Zinc absorption in adult men from a chicken sandwich made with white or wholemeal bread, measured by a double-label stable-isotope technique

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Eleven fasted adult men consumed a chicken meat sandwich made with white or wholemeal bread, extrinsically labelled with 2 mg ^{67}Zn, on two different occasions. Immediately after eating the sandwich they were given an intravenous injection of 1·5 mg ^{70}Zn. True Zn absorption (which was approximately 7% higher than apparent absorption) was determined by the faecal balance technique by making an allowance for endogenous excretion from measurements of faecal excretion of ^{70}Zn. There was no significant difference in mean true Zn absorption from the white or wholemeal bread sandwich, 33·6 and 25·4% respectively. It was concluded that the substitution of wholemeal for white bread does not reduce Zn absorption from meat-based sandwiches.

Zinc absorption: Chicken meat: Bread: Stable isotope

The amount of zinc that is available for absorption from a meal depends on the chemical composition of its constituent foods. There are a number of known inhibitors of Zn absorption of which phytate (*myo*-inositol hexaphosphate) is probably the most biologically significant. Phytate is the principal storage form of phosphorus in plants, concentrated in the aleurone layer of cereals, but animals products contain little or no phytate. This is one of the reasons why diets high in cereals but low in animal protein contain Zn of lower bioavailability (Navert *et al.* 1985).

Current dietary guidelines for developed countries emphasize an increased intake of complex carbohydrates. Many consumers have responded to these recommendations by replacing white bread by wholemeal bread. The precise effects of such a change on Zn bioavailability are not known, although there are indications that a moderate increase in complex carbohydrates has a minimal effect on Zn nutrition (Mason *et al.* 1990). The aim of the present study was to determine the precise effect of consuming wholemeal instead of white bread on Zn absorption from a chicken-meat sandwich.

METHODS

Subjects and diets

Eleven men between 20 and 54 (mean 34) years of age volunteered for the study. They all appeared to be in good health, and reported no history of gastrointestinal dysfunction. Measurements of height and weight (Table 1) were within normally accepted limits. For the duration of the study, in order to prevent any major changes in Zn metabolism and homeostasis, each subject was asked to maintain his usual dietary pattern using a self-

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Table 1. *Details of subjects studied: age (years) height (m), weight (kg) and mean daily zinc intake (mg/d)*

Subject no.	Age	Height	Wt	Analysed mean Zn intake		Calculated mean Zn intake
				Mean	SD	
1	41	1·928	67·2	*10·5	3·5	†11·3
2	22	1·711	70·6	*9·9	3·4	†11·6
3	20	1·747	72·6	*5·4	1·5	†6·1
4	26	1·755	74·8	*7·4	3·5	†8·1
5	28	1·800	87·3	†6·3	2·0	*5·4
6	52	1·822	81·4	†11·9	2·0	*10·5
7	32	1·710	67·5	†9·1	1·5	*7·9
8	54	1·680	78·5	†6·9	4·5	*6·0
9	24	1·799	66·7	*13·8	3·9	†15·0
10	52	1·657	63·5	†6·6	2·3	*5·1
11	23	1·846	71·8	*9·6	3·1	Not available
Mean	34	1·769	72·9	8·9		
SD	13	0·080	7·1	2·6		

* White bread.
† Wholemeal bread.

selected 7 d rotating cycle. This was considered to be a more important feature of the experimental design than inter-subject differences in Zn intake because each subject acted as his own control. However, one change to the habitual diet was made over the period of study, namely the type of bread eaten; subjects were told to consume white bread during the week before the white-bread test meal, and wholemeal bread during the week before the wholemeal-bread test, thus allowing a period of adaptation. Each subject was asked to weigh and record all food and drinks for the 7 d period preceding the first test meal. The 7 d menu was consumed the week leading up to and following each test meal. In order to measure Zn intakes accurately, a 7 d duplicate diet collection was made during the week of the second test meal. The subjects were given written information about the study which had been approved by the Institute of Food Research Ethics Committee.

