26.6[a]
		200–299	65	59.6	86.2
		300–399	14	12.8	99.1
		400–499	1	0.9	100.0
Karak	31	< 200 (deficient)	8	25.8	25.8[a]
		200–299	20	64.5	90.3
		300–399	3	9.7	100.0
Ma'an	77	< 200 (deficient)	16	20.8	20.8[a]
		200–299	50	64.9	85.7
		300–399	11	14.3	100.0
Tafeelah	229	< 200 (deficient)	11	4.8	4.8[a]
		200–299	125	54.6	59.4
		300–399	78	34.1	93.4
		400–499	14	6.1	99.6
		500–599	1	0.4	100.0
Dair-Alla	120	< 200 (deficient)	24	20.0	20.0[a]
		200–299	71	59.2	79.2
		300–399	20	16.7	95.9
		400–499	3	2.4	98.3
		500–599	2	1.7	100.0
South Shoenah	151	< 200(deficient)	38	25.2	25.2[a]
		200–299	91	60.3	85.4
		300–399	19	12.6	98.0
		400–499	3	2.0	100.0
North Badia	252	< 200(deficient)	85	33.7	33.7[a]
		200–299	154	61.1	94.8
		300–399	13	5.2	100.0

a. Prevalence of subclinical VAD.

the poorest, and have the least fertile soil for agriculture. Beside poverty linked-shortages of foods from animal sources, choices of vegetables and fruits were found to be inappropriate. Apart from tomatoes, which are the most popular vegetable consumed by Jordanian households, the vegetable choices are generally carotenoids-poor food items.

Without the vitamin A supplement-capsules, the school snack as it was formulated and distributed by the SSS program was not sufficient to overcome VAD among the young schoolchildren.The overall supplementation trial, however, succeeded in alleviating anemia and in peventing further stunting. Therefore, and upon the completion of the 'before' and 'after' snack assessments, a conclusive report was submitted by the Ministry of Education at the end of December 2000. The study report recommended the continuation and expansion of the snack program, with some reasonable modifications. Children under five years old, who are generally known to be at greater risk of developing VAD, need to be studied for this hidden VAD problem. From a national perspective, it is a peculiar finding to have VAD becoming endemic while

TABLE 6. Distribution of the children according to serum retinol levels (μg/dl) in both phases of the study

	Phase 1 (Before)		Phase 2 (After)	
Serum retinol level (μg/dl)	Valid %	Cumulative %	Valid %	Cumulative %
Aqaba				
< 200 (deficient)	26.6	26.6[a]	16.2	16.2
200–299	59.6	86.2	59.6	75.8
300–399	12.8	99.1	23.2	99.0
400–499	0.9	100.0	1.0	100.0
Karak				
< 200 (deficient)	25.8	25.8[a]	10.0	10.0[a]
200–299	64.5	90.3	53.3	63.3
300–399	9.7	100.0	33.3	96.7
400–499			3.3	100.0
Ma'an				
< 200 (deficient)	20.8	20.8[a]	17.9	17.9[a]
200–299	64.9	85.7	46.3	64.2
300–399	14.3	100.0	29.9	94.0
400–499			6.0	100.0
Tafeelah				
< 200 (deficient)	4.8	4.8[a]	2.8	2.8[a]
200–299	54.6	59.4	45.6	48.4
300–399	34.1	93.4	41.4	89.8
400–499	6.1	99.6	9.3	99.1
500–599	0.4	100.0	0.9	100.0
Dair-Alla				
< 200 (deficient)	20.0	20.0[a]	11.3	11.3[a]
200–299	59.2	79.2	46.1	57.4
300–399	16.7	95.9	40.0	97.4
400–499	2.4	98.3	1.7	99.1
500–599	1.7	100.0	0.9	100.0
South Shoenah				
< 200 (deficient)	25.2	25.2[a]	4.8	4.8[a]
200–299	60.3	85.4	58.6	63.4
300–399	12.6	98.0	32.4	95.9
400–499	2.0	100.0	4.1	100.0
North Badia				
< 200 (deficient)	33.7	33.7[a]	18.1	18.1[a]
200–299	61.1	94.8	62.9	81.0
300–399	5.2	100.0	17.2	98.3
400–499			1.7	100.0

a. Prevalence of subclinical vitamin A deficiency.

TABLE 7. Baseline-comparative analyses of vitamin A and growth status of school children by frequency of consumption of food groups (*t*-test)

Foods	Frequency of consumpion	*N*	Mean (SD)	*p*
Serum retinol concentration (μg/L)				
Meats	≥ once per wk	116	242 (87)	< .05*
	< once per wk	60	219 (67)	
Vegetables	≥ 4 times per wk	81	244 (104)	< .17
	< 4 times per wk	97	226 (55)	
Height-for-age Z score				
Meats	≥ once per wk	114	–1.16 (.83)	< .01*
	< once per wk	60	–1.65 (1.08)	
Vegetables	≥ 4 times per wk	81	–1.39 (.96)	< .44
	< 4 times per wk	95	–1.28 (.97)	

* Significant difference detected.

Table 8. Comparative analysis of 'baseline' and 'final' values of main study varibles (paired sample *t*-test)

Variables	N	Mean (SD)	*p*
Serum retinol concentration (μg/L)			
Baseline	897	248 (67.3)	< .001*
Final		278 (65.8)	
Hemoglobin (g/dl)			
Baseline	917	11.9 (1.035)	< .001*
Final		12.2 (.955)	
Serum ferritin (ng/ml)			
Baseline	998	23.5 (15.3)	< .01*
Final		27.8 (15.9)	
Height-for-age (Z score)			
Baseline	938	–1.297 (.893)	< .32
Final		–1.291 (.860)	
Weight-for-height (Z score)	912	–.1206 (.895)	< .001*
		.2356 (.906)	

* Significant difference detected, possibly attributed to snack and vitamin A capsules.

the country is famous for vegetable production and exportation. The proportion of the childs' diet derived from animal sources is not only a determinant of iron and zinc status, but also a support for serum vitamin A sufficiency. The abundance of vegetables in the Jordanian childs' diet seemed to fail to provide an adequate vitamin A supply.

Further methodical investigations with emphasis on the VAD issue are warrented. This study needs to be repeated, but with the use of a contol group. The prospective controlled study should continue the use vitamin A capsules as basic supplements, and should be conducted in other areas and on different sectors of the population. The capsules are helpful tools for checking the vitamin A status of the recipients as assessed by their serum retinol concentrations. With capsule supplementation, retinol values would be expected to remain relatively stable for at least two to three months. On measuring serum retinol concentrations, the values would be higher than baseline if recipients were depleted prior to supplementation or near baseline levels if they were not depleted. By the fourth month, it is likely that there would be a decline toward the baseline level among populations whose underlying diet was constant and another supplement had not been given. According to these guidelines, it would be necessary to wait for one month after supplementation before taking the follow-up blood samples (B. Underwood, personal communication, 2000). Launching a tracer based- community study should also be considered, whenever a well-developed and safe methodolgy becomes available.This sort of tracer-research work is indicated since it may help ruling out impairment of intestinal absorption and assimilation of carotenoids in the affected children.

TABLE 9. Distribution of study subjects by categories of main indicators

Indicator and category	Baseline	Final
	Valid %	
Height-for-age		
Stunted	19.9	19.9
Normal, below median	72.1	73.0
Normal, above median	8.0	7.1
Hemoglobin (g/dl)		
< 10 (moderate to severe anemia)	3.5	2.8
10–10.9 (mild anemia)	15.0	12.3
≥ 11	81.5	84.9
Serum retinol concentration (μg/L)		
< 200 (subclinical VAD)	21.8	11.0
200–299	59.4	54.0
300–399	16.3	30.5
400–600	2.5	4.5
Serum ferritin level (ng/ml)		
≤ 10 (iron deficient)	15. 2	10.1
> 10	84.8	89.9

TABLE 10. Final phase comparative analyses of main variables by sex (*t*-test)

Variable and gender	Mean (SD)	*p*
Serum retinol concentration (μg/L)		
Males	283 (71)	.07
Females	275 (63)	
Hemoglobin (g/dl)		
Males	12.0 (.87)	.005*
Females	12.2 (1.01)	
Serum ferritin (ng/ml)		
Males	30.0 (18.2)	.485
Females	27.5 (16.7)	
Height-for-age (Z score)		
Males	–1.26 (.98)	.32
Females	–1.32 (.83)	
Weight-for-height (Z score)		
Males	.10 (.87)	.001*
Females	.32 (.92)	

*Significant difference detected.

TABLE 11. Prevalence of major nutritional disorders[a] in various districts before and after the study

Site	Vitamin A deficiency (%)		Anemia (%)		Stunting (%)	
	Baseline	Final	Baseline	Final	Baseline	Final
Aqaba	26.6	16.2	14.7	9.9	27.3	27.5
Karak	25.8	10.0	3.2	6.7	25.8	33.3
Maan	21.1	17.9	10.3	11.8	24.4	20.6
Tafeelah	4.8	2.8	7.9	3.7	9.6	9.1
Dair Alla	19.3	11.3	34.9	38.9	20.0	22.2
South Shoenah	25.2	4.8	28.2	14.8	16.9	16.6
North Badia	33.7	18.1	20.9	9.9	25.8	25.8
All sites	21.8	11.0	18.8	15.1	19.9	19.9

a. Subclinical VAD, serum retinol < 200 μg/L; anemia, Hb < 11g/dl; stunting, height-for-age Z score < –2.

