

## Assessment of zinc bioavailability: studies in rats on zinc absorption from wheat using radio- and stable isotopes

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Absorption from wheat intrinsically and extrinsically labelled with  $^{67}\text{Zn}$  and extrinsically labelled with  $^{65}\text{Zn}$  was measured from  $^{67}\text{Zn}$  faecal excretion and  $^{65}\text{Zn}$  whole-body retention in rats. There were significant differences between the extrinsically- and intrinsically-labelled  $^{67}\text{Zn}$  ( $P < 0.001$ ), but not between the extrinsically-labelled  $^{65}\text{Zn}$  and intrinsically-labelled  $^{67}\text{Zn}$ . The effect of chicken meat on the absorption of Zn from intrinsically-labelled wheat was also studied in the rat. Mean Zn absorption from wheat and chicken meat fed separately was 18.5 and 68.2% respectively, and from a mixture of the two containing the same level of Zn was 50.1%. The apparent absorption of Zn from the composite meal was significantly higher than predicted from the results of the foods on their own ( $P < 0.001$ ).

### **Zinc: Absorption: Stable isotopes**

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The use of radioisotopes as extrinsic labels for Zn bioavailability studies is generally accepted as a valid technique (Evans & Johnson, 1977; Meyer *et al.* 1983; Flanagan *et al.* 1985; Gallagher *et al.* 1988), although some reports have questioned the method (Ketelson *et al.* 1984). In recent years there has been interest in the use of stable isotopes as a safe alternative to study Zn metabolism, particularly in infants and pregnant women. However, there are difficulties associated with the use of stable isotopes, notably the assumption that they can be used like radioisotopes to label foods extrinsically (Janghorbani *et al.* 1982; Serfass *et al.* 1989; Egan *et al.* 1991; Fairweather-Tait *et al.* 1991). First, there is the question as to whether or not extrinsic labels mimic native food Zn in the gastrointestinal tract during the process of digestion and absorption. Recent studies suggest that the type and physical state of the food has a more important bearing on the behaviour of the isotope and that it is this food-label interaction which determines its absorption process (Fairweather-Tait *et al.* 1991). Second, there is a large difference in the amounts of Zn added in the process of extrinsic labelling when using radio- and stable isotopes. The present study was designed to compare the absorption of stable and radio-Zn used as extrinsic labels in wheat, with intrinsically-incorporated stable Zn.

Many dietary factors affect Zn bioavailability (Solomons, 1982) and there is some evidence to suggest that meat enhances Zn absorption from non-meat sources (Shah & Belonje, 1981). This has been attributed to the level of protein in the diet and, more specifically, to the level and type of amino acids present. A number of workers have tried to demonstrate changes in Zn absorption with various protein diets with conflicting results (Snedeker & Greger, 1981, 1983; Colin *et al.* 1983; Greger & Mulvaney, 1985). Using whole-wheat and chicken meat, both intrinsically labelled with  $^{67}\text{Zn}$ , we conducted an experiment to see what influence chicken meat had on the absorption of wheat Zn in a mixed meal fed to rats.

## MATERIALS AND METHODS

*Animals and diets*

Male Wistar weanling rats, weighing 50 g each, were randomly allocated into groups of fourteen (Expts 1 and 3) or nineteen (Expt 2), and housed individually in stainless-steel wire-bottomed plastic cages. They were fed *ad lib.* on a semi-synthetic diet (Table 1), for 1 week, followed by 2 weeks of meal-feeding (two 1 h meal feeds daily) before the test meal. In all the experiments the rats were fasted overnight before administration of the test meal the following morning, and weighed 210–228 g. During the period of faecal collection the rats were fed on a low-Zn semi-synthetic diet which was similar to that described in Table 1, but without the added Zn. This minimized re-excretion of absorbed Zn isotope from the test meal (Fairweather-Tait *et al.* 1985). Any test meal not completely eaten by the rats was dried and weighed and deducted from the original weight given to the rat to calculate the administered dose.

Two experiments were performed to investigate the effect of labelling techniques of Zn on Zn absorption in a wheat meal fed to rats: Expt 1, two groups of fourteen rats were fed on a meal of 7 g wheat with  $^{67}\text{Zn}$  either as an extrinsic or as an intrinsic label; Expt 2, one group of nineteen rats was fed on a meal of 7 g wheat intrinsically labelled with  $^{67}\text{Zn}$ , to which  $^{65}\text{Zn}$  was added as an extrinsic label.

A third experiment was performed in which the effect of a dietary modifier of Zn absorption (meat) on wheat was investigated: Expt 3, the effect of chicken meat on Zn absorption from wheat was studied using chicken meat and wheat intrinsically labelled with  $^{67}\text{Zn}$  prepared as described elsewhere (Fox *et al.* 1991). Three groups of fourteen rats each were used: group 1, fed on 6.0 g wheat; group 2, 7.2 g chicken meat; group 3, 3.6 g chicken meat mixed with 3.0 g wheat. These quantities were calculated to obtain the same total amount of Zn in each meal.

