

Total zinc absorption in young women, but not fractional zinc absorption, differs between vegetarian and meat-based diets with equal phytic acid content†

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Zn bioavailability is often lower in vegetarian diets mainly due to low Zn and high phytic acid contents. The objective of the present study was to determine the fractional and total absorption of Zn from a vegetarian diet in comparison with meat diets with equal concentrations of phytic acid. A randomized cross-over design, comprising three whole-day diet periods of 5 d each, with a vegetarian diet or diets containing Polish-produced meat or Danish-produced meat, was conducted. Twelve healthy female subjects completed the study. All diets had a high content of phytic acid (1250 µmol/d) and in the meat diets the main meals contained 60 g pork meat. All main meals were extrinsically labelled with the radioactive isotope ⁶⁵Zn and absorption of Zn was measured in a whole-body counter. The mean Zn content of the whole-day diet was: Polish meat diet 9.9 (SE 0.14) mg, Danish meat diet 9.4 (SE 0.19) mg and vegetarian diet 7.5 (SE 0.18) mg. No difference was observed in the fractional absorption of Zn (Polish meat diet: 27 (SE 1.2)%, Danish meat diet: 27 (SE 1.9)% and vegetarian diet: 23 (SE 2.6)%). A significantly lower amount of total Zn was absorbed from the vegetarian diet (mean Zn absorption of Polish meat diet: 2.7 (SE 0.12) mg/d ($P < 0.001$), Danish meat diet: 2.6 (SE 0.17) mg/d ($P = 0.006$) and vegetarian diet: 1.8 (SE 0.20) mg/d). In conclusion, the vegetarian diet compared with the meat-based diets resulted in lower amounts of absorbed Zn due to a higher content of Zn in the meat diets, but no difference was observed in the fractional absorption of Zn.

Radioisotope: ⁶⁵Zn. Bioavailability: Micronutrients: Minerals

Zn is an essential mineral, incorporated in a substantial number of enzymes with important biological functions. A sub-optimal Zn status is not easily determined because there is no reliable biochemical indicator for Zn status and clinical symptoms of deficiency are subtle. The global prevalence of Zn deficiency is therefore difficult to estimate. Estimates have been made using indirect measures of Zn consumption patterns and the Zn content of the global food supply, indicating that approximately 50% of the world's population is at risk of inadequate Zn intake (Brown & Wuehler, 2000). Individuals at risk for low Zn status include people with low absorption, increased losses or increased needs due to growth or pregnancy, enforced by a low intake of Zn or diets with low bioavailability.

The bioavailability of Zn is greatly influenced by the composition of the diet, regarding both the concentration of Zn and the presence of enhancers and inhibitors of Zn absorption. Animal protein is a rich source of Zn and in addition has a possible enhancing effect on the overall absorption of Zn from the diet (Sandström & Cederblad, 1980; Sandström *et al.* 1989; Hunt *et al.* 1991, 1995). It is well known that phytic acid is a strong inhibitor of Zn absorption (Nävert *et al.* 1985; Sandström *et al.* 1987a) and a concomitant intake of protein seems to counteract the negative

effects on absorption induced by high intakes of phytic acid (Sandström *et al.* 1980, 1987a,c, 1990).

It is generally accepted that appropriately planned vegetarian diets can be healthy and nutritionally adequate, and that vegetarians have a lower prevalence of certain life-style diseases (Jenkins *et al.* 2003; Position of the American Dietetic Association and Dietitians of Canada, 2003). However, there are some concerns as to whether vegetarian diets adequately cover the requirements for selected minerals, especially Fe and Zn. The dietary intake of Zn in vegetarian diets is lower than or equivalent to the dietary intake of Zn of omnivores, but the bioavailability in vegetarian diets is generally lower, mainly due to a higher intake of phytic acid (Gibson, 1994; Hunt *et al.* 1998; Hunt, 2002, 2003; Murphy & Allen, 2003; Waldmann *et al.* 2003).

