Folic acid enrichment of bread does not appear to affect zinc absorption in young women[1–3]

Marianne Hansen, Samir Samman, Lene T Madsen, Mikael Jensen, Sven S Sørensen, and Brittmarie Sandström

ABSTRACT

Background: In several countries cereals are now enriched with folic acid to reduce the risk of neural tube defects. Human studies suggest that folic acid interferes with zinc absorption. This raises concerns about the zinc status of high-risk groups such as infants, pregnant women, and older persons.

Objective: We sought to determine the effect of added folic acid on zinc absorption from white bread with high and low zinc contents.

Design: Zinc absorption was measured in 15 healthy women (22–33 y), each of whom consumed 4 single meals spaced 2 wk apart in a randomized crossover design. The servings of bread (100 g) differed in zinc and folic acid contents as follows: A, 1.2 mg Zn and 17 µg folic acid; B, 1.2 mg Zn and 144 µg folic acid; C, 3.0 mg Zn and 17 µg folic acid; and D, 2.9 mg Zn and 144 µg folic acid. Meals were extrinsically labeled with ^{65}Zn and absorption was estimated from whole-body retention measurements. Folate status was assessed by measuring plasma and erythrocyte folate and plasma homocysteine concentrations.

Results: Mean (±SD) zinc absorption did not differ significantly in relation to the folate content of the breads at either the low zinc content (38.8 ± 13.5% and 40.6 ± 16.5% for A and B, respectively; $P = 0.74$) or the high zinc content (26.7 ± 9.3% and 22.7 ± 6.6% for C and D, respectively; $P = 0.16$). There was no significant correlation between folate status and zinc absorption ($r < 0.3$, $P > 0.1$).

Conclusion: Fortification of white bread with a commonly used amount of folic acid did not appear to influence zinc absorption at either a high or a low zinc content. *Am J Clin Nutr* 2001; 74:125–9.

INTRODUCTION

The fortification of cereals with folic acid has been implemented in several countries, such as the United Kingdom, Canada, Australia, and recently the United States. The purpose of folic acid fortification is to reduce the risk of neural tube defects in newborns. There is convincing evidence that folic acid supplementation, when taken from the onset of pregnancy, reduces the prevalence of neural tube defects (1). An additional effect of folic acid fortification may be a lowered risk of cardiovascular diseases. Supplementation with folic acid has been found to decrease the serum homocysteine concentration, an independent risk factor for cardiovascular disease (2).

Increasing the intake of folic acid through supplementation or fortification is generally considered safe. However, concern was raised in some (3–10) but not all (11–16) studies about a potential negative effect of folic acid supplementation on zinc absorption. Decreased zinc absorption could have adverse effects, particularly in individuals with increased requirements, such as children, adolescents, and pregnant or lactating women. Furthermore, many elderly persons may already be at risk of marginal zinc status because of poor appetites and inadequate dietary intakes.

Supplementation with 400 µg folic acid every other day was found to influence zinc homeostasis in men during a long-term, parallel balance study. At this dose of folic acid, fecal zinc increased when the diet had a low or moderate zinc content, but not when the diet was high in zinc. Urinary zinc decreased during the low- and moderate-zinc diets, which partially compensated for the fecal losses (5). In another study, 800 µg folic acid/d was suggested to have a negative effect on zinc absorption (measured by ^{65}Zn whole-body counting) only in subjects with a fractional zinc absorption >30%. These subjects also tended to have lower zinc intakes and lower plasma zinc concentrations (6). These studies suggest that folic acid inhibits zinc absorption and that this inhibition is more pronounced with low-zinc meals than with high-zinc meals.

The results of plasma response studies are conflicting. Plasma zinc response to a 25-mg dose of zinc was reduced to 49% in

10 pregnant women after 2 wk of supplementation with 350 μg folic acid and 100 mg Fe/d compared with the baseline response before supplementation (7). In the same study, the plasma zinc response to a 50-mg dose of zinc was reduced to 79% of the baseline value after 2 wk of supplementation with 350 μg folic acid/d in 10 young men and women. Other authors who used the plasma zinc response method found that megadoses of folic acid [10 mg (11) and 200 mg (17)] had no effect on zinc absorption.