It is well-known that there are similarities between Jordan and some neighboring countries.These include ethnicity, cultural background, socioeconomic structure, climate, soil, traditional dishes and eating habits, and other socioecological features. Based on this fact, it is likely that subclinical VAD also affects these countries, with a more or less similar pattern as in Jordan. This study, therfore, signifies the importance of united efforts among researchers in the Eastern Mediterranean region.They ought to agree to initiate a coordinated international VAD-research effort in the region to investigate the epidemiology of the disorder and adopt a sound and cost-effective strategy to overcome it. Within this context, each party will benefit, at least, from exchanging views and experiences. Such a regional campaign would gain a lot of support from many international agencies involved in the combat against VAD in the world.

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References

1. Underwood BA, Arthur P. The contribution of vitamin A to public health. FASEB J 1996 Jul;10(9):1040–8.
2. McLaren DS, Shirajian E, Tchalian M, Khoury G. Xerophthalmia in Jordan. Am J Clin Nutr 1965;17:117–30.
3. The Interdepartmental Committee on Nutrition for National Defense, and the Interdepartmental Committee on Nutrition for Jordan. The ICNND-ICNJ nutrition survey on infants and preschool children in Jordan (1962–63). Washington, D C.: US Government Printing Office, 1964.
4. Hijazi SS. Child growth and nutrition in Jordan. Amman, Jordan: The Royal Scientific Society (RSS) Pubications, 1977:96-7.
5. Hijazi S, Abu-Laban A, Waterlow JC. The duration for which breast feeding is adequate. Acta Paediatr Scan 1989; 78:23–8.
6. Hijazi SS, Abul-Dahab M. A national multipurpose study of preschool children in Jordan: health aspects. Study report. Amman, Jordan: Ministry of Social Development, 1987.
7. Hijazi S, Faqih A, Weisell R. Growth pattern of Jordanian school children, ages 6–8 years: changes between 1962–81. JMJ 1986;20:25–43.
8. Grant J. The state of the world's children 1990. A UNICEF report. New York: Oxford University Press, 1990.
9. Grant J. The state of the world's children 1994. A UNICEF report. New York: Oxford University Press, 1994.
10. Brown KH. The importance of dietary quality versus quantity for weanlings in less developed countries: A framework for discussion. Food Nutr Bull 1991;13: 86–94.
11. Messr E. Ending hidden hunger: A policy conference on micronutrient malnutrition. Food Nutr Bull 1992;14(1): 62–4.
12. Khatib I, Hijazi S. JUST-Percentiles: a cross-sectional study of the declination observed in the Jordanian child growth curve and its association with the local patterns of infant weaning. Irbid, Jordan: Jordan University of Science & Technology (JUST), 1992.
13. Khatib I, Hijazi S, Aggett P. A longitudinal -interventional-study of the impact of zinc fortified weaning food on linear growth of infants in northern Jordan. Amman, Jordan: The Higher Council for Science and Technology Publications,1995.
14. Khatib IMD, Hijazi SS, Zou'bi AA. Nutritional status of Jordanian Children: Analysis of anthropometric data of Jordan's 'Population and Family Health Survey, JPFHS,1990'. Columbia, Md,, USA: Institute of Research Development and Macro International Inc, 1993.
15. The IDD National Committee: Iodine deficiency in Jordan. A national study. Amman, Jordan: UNICEF, 1995.
16. Khatib I, Kharabsheh S , Badran U, Qarqash W. An HPLC-based survey of vitamin A status of young Jordanian children. A draft report of collaborative research with the Ministry of Health and UNICEF. Amman, Jordan: UNICEF, 1997.
17. Catignani GL, Bieri JG. Simultaneous determination of retinol and alpha-tocopherol in serum or plasma by liquid chromatography. Clin Chem 1983;29; 4:708–12.
18. WHO-Staff Members. Measuring change in nutritional status. Guidelines for assessing the nutritional impact of supplementary feeding programmes for vulnerable groups. Geneva: World Health Organization, 1983.
19. Sullivan KM, Gorsein J. ANTHRO documentation, version 1.01. Joint Collaboration of the Division of Nutrition-Center for Chronic Disease Prevention and Health Promotion/CDC, USA, and the Nutrition Unit, WHO. Geneva: WHO, 1990.
20. WHO Group. Indicators for assessing vitamin A deficiency and their application in monitoring and evaluating and evaluating intervention programmes. Geneva: WHO, 1996.

Study of essential elements in cattle tissues from a tropical country using instrumental neutron activation analysis

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Abstract

There has been increasing interest in the elemental composition of animal tissues to support health and nutritional studies. Determining the elemental concentration in cattle tissues is especially important because these materials are used for multipurpose objectives such as the assessment of animal health, the quality of human foods consumed, and as a potential environmental biomonitor. Chromium, copper, sodium, potassium, iron, and zinc levels were determined in bovine tissues—kidney, liver and muscle—from cattle bred and raised in a potentially metal contaminated region because of mineral activities. The Brazilian data were obtained using k_0-instrumental neutron activation analysis, performed at the Nuclear Development Technology Centre/Nuclear Energy National Commission (CDTN/CNEN) in Minas Gerais State. The values of international organizations and the Brazilian analytical data are compatible. This study indicates that the nuclear technique is an efficient tool to determine elemental concentration in animal biological samples.

Key words: Trace elements, cattle tissue, neutron activation analysis

Introduction

Trace elements are classified by the simplest definition. For each element there is a range of safe and adequate exposures, within which homeostasis is able to maintain optimal tissue concentrations and functions. But every element is potentially toxic when the range of safe and adequate exposure is exceeded [1]. It is important to examine the mineral composition of animal tissues from nutritional and toxicological points of view.

The Brazilian daily diet is usually composed of vegetables, rice, beans, and meat. Beef is the main protein source for Brazilians, because it is abundant and cheap. Minas Gerais State has the largest national herd of cattle. However, some animals are raised in potentially metal contaminated regions such as Curvelo City and its surrounding areas. This region was chosen as the sampling area due to its intensive mining activities. The Das Velhas River, the second largest in Minas Gerais State flows through this region, carrying out industrial residues which were previously detected in local water, soil, and forage samples [2].

The elemental determination was carried out in the Radiochemical Laboratory using k_0-instrumental neutron activation analysis.

Objective

We determined the concentration of elements in cattle tissues in order to verify the levels of essential elements and possible contamination by toxic elements and their impact on human health in future research. This paper is related to the first sampling evaluation of cattle tissue from animals raised in Curvelo and surrounding areas.

Experimental procedures

Sample preparation

Cattle tissues—muscle, kidney, and liver—were analyzed. Brazilians consume kidney and liver; it is a cultural and inexpensive habit. The beef cuts and

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bovine organs used as samples were chosen at random and purchased at butcher shops located in downtown Curvelo. The source of the animal product purchased was previously checked. Approximately 200 g of each specimen was frozen at –70°C and lyophilized. Each freeze-dried sample was powdered and homogenized and around 300 mg was weighed into polyethylene irradiation vials.

Quality control

Samples were replicate for quality control. Biological reference materials were analyzed in order to verify the efficiency of the method and the traceability of element level determinations. The reference materials used were bovine muscle powder—NIST-SRM 8414 [3] and bovine liver powder—NIST-SRM 1577b, [3], National Institute of Standards and Technology, USA. These reference materials were also weighed, around 300 mg, into polyethylene irradiation vials.

Analytical technique applied on elemental determination

The neutron activation technique (NA) is based on nuclear properties of the nucleus of the atom, radioactivity, and the interaction of radiation with matter [4]. The simplest description of the technique says that when one natural element is submitted to a neutron flux, the reaction (n,γ) occurs. The radionuclide formed emits gamma radiation, which can be measured by suitable equipment. About 70% of the elements have nuclides possessing properties suitable for neutron activation analysis. At the Nuclear Technology Development Centre (CDTN), there is a nuclear reactor TRIGA MARK I IPR-R1 that allows the application of this technique [5].

The k_0-instrumental neutron activation analysis (k_0-INAA,)[5,6] a variation of NA in which the sample is irradiated without previous chemical preparation was used in this study. This specific method is based on nuclear constants—the k_0 factors and some reactor parameters. The elemental concentration of the sample is calculated by means of the k_0 fundamental equation:

$$m_a = \frac{m_p C_{n,a} \varepsilon_p F_p S_p C_a D_a H_a}{k_0 C_{n,p} \varepsilon_a F_a S_a C_p D_p H_p} \quad (1)$$

where

$$k_0 = \frac{M_p \theta_a P_{\gamma,a} \sigma_{0,a}}{M_a \theta_p P_{\gamma,p} \sigma_{0,p}} \quad (2)$$

Considering the subscript a, sample, and p standard, in Equation (1): m is the mass of the studied element; C_n is the number of counts in the full-energy peak, corrected for pulse losses (dead time, random, and true coincidence); ε is the full-energy peak detection efficiency, including correction for gamma-attenuation; F is $[f + Q_0(\alpha)]$, where f is the subcadmium to epithermal neutron flux ratio, and $Q_0(\alpha)$ is the $I_0(\alpha)$, resonance integral, to σ_0, cross-section thermal neutron, ratio, and α is the parameter describing the real epithermal neutron flux distribution; S is the saturation factor, function of irradiation time; C is the decay factor during the counting; D is the decay factor between irradiation and counting; H is the dead time during the counting. In Equation (2), k_0 is defined by: M, the molar mass; θ, the isotopic abundance; P_γ, the gamma-emission probability; and σ, cross-section thermal neutron.

