

## Effect of dietary phytic acid on zinc absorption in the healthy elderly, as assessed by serum concentration curve tests

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Zn absorption was investigated in healthy elderly subjects aged 71–78 years and in young subjects aged 23–43 years using serum concentration curve (SCC) tests. Both groups had similar Zn and protein status. The increase in serum Zn was monitored for 180 min after ingestion of 200 ml of soya milk enriched with 50 mg of Zn. Three levels of phytic acid were used: 0 g/200 ml (totally dephytinized soya milk), 0.13 g/200 ml (half dephytinized), and 0.26 g/200 ml (natural phytic acid content). In a first study the effect of 0 v. 0.26 g/200 ml phytic acid was compared in 10 elderly and 10 young subjects, each subject receiving both treatments. In a second study soya milks with 0 and 0.13 g/200 ml were tested in nine elderly and ten young subjects, again receiving both treatments. Mean areas under the curve of the SCC tests conducted with the 0 g/200 ml soya milk were found to be the same in both studies. Phytic acid strongly depressed Zn absorption in both studies ( $P \leq 0.05$ ), but to a greater extent at the 0.26 g/200 ml level. No difference was found between the groups of young and elderly subjects. Therefore, no significant effect of aging on Zn absorption, as evaluated by the SCC test, or on the inhibitory effect of phytic acid was detected.

### Zinc absorption: Elderly: Phytic acid

Zn is an essential trace element, a cofactor of several key enzymes (Williams, 1989). It is also involved in the immune function (Kruse-Jarres, 1989), and this has been studied in the elderly (Swanson *et al.* 1988; Bogden *et al.* 1990). Elderly humans may be more susceptible to becoming marginally Zn deficient, for their intakes are quite low; mean intakes of 9–13 mg Zn/d have been reported (Bunker *et al.* 1984; Baghurst & Record, 1987; Bogden *et al.* 1987; Sahyoun *et al.* 1988), which are associated with lower energy intakes. Although these levels seem to maintain Zn balance (Bunker *et al.* 1984), they are lower than the USA recommended dietary intakes of 15 and 12 mg/d for men and women respectively, aged 51 and over (National Research Council, 1989).

Aging also results in progressive alterations of the organs involved in the digestive functions (Thomson & Keelan, 1986). The resulting loss of functionality may even lead to decreased absorption capacity, as may be the case with gastric atrophy (Russell, 1986). Zn absorption may also be altered by aging, since several studies have already shown that the absorption of Zn from a test diet devoid of inhibitors of Zn absorption (Turnlund *et al.* 1986; August *et al.* 1989)

or from a Zn-salt given with water alone (Aamodt *et al.* 1983; Bales *et al.* 1986) is decreased in the elderly, as compared with younger control subjects. However, Zn and protein-energy status were not reliably assessed. Since Zn status partly regulates the amount of Zn that is absorbed (Wada *et al.* 1986), this may have caused some interference. Moreover, whether the effect of dietary inhibitors of Zn absorption is altered by aging, is not known. Any combination of reduced intake and greater susceptibility to inhibitors could be expected to put the elderly at greater risk of Zn deficiency.

A previous study (Couzy *et al.* 1993) showed that the absorption of a stable isotope of Zn given with a standard test-meal was not decreased in elderly volunteers. Since this was the only published report showing no effect of aging on Zn absorption, the present study was undertaken to confirm this observation. Therefore, we report here the results of a study on Zn absorption in young and elderly healthy human subjects, whose Zn and protein-energy status were carefully assessed. Zn status is known to affect Zn absorption and protein-energy status was assessed so as to detect any malnourished subject. A serum concentration curve (SCC)

**Abbreviations:** AUC, area under curve; AAG,  $\alpha$ -1 acid glycoprotein; CRP, C-reactive protein; SCC, serum concentration curve.

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test, also frequently called zinc tolerance test in the literature, was used; Zn absorption was evaluated from the rise of serum Zn after ingestion of a soya milk enriched with 50 mg Zn after complete hydrolysis of its phytic acid. Susceptibility of Zn absorption to phytic acid, which is the most common and potent dietary inhibitor of Zn absorption, was also tested using a Zn-enriched soya milk that differed only by its phytic acid content.