Preparation and administration of test meals

Chicken meat was prepared for another study using rats (Fairweather-Tait *et al.* 1991). It was labelled intrinsically with the stable isotope ^{67}Zn by injecting the brachial vein in alternate wings of ten Ross I broiler-type cockerels (mean weight 2·025 kg) with 1 ml of a solution of ^{67}Zn (1·84 mg ^{67}Zn/ml) daily for 5 d (Fox *et al.* 1991). After 1 week the birds were killed, bled, defeathered and eviscerated. The skin and bone were removed and the breast and leg meat cooked in a microwave oven at 400 W for 10 min. The leached juices were discarded and the cooked meat was minced in a food blender (Magimix, Godalming, Surrey) and stored at −18° until use. On analysis it was found that the chicken meat was not sufficiently enriched with ^{67}Zn to provide enough ^{67}Zn-labelled meat in one meal for absorption to be measured by faecal balance. Therefore, an additional 2·106 mg ^{67}Zn (as zinc chloride in distilled water) was consumed with each test meal as an extrinsic label.

The day before the test meal the meat was defrosted and weighed portions were made into sandwiches with wholemeal or white bread spread with margarine (Flora; Van den Berghs & Jurgens, Sussex). After an overnight fast the subjects were given accurately

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weighed quantities of sandwiches made from a mean of 125 (SD 7) g bread, 23 (SD 3) g margarine and 104 (SD 9) g chicken meat. Each subject received both wholemeal and white sandwiches, in random order, 1 week or more apart. Malvern water was allowed *ad lib.* with the meal.

Radio-opaque markers were taken with the meal to check on the completeness of the subsequent faecal collection (Fairweather-Tait *et al.* 1989*b*). No food or drink was allowed for 4 h, after which time the subjects returned to their habitual diet. Carmine (500 mg) was taken in two capsules with the evening meal.

In addition to measuring apparent absorption of Zn from the test meals, an attempt was made to estimate endogenous excretion of Zn so as to correct the apparent absorption value to calculate true absorption. The method employed was analogous to that used for calcium (Fairweather-Tait *et al.* 1989*a*). Each subject was given an intravenous injection of 1·5 mg ^{70}Zn immediately after consuming the first ^{67}Zn-labelled test meal, and faecal and urinary excretion of the ^{70}Zn was measured as an index of endogenous Zn excretion.

Stable isotopes

The isotope used to label the chicken meat was prepared from ^{67}Zn-enriched elemental Zn (91·9 atom %; Technical and Optical Equipment, Edgware Road, London) dissolved in concentrated AnalaR hydrochloric acid and adjusted to pH 7·4 with sodium bicarbonate and trisodium citrate to a final solution containing 1·84 mg ^{67}Zn/ml.

The ^{70}Zn solution injected intravenously into the subjects was prepared as zinc citrate from ^{70}Zn-enriched zinc oxide (65·51 atom %; Oak Ridge National Laboratory, TN, USA). The ZnO (51·5 mg) was dissolved in Aristar HCl, evaporated to dryness, the precipitate taken up in trisodium citrate, and the solution titrated to pH 7·0 using sodium bicarbonate. The final solution was divided into fifteen portions of 5 ml, sealed in glass ampoules, autoclaved and subjected to routine sterility testing in the Pharmacy Department of the Norfolk & Norwich Hospital (Norwich).

Absorption measurement

A complete faecal collection was made from the time of the test meal until the carmine had been excreted, plus one further collection, as described elsewhere (Fairweather-Tait *et al.* 1987). Stools were stored at −18° until the completion of the study when all collections from each subject for each period were combined and prepared for analysis as in previous studies (Fairweather-Tait *et al.* 1989*b*). Total Zn was determined by atomic absorption spectrophotometry (AAS) and ^{67}Zn and ^{70}Zn by thermal-ionization mass spectrometry (TIMS), as described on p. 414.

Urine was collected for 24 h after the test meal and a portion evaporated to dryness in a silica crucible, heated to 480° for 48 h in a muffle furnace, and the ash taken up in concentrated HCl and analysed for Zn by AAS and ^{67}Zn and ^{70}Zn by TIMS, as described on p. 414.

Apparent absorption from the meal was calculated by deducting faecal ^{67}Zn from the administered dose, after making due allowance for naturally occurring ^{67}Zn (Fairweather-Tait *et al.* 1991). True absorption was estimated by taking into account endogenous excretion of ^{67}Zn using the faecal ^{70}Zn enrichment values obtained from the first faecal collection following the intravenous ^{70}Zn injection. As subjects did not alter their diets during the course of the study it was assumed that endogenous excretion remained constant, and the correction factor applied to both test meals for each subject was the same. The calculations were as follows:

$$E = I - A + S,$$

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where E is faecal ^{67}Zn (mg), I is ^{67}Zn intake (mg), A is ^{67}Zn absorption (mg), and S is faecal ^{67}Zn secretion (mg).