In general, the results of research in this area are inconsistent, which could be the result of inappropriate methodology and incomparability of study designs. There is still no scientific basis for a conclusion regarding the potential effect of folate on zinc absorption and metabolism.

The aim of the present study was to determine the effect of folic acid fortification of bread on zinc absorption by using a radioisotope technique with measurements of whole-body retention. We chose women of childbearing age as subjects because they represent a specific target group that is thought to benefit from fortification with folic acid and that may also be vulnerable if zinc absorption is compromised.

SUBJECTS AND METHODS

Experimental design

Each subject consumed 4 different bread meals that were served in random order and spaced 2 wk apart. Two meals had a low zinc content and 2 had a high zinc content, with or without added folic acid (2 × 2 factorial design) in amounts commonly used in fortification. The meals were extrinsically labeled with the radioisotope ^{65}Zn. Zinc absorption was calculated by measuring whole-body retention of the isotope. A blood sample was obtained from each subject before each of the 4 test meals to measure plasma folate, erythrocyte folate, and serum zinc concentrations. Homocysteine concentrations were measured in the first and last blood samples. The habitual dietary intake of selected nutrients was assessed by using four 2-d weighed-food records spread over 2 mo. Nutrient intakes were calculated by using a national food-composition database (DANKOST 2000, version 1.4c; Dansk Catering Service, Herlev, Denmark).

Subjects

Fifteen women aged 22–33 y participated in this study; the subjects' mean (±SD) body weight was 66.3 ± 7.9 kg and their mean body mass index (in kg/m^2) was 22.7 ± 3.1. All subjects were apparently healthy, and none were pregnant or lactating. None of the subjects took any vitamin or mineral supplements or donated blood during the study or in the previous 2 mo. None used medicine regularly or took oral contraceptives. Five smoked occasionally, whereas one was a regular smoker (<10 cigarettes/d for all).

The study was approved by the Municipal Ethical Committee of Copenhagen and Frederiksberg (KF 01–238/98) and the National Institute of Radiation Hygiene, Denmark. The subjects were given written and oral information about the study and written consent was obtained from all subjects.

Formulation of test meals and serving procedure

Each test meal consisted of 2 rolls made from white (refined) wheat flour (total wt: 100 g), 30 g raspberry jam, 10 g butter, and 300 g ultrapure water. The rolls were formulated with 2 different amounts of zinc in the presence or absence of added folic acid as follows: A, low zinc; B, low zinc + folic acid; C, high zinc; and D, high zinc + folic acid. Each type of roll was prepared in one batch and stored at −20 °C. The basic recipe (without zinc or folic acid added) was 1500 g white wheat flour, 900 g ultra-pure water, 30 g fresh yeast, 24 g butter, and 21 g NaCl. For the batches fortified with folic acid, 10.8 mg crystalline folic acid (Sigma Chemical Co, St Louis) was mixed with 3000 g flour in a food processor for 90 min at low speed. This equals 262 μg folic acid/serving (2 rolls), calculated as the value before baking, ie, without baking loss. The zinc content was adjusted to 1 and 3 mg/serving in the low-zinc (A) and high-zinc (C) rolls, respectively, by adding a ZnCl$_2$ solution (prepared from ZnO and 37% HCl; Merck, Darmstadt, Germany) to the dough. After mixing, the dough was allowed to rise for 1 h at room temperature and rolls consisting of 60 ± 0.5 g dough were prepared. The subjects received the meal after fasting for 12 h and were instructed to consume it within 15 min and to alternate between eating and drinking. The meals were served on disposable material. The subjects were told not to eat or drink for 4 h after the meal.