### Subjects and methods

Two series of experiments were conducted (studies 1 and 2). In each of them, two SCC tests were performed in each subject at a 3-week interval. A SCC test consisted of the ingestion of 200 ml soya milk with either a non-detectable or its natural phytic acid content (0.26 g; study 1), or a non-detectable or a 50% reduction in natural content (0.13 g; study 2). In all cases, the 200 ml soya milk serving was fortified with 50 mg Zn so as to induce a measurable increase in serum Zn.

#### Study 1

**Subjects.** Ten elderly men and women (five men and five women) aged 71–77 years, were recruited among the members of a local senior citizen's club. None of them had a disease or took medications which were known to interfere with Zn absorption and/or metabolism (e.g. nephrotic, wasting, digestive or liver diseases). They were all fully autonomous and living in their homes. Ten younger healthy men aged 23–39 years served as reference subjects. Most of them were recruited among the staff of the Research Centre. All subjects filled in a questionnaire and underwent a medical examination by a physician. Height and weight (in light clothes, shoes off) were measured in the fasting state immediately before the first SCC.

**Protocol.** Two soya milks differing only by their phytic acid content were prepared for the study. One batch of soya milk was prepared from soyabeans and tap water and was divided into two parts. One of these was treated with a commercial phytase (ALKO, Finland), so as to hydrolyse all phytic acid to a non-detectable level (< 1 mg/100 g). ZnSO<sub>4</sub> was then added to each part so as to reach a concentration of about 50 mg Zn/200 ml. Each part was then sterilized and packed aseptically in 200 ml containers (Tetrapak, Lausanne, Switzerland). The compositions of the soya milks are given in Table 1.

**Table 1.** Composition of phytase-treated and untreated-soya milk, as determined by chemical analysis

Component	Natural phytic acid	Dephitynized
Dry matter (%)	9.4	9.4
Energy (kJ/200 ml)	401	401
Protein (g/200 ml)	8.8	8.9
Fat (g/200 ml)	5.1	5.2
Carbohydrate (g/200 ml)	3.7	3.4
Calcium (mg/200 ml)	26	26
Phytic acid (g/200 ml)	0.262	ND
Zn (mg/200 ml)	51.2	51.6
Phytic acid/Zn (mol/mol)	0.51	0

ND, not detectable (< 1 mg/100 g).

Between 07.00 and 08.00 hours, a catheter was inserted into an arm vein and slowly flushed with sterile saline. At 08.00 hours, each subject drank 200 ml of either formula A or B at room temperature. Formula allocation was randomized. Blood (5 ml) was sampled at time of consumption (*t* 0), and at 30, 60, 90, 120 and 180 min thereafter. Only water was allowed until the end of the test. Subjects were seated during the whole test to avoid shifts of body fluids. A standard meal of known composition was administered on the evening before each test so as to prevent ingestion of foods that might interfere with the test.

Serum Zn, total protein, albumin, and transthyretin were measured at the beginning of the test (at *t* 0), for assessment of Zn and protein nutritional status. C-reactive protein (CRP) and  $\alpha$ -1 acid glycoprotein (AAG) were also measured to detect any infectious or inflammatory condition. All materials used were free of trace element contamination. Serum Zn only was measured in blood samples drawn after *t* 0.

#### Study 2

Study 2 was undertaken when the results of study 1 showed that the natural phytic acid content of formula A was too high for the purposes of the test. It was conducted in 9 healthy elderly subjects (age 72–78 years, five men and four women) and in ten young reference adults (age 29–43 years). The protocol was identical to that of study 1 in every aspect, except that the soya milk with the natural phytic acid content (0.26 g/200 ml) was replaced by a mixture of 100 ml of this milk and 100 ml of the dephytized milk. Thus, the level of phytic acid administered was reduced by 50%. The other SCC test was conducted with the dephytized soya milk, as in study 1. Two of the control and eight of the elderly subjects participated in both studies.