*Preparation of test meals*

The test meals were prepared by adding an equal weight of distilled water to the dried food to which a faecal marker of  $\text{Cr}_2\text{O}_3$  (5 mg/g) had been added, and mixing thoroughly to a paste a few hours before being offered to the rat. Extrinsically-labelled  $^{67}\text{Zn}$  wheat was prepared by adding a solution of elemental Zn (91.9%  $^{67}\text{Zn}$  (atom %); Technical and Optical Equipment, London) dissolved in a minimum volume of HCl (AR) and made up to a suitable volume with distilled water to non-enriched wheat 2 h before consumption. The test meal labelled with the radioisotope  $^{65}\text{Zn}$  was prepared by adding 18.5 kBq  $^{65}\text{Zn}$  ( $\text{ZnCl}_2$ ; Amersham International) to 7 g  $^{67}\text{Zn}$ -enriched wheat, prepared as described previously (Fox *et al.* 1991). The non-enriched wheat was grown from the same batch of seeds and under the same conditions as the enriched wheat. Both the enriched and non-enriched forms of wheat were milled through a 250  $\mu\text{m}$  gauge sieve after air-drying.

*Measurement of apparent  $^{67}\text{Zn}$  absorption*

Individual faecal collections were made for each rat, starting on the day of the test meal and finishing 36 h after all the  $\text{Cr}_2\text{O}_3$  had been excreted, a period usually lasting 5–6 d. The faeces were dried at 80°, ground and heated to 480° for 48 h in silica crucibles. Portions of the ash (0.025 g) were weighed, taken up in HCl and analysed by atomic absorption spectrophotometry for total Zn (Fairweather-Tait & Southon, 1989). Similarly, 0.2 g of the ash was taken up in HCl, concentrated and purified on an ion-exchange column (Fairweather-Tait *et al.* 1989) and eluted for isotope analysis by thermal-ionization mass spectrometry using a Finnigan MAT Thermoquad mass spectrometer (Eagles *et al.* 1989).

Table 1. *Composition of the semi-synthetic diet given to the rats (g/kg)*

Maize starch	309
Sucrose	309
Casein	200
Cellulose	40
Maize oil	80
Mineral mix*	40
Vitamin mix*	20
DL-Methionine	2.5

\* Micronutrients were formulated to provide normal requirements for growth (Fairweather-Tait *et al.* 1991).

Table 2. *Analysis of test meals*

Food	Isotope label	<sup>67</sup> Zn (μg/g)	Zn (μg/g)	Total Zn in meal (mg)
Wheat (Expts 1 and 2)	Intrinsic	14.86	62.75	0.44
Wheat (Expt 1)	Extrinsic	4.23	60.63	0.42
Wheat (Expt 3)	Intrinsic	10.14	66.00	0.40
Chicken (Expt 3)	Intrinsic	5.71	48.06	0.35
Wheat and chicken (Expt 3)	Intrinsic	8.17	59.32	0.37

Total <sup>67</sup>Zn, over and above natural abundance, was calculated for the test meal (Table 2) and the faecal samples (Fairweather-Tait *et al.* 1991), and apparent absorption calculated by deducting faecal <sup>67</sup>Zn from the administered dose.

#### *Measurement of <sup>65</sup>Zn absorption*

In Expt 2 the animals were counted in a small-animal whole-body counter (NE 8112; Nuclear Enterprises, Beenham, Berks.) immediately after they had consumed the test meal on day 1 and again on each day between day 7 and day 14 (Fairweather-Tait & Wright, 1984). Each time the animals were counted the results obtained were corrected for background radiation, decay and counting efficiency and the resulting values expressed as a percentage of those obtained on day 1.

#### *Statistical analysis*

Results from Expts 1 and 2 were compared by *t* tests, and Expt 3 by  $\chi^2$  test.

### RESULTS

The results for all three experiments are illustrated in Fig. 1. In Expt 1 mean apparent Zn absorption results for the wheat extrinsically and intrinsically labelled with <sup>67</sup>Zn were 30.3 (SD 3.7) and 22.3 (SD 4.1)% for the two groups of rats respectively, demonstrating a highly significant difference in absorption ( $P < 0.001$ ) between the differently labelled forms of wheat.