Previous studies identifying an enhanced absorption of Zn with an additional intake of protein were all meals with varying contents of phytic acid, which is known to be an important determinant for Zn absorption (Sandström & Cederblad 1980; Sandström *et al.* 1987c, 1989; Hunt *et al.* 1991; Johnson & Walker, 1992; Zheng *et al.* 1993; Hunt *et al.* 1995). With a few exceptions (Hunt *et al.* 1995, 1998) all previous studies have been based on a single meal. In relation to Fe absorption, which in some

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regards can be compared with Zn absorption, a potential discrepancy has been identified between single meal studies and whole meal studies, expressed as a higher absorption of Fe from single meal studies than from whole diet studies (Cook *et al.* 1991). To our knowledge, no whole-day absorption studies have investigated the bioavailability of Zn in vegetarian diets compared with meat diets with similar phytic acid contents over five consecutive days.

The aim of the present study was to determine the absorption of Zn from a vegetarian diet in comparison with a meat diet, with equal concentrations of phytic acid in all diets. The pork meat was produced by a conventional production system in Denmark and Poland.

Subjects and methods

Subjects

Sixteen healthy women, mean age 24.2 (SD 3.6) years, with a mean BMI of 21.6 (SD 1.8) kg/m², were included in the study. All subjects were omnivores, non-smoking, not pregnant or lactating nor exercising heavily. The subjects did not take any medication or vitamin, mineral or other supplements, and did not donate blood, during and for at least 2 months prior to the study. Eleven subjects took oral contraceptives. Four subjects dropped out of the study; one moved out of the area and the remaining three due to personal reasons.

All subjects were informed, orally and in writing, about the study. Written consent of participation, stating that the subject participated voluntarily and was free to withdraw from the study at any time, was obtained before enrolment. The study protocol was approved by the Ethics Committee of Copenhagen and Frederiksberg (authorization number (KF) 01-197/02) and the National Institute of Radiation Hygiene, Denmark.

Study design

The absorption of Zn was measured in a cross-over design with three types of full-day diets, two meat diets and a vegetarian diet, during three periods of 5 d each (Monday–Friday). The study is a parallel design to the Fe absorption study from vegetable- and meat-based diets by Bach Kristensen *et al.* (2005). The study design, composition of the test meals and serving procedures are thoroughly described in the mentioned article (Bach Kristensen *et al.* 2005). All diets consisted of a basic diet, containing relatively high amounts of phytic acid. The two meat diets contained either of two meat sources (longissimus dorsi from pigs produced in Poland or Denmark) and breakfast, lunch and dinner each contained 60 g meat.

All breakfast, lunch and dinner meals were extrinsically labelled with ⁶⁵Zn and served at the Department of Human Nutrition. A 2 d menu cycle was served throughout the week (Table 1) and did not differ between study periods. The study design is illustrated in Fig. 1. The subject's individual weight was recorded at baseline and prior to the final whole-body counting, using the same digital scale.

Meat was obtained from The Danish Institute of Agricultural Sciences, Foulum and The Kielanowski Institute of Animal Physiology and Nutrition as part of the 5th Framework Project SUSPORKQUAL, where it was previously shown that the pork meat from Poland had a higher Zn content than the Danish

Table 1. Composition of the whole-day diet of 10MJ*

| | Meat diet (g) | Vegetarian diet (g) |
|---------------------------------|---------------|---------------------|
| Breakfast | | |
| Whole-grain wheat bread | 100 | 100 |
| Pâté† | 106 | 54 |
| Butter | 15 | 15 |
| Marmalade (raspberry/apricot)‡ | 20 | 20 |
| Raisins | 25 | 25 |
| Carbonated drinks | – | 195 |
| Lunch | | |
| Whole-grain wheat bread | 100 | 100 |
| Pâté | 106 | 54 |
| Butter | 15 | 15 |
| Beetroot (pickled)/carrots‡ | 30/40 | 30/40 |
| Carbonated drinks | – | 195 |
| Dinner | | |
| Whole-grain pasta /brown rice‡§ | 80 | 80 |
| Casserole | 250 | 205 |
| Whole-grain wheat bread | 40 | 40 |
| Carbonated drinks | – | 195 |
| Snack | | |
| Chocolate | 49 | 49 |
| Dried apricots | 75 | 75 |
| Apple/pear‡ | 160 | 160 |

* Breakfast, lunch and dinner each contained 60 g pork meat in the meat diets (raw weight).