#### Sample analysis

Blood was allowed to clot for 60 min, and centrifuged at 1800 g for 10 min. It was then kept frozen at –70° until analysis. Serum Zn was measured in duplicate by flame atomic absorption spectrometry (Varian Pty, Mulgrave, Australia) following protein precipitation with 0.4 M-trichloroacetic acid (Prasad *et al.* 1965). This method was validated using a standard reference material (Bovine Serum Standard, NIST, Gaithersburg, MD, USA). Measured values were 14.5 (SD 0.2, *n* 3) and 14.2 (SD 0.2, *n* 6)  $\mu$ mol Zn/l for studies 1 and 2, respectively, as compared with a certified value of 14.0 (SD 0.9)  $\mu$ mol Zn/l. Within-run accuracy was also checked, using a commercial human serum (Seronorm, Nycomed, Norway). Serum total protein content was measured with a COBAS analyser (Hoffmann-LaRoche, Basle, Switzerland) and serum specific proteins (albumin, transthyretin, AAG, and CRP) using a BNA nephelometer (Behring, Marburg, Germany). The methods, the reagents, and the quality control sera were supplied by the manufacturers. Composition of soya milk formulas was determined using standard procedures. Phytic acid was measured according to Makower (1970), except that Ce IV replaced Fe III in the precipitation step. Detection limit was 1 mg/100 g soya milk. Zn and Ca were measured by flame atomic absorption spectroscopy, after wet digestion

**Table 2.** Anthropometric features of subjects who participated in Studies 1 and 2

Variable	Study 1*				Study 2*			
	Young (n 10)		Elderly (n 10)		Young (n 10)		Elderly (n 9)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age (years)	31.4	5.9	73.7	2.0	33.9	4.8	74.3	1.8
Weight (kg)	77.5 <sup>a</sup>	12.3	62.6 <sup>b</sup>	12.4	71.7	8.4	64.0	12.3
Height (m)	1.81 <sup>a</sup>	0.08	1.65 <sup>b</sup>	0.09	1.77 <sup>a</sup>	0.06	1.66 <sup>b</sup>	0.09
BMI (kg/m <sup>2</sup> )	23.6	3.6	22.9	3.1	23.0	2.7	23.1	3.5

\* For details of studies 1 and 2, see p. 178.

<sup>a,b</sup> Mean values within a study which unlike superscripts were significantly different (*t* test, *P* = 0.05).

with 14.4 M-HNO<sub>3</sub> (Suprapur, Merck, Darmstadt, Germany) in a low-pressure digestion system (Seif, Unterschleissheim, Germany).

#### Calculation of the area under the curve

The area under the curve (AUC) of serum Zn concentration plotted against time was calculated by triangulation. For each subject the baseline serum Zn concentration was subtracted from subsequent concentrations so as to lower inter-individual variability. Since the SCC test lasted 180 min, as compared with a more usual duration of 4–6 h, the results will be reported under the denomination 'AUC 0–180'.

Normalization for weight of AUC values has been proposed in order to reduce variability introduced by differences in body weights. Since weights differed markedly among the subjects involved in the present study, AUC 0–180 normalized for weight was also calculated, according to the formula (Watson, 1988):

$$\text{Normalized AUC 0–180} = \frac{\text{AUC 0–180} \times \text{body weight}}{70}$$

#### Statistical analyses

Anthropometric and biochemical values were compared by *t* test. The effect of age and phytic acid on Zn absorption, as evaluated by the AUC, was evaluated by ANOVA with

repeated measurements (BMDP, University of California, Berkeley, CA, USA). Significance level was chosen at *P* ≤ 0.05.

#### Ethical considerations

The subjects were fully informed of the aims and purposes of the study, and signed an informed consent. The protocol was approved by the ethical committee of the Research Centre.

#### Results

Anthropometric measurements of young and elderly subjects are reported in Table 2. No major differences were found between subjects from studies 1 and 2. Elderly subjects were statistically significantly lighter (in study 1 only) and shorter than the controls, mainly because the group included women. However, mean BMI values were identical. Values of parameters of nutritional and infectious and/or inflammatory status are reported in Table 3. Mean serum Zn and albumin were significantly lower in the elderly subjects in both experiments. However, the mean serum Zn:albumin ratios did not differ significantly in both experiments between young and elderly subjects. Serum transthyretin was significantly lower in the elderly subjects in study 1 only, and serum total protein was lower in the elderly subjects in study 2 only. Broadly, protein–energy status was adequate in all subjects; none exhibited an abnormally low serum transthyretin value (<150 mg/l) or