In Expt 2 statistical analysis of the data using the day 7 apparent absorption results in animals given the wheat meal labelled with two isotopes (<sup>67</sup>Zn intrinsic, <sup>65</sup>Zn extrinsic) showed no difference. Mean Zn absorption by the rats consuming a test meal containing 0.44 mg Zn (of which 103 μg was <sup>67</sup>Zn) was calculated to be 20.7 (SD 4.4)% from the <sup>65</sup>Zn data and 19.8 (SD 6.2)% from the <sup>67</sup>Zn data.

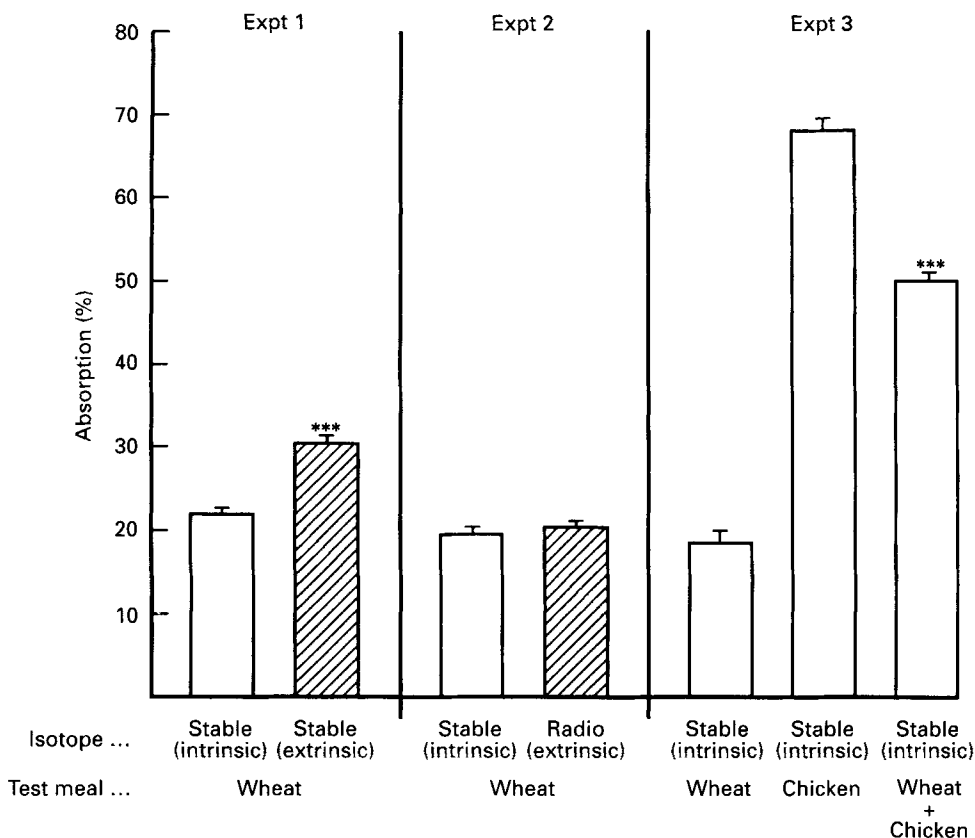


Fig. 1. Zn absorption (% of dose) from  $^{67}\text{Zn}$  intrinsically- ( $\square$ ) and extrinsically- ( $\blacksquare$ ) labelled wheat (Expt 1),  $^{67}\text{Zn}$  intrinsically- ( $\square$ ) and  $^{65}\text{Zn}$  extrinsically- ( $\blacksquare$ ) labelled wheat (Expt 2), and  $^{67}\text{Zn}$  intrinsically-labelled ( $\square$ ) wheat, chicken and wheat and chicken mixture (Expt 3). For details of test meals and procedures, see Tables 1 and 2 and pp. 96–97. Values are means and standard deviations represented by vertical bars. In Expt 1 mean values were significantly different: \*\*\*  $P < 0.001$ . In Expt 3 the mean for the chicken and wheat meal was significantly higher than that predicted from values for the individual chicken and wheat meals: \*\*\*  $P < 0.001$ .

In Expt 3 the mean apparent absorption of Zn for the three groups of rats consuming test meals of intrinsically-labelled wheat, chicken meat and a mixture of both were 18.5 (SD 6.6), 68.2 (SD 5.6) and 50.1 (SD 4.3) % respectively. The Zn content of the three test meals were similar.