If S is $x\%$ of A, where x is assumed to be the same values as obtained for faecal ^{70}Zn (expressed as a percentage of dose administered), then by substitution

$$A = I - E + \frac{x}{100} A,$$

$$= \frac{I - E}{1 - x\%},$$

and percentage true absorption from the test meal is $A/I \times 100\%$.

Faecal analysis

Pooled faecal samples were heated in an autoclave at 0·776 mmHg and 121° for 20 min, freeze-dried, ground to a fine powder in a Moulinex coffee grinder (Moulinex Ltd, Coulsdon, Surrey) and passed through a 30 mm sieve to recover all the radio-opaque markers. After mixing in a powder homogenizer (Pascall Engineering, Crawley, Sussex) for 30 min, subsamples were taken for analysis. The total Zn content was determined by AAS (PU9000; Philips, Cambridge) after dry-ashing at 480°, using bovine liver and wheat flour as reference standards (National Bureau of Standards, Gaithersburg, MD, USA).

The isotopic enrichment of the samples was determined by TIMS (THQ; Finnegan-MAT GmbH, Bremen, Germany) after selective extraction of Zn (Fairweather-Tait *et al.* 1989*b*), as described previously (Fairweather-Tait *et al.* 1991).

Food analysis

Daily duplicate diet collections (solids and liquids) were homogenized in a Waring food blender (Jennings, Nottingham), made up to a known volume and a portion was freeze-dried. The freeze-dried powder was mixed and subsamples were analysed for Zn by AAS. The test meals were analysed for Zn and Ca by AAS.

Phytate

The phytate content of the wholemeal and white breads used to make the chicken sandwiches was measured, separating the phytate by the method of Harland & Oberleas (1986). Hydrolysis of phytate eluate was carried out with sulphuric acid for 3 h at 140° and P was determined by the method of Parker & Peterson (1965).

Statistical analysis

Results for Zn absorption were tested by Student's paired t test (Snedecor & Cochran, 1967). The relationships between Zn intake and Zn absorption and excretion were examined by regression analysis using a GENSTAT program (Payne *et al.* 1987).

RESULTS

Mean daily Zn intakes of the subjects (measured over a 7 d period) on diets containing white or wholemeal bread are given in Table 1, together with their standard deviations. The overall mean for the group was 8·9 (SD 2·6) mg/d, and values ranged from 5·4 to 13·8 mg/d. Although individual subjects generally followed a high, medium or low pattern of intake, where dietary patterns differed greatly from day to day the variation in Zn intake also fluctuated. For example, Zn intake by subject no. 8 was low (4–5 mg/d) for 5 d of the week, but higher on the other 2 d (8·2 and 16·6 mg/d). The type of bread consumed affected Zn

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Table 2. *Total zinc, ^{67}Zn, calcium and phytate content of chicken meat and bread used to make test meals**

	Chicken meat	White bread	Wholemeal bread
Zn: μg/g dry wt	44·8	10·4	35·4
mg/average portion	1·50	0·78	2·66
^{67}Zn enrichment: μg/g dry wt	6·25	0	0
mg/average portion	0·208	0	0
Ca: mg/g dry wt	0·3	1·2	0·2
mg/average portion	11·0	96·4	15·4
Phytic acid: mg/g dry wt	0	1·15	5·77
mg/average portion	0	87	433

* For details of procedures, see pp. 412–414.

Table 3. *Faecal and 24 h urinary excretion of intravenous ^{70}Zn administered to adult male subjects*

	After the first test meal			After the second test meal	
Subject no.	Faecal collection (no. of days)	Faecal excretion (% of dose)	Urinary excretion (% of dose)	Time interval between test meals (d)	Daily excretion of ^{70}Zn (% of dose)
1	5	11·0	0·2	7	1·0
2	6	7·3	0·3	21	0·5
3	4	5·2	0·3	7	1·5
4	6	9·3	0·5	7	0·7
5	5	9·6	0·5	7	0·7
6	5	9·4	0·4	7	0·8
7	6	6·4	0·4	20	0·1
8	6	8·3	0·4	20	0·4
9	4	5·4	0·4	7	0·5
10	4	5·5	0·4	7	0·9
11	5	4·6	1·0	21	0·2
Mean		7·5	0·4		0·7
SD		2·2	0·2		0·4

intake since wholemeal bread contains 2·5 times more Zn than white bread. The Zn intake on the alternative diet was calculated from a knowledge of the weight of bread consumed, and this is given in Table 1.