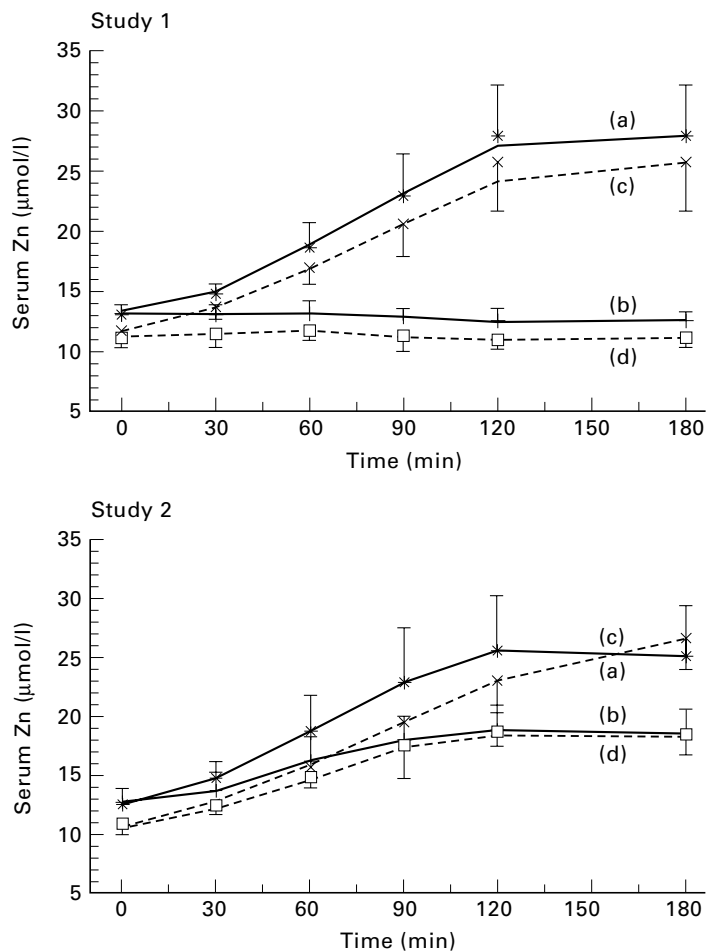
**Table 3.** Serum concentrations of parameters of zinc (zinc, zinc/albumin) and protein–energy (total protein, albumin, transthyretin) nutritional status, and of infectious and/or inflammatory status (1-α acid glycoprotein, C-reactive protein)

Indicator of nutritional status	Study 1*				Study 2*			
	Young (n 10)		Elderly (n 10)		Young (n 10)		Elderly (n 9)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Zn (μmol/l)	133 <sup>a</sup>	1.3	11.5 <sup>b</sup>	0.8	12.7 <sup>a</sup>	1.3	10.9 <sup>b</sup>	0.7
Zn/albumin (μmol Zn/g albumin)	0.31	0.02	0.30	0.02	0.28	0.03	0.27	0.02
Total protein (g/l)	69.3	2.8	66.8	3.2	69.5 <sup>a</sup>	2.1	65.5 <sup>b</sup>	3.0
Albumin (g/l)	42.7 <sup>a</sup>	2.0	38.6 <sup>b</sup>	1.7	44.9 <sup>a</sup>	1.9	40.5 <sup>b</sup>	2.3
Transthyretin (mg/l)	297 <sup>a</sup>	35	253 <sup>b</sup>	24	278	37	248	25
AAG (mg/l)	602	179	636	118	695	145	661	142
CRP (no. values > 20 mg/l)	0		0		0		0	

\* For details of study 1 and 2, see p. 178.

AAG, 1-α acid glycoprotein; CRP, C-reactive protein.

<sup>a,b</sup> Mean values within a study with unlike superscripts were significantly different (*t* test, *P* = 0.05).



