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# *In vitro* availability of iron and zinc from some Indian vegetarian diets: correlations with dietary fibre and phytate

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Sixty vegetarian diets consumed by children, adolescents, adults and older adults from rural families living in northern India were studied for Fe and Zn availability (*in vitro*). The method measures ionizable Fe at pH 7.5 in the diets, simulating conditions prevailing in the intestine and correlates highly with per cent Fe absorption in humans. Zinc availability is measured in the same sample by following the same procedure based on the fact that most of the Zn is absorbed in the intestine under similar conditions. The availability of these minerals was poor, ranging from 7.8 to 8.7% and from 3.3 to 4.4% for Zn and Fe, respectively. The poor availability could be related to the compositions of these diets, which included mainly cereals, and these contributed high amounts of phytate as well as fibre. A negative and significant correlation coefficient between dietary fibre or phytate content and Fe and Zn availability in these diets suggests some possible interactions. Copyright © 1996 Published by Elsevier Science Ltd

## INTRODUCTION

Vegetarian diets (rich in cereals, legumes) appear, on the basis of chemical analyses, to be good sources of trace minerals but, in marked contrast to foods derived from animal sources, such minerals are generally poorly utilized by man and other monogastric animals (O'Dell, 1969).

Both endogenous and erogenous factors have been implicated in such reduction of bioavailability and, amongst the former, are phytate (Zemel, 1989), fibre components (Toma & Curtis, 1986) and tannins (Erdman, 1981); these are all present in considerable amounts in cereals and pulses. However, according to Barbara (1989) most of the effects of dietary fibre on mineral binding are owing to the presence of phytate. Interactions of dietary substances during food processing, and the thermal effects of heating and cooking foods may positively or negatively affect mineral availability (Erdman, 1981).

The objective of the present study was to measure the availability of iron and zinc in vegetarian diets consumed by children, adolescents, adults and older adults in northern India, to compare these data with the levels measured previously in the consumed foods (Pushpanjali & Khokhar, 1995) using atomic absorption, and to relate the figures obtained to the phytate (Khokhar *et al.*, 1994) and fibre contents of the diets (Khokhar & Pushpanjali, 1995).

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## MATERIALS AND METHODS

### Diet collection and analyses

Sixty traditional diets consumed by children (4–9 years of age), adolescents (10–19 years), adults (20–45 years) and older adults (older than 45 years) from rural villages in Hisar (Haryana State, north India) were collected. Fifteen diets representing each group were analysed in triplicate. The diets were homogenized, dried and ground to store prior to analyses.

Total iron and zinc contents of diets were determined by the method described by Pushpanjali & Khokhar (1995). The procedure involved digestion of 2 g of sample with 20 ml of diacid mixture ($HNO_3$–$HClO_4$ (5:1 v/v)) at 80°C for 2–3 h. Prior to digestion, the sample was kept in acid mixture overnight. The digestion was continued until the contents in the flask were clear and dry. The digested samples were reconstituted with distilled water and analysed for Fe and Zn using an atomic absorption spectrophotometer (Perkin-Elmer

**Table 1. *In vitro* availability of zinc and iron in the diets (per 100 g DM)**

| Group | Total zinc (mg) | Ionizable zinc (mg) | Zinc availability (%) | Total iron (mg) | Ionizable iron (mg) | Iron availability (%) |
|---|---|---|---|---|---|---|
| Children | 1.8±0.4 | 0.1±.02 | 8.0±1.9 | 8.0±1.6 | 0.4±.03 | 4.4±0.9 |
| Adolescents | 2.0±0.3 | 0.2±.03 | 8.2±1.4 | 9.1±2.5 | 0.4±.04 | 4.0±1.2 |
| Adults | 2.0±0.3 | 0.2±.02 | 8.7±2.2 | 8.7±1.9 | 0.3±.04 | 3.3±0.7 |
| Older adults | 2.1±0.3 | 0.2±.03 | 7.8±1.9 | 8.8±1.4 | 0.3±.03 | 3.6±0.7 |
| CD ($P<0.01$) | NS | NS | NS | NS | NS | NS |

Values are presented as mean of 45 replicates ± SD.