For 91 isotopes the k_0 values have been determined in several laboratories throughout the world, and the k_0 values are available in the literature with about 2% uncertainty. For 21 elements the uncertainty values are about 5%.

The cattle tissues were irradiated in the reactor TRIGA MARK I IPR-R1. At 100 kW the thermal neutron flux is 6.6×10^{11} neutrons cm^2 s^{-1}. The samples were irradiated simultaneously with standards of gold and sodium as comparators, and the reference materials. The elements were determined through three schemes of irradiation: 5 minutes to detect the short half-life radionuclides; 4 hours to detect the medium, and 20 hours, the long half-life radionuclides.

After suitable decay time, the gamma spectroscopy was performed in a HPGe detector, 10% of efficiency, FWHM 1.85 keV and ^{60}Co, 1332 keV, connected to a multichannel analyzer. The calculations were based on the reactor parameters [5, 6], k_0 constants, and the equations 1 and 2 [6]. The first scheme of irradiation was used to determine copper; the second scheme for potassium and sodium, and the third for chromium, iron and zinc.

Results and discussion

Table 1 summarizes the sample concentrations for chromium, copper, iron, potassium, sodium, and zinc, determined in certified reference materials, bovine muscle powder (NIST-SRM 8414) and bovine liver powder (NIST-SRM 1577b) from National Institute of Standards and Technology, Gaithersburg, USA. A good agreement was found between the experimental and certified values.

The same elements were also determined in cattle samples. Tables 2, 3, and 4 show the results for kidney, liver, and muscle samples, and the values from other countries. All the results based on edible portion, according to values reported in the literature [7]. The data related to Argentina [8] and Chile [8] were supplied by FAO [5]; the data from the United States were supplied by United States Department of Agriculture

(USDA) [9] and the Subcommittee on Mineral Toxicity in Animals [10]. The data from Italy were supplied by INRAM [11].

In general the kidney samples had similar levels of concentration (table 2). In spite of showing the same value for iron as reported in the United States [9] both results were half the concentration observed in Argentina [9]. It is possible to verify a considerable accumulation of chromium for the Brazilian sample. This result may suggest contamination in the environment [2].

The Brazilian liver samples had a higher copper concentration than the USA results, while zinc was lower. Zinc deficiency is one of the most important deficiencies worldwide. A moderate zinc deficiency is hardly recognized by the cattle breeder. The levels of the other elements reported were comparable.

The concentration of elements in the muscle samples, including the Italian report [11] are shown in table 4. In general the values for muscle were similar.

Variations in elemental tissue concentrations could be credited to the animal's age, variable input and output of inorganic elements, non-homogeneous composition of tissues [12], local water and soil conditions, as well as diet and environment. The variation in measurement methods [8–11] should be emphasized.

Conclusions

We verified the essential element concentration and possible contamination by toxic elements in cattle tissues that can affect human nutrition. There was a

TABLE 1. Elemental concentrations determined in certified reference materials

	Bovine muscle powder (NIST SRM 1577–b)		Bovine liver powder (NIST SRM 8414)	
Element	Experimental ($mg.kg^{-1}$)	Certified ($mg.kg^{-1}$)	Experimental ($mg.kg^{-1}$)	Certified ($mg.kg^{-1}$)
Chromium	ND	NR	153 ± 6	160 ± 8
Copper	ND	NR	ND	NR
Iron	205 ± 20	184 ± 15	ND	NR
Potassium	ND	NR	15,000 ± 170	15,170 ± 300
Sodium	1,953 ± 150	2,100 ± 100	2,193 ± 230	2,420 ± 60
Zinc	122 ± 5	127i	ND	NR

ND, not detected; NR, not reported; i, information value.

TABLE 2. Elemental concentrations in samples of cattle tissues: kidney

	This work (Brazil)	USA [9]	Chile [8]	Argentina [8]
Element	Concentration ($mg.kg^{-1}$)	Concentration ($mg.kg^{-1}$)	Concentration ($mg.kg^{-1}$)	Concentration ($mg.kg^{-1}$)
Chromium	1.2 ± 0.4	0.01–0.50	NR	NR
Copper	ND	NR	NR	NR
Iron	72 ± 20	73.60	NR	150
Potassium	2,430 ± 325	2,570	2,870	2,310
Sodium	1,545 ± 180	1,790	2,500	2,450
Zinc	19 ± 7	18.50	NR	NR

ND, not detected; NR, not reported.

TABLE 3. Elemental concentrations in samples of cattle tissues: liver

	This work (Brazil)	USA [9,10]	Chile [8]	Argentina [8]
Element	Concentration ($mg.kg^{-1}$)	Concentration ($mg.kg^{-1}$)	Concentration ($mg.kg^{-1}$)	Concentration ($mg.kg^{-1}$)
Chromium	ND	NR	NR	NR
Copper	73 ± 1	33.39	NR	NR
Iron	< 70	68.20	NR	100
Potassium	3,080 ± 370	3,230	3,240	3,200
Sodium	810 ± 100	730	790	860
Zinc	18 ± 2	39.20	NR	NR

ND, not detected; NR, not reported.

TABLE 4. Elemental concentrations in samples of cattle tissues: muscle

	This work (Brazil)	USA [9,10]	Chile [8]	Argentina [8]	Italy [11]
Element	Concentration ($mg.kg^{-1}$)	Concentration ($mg.kg^{-1}$)	Concentration ($mg.kg^{-1}$)	Concentration ($mg.kg^{-1}$)	Concentration ($mg.kg^{-1}$)
Chromium	ND	NR	NR	NR	NR
Copper	ND	NR	NR	NR	NR
Iron	< 70	NR	32	27	19
Potassium	3,023 ± 260	3,490	NR	3,690	3,300
Sodium	806 ± 50	590	630	790	410
Zinc	20 ± 4	36.40	NR	NR	28

ND, not detected; NR, not reported.

good agreement the between values reported international organizations and the Brazilian analytical results obtained; the required data quality was also achieved. The higher iron, chromium, and copper concentrations can reflect the influence of environmental contamination in the animal tissues. It may have been caused by the pollutants in the environment reaching the livestock through water and forage. In this first study it is not possible to affirm that the higher iron, chromium, and copper concentrations mean that these elements are playing the role of toxic elements because it was a preliminary sampling. However, the presence of such elements which is not reported elsewhere [8] should be verified in detail during other studies.

It is important to analyze cattle tissues since these materials can be used to assess the animal's health, the quality of human foods, and can be a potential environment biomonitor.

The application of k_0-instrumental neutron activation analysis was an effective multi-elemental method used to determine mineral concentration of biological material. Iyengar [7] has been defending the argument—while one is dealing with methods for elemental analysis—that the ideal method would be a technique applicable to all elements. The k_0-INAA as a multi-element technique approaches this ideal method.

References

1. Sigel H. Concepts on metal ion toxicity. New York: Marcel Dekker, 1986.
2. Veado MARV. Application de l'analyse par activation neutronique et de la spectométrie de masse associée à un plasma dárgon pour l'identification et le dosage des éléments métalliques apportés par les rejets miniers dans la rivière Das Velhas. Doctoral thesis. Orsay, France: Thesis in Université de Paris XI, 1997.
3. IAEA. Survey of reference material—biological and environmental reference materials for trace elements, nuclides and microcontaminants. Vienna: IAEA, 1995.
4. Ehmann W, Ehmann DE. Radiochemistry and nuclear methods of analysis. New York: Wiley Interscience, 1991.
5. Sabino CVS, Montoya H, Rossi, Kastner G F, Franco MB. Testes relativos a implantação do método k_0 no reator IPR R1. CDTN-805/95. Belo Horizonte, Brazil: Nuclear Technology Development Center/Nuclear Energy National Commission 1995.
6. DeCorte, F. The k_0-standardisation method; a move to the optimisation of neutron activation analysis. Ryksuniversiteit Gent, Faculteit Van de Wetenschappen, Netherlands, 1986.
7. Iyengar GB. Elemental analysis of biological systems. Boca Raton, Fla., USA: CRC Press, 1992.
8. FAO. Tabla de Composicíon de Alimentos de America Latina, 1998. FAO's Official Web Site, http://www.rlc.fao.org/bases/alimento/comp.htm, accessed 04/20/2001.
9. United States of America Department of Agriculture. Official Web Site: http://www.nal.usda.gov, accessed 04/20/2001.
10. Subcommittee on Mineral Toxicity in Animals. Mineral tolerance of domestic animals. Washington D.C.: Library of Congress, 1980.
11. INRAM, Istituto Nazionale di Ricerca pergli Alimenti e la Nutrizione Banca Dati Interativa Composizione degli Alimenti Web Site: http://www.inram.org, accessed 12/14/2001.
12. Iyengar GV, Kollmer WE, Bowen HJM. The elemental composition of human tissues and body fluids: a compilation of values for adults. Weinheim: Verlag Chemie. 1978.