† Pork (only in meat diets), rapeseed oil, eggs, spinach, mushroom, almond, onion, coriander, gelatine, salt.

‡ The variation of the 2 d menu cycle.

§ Raw weight.

|| Pork (only in meat diets), rapeseed oil, mushroom, carrot, canned tomato, tomato purée, onion, garlic, beer, salt.

meat. Breakfast, lunch and dinner meals were extrinsically labelled by adding 1 ml radioisotope solution (Risø National Laboratory, Roskilde, Denmark) to the main meal > 16 h prior to consumption. Each subject received a total dose of 120 kBq ⁶⁵Zn from the forty-five meals.

Content of protein, fat, carbohydrate, vitamin C and calcium was calculated using the Dankost 2000 dietary assessment software (Danish Catering Center, Herlev, Denmark).

Determination of zinc absorption

All subjects' individual background radiation was measured at baseline by whole-body retention and Zn absorption from the full-day diets was estimated by whole-body retention of ⁶⁵Zn 21 d after consumption of extrinsically labelled meals, which also accounted for baseline for the following intervention week. The natural decay of the radioisotope was accounted for in the calculations of the amount of absorbed isotope. The measurements were performed in a lead-lined steel chamber with four NE110 plastic scintillator blocks (Nuclear Enterprises Ltd, Edinburgh, UK) connected to conventional nuclear electronic modules and a multichannel analyser system. The counting efficiency and energy window settings were established through measurements of water-filled phantoms whose outlines and weights were approximately equal to those of the subjects of the present study. The phantoms were filled with the amount of isotope corresponding to the amount received by each subject. In the actual set-up and with the energy window used, the overall counting efficiency for ⁶⁵Zn evenly distributed in a 77 kg phantom was approximately 20%. To minimize contamination by atmospheric background activity, all subjects had a shower, washed their hair and were dressed in hospital clothing

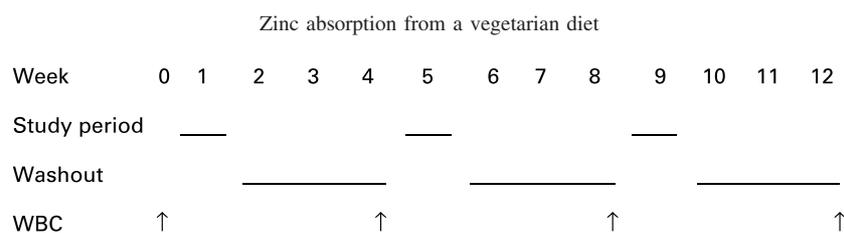


Fig. 1. Design of the study, indicating three study periods of 5 d with 21 d of washout and four whole-body countings (WBC) for measurements of zinc absorption. Isotope ^{65}Zn was administered to all main meals on all five days of the study periods.

before each measurement. The counting time was 10 min, and the results were corrected for chamber background radiation and the subject's background radiation radioisotope level measured 3 d before the beginning of the first intervention period. All results from the determination of radioactivity measurements were corrected for the physical decay of the isotope. Results are expressed as fractional absorption (percentage of ingested amount) and total absorption.

Chemical analysis

Zn content was analysed in triplicate portions of complete 10 MJ diets (Knudsen *et al.* 1996). The foods were homogenized, lyophilized and microwave-digested (MES-1000; CEM Corporation, Matthews, NC, USA) with HNO_3 65% suprapur (Merck, Darmstadt, Germany) and 30% H_2O_2 suprapur (Merck). Zn was measured by atomic absorption spectroscopy (Spectra AA-200 VARIAN; Varian Techtron Pty Ltd, Victoria, Australia). The Zn standard was prepared from a standard solution of 1000 mg Zn/l (Tritisol[®]; Merck) and a reference diet (Standard Reference Material 1548a, Typical Diet; National Institute of Standards and Technology, Gaithersburg, MD, USA) was analysed in the same run with a certified value of 24.6 (SD 1.79) $\mu\text{g Zn/g}$. Analysed values were (n 2) 22.7 $\mu\text{g/g}$ and 25.3 $\mu\text{g/g}$. The concentration of phytic acid was determined using the method of Carlsson *et al.* (2001).