**Fig. 1.** Changes in serum Zn concentration in young subjects (a,b) and elderly subjects (c,d) after ingestion of 200 ml soya milk containing 0 g (a,c) or 0.26 g (b,d) phytic acid (Study 1) or 0 g (a,c) or 0.13 g (b,d) phytic acid (Study 2). Data are means with their standard deviations shown as vertical bars.

a low albumin value (<35 g/l) (Haider & Haider, 1984). Individual AAG and CRP values also were all in the normal range (<1400 mg/l for AAG and <20 mg/l for CRP). This confirmed that the subjects were free of infectious or inflammatory disease. Since CRP could not be reliably assessed at concentrations below 1 mg/l, no quantitative value is provided in Table 3.

The evolution of the mean serum Zn concentrations during the SCC tests in studies 1 and 2 is shown in Fig. 1. In both cases, curves are very similar for both groups. However, they are shifted to lower levels for the elderly groups because of the lower initial serum Zn. Fig. 1 also shows that the natural phytic acid content of the soya milk (0.26 g/200 ml) dramatically reduced absorption of a

**Table 4.** Area under the curve (plasma Zn v. time, 0–180 min following serum concentration curve test) in young and elderly healthy subjects, as affected by dietary phytic acid

	Study 1*				Study 2*			
	Young (n 10)		Elderly (n 10)		Young (n 10)		Elderly (n 9)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
AUC 0-180 (µmol Zn/min per l)								
No phytic acid	1558 <sup>a</sup>	502	1472 <sup>a</sup>	421	1503 <sup>a</sup>	520	1471 <sup>a</sup>	284
With phytic acid †	-48 <sup>b</sup>	131	20 <sup>b</sup>	52	727 <sup>b</sup>	263	916 <sup>b</sup>	185
AUC 0-180 normalized for weight (µmol Zn/min per l)								
No phytic acid	1725 <sup>a</sup>	633	1585 <sup>a</sup>	514	1534 <sup>a</sup>	578	1355 <sup>a</sup>	389
With phytic acid †	-50 <sup>b</sup>	139	20 <sup>b</sup>	46	732 <sup>b</sup>	248	826 <sup>b</sup>	174

<sup>a,b</sup> Mean values in the same study with unlike superscripts were significantly different (ANOVA with repeated measurements,  $P = 0.005$ ).

AUC 0–180, area under the curve (plasma Zn v. time, 0–180 min) following serum concentration curve test (for details of test see pp. 178–179).

\* For details of studies 1 and 2, see pp. 178–179.

† Study 1, 0.26 g phytic acid in untreated soya milk; study 2, 0.13 g phytic acid in partially phytase-treated soya milk.

**Table 5.** Relative reduction of area under curve (plasma Zn v. time, 0–180 min following serum concentration curve test) by phytic acid in young and elderly healthy subjects

Subjects	Study 1*		Study 2*	
	Mean	SD	Mean	SD
AUC 0–180 ( $\mu\text{mol Zn/min per l}$ )				
Young ( $n$ 10)	103	9	47	21
Elderly ( $n$ 10)	99	4	37	13

\* For details of studies 1 and 2, see pp. 178–179.

dose of 50 mg Zn, to a level undetectable using this test (study 1). Mean AUC 0-180 values in the young and elderly groups did not differ significantly in both experiments (Table 4). Negative AUC 0-180 values in study 1 are due to the combination of the very strong inhibition of Zn absorption by the high level of phytic acid, and of the serum Zn decrease during the morning which is part of the postprandial decline (Hambidge *et al.* 1989). Phytic acid significantly reduced the mean AUC 0–180 values in studies 1 and 2. In contrast, the effect of age was not found to be statistically significant. No further information was brought by normalization for body weight (Table 4).

The effect of phytic acid on Zn absorption was also not significantly dependent on age when expressed as the percentage reduction of AUC 0-180 values (Table 5).

### Discussion

Nutritional status was assessed in this study in order to be able to exclude possible effects of malnutrition or of inflammation and/or infection, or of Zn deficiency, on the results of the SCC tests. Anthropometric values were in good agreement with those measured in a similar French population (Rolland-Cachera *et al.* 1991). Generally, nutritional status, as assessed here using anthropometric and biological parameters, was adequate and similar for the young and the elderly subjects who were involved in this study.