The Zn, ^{67}Zn, Ca and phytate content of the chicken meat and breads used to prepare the test meals are given in Table 2. The contribution made by margarine to the Zn and phytate intake is not shown as it was negligible.

Faecal and urinary excretion of the injected ^{70}Zn is given in Table 3, expressed as a percentage of administered dose. Mean excretion was 7·5 (SD 2·2)% of the dose in the pooled faeces collected for about 5 d and 0·4 (SD 0·2)% of the dose in the urine collected for 24 h following the injection. Not all the subjects were able to participate at the same time, so the time interval between the two test meals varied from 7 to 21 d. Daily faecal excretion of ^{70}Zn was 0·9% 7 d post injection, whereas it had fallen to 0·3% at 20–21 d. Urinary excretion of ^{67}Zn was negligible.

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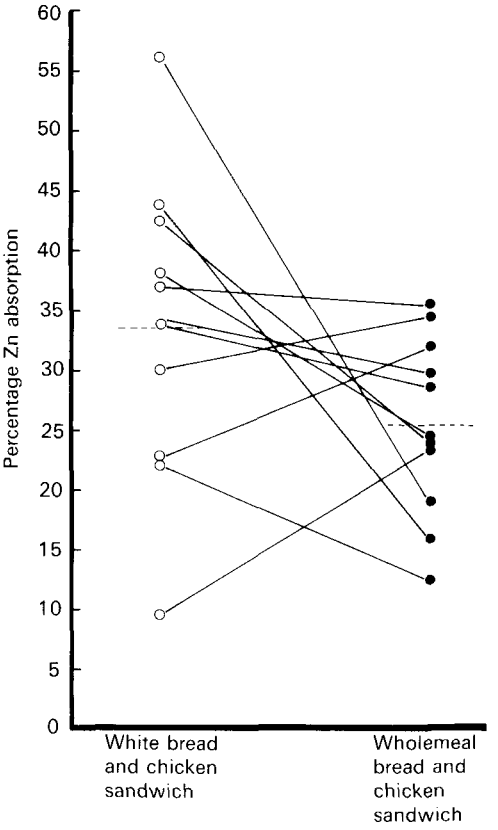

Fig. 1. Mean percentage ^{67}Zn absorption in adult men from sandwiches made from chicken meat in white (○) or wholemeal (●) bread, enriched with ^{67}Zn. Individual results with group means (– – –).

Individual results for ^{67}Zn absorption from the chicken-meat sandwich in white and wholemeal bread are shown in Fig. 1. There was no significant difference between the two test meals. Regression analysis of mean daily Zn intake (Table 1), Zn intake the day before (day − 1) and the day of the test meals (day 0) and Zn absorption showed no significant relationships. Nor was there any correlation between Zn intake (mean, day −1, day 0) and Zn excretion.

DISCUSSION

The likely variation in Zn absorption rates for normal subjects consuming identical stable isotopically labelled test meals on two occasions whilst consuming the same dietary intake of Zn has not yet been firmly established. Undoubtedly there are fluctuations, as described for example by Solomons *et al.* (1982) who found the mean Zn absorption from replicate ^{70}Zn-labelled meals given on two occasions to be 32 (SD 9) and 38 (SD 4) % in four subjects. Thus, small differences in absorbability of Zn from foods may be obscured owing to the 'noise' factor. This is a problem common to most studies of mineral bioavailability and is generally addressed by using a large enough group of subjects to test for differences at the appropriate level of significance. In the present study the difference between the two test meals was not statistically significant, although eight of the eleven subjects had a lower percentage ^{67}Zn absorption from the wholemeal-bread sandwich.