Effect of traditional fermentation and malting on phytic acid and mineral availability from sorghum *(Sorghum bicolor)* and finger millet *(Eleusine coracana)* grain varieties grown in Kenya

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Abstract

Sorghum and finger millet grains are traditional staple foods in Kenya. However, they have naturally occurring anti-nutritional factors, such as phytic acid, that decrease their dietary availability. This work determined the effect of fermentation and malting on the phytic acid content of, and mineral availability in five varieties of sorghum and four varieties of finger millet grain grown in Kenya. Phytic acid ranged from 875.1 to 2,211.9 mg/100 g in sorghum. The levels in finger millet ranged from 851.6 to 1,419.4 mg /100 g grain. Fermentation resulted in a mean decrease of phytic acid in of 64.8% after 96 hours and 39.0% after 72 hours in sorghum grain. In finger millet, there was a mean decrease of 72.3% and 54.3% after 96 and 72 hours, respectively. Malting also resulted in a mean decrease of 23.9 and 45.3% after 72 and 96 hours, respectively. The extent of decrease of phytic acid differed among the grain varieties. Fermentation increased the rate of available iron, manganese, and calcium in both sorghum and finger millet. The available minerals were generally higher in finger millet than in sorghum after fermentation. Fermentation was also more effective than malting in reducing phytic acid in sorghum and finger millet. Simple traditional food processing methods can therefore be used to increase mineral availability.

Key words: fermentation, malting, phytic acid, sorghum, finger millet

Introduction

Sorghum, *Sorghum bicolor* (L) Moench and finger millet, *Eleusine coracana,* have been staple foods in Kenya and many other African countries for centuries [1]. These crops are still the principal source of energy, and even of protein and minerals for a large segment of population in these regions. They are better adapted than many other crops to these regions. For instance sorghum is fairly drought tolerant and can grow relatively well in the arid and semi-arid land zones. This is an important consideration in Kenya, where about 80% of the landmass fall within the arid and semi-arid land zones. They are also grown with relatively little application of inputs such as fertilizer by many small holder farmers. Finger millet can be stored for long periods, without insect damage [2]. This is important during drought and famine periods, as insect pests destroy most other crops during periods of long storage.

As with many other foods, sorghum and millet grain are associated with some naturally occurring anti-nutritional factors, which reduce their nutritional value. Notable among these factors are phytic acid and polyphenols or tannins. Although the presence of these factors in the grain have been known for over a century, their biological role is not completely understood.

Phytic acid is a strong acid, which binds to many types of bivalent and trivalent cations, including mineral nutrients such as phosphorous and iron. Most of the phytate-metal complexes are insoluble at the physiological pH in the human body. Therefore phytate binding renders some minerals biologically unavailable to the body. Doherty et al. [3] observed that over 85% of the total phosphorous in sorghum grain was bound as phytin phosphorous. Wang et al. [4] reported earlier that the phytin was mainly distributed in the germ and bran of the grain, while the endosperm had the least.

Bioavailability of iron from sorghum grain for human subjects was found to be affected more by the phytin phosphorous than by the tannin content of the sorghum grain [5]. Ionizable iron was inversely correlated and soluble zinc negatively correlated with phytin phosphorous [6]. It has been reported elsewhere that germination of sorghum increased its relative nutritive value, mainly by reducing the level of phytate [7, 8]. Similar observations were also made for finger millet [9–11]. Fermentation in millet has also been reported

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to reduce phytin phosphorous [12].

In Kenya, traditional processing of finger millet and sorghum may involve fermentation or malting. However, there is little information on the effect of these processes on the nutritional quality of the many varieties of these grains. The main objective of the study was to determine the effect of natural fermentation and malting on phytate and mineral availability in sorghum and finger millet grain grown in Kenya.

Materials and methods

The sorghum and finger millet varieties were obtained from the Kenya Agriculture Research Institute (KARI), Kakamega research station in western Kenya. The sorghum varieties were ICS 3, ICS 4, DC 75, DC 8602, and Serena. The finger millet varieties were EKR 227, EKR 228, U 15, and Ikhulule. The grain was cleaned by sieving and then finely milled to obtain a fine ground flour.

Malting was done as described by Gomez et al. [13]. Clean whole grain was steeped for 24 hours, then germinated for 72 or 96 hours. (Traditionally, it is done for three to four days). It was then dried at 50°C for 24 hours. For fermentation the clean grain was finely ground into flour. The flour was then mixed into dough with an equal amount of distilled water on weight basis. The dough was allowed to ferment naturally for 48, 72, or 96 hours. (Traditionally, it ferments for two to four days). Moisture was determined using an air oven [14].

Iron, zinc, copper, manganese, and magnesium in the malted and fermented grain samples were determined using an atomic absorption spectrophotometer [14, 15]. The available minerals in, malted and fermented grain samples were determined by the HCl extraction method [16]. Phytic acid was determined by extraction in HCl, centrifugation, and then measurement by a colorimeter [17].

Results and discussion

Mineral content of sorghum and finger millet

The mineral content of sorghum and finger millet is shown in tables 1 and 2, respectively. There was significant difference ($p < .05$) in the content of each mineral among the sorghum varieties. The manganese content in sorghum varied from 2.0 in DC 8602 to 3.2 mg per 100 g in ICS 4, with a mean of 2.6mg per 100 g dry matter (DM). The minimum iron content was 4.0 in DC 8602, while the maximum was 6.5 in ICS 3. The mean iron content was 5.5 mg per 100 g DM. Calcium was generally low and varied from 4.0 mg per 100 g DM in Serena to 6.8 mg per 100 g DM in ICS 4, with a mean of 4.9 mg per 100 g DM. Sodium varied widely from 66.7 in serena to 150.0 in ICS 3. The mean was 97.5 mg per 100 g DM. The lowest potassium content was 360.0 in ICS 4 while the highest was 650.0 mg per 100 g DM in ICS 3. The mean potassium content was 444.0 mg per 100 g DM. Magnesium varied widely from a minimum of 25.0 in ICS 3 to a maximum of 300 mg per 100 g DM. These mineral contents are within the ranges elsewhere [1,18].

For finger millet, manganese varied from a minimum of 3.7 mg per 100 g DM in U 15 to a maximum of 121.3

TABLE 1. Mineral content in sorghum grain (mg/100 g dry matter)

Variety	Manganese	Iron	Calcium	Sodium	Potassium	Magnesium
ICS 3	3.0^{b} *	6.5^{b}	4.5^{a}	150.0^{b}	650.0^{b}	25.0^{a}
ICS 4	3.2^{b}	5.2^{ab}	6.8^{b}	80.0^{a}	360.0^{a}	120.0^{a}
DC 75	2.7^{ab}	5.9^{ab}	4.5^{a}	90.9^{a}	409.9^{a}	272.7^{b}
DC 8602	2.0^{a}	4.0^{a}	4.5^{a}	100.0^{a}	400.0^{a}	300.0^{b}
Serena	2.3^{a}	6.0^{ab}	4.0^{a}	66.7^{a}	400.0^{a}	200.0^{ab}
Mean	2.6 ± 0.49	5.5 ± .097	4.9 ± 1.22	97.5 ± 31.86	444.0 ± 116.75	228.5 ± 112.87

* Values in the same column followed by the same letter are not significantly different ($p < .05$) from each other. They differ significantly ($p < .05$) with values that do not share a similar letter.

TABLE 2. Mineral content of finger millet grain (mg/100 g dry matter)

Variety	Manganese	Iron	Calcium	Sodium	Potassium	Magnesium
U 15	3.7^{a} *	1.9^{a}	105.8^{b}	311.9^{ab}	391.1^{a}	128.7^{a}
EKR 228	15.6^{a}	4.5^{b}	26.6^{a}	380.6^{b}	484.9^{b}	172.1^{ab}
EKR 227	121.3^{b}	4.6^{b}	132.2^{b}	274.0^{a}	472.8^{ab}	179.3^{b}
Ikhulule	4.4^{a}	2.6^{ab}	112.2^{b}	346.9^{ab}	489.0^{b}	150.5^{ab}
Mean	36.3 ± 56.96	3.4 ± 1.36	109.2 ± 46.45	328.4 ± 45.82	459.5 ± 46.08	157.7 ± 22.85

* Values in the same column followed by the same letter are not significantly different ($p < 05$) from each other. They differ significantly ($p < .05$) with values that do not share a similar letter.

mg per 100 g DM in EKR 227. The mean manganese content in the finger millet grain was 36.3 mg per 100 g DM. The iron content was lowest at 1.9 mg per 100 g DM in U 15, and highest in EKR 227 at 4.6 mg per 100 g DM. The mean iron content was 3.4 mg per 100 g DM. EKR 228 had the lowest calcium content at 26.6 mg per 100g while EKR 227 had the highest at 132.2 mg per 100g DM. The mean calcium content was 109. 2 mg per 100 g DM. The minimum sodium content was 274.0 in EKR 227, while the highest was 380.6 in EKR 228. The mean sodium content was 328.4 mg per 100 g DM. Potassium levels varied from 391.1 in U 15 to 489.0 mg per 100 g DM in Ikhulule. The mean was 459.5 mg per 100 g DM. The minimum magnesium content was 128.7 in U 15, and the highest was 179.3 mg per 100 g DM in EKR 227. There was a significant difference ($p < .05$) in the content of each mineral among the finger millet varieties. The mineral composition in the finger millet is also generally similar to that reported earlier [18]. Finger millet varieties have higher levels of calcium, manganese, and sodium than sorghum grain. On the other hand, the sorghum grain samples have a mean magnesium content of 228.5, which is much higher than that of finger millet at 157.7 mg per 100 g DM.