#### DISCUSSION

The explanation for the difference in apparent absorption in Expt 1 between the wheat meals containing extrinsic or intrinsic  $^{67}\text{Zn}$  probably lies in the addition of the isotopic Zn as an extrinsic label. In Expt 2, where the contribution made by  $^{65}\text{Zn}$  extrinsic label to the overall Zn content was negligible, no difference was found between the intrinsically- and extrinsically-labelled wheat. The native Zn, labelled or otherwise, was chemically and physically associated within the matrix of the food, whereas the extrinsic stable isotope was added in an aqueous phase. Absorption may have taken place, therefore, at a different time and site within the small intestine. It is possible that the extrinsic  $^{67}\text{Zn}$  label was more available for absorption, provided that it was not involved in chemical interactions and associations leading to the formation of complexes that would render it unavailable for absorption. The radioisotope, on the other hand, was virtually carrier-free and, with an

extremely low quantity of Zn, was more likely to behave in the same manner as the intrinsic Zn. It would appear that isotopic exchange that occurred during mixing of the test meal allowed complete exchange of the small amount of  $^{65}\text{Zn}$  present, resulting in the formation of a common pool of exchangeable Zn, in which the behaviour of the radioisotope represented that of the native Zn.

Using stable isotopes to label foods extrinsically for bioavailability studies has the disadvantage of the need to add relatively large quantities of Zn to a food or meal which already has its full complement of Zn within the food. It has been shown that Zn absorption from the gastrointestinal tract is not a linear function of Zn content and that the fractional absorption of Zn decreases markedly at concentrations above  $70\ \mu\text{mol}$  in humans (Sandstrom & Cederblad, 1980; Wada *et al.* 1985). It is impossible to avoid adding significant quantities of isotopically-enriched Zn in order to enrich the faeces sufficiently after dilution with dietary and endogenous Zn. Multi-labelling techniques, using smaller quantities of isotope over longer periods of time, can be employed to avoid the problem of giving excessive amounts of Zn in individual meals. However, it is still not certain that the isotope will behave in exactly the same way as the native Zn bound in the food matrix, and re-excretion of the absorbed label over a longer time-period would have to be taken into account, making interpretation of results difficult.

By using the  $^{65}\text{Zn}$  whole-body retention data from day 7 to day 14 in Expt 2 it is possible to estimate endogenous excretion of Zn during the first 7 d and, hence, calculate true absorption (Fairweather-Tait & Southon, 1989). No significant difference was found between true absorption (21.5%) and retention on day 7 (20.7%) when compared with retention calculated from faecal monitoring following administration of intrinsically-labelled  $^{67}\text{Zn}$  (19.8%). This was not surprising since the study was designed to minimize endogenous Zn excretion by feeding a low-Zn diet during the period of faecal collection. However, since endogenous excretion increases with dietary Zn (Turnlund *et al.* 1986) higher intakes of Zn could lead to an underestimation of absorption and, hence, incorrect assessment of Zn bioavailability.

The results from the wheat and chicken mixed meal demonstrated a significantly higher Zn absorption from the composite test meal (50.1%) than that predicted (43.4%) from the results of the individual wheat and chicken test meals ( $P < 0.001$ ). This increase in absorption could be due to either of the two sources of Zn being more efficiently absorbed. However, it would seem more likely that the observed increase in Zn absorption was due to an increase in absorption of Zn from the wheat fraction since Zn absorption from chicken meat was already 68%, a value similar to previous findings for the absorption of chicken meat in rats (Fairweather-Tait *et al.* 1990). There is some evidence to suggest that the inhibitory effect of phytate on Zn absorption from wheat is reduced in the presence of protein. Shah & Belonje (1981), using a rat femur assay technique, found that mixing beef with textured soya-bean protein increased the relative biological value of Zn from the soya-bean product from 47 to 94%, and beef mixed with rapeseed protein concentrate increased from 53 to 83%. Other work by Sandstrom *et al.* (1989) in human subjects demonstrated a 50–70% increase in Zn absorption from a bean meal as a result of doubling the protein in the meal. Both wheat and beans contain phytate which is known to inhibit Zn absorption and it has been suggested that protein or peptides form complexes with Zn, thereby preventing the formation of Zn phytate (Sandstrom *et al.* 1980). Other possibilities are that natural mineral chelates exist in animal protein (Scott & Zeigler, 1963), or that peptides and amino acids, formed during digestion, increase Zn solubility and facilitate its absorption.

These studies demonstrate that there is a need for caution when employing a stable isotope of Zn as an extrinsic label for Zn bioavailability studies. However, the use of radio-Zn ( $^{65}\text{Zn}$ ) to label wheat extrinsically appears to be a valid technique. The advantage of

using stable isotopes to label foods is that multi-isotope studies can be carried out. Interactions between three foods within the same meal, each labelled with a different isotope of Zn ( $^{67}\text{Zn}$ ,  $^{68}\text{Zn}$ ,  $^{70}\text{Zn}$ ) can be investigated, and by using  $^{65}\text{Zn}$  a further food can be labelled. The results presented in the present paper have also demonstrated that the absorption of Zn from a meal cannot be taken to be the sum of the individual foods. In conclusion, the assessment of Zn bioavailability in a food or meal is influenced by the presence of other dietary constituents and its measurement is affected by the isotopic-labelling techniques employed.

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