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Fractional Zn absorption has been shown to be higher from white than wholemeal bread (Sandstrom *et al.* 1980), and this is thought to be related to the very different levels of phytate (Lonnerdal, 1989). However, wholemeal bread contains approximately three times more Zn than white bread, and Zn absorption is dose-dependent (Fairweather-Tait, 1988; Fairweather-Tait & Southon, 1989). Therefore, despite a fall in fractional absorption with increasing dose, absolute absorption may in fact rise. For example, Sandstrom *et al.* (1980) found a higher absolute absorption of Zn from wholemeal than white bread, but when they attempted to eliminate the dose–response effect by adding zinc chloride to the white bread there was a higher absorption from white than wholemeal bread.

The amount of Ca and phytate in a meal undoubtedly influences Zn bioavailability (Ellis *et al.* 1987). Current literature suggests that the critical values for phytate:Zn and phytate × Ca:Zn millimolar ratios are > 15 and > 200 respectively (Ferguson *et al.* 1989). In the present study the phytate:Zn and phytate × Ca:Zn ratios in the test meals were 1·95 and 6·55 for the white-bread sandwich and 6·1 and 6·46 for the wholemeal-bread sandwich respectively. The results of the present study provide further confirmation that Zn absorption is not reduced in the presence of low ratios, as found in sandwiches containing meat which is a reasonably rich source of Zn.

Protein also modifies the bioavailability of Zn from foods (Solomons, 1982). Sandstrom *et al.* (1980) used ^{65}Zn to label extrinsically meals based on bread and demonstrated an enhancing effect of beef, cheese and egg on Zn absorption from bread in human subjects. In a more recent study using similar techniques (Sandstrom *et al.* 1987) the absorption of Zn from a meal of chicken plus white (but not wholemeal) bread was measured and found to be 46·1 %, equivalent to an absolute value of 0·565 mg Zn. The mean Zn absorption from the chicken sandwich in white bread in the present study was 33·6 %, which is equivalent to 1·47 mg Zn. The latter value is somewhat higher than that of Sandstrom *et al.* (1987) because of the added (extrinsic) Zn isotope which increased the Zn concentration of the test meal. Mean absorption from the wholemeal-bread sandwich was lower (albeit not statistically different) at 25·4 %, which was equivalent to 1·59 mg Zn. Thus, the higher Zn intake associated with wholemeal-bread more than compensated for the lower percentage absorption.

The previously mentioned calculations depend on the assumption that the added (extrinsic) isotopic label fully exchanges with endogenous Zn in the meal. The validity of this assumption is questionable. Gallaher *et al.* (1988) found no difference in absorption by adults given beef labelled intrinsically and extrinsically with ^{65}Zn. Using stable isotopes, Flanagan *et al.* (1985) found no difference in intrinsically and extrinsically labelled turkey meat, but Janghorbani *et al.* (1982) found consistently lower absorption from the extrinsic than intrinsic label in chicken meat. Our own experience with chicken is that the extrinsic label closely models the intrinsic label, but exchange is not 100 % (Fairweather-Tait *et al.* 1991). However, the behaviour of the extrinsic and intrinsic labels was closer in chicken meat than any of the other foods we tested. When more than one food is given, the isotope must exchange to some extent with all the Zn present in the meal. Preliminary results from our wheat studies again indicate incomplete exchange of extrinsic and intrinsic label, but with respect to Zn absorption the food effect far outweighs the labelling effect. In the present study, for reasons already stated, the quantity of extrinsic label in each meal (2·106 mg ^{67}Zn) far outweighed the amount of intrinsic label (0·208 mg ^{67}Zn). Thus, the absorption measurements are primarily measurements from extrinsically-labelled material. However, since the behaviour of the extrinsic isotopic label has been shown to give a good indication of the movement of endogenous Zn, and because the study was designed as a direct comparison between two meals similarly labelled, we believe that the method of labelling did not prejudice the results nor the validity of the conclusions reached.