Effect of natural fermentation on mineral availability

Mineral availability was determined in the fresh grain samples and after fermentation periods of 72 or 96 hours. Except for sodium and potassium, fermentation increased the availability of all the other minerals in the sorghum (table 3). Increasing the fermentation time from 72 to 96 hours resulted in a further increase in the availability of the minerals. However, it was noted that at each period, the percent increase in the availability of each mineral differed among the sorghum varieties. Expectedly, fermentation did not significantly affect the availability of sodium and potassium since they are monovalent cations and are not bound by phytic acid.

Fermentation of finger millet dough similarly resulted in an increase in availability of iron, manganese, calcium, and magnesium (table 4). The mineral availability increased with longer fermentation periods.

Effect of fermentation on phytic acid in sorghum grain

The effect of fermentation on the phytic acid content in sorghum grain is shown in table 5. The mean phytic acid content was 1,657.1 mg per 100 g DM in the five sorghum grain samples. These results were comparable to those reported by Doherty et al. [3]. There was a significant difference ($p < 0.05$) in the phytic acid content among sorghum varieties. DC 75, ICS 4, and DC 8602 had phytic acid contents of 2,211.9, 1,903.1 and 1,826.5 mg per 100 g DM, respectively. These were significantly higher ($p < .05$) than those in ICS 3 and Serena, which were 875.1 and 1,468.8 mg per 100 g DM, respectively. In all cases, fermentation resulted in a decrease in phytic acid. The reduction was more when the fermentation time was increased from 72 to 96 hours. The percentage decrease differed with varieties ($P < 0.05$), with DC 8602 having the highest decrease of 76.7% while Serena had the lowest of 51.6% after a fermentation period of 96 hours.

Effect of fermentation and malting on phytic acid content in finger millet

The effect of fermentation and malting on the phytic acid content in finger millet is shown in tables 6 and 7, respectively. The mean phytic acid content of finger millet grain was 1,171.0 mg per 100 g DM. The minimum content was 851.6 mg per 100 g DM in Ikhulule while the maximum content was 1,419.4 mg per 100 g DM in EKR 227. The mean decreases after 72 and 96 hours fermentation were 54.3% and 72.3%, respectively. The decrease did not appear to differ significantly ($p < .05$) among finger millet varieties after 96 hours of fermentation. This may indicate that at

TABLE 3. Effects of fermentation on HCl extractable minerals in sorghum grain (% of total minerals)

	Iron		Manganese		Calcium		Sodium		Magnesium		Potassium	
Variety	72 hr	96 hr	72 hr	96 hr	72 hr	96 hr	72 hr	96 hr	72 hr	96 hr	72 hr	96 hr
ICS3	21.7^{a*}	41.7^{a}	17.1^{a}	37.1^{a}	19.3^{a}	40.0^{a}	0.8^{a}	1.8^{a}	20.9^{a}	41.8^{a}	3.0^{b}	6.0^{b}
ICS4	27.9^{a}	46.9^{ab}	26.9^{ab}	43.1^{ab}	37.0^{ab}	44.2^{ab}	2.3^{ab}	5.8^{ab}	36.2^{ab}	39.9^{a}	1.1^{a}	1.9^{a}
DC8602	37.4^{ab}	55.3^{b}	29.9^{ab}	42.9^{ab}	44.4^{b}	51.0^{b}	7.3^{b}	11.1^{b}	48.2^{b}	51.6^{ab}	3.8^{b}	5.1^{ab}
DC74	40.9^{b}	58.9^{b}	39.4^{b}	51.0^{b}	43.0^{b}	53.4^{b}	3.3^{ab}	7.1^{ab}	44.8^{b}	49.5^{ab}	2.2^{ab}	2.3^{a}
Serena	34.8^{ab}	45.6^{ab}	36.5^{b}	46.5^{b}	38.1^{ab}	47.4^{ab}	2.5^{ab}	3.3^{a}	38.0^{ab}	56.0^{b}	3.0^{b}	4.3^{a}
Mean	32.5 ± 6.89	49.7 ± 6.40	30.0 ± 7.83	44.1 ± 4.58	36.4 ± 8.98	47.2 ± 4.77	3.2 ± 2.19	5.8 ± 3.22	37.6 ± 9.44	47.8 ± 6.05	2.6 ± 0.91	3.9 ± 1.59

* Values in the same column followed by the same letter are not significantly different ($p < 05$) from each other. They differ significantly ($p < .05$) with values that do not share a similar letter.

this fermentation period, maximum hydrolysis of the acid had occurred.

Malting of finger millet resulted in a mean decrease of 23.9% at 72 hours and 45.3% at 96 hours (table 7). There was significant difference ($p < .05$) in the decrease among varieties. After 96 hours of germination, U15 had decreased by 60.0%, while EKR 228 had decreased by only 20.0%.

TABLE 5. Effect of fermentation after 72 and 96 hours on the phytic acid content of sorghum grain

Variety	Phytic acid content mg / 100 g dry matter	Phytic acid hydrolyzed after 72 hours (%)	Phytic acid hydrolyzed after 96 hours (%)
DC8602	$1{,}826.5^{b}$ *	52.0^{b}	76.7
DC75	$2{,}211.9^{b}$	52.7^{b}	65.5
ICS3	875.1^{a}	36.3^{ab}	67.6
ICS4	$1{,}903.1^{b}$	41.3^{ab}	62.7
Serena	$1{,}468.8^{ab}$	12.5^{a}	51.6
Mean	1,657.1 ± 456.9	39.0 ± 14.6	64.8 ± 8.10

* Values in the same column followed by the same letter are not significantly different ($p < .05$) from each other. They differ significantly ($p < .05$) with values that do not share a similar letter.

TABLE 6. Effect of fermentation on the phytic acid content of finger millet grain

Variety	Phytic acid content mg / 100 g dry matter	Phytic acid hydrolyzed after 72 hours (%)	Phytic acid hydrolyzed after 96 hours (%)
U 15	$1{,}135.6^{ab}$ *	51.3^{a}	75.5
EKR 228	$1{,}277.5^{b}$	60.0^{b}	75.0
EKR 227	$1{,}419.4^{b}$	58.3^{ab}	69.8
Ikhulule	851.6^{a}	47.5^{a}	69.2
Mean	1,171.0 ± 187.8	54.3 ± 5.88	72.3 ± 3.18

* Values in the same column followed by the same letter are not significantly different ($p < .05$) from each other. They differ significantly ($p < .05$) with values that do not share a similar letter.

TABLE 7. Effect of malting on phytic acid content of finger millet grain

Variety	Phytic acid content mg/100 g dry matter	Phytic acid hydrolyzed after 72 hours (%)	Phytic acid hydrolyzed after 96 hours (%)
U 15	$1{,}135.6^{a}$ *	40.0^{ab}	60.0
EKR 228	$1{,}277.5^{ab}$	16.7^{a}	20.0
EKR 227	$1{,}419.4^{b}$	15.0^{a}	56.0
Mean	1,277.5 ± 115.86	23.9 ± 11.41	45.3 ± 18.00

* Values in the same column followed by the same letter are not significantly different ($p < .05$) from each other. They differ significantly ($p < .05$) with values that do not share a similar letter.

TABLE 4. Effect of fermentation on HCl extractable minerals from finger millet grain (% of total minerals)

	Iron			Manganese			Calcium			Sodium			Magnesium			Potassium		
Variety	48 hr	72 hr	96 hr	48 hr	72 hr	96 hr	48 hr	72 hr	96 hr	48 hr	72 hr	96 hr	48 hr	72 hr	96 hr	48 hr	72 hr	96 hr
U15	22.0	34.9^{ab}	42.9^{b}	15.1^{a}	30.7^{ab}	32.7^{a}	26.7	27.9	31.7	6.1^{b}	10.2^{b}	17.1^{b}	18.0^{a}	24.5^{a}	27.1^{a}	2.9^{ab}	5.8^{ab}	6.8^{ab}
EKR228	19.3	37.0^{b}	41.4^{a}	21.3^{ab}	31.7^{ab}	36.7^{ab}	19.0	23.4	31.0	2.2^{ab}	3.0^{a}	5.2^{a}	17.0^{a}	30.4^{ab}	31.8^{ab}	4.2^{b}	7.1^{b}	9.1^{b}
EKR227	19.7	29.2^{a}	41.4^{a}	15.4^{a}	28.4^{a}	32.7^{a}	31.1	34.7	37.7	4.9^{ab}	9.1^{b}	10.9^{ab}	22.5^{ab}	27.6^{a}	33.4^{ab}	1.7^{ab}	1.7^{a}	5.0^{ab}
Ikhulule	19.2	32.2^{a}	41.6^{a}	23.1^{b}	34.1^{b}	37.1^{b}	18.9	27.1	30.2	1.0^{a}	5.9^{a}	8.0^{a}	24.2^{b}	37.9^{a}	39.8^{b}	0.0^{a}	0.9^{a}	1.5^{a}
Mean	20.1 ± 1.14	33.3 ± 2.93	41.8 ± 0.63	18.7 ± 3.53	31.2 ± 2.05	34.8 ± 2.10	23.9 ± 5.21	28.3 ± 4.07	32.7 ± 2.96	3.6 ± 2.04	7.1 ± 2.82	10.3 ± 4.41	20.4 ± 3.01	30.1 ± 4.96	33.0 ± 4.55	2.2 ± 1.55	3.9 ± 2.63	5.6 ± 2.78

* Values in the same column followed by the same letter are not significantly different ($p < .05$) from each other. They differ significantly ($p < .05$) with values that do not share a similar letter.