Isotopes and labeling procedures

For each test meal, the rolls were extrinsically labeled with 0.04 MBq ^{65}Zn by adding drops of the isotope solution (1 mL total) to the top of the baked rolls. The radioisotopes were purchased at Risø National Laboratory (Roskilde, Denmark) as ^{65}ZnCl$_2$ in 0.1 mol HCl/L with a specific activity of 35 MBq/mg Zn. The total zinc contribution to each meal from the radioisotope was in the order of 1 μg, which represents <0.1% of the total zinc. The labeling was undertaken in the presence of an observer ≈18 h before the test meal was served. The calculated total radiation dose from the 4 meals for each subject was 0.56 mSv.

Whole-body counting and calculation of zinc absorption

Whole-body retention of ^{65}Zn was measured in a whole-body counter on day 12 or 13 after each test meal. This time period was chosen to allow for the excretion of unabsorbed isotope. The results were corrected for background radiation in the chamber and the subject's background radioisotope level (^{40}K). Zinc absorption was calculated from the whole-body retention value, which was corrected for endogenous excretion from days 0 to 12 or 0 to 13. This correction was performed by using a mean retention function developed from measurements of whole-body retention over time in a group of healthy subjects after they had received an intravenous dose of ^{65}Zn (18). When performing calculations for the second, third, and fourth meals, allowance was made for residual activity in the subjects from the preceding test meals by using the same equation. All values were corrected for physical decay.

The whole-body counter, located at the National University Hospital in Copenhagen, consisted of a lead-lined steel chamber with 4 plastic scintillator blocks (NE110; Nuclear Enterprises Limited, Edinburgh), 2 placed above the subject and 2 placed below. The counting efficiency and the setting of the energy window were established by measuring 3 water-filled phantoms, each containing 0.04 MBq ^{65}Zn. The phantoms weighed 55, 66, and 77 kg and had outlines of typical humans of corresponding weights. A slight decrease in counting efficiency was observed with increasing weight. A linear interpolation was used to calculate the actual counting efficiency for each individual subject according to her weight. The overall counting efficiency for the 66-kg phantom was 0.2 counts·s^{-1}·Bq^{-1} ^{65}Zn. The energy

TABLE 1
Folate and zinc contents of the test meals (bread rolls) per serving and zinc absorption

Test meals	Folate[1]	Zinc[2]	Zinc absorption[3]
	μg	mg	% (mg)
A: Low zinc	17	1.2	38.8 ± 13.5 $(0.47 \pm 0.16)^{a,4}$
B: Low zinc + folic acid	144 ± 13	1.2	40.6 ± 16.5 $(0.49 \pm 0.20)^{a}$
C: High zinc	17	3.0	26.7 ± 9.3 $(0.78 \pm 0.27)^{b}$
D: High zinc + folic acid	144 ± 13	2.9	22.7 ± 6.6 $(0.68 \pm 0.20)^{a,b}$

[1] Endogenous folate was analyzed in unfortified bread (A and C), $n = 2$. Folate in B and D ($n = 5$) includes endogenous folate and added folic acid.

[2] $n = 2$ for each kind of bread.

[3] For % absorption, there were no significant differences between A and B or between C and D (paired t tests). For mg absorption, values with different superscript letters are significantly different, $P < 0.05$ (ANOVA followed by multiple comparisons). The differences between A and D and between B and D were marginally significant ($P = 0.05$).

[4] $\bar{x} \pm SD$.

window was set at a lower level of ≈ 80 keV, allowing detection of the compton events (inelastic scattered γ-photons) in the plastic scintillators from both 511 and 1115 keV radiation from ^{65}Zn. No upper energy limit was used. To minimize contamination by atmospheric background activity, the subjects showered and washed their hair and then dressed in hospital clothing before each measurement. Each measurement of whole-body retention lasted for 10 min.