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The use of intravenously administered ^{70}Zn to quantify the loss of endogenous Zn was based on a method devised to measure true Ca absorption from foods (Fairweather-Tait *et al.* 1989*a*). This technique assumes that the intravenous Zn is handled in the same manner as the absorbed ^{67}Zn. However, it is not possible to mimic gastrointestinal Zn absorption, which takes place over a varying time period of several hours after ingestion of a test food (Valberg *et al.* 1985). Nor is it possible to validate the assumption that the two modes of administered Zn are handled in the same way. It is, of course, important not to perturb Zn metabolism; immediately after infusion the plasma Zn will be equilibrating with the very rapidly exchanging body pools, with enrichment falling in an exponential manner. We used a dose of 1·5 mg Zn (as ^{70}Zn) and were reluctant to give a very much smaller dose because of uncertainty about the rate of excretion and the consequent difficulties of detecting and quantifying small amounts of isotope. In fact, only 7·5% of the intravenous dose was excreted in the faeces collected 4–6 d post injection and 0·4% in the urine collected 24 h post injection, which indicates that plasma capacity for Zn was not exceeded and that the intravenous ^{70}Zn was rapidly moved into body pools. The intravenous dose of Zn was in fact similar to the amount of Zn absorbed from the test meal, i.e. 1·5–1·6 mg, albeit over a different time interval.

When endogenous losses are small, as found with low Zn intakes (Jackson *et al.* 1988), there is little difference between apparent Zn absorption (calculated as the difference between intake and excretion) and true Zn absorption. In the present study, the habitual Zn intakes of the subjects were not particularly high (mean 8·9 mg/d), and perhaps as a consequence of this the ^{70}Zn excretion was fairly low. Apparent mean Zn absorption from the white-bread sandwich was 31·1 (SD 11·8)% and from the wholemeal-bread sandwich 23·5 (SD 6·7)%, which is a 7% underestimate of true absorption. No allowance was made for urinary losses of isotopes in any of the calculations because there was no measurable enrichment with ^{67}Zn, and the ^{70}Zn was considered to make a negligible contribution to the total. It was interesting to note the prolonged faecal excretion of ^{70}Zn. However, since the stool collections were bulked before analysis, we could not make any detailed deductions about the rate of fall over time.

In conclusion, it would appear from the results of the present study that Zn absorption from a chicken-meat sandwich was not adversely affected by substituting wholemeal for white bread. True Zn absorption, measured using a double-label isotope technique, was 7% higher than apparent absorption (intake minus faecal excretion) in male subjects whose mean habitual Zn intake was 8·9 mg/d.

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REFERENCES

Ellis, R., Kelsay, J. L., Reynolds, R. D., Morris, E. R., Moser, P. B. & Frazier, C. W. (1987). Phytate:zinc and phytate × calcium: zinc millimolar ratios in self-selected diets of Americans, Asian Indians, and Nepalese. *Journal of the American Dietetic Association* **87**, 1043–1047.

Fairweather-Tait, S. J. (1988). Zinc in human nutrition. *Nutrition Research Reviews* **1**, 23–37.

Fairweather-Tait, S. J., Fox, T. E., Wharf, S. G., Eagles, J., Crews, H. & Massey, R. (1991). Apparent zinc absorption by rats from foods labelled intrinsically and extrinsically with ^{67}Zn. *British Journal of Nutrition* **66**, 65–71.

Fairweather-Tait, S. J., Johnson, A., Eagles, J., Ganatra, S., Kennedy, H. & Gurr, M. I. (1989*a*). Studies on calcium absorption from milk using a double-label stable isotope technique. *British Journal of Nutrition* **62**, 379–388.

Fairweather-Tait, S. J., Minski, M. J. & Singh, J. (1987). Nonradioisotopic method for measuring iron absorption from a Gambian meal. *American Journal of Clinical Nutrition* **46**, 844–848.

https://doi.org/10.1079/BJN19920046 Published online by Cambridge University Press

Fairweather-Tait, S. J., Portwood, D. E., Symss, L. L., Eagles, J. & Minski, M. J. (1989*b*). Iron and zinc absorption in human subjects from a mixed meal of extruded and nonextruded wheat bran and flour. *American Journal of Clinical Nutrition* **49**, 151–155.

Fairweather-Tait, S. J. & Southon, S. (1989). Studies of iron:zinc interactions in adult rats and the effect of iron fortification of two commercial infant weaning products on iron and zinc status of weanling rats. *Journal of Nutrition* **119**, 599–606.

Ferguson, E. L., Gibson, R. S., Thompson, L. U. & Ounpuu, S. (1989). Dietary calcium, phytate, and zinc intakes and the calcium, phytate, and zinc molar ratios of the diets of a selected group of East African children. *American Journal of Clinical Nutrition* **50**, 1450–1456.

Flanagan, P. R., Cluett, J., Chamberlain, M. J. & Valberg, L. S. (1985). Dual-isotope method for determination of human zinc absorption: the use of a test meal of turkey meat. *Journal of Nutrition* **115**, 111–122.