Statistical analysis

All analyses and calculations were performed using the Strategic Analysis System software package, version 8.2 (SAS Institute, Cary, NC, USA). Univariate mixed-model ANOVA were performed in the procedure MIXED. In the statistical model, the Zn absorption measured at the end of each period was evaluated as the dependent variable. Treatment, order, period and treatment \times order were included as independent fixed variables. Subjects were included as a random effect. Homogeneity of variance and normal distribution among random effects were investigated by plots of residuals. The Shapiro–Wilk test for normal distribution was performed.

Results

Breakfast, lunch and dinner meals were all extrinsically labelled and consumed at the department under supervision of a staff member. Compliance to the dietary intervention was evaluated to be high. All subjects remained weight-stable throughout the study.

The dietary composition of the whole-day diet (10 MJ) is outlined in Table 2. Content of protein and fat was higher in the

meat diets, since carbonated drinks substituted the equivalent energy contribution from the meat. The evening snack, the only meal not extrinsically labelled, contained approximately 10% of the daily intake of Zn in the meat diets and 18% of the Zn content in the vegetarian diet. The amount of Zn was evenly distributed in the three main meals.

The average daily energy intake was 10 (SE 0.18) MJ. The intake, fractional absorption and total absorption of Zn are outlined in Table 3. The Zn intake from the meat diets was 20–24% higher than that from the vegetarian diet. Fractional Zn absorption from the meat diets was not different from the vegetarian diet. However, the total Zn absorption from the meat diets increased significantly, by 45% ($P=0.009$) and 50% ($P<0.001$) for the Danish meat diet and Polish meat diet, respectively, compared with the vegetarian diet. No difference was observed in either fractional or total Zn absorption between the two meat diets.

Discussion

A meat intake of 60 g in each of the three main courses of the day did not lead to increased fractional absorption of Zn but resulted in increased total absorption compared with the vegetarian diet. The fractional and total absorption of Zn from the three diets was in the range of 24–27% and 1.8–2.7 mg/d, respectively. Several other studies investigating absorption of Zn from diets high in phytic acid have found similar absorption ratios (Sandström *et al.* 1987b, 1989; Hunt *et al.* 1995, 1998).

The three principal dietary factors influencing absorption and utilization of Zn are the content of inositol phosphates (phytic acid; Nävert *et al.* 1985; Sandström *et al.* 1987a), the total Zn content of the meal (Sandström *et al.* 1980, 1987a) and the amount and source of protein (Sandström *et al.* 1980, 1987c, 1989). Previous studies investigating the potential enhancing

Table 2. Energy, macronutrient, micronutrient and phytic acid content of the 10 MJ diet

| | Meat diets | Vegetarian diet |
|----------------------------------|------------|-----------------|
| Protein (g) | 84 | 48 |
| Fat (g) | 85 | 76 |
| Carbohydrate (g) | 313 | 371 |
| Dietary fibre (g) | 29 | 29 |
| Vitamin C (mg) | 51 | 51 |
| Ca (mg) | 430 | 540 |
| Fe (mg)* | 10.3 | 9.5 |
| Phytic acid (μmol)* | 1280 | 1160 |
| Phytic acid:Zn | 9:1 | 10:1 |

* Analysed values.

Table 3. Content of analysed zinc, the average fractional absorption and total absorption of zinc in whole-day diets from two meat-based diets and the same diet without meat (vegetarian diet)

(Mean values with their standard error for twelve subjects per group)

| | Total Zn intake (mg/d) | | Fractional Zn absorption (%) | | Total Zn absorption (mg/d) | |
|------------------|------------------------|------|------------------------------|-----|----------------------------|-------------------|
| | Mean | SE | Mean | SE | Mean | SE |
| Danish meat diet | 9.4 | 0.19 | 27 | 1.9 | 2.6 | 0.19 ^a |
| Polish meat diet | 9.9 | 0.14 | 27 | 1.2 | 2.7 | 0.14 ^a |
| Vegetarian diet | 7.5 | 0.18 | 24 | 2.6 | 1.8 | 0.21 ^b |

^{a,b} Mean values within a column with unlike letter superscripts were significantly different ($P < 0.05$).

factor of an additional protein intake have all been based on meals or diets with different contents of phytic acid (Sandström & Cederblad, 1980; Sandström *et al.* 1989; Hunt *et al.* 1998), whereas, in the present study, the content of phytic acid was kept at a constant level in all three meals to ensure that this contributing effect on Zn absorption was equal in all diets.