Dietary analyses

Two rolls from each batch were pooled, freeze-dried, homogenized, and analyzed in duplicate for zinc content by using atomic absorption spectrometry (Spectr-AA 200; Varian, Mulgrave, Victoria, Australia) after microwave digestion. A reference material [Standard Reference Material 1548, total diet (mixed food sources); National Institute of Standards and Technology, Gaithersburg, MD] was analyzed for zinc content in the same run as the meal samples. The zinc content of the standard was measured as 26.45 μg/g, compared with the certified value of 24.6 $\pm$ 1.79 μg/g. The folate-fortified ($n = 5$) and unfortified ($n = 2$) rolls were analyzed in duplicate for folic acid content by turbidimetric measurement on a spectrophotometer (UV160A; Shimadzu Corp, Kyoto, Japan) by using *Lactobacillus casei* as a test organism, according to the method of Finglas et al (19). The analyses of the unfortified rolls represented the background analytic amounts of folate equivalents (analyzed as folic acid) in the rolls. An internal reference was included (0.68 μg/g with an average laboratory value of 0.62 μg/g).

Blood analyses

Four blood samples (1 for each of the 4 test meals) were obtained from each subject after a 12-h fast and 10 min of rest in the supine position. Subjects were asked to refrain from physical exercise for 36 h and to refrain from using alcohol or medication for 24 h before blood sampling. Serum zinc concentrations were analyzed by using flame atomic absorption spectrometry (Spectra AA-200; Varian). The intraassay CV was 2.0% and the interassay CV was 7.6%. An internal control was included in all runs (15.3–15.5 μmol/L with an average laboratory value of 15.2 μmol/L). Hemoglobin concentrations and hematocrit values were determined on a Cobas Minos ABX automatic cell counter (Groupe Hoffmann, Montpellier, France).

The intraassay CVs were 1.0% and 1.6% for hemoglobin and hematocrit, respectively, and the corresponding interassay CVs were 1.7% and 2.4%, respectively. Blood collected in heparin-containing tubes was used for plasma and erythrocyte folate analyses. For erythrocyte folate, 50 μL blood was incubated with 1 mL ascorbic acid (L-ascorbic acid; Merck) solution (0.5% wt:vol in distilled water) for 20 h in the dark at room temperature. Erythrocyte and plasma folate concentrations were analyzed fluorometrically (Delfia 1232; Wallac OY, Turku, Finland) with a time-resolved fluoroimmunoassay kit (Delfia Folate Kit A072–101; Wallac OY) (20). The interassay CVs were 6.5% and 3.4% for plasma and erythrocyte folate, respectively. Plasma homocysteine concentrations were analyzed fluorometrically with a fluorescence polarization immunoanalysis kit (IMx Homocysteine kit 3D3920–20; Abbott Laboratories, Chicago) (21). An internal control was included (12.3 μmol/L with an average laboratory value of 12.5 μmol/L; intraassay CV, 1.9%; interassay CV, 4.1%).

Statistical analyses

Values are expressed as means $\pm$ SDs. To compare fractional zinc absorption, we performed pairwise comparisons (paired t tests) between the 2 high-zinc meals and also between the 2 low-zinc meals. Pairwise comparisons were performed because of the inherent inverse relation between zinc dose and fractional absorption. Absolute zinc absorption values for all 4 meals were compared by using a repeated-measures two-factor analysis of variance with an interaction term. When significance was reached, we performed a modified t test according to the Bonferroni method. MICROSOFT EXCEL 2000 software (Microsoft Corp, San Francisco) and the STATISTICAL ANALYSIS SYSTEM (SAS) statistical package, version 6.12 (SAS Institute Inc, Cary, NC) were used for the statistical analyses.

RESULTS

The zinc and folate contents of the test meals are shown in **Table 1**. We had added 262 μg folic acid to each serving of rolls, and 144 μg folic acid was recovered in the rolls after baking. After we accounted for the endogenous folate content (17 μg folate equivalents) of the unenriched rolls, the recovery of added folic acid was calculated to be 48%. Consequently, the baking loss was $\approx 52\%$.

Addition of folic acid did not influence zinc absorption from either the low-zinc or the high-zinc meal (Table 1). Fractional zinc absorption was higher from the 2 low-zinc meals (38.8–40.6%) than from the 2 high-zinc meals (22.7–26.7%), whereas in absolute amounts, zinc absorption was higher from the high-zinc meals. There was no significant interaction between zinc content and addition of folic acid.