Fox, T. E., Fairweather-Tait, S. J., Eagles, J. & Wharf, S. G. (1991). Intrinsic labelling of different foods with stable isotopes of zinc (^{67}Zn) for use in bioavailability studies. *British Journal of Nutrition* **66**, 57–63.

Gallaher, D. D., Johnson, P. E., Hunt, J. R., Lykken, G. I. & Marchello, M. J. (1988). Bioavailability in humans of zinc from beef: intrinsic vs extrinsic labels. *American Journal of Clinical Nutrition* **48**, 350–354.

Harland, B. F. & Oberleas, D. (1986). Anion-exchange method for determination of phytate in foods: collaborative study. *Journal of the Association of Official Analytical Chemists* **69**, 667–670.

Jackson, M. J., Giugliano, R., Giugliano, L. G., Oliveira, E. F., Shrimpton, R. & Swainbank, I. G. (1988). Stable isotope metabolic studies of zinc nutrition in slum-dwelling lactating women in the Amazon valley. *British Journal of Nutrition* **59**, 193–203.

Janghorbani, M., Istfan, N. W., O'Pagounes, J., Steinke, F. H. & Young, V. R. (1982). Absorption of dietary zinc in man: comparison of intrinsic and extrinsic labels using a triple stable isotope method. *American Journal of Clinical Nutrition* **36**, 537–545.

Lonnerdal, B. (1989). Food and dietary factors influencing levels and bioavailability of trace elements. In *Nutrient Availability: Chemical and Biological Aspects*, pp. 131–143 [D. Southgate, I. Johnson and G. R. Fenwick, editors]. Cambridge: Royal Society of Chemistry.

Mason, P. M., Judd, P. A., Fairweather-Tait, S. J., Eagles, J. & Minski, M. J. (1990). The effect of moderately increased intakes of complex carbohydrates (cereals, vegetables and fruit) for 12 weeks on iron and zinc metabolism. *British Journal of Nutrition* **63**, 597–611.

Navert, B., Sandstrom, B. & Cederblad, A. (1985). Reduction of the phytate content of bran by leavening in bread and its effect on zinc absorption in man. *British Journal of Nutrition* **53**, 47–53.

Parker, F. & Peterson, N. F. (1965). Quantitative analysis of phospholipids and phospholipid fatty acids from silica gel thin-layer chromatography. *Journal of Lipid Research* **6**, 455–460.

Payne, R. W., Lane, P. W., Ainsley, A. E., Bicknell, K. E., Digby, P. G. N., Harding, S. A., Leech, P. K., Simpson, H. R., Todd, A. D., Verner, P. J., White, R. P., Gower, J. C., Tunnicliffe Wilson, G. & Paterson, L. J. (1987). *Genstat 5 Reference Manual*. Oxford: Clarendon Press.

Sandstrom, B., Arvidsson, B., Cederblad, A. & Bjorn-Rasmussen, E. (1980). Zinc absorption from composite meals. I. The significance of wheat extraction rate, zinc, calcium, and protein content in meals based on bread. *American Journal of Clinical Nutrition* **33**, 739–745.

Sandstrom, B., Davidsson, L., Kivisto, B., Hasselblad, C. & Cederblad, A. (1987). The effect of vegetables and beet fibre on the absorption of zinc in humans from composite meals. *British Journal of Nutrition* **58**, 49–57.

Snedecor, G. W. & Cochran, W. G. (1967). In *Statistical Methods*, pp. 91–100. Ames, IA: Iowa State University Press.

Solomons, N. W. (1982). Biological availability of zinc in humans. *American Journal of Clinical Nutrition* **35**, 1048–1075.

Solomons, N. W., Janghorbani, M., Ting, B. T. G., Steinke, F. H., Christensen, M., Bijlani, R., Istfan, N. & Young, V. R. (1982). Bioavailability of zinc from a diet based on isolated soy protein: application in young men of the stable isotope tracer, ^{70}Zn. *Journal of Nutrition* **112**, 1809–1821.

Valberg, L. S, Flanagan, P. R., Brennan J. & Chamberlain, M. J. (1985). Does the oral zinc tolerance test measure zinc absorption? *American Journal of Clinical Nutrition* **41**, 37–42.

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