Conclusion and recommendations

Fermentation and malting under conditions similar to those applied traditionally in Kenya were effective in drastically reducing phytic acid levels in sorghum and finger millet grain. However, the extent of reduction varied among the varieties of sorghum and finger millet. Fermentation increased the availability of minerals in the sorghum and finger millet grains. There is need for analysis of more local varieties of sorghum and finger millet to determine the effect of traditional malting and fermentation on them. It would also be useful to investigate the basis for differences in the phytic acid content and availability among sorghum and finger millet varieties grown under the same conditions.

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References

1. Doggett H. Sorghum. International Development Research Centre. London: Longman Group, 1988: 428–53.
2. Purseglove JW. Tropical crops: monocotyledons, vol. 1. London: Longman Group, 1972.
3. Doherty C, Faubian JM, Rooney LW. Semi-automated determination of phytate in sorghum and sorghum products. Cereal Chem 1982;59:373–9.
4. Wang C, Michel HC, Barham HN. The phytin content of sorghum grain. Transactions, Kansas Academy of Science (USA) 1959;62:208–11.
5. Randhakrishnan MR, Sivaprasad J. Tannin content of sorghum varieties and their role in iron bioavailability. J Agric Food Chem 1980;28:55–7.
6. Simwemba CG, Hoseney RC, Varriano-Marston E, Zeleznak K. Certain B vitamins and phytic acid contents of pearl millet (*Pennisetum americanum).* J Agric Food Chem 1984;32:33–4.
7. Wang YD, Fields ML. Germination of corn and sorghum in the home to improve nutritive value. J Food Sci 1978;43:1113–5.
8. Malleshi NG, Desikachar HSR, Venkat Rao S. Protein quality evaluation of a weaning food based on malted ragi and green gram. Qualitas Plantarum Plant Foods Hum Nutr 1986;36:223–30.
9. Sankara Rao DJ, Deosthale YG. Mineral composition, ionizable iron and soluble zinc in malted grain of pearl millet and ragi. Food Chem 1983;11:217–23.
10. Udayasekara Rao P, Deosthale YG. In vitro availability of iron and zinc in coloured ragi (*Eleusine coracana*): role of tannin and phytate. Qualitas Plantarum Plant Food Hum.Nutr 1988;38:35–41.
11. Malleshi NG, Desikachar HSR. Nutritive value of malted millet flours. Qualitas Plantarum Plant Foods Hum Nutr 1986;36:191–6.
12. Dhankher N, Chauhan BM. Effect of temperature and fermentation time on phytic acid and polyphenol content of *rabadi*- a fermented pearl millet food. J Food Sci 1987;52:828–9.
13. Gomez MI, Obilana AB, Martin DF, Madzvamuse M, Monyo ES, eds. Manual of laboratory procedures for quality evaluation of sorghum and pearl millet. Patancheru, Andhra Pradesh, India: International Crops Research Institute for the Semi-arid Tropics (ICRISAT), 1997.
14. AOAC. Official methods of analysis. 14th ed. Washington, D.C.: Association of Official Analytical Chemists, 1984.
15. Osborne DR, Voogt P. The analysis of nutrients in food. London: Academic Press, 1978.
16. Khetarpaul N, Chauhan BM. HCl-extractability of minerals from pearl millet. Food Chem. 1990;37:69–75.
17. Kent JDW, Amos AJ. Modern cereal technology. London: Food Trade press, 1967.
18. Hulse JH, Laing EM, Pearson OE. Sorghum and the millets: their composition and nutritive value. London: Academic Press, 1980.

The nutritional evaluation of underutilized cereals and buckwheat

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Abstract

We evaluated the nutritional factors of underutilized cereals (spelt, emmer, einkorn, millet, foxtail millet, semiperennial rye, naked oat, and naked barley) and buckwheat. The basic food components as well as minor nutrients were determined. The analyses included dry matter, ash, protein, dietary fiber, fat, fatty acids, amino acids, minerals, and lipophilic and hydrophilic vitamins. Rutin was also determined in buckwheat. We hope to offer new recipes for the healthy food production and for special dietary use (diabetes, celiac disease, phenylketonuria diet, etc.). Use of the germinated seeds is also suggested. The examples of some healthy food products in the Czech Republic are mentioned.

Key words: cereals, buckwheat, underutilized, nutritional evaluation, organic agriculture, healthy food products, special diets

Introduction

Consumer interest in healthy food products has been increasing for the last 10 years in the Czech Republic. At the same time the growers have increased the number of the cultivated crops. In order to extend the diversity of the cultivated crops and recommend their suitable use two groups of cereals and pseudocereals were investigated. The first group is the crops grown in a small area. Their utilization by the food industry is insufficient—buckwheat *(Fagopyrum esculentum*), millet *(Panica miliaceum L.*), spelt *(Triticum spelta)*, emmer (*Triticum dicoccum Schuebl.*) [1] and naked oat *(Avena sativa var.nuda)*. These crops can be grown using organic or conventional agricultural methods. Products made from this group can be found in health foods markets—spelt products (spelt muesli, bulgur, flakes, pops, omelette mix, mushroom spelt risotto, vegetable spelt risotto, pasta), buckwheat products (pops, groat, pasta, omelette mix, crispbread, bisquits), and millet products (instant poridge, flakes, pops). The other group includes the crops not grown in the Czech Republic—einkorn (*Triticum monococcum L.*)[2, 3], naked barley *(Hordeum vulgare var.nudum*) [4], semiperennial rye *(Secale cereale var.multicaule)*, and foxtail millet *(Setaria italica*). This group was tested under various growing conditions in the particular regions of the Czech Republic.

Material and methods

Samples of cereals and buckwheat were obtained from the organic agriculture farm (Pro-Bio, Staré Město pod Sněžníkem, Czech Republic) and the Research Institute of Crop Production (Praha, Czech Republic). The grains were milled into flour using a laboratory mill (Fritsch rotor speed mill, Fritsch GmbH, Laborgerätebau, Idar-Oberstein, Germany) before analysis.

Buckwheat was soaked for three hours at laboratory temperature and then allowed to germinate 4, 6, and 8 days at 20°C and 75% relative humidity. During the germination period the grains were washed twice a day.

Dry matter was determined by the gravimetric method (drying to a constant weight at 105°C), ash by dry ashing (520°C), and fat by the Soxhlet method after acidic hydrolysis. The AOAC enzymogravimetric method was used for dietary fiber determination and Kjeldahl method for protein determination.

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The reversed-phase high performance liquid chromatography (RP-HPLC) method was used to determine thiamin and tocopherols, microbiological methods were used for niacin, pantothenic acid, and vitamin B6. Vitamin C was analyzed by the titrimetric method, carotenoids by the spectrophotometric method, and riboflavin by lumiflavin method. Rutin was determined by RP-HPLC. Amino acids were determined by ion-exchange chromatography and the fatty acids by gas chromatography. A flame atomic absorption spectrometer (AAS) was used for mineral determination for except phosphorus for which the spectrophotometric method was used.

Results and discussion

The basic foods and vitamin contents are shown in the table 1. The protein content ranged 11% to 19%. The highest protein content was found in einkorn, emmer, and spelt. Naked oat (7.4%) had the highest fat content. There were high levels of lipophilic vitamins in millet (carotenoids, 0.97 mg/100 g sample) and naked oat (tocopherols, 2.45 mg α-tocopherol equivalent/100 g sample). There were no statistically significant differences in riboflavin levels. Naked barley and emmer were found to be the best sources of niacin (up to 8 mg/100 g sample) and naked oat (0.54 mg/100 g sample) and millet (0.56 mg/100 g sample) the best sources of thiamin. Buckwheat seems to be a good source of vitamin B_6 (0.73 mg/100 g sample). Emmer had the highest level of pantothenic acid (1.14 mg/100 g sample). The levels of minerals (table 2) correspond to the those in food composition tables, the high level of magnesium was found in buckwheat only (over 200 mg/100 g sample). The limiting amino acid (table 3) for the crops studied is methionine. Fatty acids (table 4) are characterized by high levels of linoleic and oleic acids. The highest levels of linoleic acid were found in millet (64 g/100 g fatty acids), spelt (56 g/100 g fatty acids), einkorn and emmer (54 g/100 g fatty acids), the highest levels of oleic acid were found in naked oat and buckwheat (38 g/100 g fatty acids). High levels of linolenic acid were found in semiperennial rye (8.3 g/100 g fatty acids) and naked barley (5.4 g/100 g fatty acids).

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TABLE 1. Basic food components and vitamins per 100 g sample

Nutrient	Crop							
	Naked barley	Naked oat	Spelt	Einkorn	Emmer	Semiperennial rye	Millet	Buckwheat
Dry matter (g)	90.1	89.9	89.0	91.1	89.4	88.4	88.8	86.6
Ash (g)	1.7	2.0	1.9	2.2	2.2	1.9	1.3	2.2
Fat (g)	2.4	7.4	3.0	3.4	2.9	2.4	4.1	2.4
Protein (g)	11.8	13.2	17.0	18.1	17.9	13.2	14.5	14.4
Dietary fiber (g)	11.1	6.8	9.2	10.4	8.7	15.2	2.9	8.5
Rutin (mg)	—	—	—	—	—	—	—	10.1
Thiamin (mg)	0.24	0.54	0.30	0.34	0.38	0.18	0.56	0.46
Riboflavin (mg)	0.07	0.08	0.07	0.09	0.09	0.10	0.11	0.14
Niacin (mg)	7.6	0.8	6.6	5.7	8.0	1.3	5.1	4.9
Pantothenic acid (mg)	0.47	0.98	0.60	0.47	1.14	0.61	0.60	1.05
Vitamin B6 (mg)	0.46	0.17	0.36	0.40	0.39	0.29	0.51	0.73
Carotenoids (mg)	0.20	0.22	0.32	0.80	0.18	0.29	0.97	0.21
Tocopherols[a] (mg)	1.47	2.45	1.05	1.45	1.78	2.10	0.23	0.66

a. mg α-tocopherol equivalent/100 g sample.