The subjects' habitual daily intakes of energy and selected nutrients were 9.9 $\pm$ 1.2 MJ, 12 $\pm$ 2% of energy as protein, 26 $\pm$ 3% of energy as fat, 58 $\pm$ 5% of energy as carbohydrate, 23 $\pm$ 5 g fiber, 9.3 $\pm$ 2.0 mg Zn, and 308 $\pm$ 60 μg folate. Dietary zinc and folate intakes met the Nordic Nutrient Recommendations of 7 mg and 300 μg, respectively (4). The subjects' concentrations of serum zinc, plasma and erythrocyte folate, and plasma homocysteine were within reference ranges for the Danish population (**Table 2**). Zinc absorption was not affected by folate status, because neither erythrocyte folate nor plasma folate correlated with fractional zinc absorption ($r < 0.3$, $P > 0.1$).

TABLE 2
Serum zinc and blood indexes of folate status[1]

	Serum zinc[2]	Plasma folate[2]	Erythrocyte folate[2]	Plasma homocysteine[3]
	μmol/L	*nmol/L*	*nmol/L*	*μmol/L*
$\bar{x} \pm$ SD	15.4 ± 1.3	13.1 ± 3.8	436 ± 84	6.4 ± 1.9
Range	14.3–17.0	6.2–28.1	305–645	4.0–12.7
Reference range[4]	10.0–19.0	1.7–23.5	225–676	5.0–12.0

[1] $n = 15$.

[2] Average of 4 blood samples (1 obtained at each of the 4 test meals).

[3] Average of 2 blood samples (1 obtained at the beginning and 1 obtained at the end of the study).

[4] Laboratory reference range for the Danish population.

DISCUSSION

Folic acid is highly bioavailable from bread, according to previous research (22). In the present study, we added folic acid to test meals (bread rolls) in amounts typically used in fortification. The zinc contents of the test meals also corresponded to realistic dietary intakes. Two different amounts of zinc (low and high) were used because previous studies suggested that folic acid inhibits zinc absorption from low-zinc diets (5, 6). Consistent with previous results (23), the fractional absorption of zinc was substantially higher from the low-zinc meals than from the high-zinc meals (Table 1) and the absolute amount of zinc absorbed was approximately 1.5-fold greater from the high-zinc meals. The extent of zinc absorption in the present study is consistent with previous research that included a range of zinc intakes from high-bioavailability diets (24). Our results showed no significant differences in zinc absorption from bread with and without folic acid fortification at either low or high zinc contents. However, a small inhibitory effect of folic acid on zinc absorption from the high-zinc meals cannot be excluded. A posthoc power analysis showed that with a power of 80% ($\alpha = 0.05$ and $\beta = 0.8$), we would be able to detect only an 8% or larger difference. However, it is unlikely that the observed 4% lower zinc absorption would be of any nutritional significance.

The mechanism for a potential folate-zinc interaction could operate over the long term, rather than at one moment in time. If a high folate content of a single meal does inhibit zinc absorption, then it is possible that a high folate intake over the long term would lead to low zinc absorption from a single meal. In accordance with the lack of effect of folic acid content on zinc absorption in this single-meal study, there was no correlation between markers of folate status and fractional zinc absorption.

The folic acid content of the fortified bread rolls used in the present study was 144 μg/100 g. After correction for the endogenous folate content of the flour, the baking loss of added folic acid was 52%. This loss was substantially higher than the losses from bread reported by others [8–14% (25) and 30% (22)]. The baking loss of added folic acid may be related to the size and shape of the bread. The 144 μg folic acid in 100 g bread was higher than the amount that would be obtained by using flour fortified with 140 μg folic acid/100 g, the standard of the Food and Drug Administration (26). Thus, our data show that even with folic acid fortification at the high end of the recommended range, there was no significant interaction with zinc absorption.