Due to a considerable contribution of Zn from the pork meat, the concentration of Zn in the meat diets in the present study was 20–24% higher than in the vegetarian diet. The dietary Zn content has been reported in several studies to be the most important single factor for the fractional absorption of Zn, with decreased fractional absorption of Zn from meals with higher Zn content (Sandström *et al.* 1987a; Hunt *et al.* 1995; Lonnerdal, 2000). In the present study no difference was observed in the fractional absorption of Zn between the vegetarian diet and the meat diets. A higher Zn content in the meat diets has possibly counterbalanced a potential enhancing effect of meat on the absorption of Zn by lowering the fractional absorption (Table 3). An advantage of an intake of meat on the absorption of Zn is reflected in the significant increased total absorption of Zn as observed by Hunt *et al.* (1995).

Zn absorption can be measured with high precision and accuracy by the extrinsic labelling of meals with the radioisotope ⁶⁵Zn followed by analysis of the absorbed isotope in a whole-body counter. The method has been validated by Arvidsson *et al.* (1978). Use of this method assumes that isotopic exchange occurs between the added isotope and the dietary Zn, whereby a common pool is formed (Flanagan *et al.* 1985; Gallaher *et al.* 1988; Egan *et al.* 1991). In the present study, only selected parts of the meals were labelled (the pâté at breakfast and lunch and the casserole with rice or pasta in the dinner meal). All components of the meal were consumed together and, as the Zn content of the meal would have been mixed in one pool, it was assumed that complete isotopic exchange occurred.

Like Zn, Fe is a divalent ion and absorption is significantly inhibited by a concomitant intake of phytic acid (Hallberg *et al.* 1987) and possibly enhanced by intake of meat (Bæch *et al.* 2003). Differences in the absorption ratio of Fe when comparing single meal studies with whole diet studies have indicated that the former overestimate the effects of dietary enhancers and inhibitors on Fe absorption (Cook *et al.* 1991), and that short-term measurements of absorption overestimate differences in Fe bioavailability between diets (Hunt & Roughead, 2000). To our knowledge, no similar studies investigating a similar discrepancy in Zn absorption between whole meal studies and single

meal studies have been published but similar differences in absorption of Zn from whole meal studies compared with single meal studies may occur. In the present study design a whole diet on five consecutive days was carried out in order to eliminate possible effects of a single meal design and to imitate the natural diet.

To ensure that the meat diets and vegetarian diets were iso-energetic, carbonated beverage was served at all vegetarian meals. The beverage did not contain ingredients that are believed to affect Zn absorption. This design may give limitations for practical applications of the results, since vegetarians most likely consume other food items such as bread or vegetables to compensate for the lack of meat as well as sources of compensatory protein. Other food items may result in elevated phytic acid contents of the vegetarian meals and thereby a higher proportion of inhibitors in the total diet. An additional limitation to the study could be the lower Zn content in the vegetarian diet. As described previously, the Zn content in the meal influences the fractional absorption of Zn, but in order to investigate the absorption from the natural diets, Zn content was not adjusted between the three diets by addition of inorganic Zn. If the Zn content had been equal between the two diets, the total absorption of Zn might not have differed.

The average fractional absorption of Zn in the present study of 24–27% reflected the absorption reported by similar studies, but was substantially lower than the estimated 40% fractional absorption upon which recommended intakes in the Nordic Nutrition Recommendations (www.norden.org) are based. The lower absorption in the present study is most likely due to a high content of phytic acid in the diet.

In conclusion, fractional Zn absorption in this whole meal study was similar in the vegetarian and meat diets of meals with equal phytic acid contents, most likely due to the lower amount of Zn in the vegetarian diet. However, the total absorption of Zn from the vegetarian diet was significantly lower than from the meat diets, which is most likely associated with the higher intakes of protein and Zn in the meat diets. A daily intake of 180g meat resulted in a significantly increased total absorption of Zn compared with the vegetarian diet.

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