TABLE 2. Minerals (mg/100 g sample)

Mineral	Crop							
	Naked barley	Naked oat	Spelt	Einkorn	Emmer	Semiper-ennial rye	Millet	Buck-wheat
Sodium	9.3	6.0	2.9	1.8	7.1	3.3	2.5	2.8
Potassium	381	331	281	394	373	372	209	455
Calcium	28	62	28	29	26	33	6.5	20
Magnesium	90.4	124	117	131	130	108	129	206
Phosphorus	371	475	463	493	517	414	322	472
Zinc	1.4	1.6	3.1	4.2	4.0	2.6	3.6	2.8
Iron	2.8	2.9	2.9	2.9	3.6	3.0	3.7	2.3
Manganese	1.1	4.0	2.8	3.4	2.9	2.2	0.7	1.7

TABLE 3. Amino acids (g/100 g sample)

Amino acid	Crop							
	Naked barley	Naked oat	Spelt	Einkorn	Emmer	Semiper-ennial rye	Millet	Buck-wheat
Asparagic acid	0.613	0.824	0.781	0.818	0.833	0.755	0.991	1.032
Threonine	0.386	0.402	0.487	0.454	0.520	0.433	0.430	0.476
Serine	0.387	0.474	0.615	0.673	0.711	0.452	0.723	0.551
Glutamic acid	2.578	2.125	5.275	5.472	5.602	2.783	2.954	2.082
Proline	1.192	0.624	3.115	1.602	0.973	1.540	1.070	0.299
Glycine	0.398	0.540	0.560	0.620	0.650	0.442	0.309	0.646
Alanine	0.447	0.661	0.561	0.705	0.756	0.448	1.637	0.529
Valine	0.493	0.544	0.619	0.648	0.686	0.506	0.656	0.694
Methionine	0.189	0.260	0.212	0.289	0.373	0.180	0.151	0.239
Isoleucine	0.428	0.493	0.651	0.793	0.732	0.451	0.607	0.539
Leucine	0.807	0.898	1.317	1.170	1.297	0.823	1.891	1.022
Tyrosine	0.365	0.609	0.577	0.632	0.770	0.549	0.654	0.370
Phenylalanine	0.523	0.416	0.812	0.979	0.711	0.508	0.715	0.550
Histidine	0.263	0.375	0.418	0.553	0.623	0.264	0.291	0.357
Lysine	0.347	0.560	0.372	0.530	0.536	0.336	0.185	0.794
Arginine	0.586	0.999	0.780	0.974	1.087	0.478	0.511	1.438
Cysteine	0.211	0.381	0.365	0.437	0.390	0.291	0.240	0.386
Total	10.21	11.19	17.52	17.35	17.25	11.24	14.02	12.00

TABLE 4. Fatty acids (g/100 g fatty acids)

Fatty acid	Crop							
	Naked barley	Naked oat	Spelt	Einkorn	Emmer	Semiper-ennial rye	Millet	Buck-wheat
Myristic acid (14:0)	0.35	0.27	0.16	0.18	0.15	0.26	0.10	0.25
Myristoleic acid (14:1)	0.10	—	0.13	0.08	0.11	0.14	0.04	0.11
Palmitic acid (16:0)	21.9	18.0	14.4	13.9	16.7	14.7	9.4	13.2
Palmitoleic acid (16:1)	0.35	0.19	0.18	0.21	0.18	0.54	0.22	0.29
Stearic acid (18:0)	2.36	2.24	1.06	0.87	1.45	1.73	1.62	2.01
Oleic acid (18:1)	15.8	38.4	23.8	25.3	23.5	19.7	20.8	37.7
Linoleic acid (18:2)	51.1	36.9	55.7	54.0	52.6	52.3	64.4	37.5
Linolenic acid (18:3)	5.35	1.46	3.21	3.60	3.45	8.25	1.59	2.55
Arachidic acid (20:0)	0.25	0.20	0.21	0.15	0.25	0.25	0.53	1.40
Cis-11-eicosenoic acid (20:1)	0.79	0.70	0.96	1.36	1.29	1.14	0.66	3.44
Behenic acid (22:0)	0.65	0.26	0.15	0.14	0.18	0.22	0.48	1.60
Erucic acid (22:1)	1.05	1.46	0.12	0.23	0.25	0.77	0.23	—

References

1. Reddy MM, Yeagi NB, Rao M, Srinivasan CN, Hanchinal RR. Grain and gluten quality of some cultivars of wheat species and their suitability for preparation of traditional South Indian sweet products. J Food Sci Tech India 1998;35(5):441–4.
2. D'Egidio MG, Nardi S, Vallega V. Grain, flour and dough characteristics of selected strains of diploid wheat, *Triticum monococcum L*. Cereal Chem 1993;70(3):298–303.
3. Acquistucci R, D'Egidio MG, Vallega V. Amino acid composition of selected strains of diploid wheat. *Triticum monococcum L*. Cereal Chem 1995;72(2):213–6.
4. Vavreinova S, Smrčinova H, Fiedlerová V. Cereals in functional foods. Proceedings of the International Conference "Cereals for Human Health and Preventive Nutrition." Prague, Czech Republic: Food Research Institute, 1998.

Nutritional status and body composition in Chilean preschool children attending day care centers

Milagros Marcia Velasquez, Gabriela Salazar, Fernando Vio, Jimmy Hernandez, Juana Rojas

Abstract

*Obesity increased in preschool children attending day care centers in Chile from 5% in 1985 to 10.8% in 2000. We assessed the nutritional status and body composition by deuterium dilution, in preschool children attending day care centers. In the whole group (*n *= 681), 3 to 5 year old children were classified according to weight-for-height (ZWH) and height-for-age (ZHA) Z scores from the NCHS reference. We found 28.5% overweight, 13.2% obese, and 18.6% deficient in height-for-age. From this group, 239 children were evaluated for total body water (TBW) and fat percentage. Normal children in this group have a lower percentage of water (59.6 ± 3.9%) and higher fat (22.2 ± 4.7%) than the reference child. The association between the percentage of fat and skinfolds' sum (tricipital, bicipital,suprailiac, subescapular), was greater for overweight and obese children (r = 0.70). At present, validation of anthropometric models to evaluate fat and water content is underway in Chile.*

Key words: body composition, preschool children, nutritional status

Introduction

The increase of overweight children in Chile and Latin America is a health concern for South America. Obesity has increased in preschool children attending day care centers from 5% in 1985 to 10.8% in 2000, meanwhile the last figures for obesity in the whole child population (2 to 5 year olds) was 7.78% at national level [1, 2]. The nutritional transition in Chile and Latin America has modified a situation in which overweight and obesity predominate [3–7].

To implement preventive measures and promote health habits in childhood, measures are being taken to lower the calorie content of the food and to increase physical activity at day care centers. We studied the body composition of preschool children to have a basis for evaluating the impact of the changes.

Subjects and methods

Nearly 700 children were evaluated for weight-for-height (WH), height-for-age (HA), and weight-for-age (WA) from four day care centers (Areas Verdes, Copihues, Nuevo Amanecer, and Tierra de Niños) in the urban metropolitan region. Body composition was evaluated in 239 children (115 boys and 124 girls), equivalent to 48.1% and 51.9% of the sample. This sample was chosen to have a similar numbers of normal, overweight, and normal children.

The protocol was explained to the parents who signed a consent form for the child to participate in the project. The study was approved by the Ethical Committee from the Institiute of Nutrition and Food Technology (INTA), University of Chile, and by the Board from Junta Nacional de Jardines Infantiles (JUNJI). All measurements were conducted at the same day care centers.

Anthropometry

A portable balance (EXCEL, model HWGO, 100 gr. precision, Santiago, Chile) was used to measure weight. Height was measured with a metal stadiometer (precision 1.5 cm), and the same balance and stadiometer were used in all the day care centers. Skinfolds (tricipital, bicipital, suprailiac, and subscapular were measured using a Lange Skinfold Caliper, precision 1 mm, (Cambridge Scientific Industries, Inc, Md., USA);

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the measurements were conducted following the techniques of others [8–10] and evaluated by the same trained nutritionist. Nutritional status was classified according reference tables from NCHS [3], and body mass index (BMI) according to Cole et.al. [11].

Isotopic dilution

Body water was evaluated by deuterium dilution using a 50% diluted dose (1.5 gr. D_20) for all children and a rinse of 20 ml was given after the dose to assure its ingestion. The children were asked to consume only milk, two hours before the measurement. A basal saliva sample was collected immediately after they arrived at the center, the dose administered, and a saliva post-dose sampling was collected after 2.5 hours, to help children to fulfill the protocol and comply with lunch time at the day care centers.

During that time, children performed sedentary recreative activities (drawing, watching a film, etc) in the same room. Samples were analyzed in triplicate, equilibrated with 99.9% hydrogen gas for 15 hours to 3 days, depending on the catalyzer used, and frozen at –20°C until measurement in an HYDRA continuous flow, isotope-ratio mass spectrometer (Crewe, Manchester, UK).