Although several studies investigated the effect of folic acid on zinc absorption, limitations in the study designs and methodologies may have contributed to the inconsistent findings. In several studies, the plasma zinc response to an oral zinc challenge was measured to assess the effect of folic acid on zinc absorption (7, 11, 17). Some disadvantages of this method include the use of pharmacologic doses of zinc and the confounding effects of large fluctuations in the rate of gastric emptying and blood clearance and urinary excretion of zinc. These limitations are reflected by large inter- and intraindividual variations (27, 28) and poor concordance with isotope methods (27). Pharmacologic doses of folic acid were also used commonly in studies of folate-zinc interaction (11, 13, 17). At unphysiologically high doses of folate, zinc, or both, other factors may be responsible for an observed interaction. These factors may include saturation or alternation of uptake mechanisms in the brush border membrane because of stressing of the absorptive mechanisms. This may explain why inconsistent results were obtained in studies with folate-to-zinc molar ratios ranging from 1:1 (17) to 1:975 (7). Another methodologic problem is the lack of sensitive and valid indexes of zinc status; this may explain why no consistent relation between folate intake or status and zinc status has been found (10, 12–14, 29). In accordance with this, several studies found no effect of folate supplementation on serum zinc concentrations during pregnancy (13, 14, 29).

The strengths of the present study were the use of isotope labeling and a whole-body counting technique for measurement of zinc absorption and the use of nonpharmacologic doses of zinc and folic acid.
Isotope techniques generally provide more accurate and precise results than do other methods, but few published studies used isotope techniques. Milne (6) used ^{65}Zn labeling and the whole-body counting technique for determining zinc absorption from single meals. Our data are in accordance with those of Milne, who found no significant difference in zinc absorption between a control meal providing 2.7 mg Zn (equivalent to our high-zinc meal) and the same meal supplemented with 800 μg folic acid (a 5.5-fold greater dose than that used in our test meals). In Milne's study, ^{65}Zn absorption from the control meal was plotted against the difference in absorption between the folic-acid-supplemented and control meals, and a linear correlation was found. On the basis of this finding, Milne concluded that zinc absorption is inhibited by folic acid; however, by using the author's reasoning it can be argued equally well that folic acid promotes zinc absorption.

The long-term effect of folic acid on zinc status per se cannot be evaluated effectively in humans because reliable biomarkers of zinc status are not available. Therefore, we must rely on zinc absorption studies. On the basis of the present results obtained with radioisotope labeling and whole-body counting, it is not likely that fortification with the currently used concentrations of folic acid is cause for any concern. However, it would be relevant to study the effects of higher doses of folic acid, as are found in supplements, on zinc absorption with the same method.

REFERENCES

1. Scott JM, Weir DG, Kirke PN. Folate and neural tube defects. In: Bailey LB, ed. Folate in health and disease. New York: Marcel Dekker, 1995:329–60.
2. Boushey CJ, Beresford SA, Omenn GS, Motulsky AG. A quantitative assessment of plasma homocysteine as a risk factor for vascular

disease. Probable benefits of increasing folic acid intakes. JAMA 1995;274:1049–57.

3. Department of Health. Report on health and social subjects, 41. Dietary reference values for food, energy and nutrients for the United Kingdom. London: Her Majesty's Stationery Office, 1991.

4. Nordic Committee on Foods. Nordic nutrition recommendations. Copenhagen: Nordic Council of Ministers, 1996.

5. Milne DB, Canfield WK, Mahalko JR, Sandstead HH. Effect of oral folic acid supplements on zinc, copper, and iron absorption and excretion. Am J Clin Nutr 1984;39:535–9.

6. Milne DB. Effects of folic acid supplements on zinc-65 absorption and retention. J Trace Elem Exp Med 1989;2:297–304.

7. Simmer K, Iles CA, James C, Thompson RP. Are iron-folate supplements harmful? Am J Clin Nutr 1987;45:122–5.

8. Keizer SE, Gibson RS, O'Connor DL. Postpartum folic acid supplementation of adolescents: impact on maternal folate and zinc status and milk composition. Am J Clin Nutr 1995;62:377–84.