2380). Fe and Zn standards from Sigma were used for calibration according to the method of Lindsey & Norwell (1969).

### Availability of Fe and Zn

Rao & Prabhavathi (1978) proposed that ionizable Fe at pH 7.5 can be used as a reliable measure of bioavailability of non-haem Fe in foods, which was based on their findings where the per cent ionizable Fe at pH 7.5 in a number of diets was correlated highly with per cent iron absorption in humans from the same diets.

The method is based on the release of ionizable Fe from plant foods subjected to treatment with pepsin–HCl at pH 1.35 and subsequently at pH 7.5, simulating similar conditions prevailing in the stomach and in the intestine, respectively. In the present study we have also determined ionizable Zn in the same sample on the basis of the fact that the absorption of Zn also takes place from intestine under similar conditions.

For analyses, 2 g of powdered diet was first incubated with pepsin–HCl solution (0.5% pepsin in 0.1 N HCl). The mixture was adjusted to pH 1.3 and incubated in a 100 ml conical flask at 37°C in a metabolic shaker waterbath for 2 h, and subsequently the pH was raised to 7.5 before incubating for another 2 h. At the end of this incubation period, the contents of the flask were centrifuged at 5000 rpm for 30 min, and the supernatant containing the free form of these elements was collected. An aliquot, in suitable dilution, was placed in an atomic absorption spectrophotometer to determine ionizable Fe and Zn.

Phytate levels were measured by the method of Davies & Reid (1978) and Haug & Lanzsch (1983). Dietary fibre was analysed by the methods of Van Soest & Wine (1967).

Correlation coefficients were calculated among dietary fibre, phytate and availability of zinc and iron to study the effect of fibre and phytates on availability of these minerals in the diet. All the data were evaluated statistically by analysis of variance.

## RESULTS

Total Fe and Zn contents of the diets consumed by the different population groups were between 8 and 9.1 and 1.8 and 2.1 mg % and the *in vitro* availability of these minerals varied between 3.3–4.4% and 7.8–8.7%, respectively (Table 1). No significant variations were observed amongst the different diets, which is most probably a reflection of the similarity of the diets consumed by all four population (age) groups examined (Table 2). These diets were cereals (wheat) -based, thus contributing significant levels of phytate and fibre.

When correlation coefficients among dietary fibre, phytic acid, iron and zinc availability were calculated, availability of Fe or Zn was found to be negatively and significantly ($P<0.05$) correlated with phytic acid and also with dietary fibre indicating that these had adverse effects on the availability of these minerals (Table 3). Similarly, Harland (1989), found that mineral bioavailability was affected by the intake of dietary fibre in the presence of phytates. From other reports it appears that mineral availability was reduced, either in the presence of both phytate and fibre or phytate or fibre (Obzoba, 1981; Davies *et al.*, 1977). Franz *et al.* (1980) concluded that phytates rather than fibre are important in limiting

**Table 2. Composition of diets as consumed by different age groups**

| Foods | Mean daily intake (g) | | | |
|---|---|---|---|---|
| | Children | Adolescents | Adults | Older adults |
| Cereals | 141–241[a] | 217–257 | 232–442 | 257–389 |
| Legumes | 11–17 | 15–21 | 15–34 | 12–28 |
| Vegetables | 15–74 | 13–74 | 20–84 | 5–70 |
| Fruits[b] | – | – | – | – |
| Milk and milk products | 286–574 | 365–573 | 459–806 | 382–558 |
| Fats and oils | 17–37 | 32–40 | 41–56 | 30–44 |
| Sugar and jaggery | 13–23 | 12–20 | 15–30 | 14–18 |

[a]Range of values denotes average daily intake from 3 consecutive days diet collection during three seasons in a year.
[b]Fruit consumption was negligible throughout the year.