Fat was calculated from total body water determined, applying Fomon coefficients (sex, age) [12] and corrected by Schoeller[13], assuming an overestimation of 4% of water measured.

Statistical methods

EpiInfo was used to classify nutritional status. Descriptive statistics were used to evaluate means, percentage and standard deviation. Analysis of variance (Anova-Manova), comparisons of samples by T-Student and Kruskal Wallis, were used to evaluate homogeneity of variance and significance [14].

Results and discussion

The physical characteristics of the children are presented in table 1. The mean age was 4.1 years, and mean weight and height was similar for both boys and girls (18.1 vs.17.8 kg, and 102.95 vs. 101.86 cm).

The distribution of nutritional status is shown in table 2. More girls were overweight than boys (16.0% vs. 12.5%) and both sexes had a similar distribution of obesity. This data coincides with the national data from the National Grant Board (JUNAEB), in which the incidence of obesity in children has doubled (boys from 6,5% to 13.1% and girls from 7.7% to 14.7%, between 1987 and 1996 [15]. Similar data was reported for German children (1975–1995) [16]. Kotani et.al, reported that obesity in Japanese children has increased from 5% to 10% in the last two decades [17]. It is evident that Chilean children are getting obese at a faster pace than in Germany and Japan.

In relation to HA (table 3), 18.6% still have a height deficiency and only 12.5% are over the median. This may be due to ambient factors and secondary malnutrition still present in Chilean children. Similar values were reported by Bustos et al., who noted that the early deficit in height is present in national data from the Ministry of Health, particularly in vulnerable sectors of the population [18].

Table 4 shows the body composition in these children. Obese children have a higher fat-free mass, but a lower body water percentage ($p = .0001$). The main differences were encountered between normal and obese children.

TABLE 1. Anthropometric characteristics of whole group (n = 681)

Variables	Boys Mean ± SED ($n = 353$)	Girls Mean ± SED ($n = 328$)
Age (yr)	4.12 ± 0.74	4.15 ± 0.78
Weight (kg)	18.16 ± 3.29	17.80 ± 3.08
Height (cm)	102.95 ± 6.72	101.86 ± 6.79
BMI (kg/m^2)	17.03 ± 1.65	17.06 ± 1.64
Weight-for-age	0.87 ± 1.13	1.01 ± 1.03
Weight-for-height	0.55 ± 1.30	0.60 ± 1.07
Height-for-age	–0.13±1.03	0.12 ± 1.01

TABLE 2. Nutritional status by weight-for-height and sex ($n = 681$)

Weight-for-height Z score	Boys		Girls		Total	
	n	%	*n*	%	N	%
Risk	4	0.6	3	0.4	7	1.0
Normal	218	32.0	172	25.3	390	57.3
Overweight	85	12.5	109	16.0	194	28.5
Obese	46	6.8	44	6.5	90	13.2
Total	353	51.8	328	48.2	681	100.0

TABLE 3. Nutritional status by height-for-age ($n = 681$)

Height-for-age Z score	Boys		Girls		Total	
	n	%	*n*	%	N	%
Low height	66	9.7	61	9.0	127	18.6
Normal	243	35.7	226	33.2	469	68.9
High height	44	6.5	41	6.0	85	12.5
Total	353	51.8	328	48.2	681	100.0

Table 4. Body composition by deuterium dilution (n=239)

Variables	Normal $n = 96$	Overweight $n = 90$	Obese $n = 53$	p
	Mean ± SD			
Total body water (l)	10.5 ± 1.3[a]	11.2 ± 1.4[a]	12.1 ± 1.2[a]	.0001
Total body water (%)	59.6 ± 3.9[a]	57.3 ± 3.2	55.5 ± 3.8[a]	.0001
Fat mass (kg)	3.9 ± 1.0[a]	5.0 ± 0.9	6.2 ± 1.5[a]	.0001
Fat mass (%)	22.2 ± 4.7[a]	25.4 ± 4.2	28.2 ± 4.6[a]	.0001
Fat-free mass (kg)	13.6 ± 1.8[a]	14.6 ± 1.9	15.7 ± 1.6[a]	.0001
Fat-free mass (%)	77.7 ± 4.7[a]	74.5 ± 4.2	71.8 ± 4.7[a]	.0001

a. Difference.

The skinfolds' sum correlated well with the fat percentage (0.70) (fig. 1). Although skinfolds represent only subcutaneous fat, they may be very useful to construct anthropometric models for body composition evaluation, as there are no cutoff limits for children of this age (skinfolds) nor is there a validated body composition model for our children [7, 19, 20].

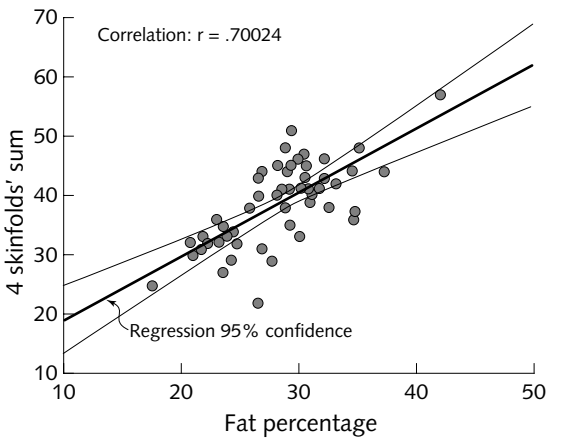

FIG 1. Association between fat percentage and skinfolds' sum

Sex, growth, ethnic background, and physiological factors may affect body composition, which strengthens the need to assess correctly body composition of preschool children.

A proposal for body composition determination is being validated at present in Chilean preschool children (3 to 5 years of age). As such, any intervention (intake, physical activity, nutritional education) could be evaluated and reformulated, if necessary. This step is important to help programs to be cost-effective and fulfill the aims for which they were formulated.

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References

1. Kain J, Vio F, Albala C. Childhood nutrition in Chile: from deficit to excess. Nutr Res 1998;18(11)1825–37.
2. Ministry of Health (MINSAL). Diagnóstico nutricional integrado de la población menor de 6 años. Santiago: MINSAL, Chile, 2000.
3. Vio F, Albala C, Crovetto M. Health promotion in the Chilean epidemiological transition. Rev Chil 2000;27(1): 21–2.
4. Albala C, Vio F, Kain J. Obesity, an unresolved challenge in Chile. Rev Med Chile 1998;26(8):1001–9.
5. Vio F, Albala C. Obesity in Chile: an epidemiological look. In: Albala C, Kain J, Barrows R, Díaz E, eds. Obesity, a pending challenge. Santiago: Editorial Universitaria, 2000:31–43.
6. Alegría A, Ordoñez E, Verdugo E, Verdugo P, Sepulveda H, López I. Obesidad y sobrepeso en menores de 6 años. Rev Pediatr 1988;(31):140–5.
7. Rolland-Cachera M, Bellisle F, Deheeger M, Pequignot F, Sempe M. Influence on body fat distribution during childhood on body fat distribution in adulthood: a two-decade follow-up study. Int J Obes 1990;14:473–81.
8. Frisancho R. Anthropometric standards for the assessment of growth and nutritional status. Ann Arbor, Mich., USA: University of Michigan Press, 1990.
9. Velasquez RMM. Métodos antropometricos para la evaluación del estado nutricional en adultos. La Paz, Bolivia: Ministerio de Desarrollo Humano, Secretaria Nacional de Salud, Unidad de Política Alimentaria Nutricional, Organización Panamericana de Salud (UPAN/OMS), 1995.
10. Quinteros SD. Técnicas para las medidas antropometricas. Medellin: Centro de Atención Nutricional, 1992.
11. Cole J, Bellizi M, Flegal K, Dietz W. Establishing a standard definition for child overweight and obesity worldwide: International survey. BMJ 2000;320:1240–50.
12. Fomon S, Haschke F, Ziegler EE, Nelson SE. Body composition of reference children from birth to age 10 years. Am J Clin Nut 1998;35:1169–75.

13. Schoeller AD. Hydrometry. In: Lohman T, ed. Human body composition. Champaign, Ill., USA: Human Kinetics, 1996(2):25–43.
14. Taucher E. Bioestadística. Santiago: Santiago Editorial Universitaria. 1997:15–30
15. Albala C, Vio F, Kain J. Obesity: a pending challenge in Chile. Rev. Méd. Chile 1998;126:1001–9.
16. Kromeyer-Houschild K, Zellner k, Jaeger V, Hoyer H. Prevelence of overweight and obesity amongschool children in Jena (Germany). Int J Obes Rel Met Dis 1999;23(11):1143–50.
17. Kotani K, Nishida M, Yamashita S, Fumahashi T, Fujioka S, Tokunaga K, Ishikawa K, Tarui S, Matsuzawa Y. Two decades of annual medical examinations in Japanese obese children: do obese children grow into obese adults?. Int J Obes Rel Met Dis 1997;21:912–21.
18. Bustos P, Estado Nutricional al ingreso a la escuela. Editores: Amigo H, Bustos P, Erazo. Niños Mapuches. Crecimiento y nutrición y salud. Universidad de Chile, 2001:51–48.
19. Gibson R. Principles of nutritional assessment. New York: Oxford University Press, 1990.
20. Lean M, Han T, Deurenberg P. Predicting body composition by densitometry from simple anthropometric measurements. Am J Clin Nutr.1996;63:4–14.