9. Butterworth CE Jr, Tamura T. Folic acid safety and toxicity: a brief review. Am J Clin Nutr 1989;50:353–8.

10. Mukherjee MD, Sandstead HH, Ratnaparkhi MV, Johnson LK, Milne DB, Stelling HP. Maternal zinc, iron, folic acid, and protein nutriture and outcome of human pregnancy. Am J Clin Nutr 1984; 40:496–507.

11. Keating JN, Wada L, Stokstad EL, King JC. Folic acid: effect on zinc absorption in humans and in the rat. Am J Clin Nutr 1987;46:835–9.

12. Kauwell GP, Bailey LB, Gregory JF, Bowling DW, Cousins RJ. Zinc status is not adversely affected by folic acid supplementation and zinc intake does not impair folate utilization in human subjects. J Nutr 1995;125:66–72.

13. Butterworth CE Jr, Hatch K, Cole P, et al. Zinc concentration in plasma and erythrocytes of subjects receiving folic acid supplementation. Am J Clin Nutr 1988;47:484–6.

14. Tamura T, Goldenberg RL, Freeberg LE, Cliver SP, Cutter GR, Hoffman HJ. Maternal serum folate and zinc concentrations and their relationships to pregnancy outcome. Am J Clin Nutr 1992;56:365–70.

15. Fuller NJ, Evans PH, Howlett M, Bates CJ. The effects of dietary folate and zinc on the outcome of pregnancy and early growth in rats. Br J Nutr 1988;59:251–9.

16. Krebs NF, Hambidge KM, Hagerman RJ, et al. The effects of pharmacological doses of folate on zinc absorption and zinc status. Am J Clin Nutr 1988;47:783 (abstr).

17. Arnaud J, Favier A, Herrmann MA, Pilorget JJ. Effect of folic and folinic acids on zinc intestinal absorption. Ann Nutr Metab 1992;36: 157–61.

18. Arvidsson B, Cederblad Å, Björn-Rasmussen E, Sandström B. A radionuclide technique for studies of zinc absorption in man. Int J Nucl Med Biol 1978;5:104–9.

19. Finglas PM, Faure U, Southgate DAT. First BCR-intercomparison on the determination of folates in food. Food Chemistry 1993;46: 199–213.

20. Lövgren T, Hemmilä I, Petterson K, Halonen P. Time-resolved fluorometry in immunoassays. In: Collins WP, ed. Alternative immunoassays. Chichester, United Kingdom: John Wiley & Sons Ltd, 1985: 203–19.

21. Shipchandler MT, Moore EG. Rapid, fully automated measurement of plasma homocyst(e)ine with the Abbott IMx Analyzer. Clin Chem 1995;41:991–4.

22. Pfeiffer CM, Rogers LM, Bailey LB, Gregory JF III. Absorption of folate from fortified cereal-grain products and of supplemental folate consumed with or without food determined by using a dual-label stable-isotope protocol. Am J Clin Nutr 1997;66:1388–97.

23. Zinc. In: Trace elements in human nutrition and health. Report of a Joint Food and Agriculture Organization/International Atomic Energy Agency/World Health Organization Expert Consultation. Geneva: World Health Organization, 1996:72–105.

24. Sandström B, Cederblad Å. Zinc absorption from composite meals. II. Influence of the main protein source. Am J Clin Nutr 1980; 33:1778–83.

25. Keagy PM, Stokstad ELR, Fellers DA. Folacin stability during bread processing and family flour storage. Cereal Chem 1975;52:348–56.

26. Food and Drug Administration. Food standards: amendment of standards of identity for enriched grain products to require addition of folic acid. Fed Regist 1996;61:8781–97.

27. Valberg LS, Flanagan PR, Brennan J, Chamberlain MJ. Does the oral zinc tolerance test measure zinc absorption? Am J Clin Nutr 1985;41:37–42.

28. Samman S, van-het-Hof K, Snitch P. The reproducibility of the plasma response to a physiological dose of zinc in healthy subjects. Implications for study design. Biol Trace Elem Res 1993;37: 201–7.

29. Hambidge M, Hackshaw A, Wald N. Neural tube defects and serum zinc. Br J Obstet Gynaecol 1993;100:746–9.