Zn status remains difficult to assess in humans, especially in the elderly. Serum Zn is the most frequently used parameter, but it lacks sensitivity and specificity. The use of other parameters of Zn status has been proposed, e.g. hair or leucocyte Zn. However, none has yet been shown to bring definite advantage over serum Zn. Levels of metallothioneins have also been proposed (Bremner & Beattie, 1990), but the technique is not yet widely available and also has its limitations. The major disadvantage of serum Zn is that it is very sensitive to any change in the concentration of the serum proteins, as is the case after surgery (Puri *et al.* 1981; Faure *et al.* 1990), or in athletes during training (Couzy *et al.* 1990). Since about two-thirds of serum Zn is bound to albumin (Gardiner *et al.* 1981) and since serum albumin is lower in the elderly as a result of a decreased hepatic protein synthesis (Gersovitz *et al.* 1981), the serum Zn:albumin ratio can be calculated as an attempt to compensate for these changes in albumin concentration.

The Zn:albumin ratios found here show that Zn status was similar in the young and elderly participants in both studies; lower serum Zn values found in the elderly are linked to the decrease in serum albumin. This is of importance, since studies using stable or radioactive Zn isotopes

have shown that Zn status regulates the absorption of Zn in the rat (Weigand & Kirchgessner, 1980) and in man, either young (Wada *et al.* 1985), or elderly (August *et al.* 1989). Furthermore, it has been shown that the Zn:albumin ratio is inversely related to the fractional absorption of Zn from a standard test meal, better even than serum Zn (Couzy *et al.* 1993). This result was obtained using a stable isotope technique in healthy young and elderly volunteers. However, whether a change in Zn status would have affected the results of a SCC test is not known, since the only study that investigated the effect of Zn status on SCC test values provided conflicting results (Fickel *et al.* 1986).

The health status of the subjects was also evaluated using clinical examination by the physician and the measurement of AAG and CRP values in serum. AAG and CRP are both acute phase reactants, and their concentration increases in case of infectious or of inflammatory disease. This assessment showed that all subjects involved in these studies were healthy. This is of importance, since infection and/or inflammation considerably alter the metabolism of several trace elements, including Zn (Pekarek *et al.* 1973; Srinivas *et al.* 1988). Therefore, age was the only parameter that differentiated the two populations studied.

No effect of age on Zn absorption, as assessed by the AUC 0-180 values of the test without phytic acid, was found. This is in contrast with a SCC test study where 25 mg Zn, as the acetate salt, were administered in two groups of subjects (Bales *et al.* 1986): the AUC value was found to be significantly decreased in the group aged 60 and over. Several differences exist between this study and the present study. First, the dose of Zn was lower (25 mg Zn v. 50 mg Zn in the present study). Second, Zn was administered as Zn acetate in a gelatin capsule, in contrast with the present study where it was incorporated in a food. Third, nutritional status was not measured in spite of the much larger prevalence of overweight among the elderly subjects (52 %) than among the young subjects (7 %).

Faecal monitoring of Zn-stable isotopes was also used in the elderly to assess Zn absorption from semi-purified diets low in inhibitors of Zn absorption (Turnlund *et al.* 1986; August *et al.* 1989). Both studies found that aging resulted in a significant reduction of Zn absorption, by about 45 %. A similar, but much less dramatic effect was also shown using the retention within the body of a Zn-radioisotope administered with water (Aamodt *et al.* 1983). However, Zn status was not reliably assessed in all these studies, and therefore the effect of age can not be isolated. When Zn absorption from foods was measured in young and elderly healthy volunteers of identical Zn and nutritional status, using stable isotopes, no difference was found (Couzy *et al.* 1993). This finding is supported by the results of the present study.

Phytic acid is a well-known and potent inhibitor of Zn absorption (Harland & Oberleas, 1987). Its effect has been found in the present study not to be significantly affected by aging, which confirms our previous findings (Couzy *et al.* 1993).

Finally, these results show that Zn absorption does not seem to be significantly, if at all, altered by aging, at least in healthy humans aged less than 80 years. The fact that phytic acid was shown to exert the same inhibitory effect seems to indicate that aging *per se* does not lead to a greater

susceptibility of Zn absorption to its most common dietary inhibitor.

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