**Table 3. Correlation coefficient of iron and zinc availability with phytic acid and dietary fibre in the diets (per 100 g DM)**

| Group | Phytic acid(g) | Dietary fibre(g) | Fe availability | | Zn availability | |
|---|---|---|---|---|---|---|
| | | | Phytic acid | Dietary fibre | Phytic acid | Dietary fibre |
| Children | 0.48 ± 0.09 | 38.0 ± 8.1 | −0.88** | −0.89** | −0.75** | −0.72** |
| Adolescents | 0.52 ± 0.07 | 41.8 ± 3.7 | −0.90** | −0.57** | −0.93** | −0.69** |
| Adults | 0.49 ± 0.07 | 39.9 ± 5.7 | −0.91** | −0.91** | −0.95** | −0.90** |
| Older adults | 0.51 ± 0.05 | 42.0 ± 3.9 | −0.87** | −0.86** | −0.91** | −0.87** |

**Significant at 1% level.

the absorption of zinc from a diet rich in both phytic acid and dietary fibre.

## DISCUSSION

In this study the per cent *in vitro* availability of Fe and Zn of the diets consumed by four different age groups has been determined by using the method, which is based upon measuring ionizable Fe in diets by simulating similar conditions as that of small intestine where most of these elements are absorbed. The validity of the method has been studied by Rao & Prabhavathi (1978) by comparing *in vivo* Fe absorption with ionizable Fe in diets at pH 7.5 which gave a correlation coefficient of 0.94.

The negative correlation between phytate or fibre and Fe or Zn availability in this study suggests that fibre-containing foods, and various types of fibres, might have an inhibiting effect on the absorption and retention of these minerals. Physicochemical and nutritional studies have shown that the penta- and hexaphosphate phytic acid species do effectively chelate non-haem iron, and thus reduce its bioavailability. According to Obzoba (1981) and Davies *et al.* (1977) mineral availability was reduced in the presence of fibre, phytate or a combination of both, whilst Harland (1989) concluded that the absorption and utilization of essential minerals was affected by dietary fibre in the presence of phytate. In contrast, Franz *et al.* (1980) considered that phytate, rather than fibre, was the more important factor limiting the availability of zinc from a diet rich in both compounds. Recently, Wise (1995) has also discussed, in his review, the possible negative effects of phytate and fibre on Zn availability in animals as well as humans. But the debate on the role of these dietary compounds in digestion, absorption and utilization of minerals is still open. It is thus likely that the availability of essential minerals from the diet will be determined by a complex set of factors, including the levels and chemical forms of the minerals present in the diet and the presence of other compounds in that diet which inhibit or enhance availability. The compounds present naturally in condiments and spices may also have a negative effect on mineral availability (Hazell & Johnson, 1988). Such compounds, including tannins (polyphenolics) might be expected to contribute to the low availability found in the present study since the foods consumed by the Indian populations are traditionally highly spiced and, in addition, tea, a source of tannins, is a very popular drink with all meals. The finding that ascorbic acid increases the absorption of Fe is not considered to be significant in the present context since the diet of the populations studied contained little vitamin C, the main sources of which are citrus fruits and uncooked vegetables.

Whilst there have been numerous studies on the availability of minerals from individual foods, there have been very few investigations on the diets across different age groups in this part of India or elsewhere. This paper indeed is the first such report from Haryana State. The low mineral availability found here does not appear to be associated with any obvious disease status or morbidity. Although the human body adapts readily to a temporary increase/decrease in food (hence nutrient) intake, prolonged nutrient imbalance will certainly result in a weakening of the system. Thus, although both cereals and legumes contain compounds which effectively lower the availability of essential minerals, significant reduction in the intake of either of the staples would have adverse nutritional consequences because of the concomitant reductions in protein and energy.

In the short to medium term, the mineral availability of Indian vegetarian diets may be improved by increasing the amounts of vegetables and sprouted legumes consumed. In time, the biologically-active forms of phytic acid are degraded through the action of phytase and the germinated seed also contains significant levels of vitamin C. It is gratifying to note that such a trend is emerging in urban regions of India. In the longer term, selection for crops showing improved agronomic performance associated with reduced levels of phytate (or increased phytase) and optimal levels of fibre should be encouraged and explored.

Unless we include the availability and utilization of nutrients in dietary studies, nutrient intake studies will make little sense in assessing the nutritional status of an individual or a population.

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