

Absorption of zinc from lupin (*Lupinus angustifolius*)-based foods

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The absorption of Zn from a lupin (*Lupinus angustifolius*) milk fortified with Ca, a bread containing lupin flour (230 g/kg), a sauce containing lupin flour and a sauce containing a lupin-protein isolate was determined in humans by measuring the whole-body retention of radioisotope from meals labelled with 0.02 MBq ⁶⁵Zn, allowing for endogenous excretion of Zn, after 14 d. The absorption of Zn from the Ca-enriched milk (16.2%) and the bread made with lupin flour (27.0%) was similar to literature figures for comparable soya-bean products. The absorption from composite meals made with lupin flour (28.2%) and protein isolate (32.7%) was significantly higher than that reported for comparable soya-bean products. In a second experiment the absorption of Zn from a lupin-milk base and a soya-bean-milk base was compared with that from Ca-supplemented bases. The absorption of Zn from the lupin-milk base (26.3%) was significantly higher than from the soya-bean-milk base (17.6%), and neither was significantly altered by the addition of Ca. Overall the absorption of Zn from lupin-protein foods was found to be higher than from comparable soya-bean products. Lupin milk could be an attractive alternative to soya-bean milk for infant formulas.

Zinc bioavailability: Lupins

Two species of lupin, *Lupinus albus* in the Mediterranean basin and *L. mutabilis* in the Andean highlands, have been cultivated for seed for over 2000 years (Gladstones, 1982). The extent of use of these seeds was greatly restricted by the high alkaloid content of the seed, often more than 20 g/kg. Low-alkaloid genes were discovered early this century in *L. luteus* and *L. angustifolius* and since then there have been great reductions in the alkaloid content of lupin seed through plant breeding programs. This has been contiguous with improved agronomic characteristics, especially for *L. angustifolius* in Australia (Gladstones, 1977, 1982). Lupin seed is now a major crop in Western Australia with annual production of about 1 million tonnes: about 40% of this is retained on-farm for summer feeding of livestock and the remainder sold, to be used mostly in formulations for intensive animal industries. Only a few thousand tonnes are used for human consumption; however, there is increasing interest in this area (Kyle *et al.* 1991). The most obvious uses would be as an alternative to the soya bean and to other grain legumes. One of the advantages lupin seed offers over these commodities is the low content of antinutritive factors; typically they

contain no detectable lectins, 0.14 mg trypsin inhibitors/g and 4.4 mg phytate/g (Horton *et al.* 1990).

The bioavailability of minerals, i.e. the absorption and utilization of the amounts present in the diet, depends on the chemical form of the element and the presence of other food components which may inhibit or stimulate absorption. In animal models the overall effects of a food component on bioavailability can be measured, e.g. by changes in growth rate or the mineral concentration of target tissues. In humans absorption can be estimated by isotope techniques while there is limited access to tissues that could mirror the overall bioavailability. For most elements, however, absorption is the major determinant of bioavailability. Phytate has been found to be a strong inhibitor of Zn absorption in man (Lönnerdal *et al.* 1984; Turnlund *et al.* 1984).

It is important to learn whether the low level of phytate in lupins will mean a higher absorption of minerals by humans. There are data showing that phytate alone is not the sole determinant of mineral absorption from grains and that the (Ca × phytate):Zn ratios (Davies & Reid, 1979), and these ratios in relation to energy content of the diet (Gibson *et al.* 1991) and mineral concentration (Sandström, 1992) can serve as indicators. The present study investigated the absorption of Zn by humans from various lupin-containing meals, and compared the absorption from lupin- and soya-bean-milk bases with and without added Ca.

MATERIALS AND METHODS

Healthy men and women volunteers were given written and oral information about the aims of the study. The study was approved by the Research Ethical Committee and the Isotope Committee of Sahlgren's Hospital, Gothenburg.

There were twelve female and twenty male volunteers of mean age 26 (SD 6, range 19–37) years. Their body mass index values were 22.3 (SD 4.4, range 19.0–32.9) kg/m²: four subjects had an index above 25. Serum Zn concentrations were 15.8 (SD 2.4) μmol/l, with the range 12.9–23.3 μmol/l being within the hospital's reference range for young subjects.

In the first experiment, three lupin-based products were tested. Four meals were prepared: (1) a 450 g serving of lupin milk; (2) three buns, net weight 87 g, made with a 3:10 mixture of lupin flour and wheat flour, served with 10 g butter and 200 g cow's milk (5 g fat/l); (3) a vegetarian sauce, net weight 250 g, based on 55 g lupin-protein isolate served with 40 g rice and water; (4) a vegetarian sauce, net weight 250 g, based on 20 g lupin-protein isolate served with 40 g rice and water. The composition of each meal was as close as possible to that of soya-bean-protein-based meals used in earlier studies (Sandström *et al.* 1983, 1987). Each meal was labelled with 0.02 MBq ⁶⁵Zn during preparation and allowed to equilibrate overnight before being offered to the subjects. Each subject was randomly served two of the test meals, a minimum of 6 weeks apart. The milk and the buns were served after an overnight fast and the sauces at lunch time, 4 h after consumption of a standardized breakfast (Arvidsson *et al.* 1978). No food or drink was allowed for 3 h after ingestion of the labelled meals. The absorption of Zn was determined by measuring the whole-body retention of ⁶⁵Zn after 14 d and correcting for endogenous excretion of Zn (Arvidsson *et al.* 1978).

In the second experiment, two lupin milks and two soya-bean milks were tested. Four meals were prepared: (1) a 250 g serving of lupin-milk base; (2) a 250 g serving of lupin-milk base fortified with Ca (approximately 45 mmol); (3) a 250 g serving of soya-bean-milk base; and (4) a 250 g serving of soya-bean-milk base fortified with Ca (approximately 45 mmol). Labelling of the meals and the test routines was as in the first experiment.

The lupin milk was prepared from kernels of *L. angustifolius* (cv. Danja). The cotyledons were hammer-milled twice, first with a 3.2 mm screen and then with a 1.6 mm screen. The

'flour' was diluted 12-fold with water and the slurry passed through a laboratory-scale Fryma carborundum stone colloid mill (Fryma-Maschinen AG, Reinfelden, Switzerland). Sucrose (29 g/l) and CaCl_2 (2 g/l) were blended into the suspension and the pH adjusted to 6.0 by adding Na_2CO_3 . The material was spray-dried in a Niro Minor (A/S Niro Atomizer, Copenhagen, Denmark), inlet temperature 190°, outlet 90°, vacuum packed and reconstituted as required.

The lupin-milk base for the second experiment was prepared at Alfa-level South East Asia Pte, Jurong, Singapore. Dehulled lupin seed, *L. angustifolius* cv. Gungurru, was soaked in five volumes of NaHCO_3 (5 g/l) and glucono- δ -lactone (10 g/l) for 1 h and drained. The soaked beans were then fed into a Fryma perforated disk mill, 4 mm holes, with 3.5 volumes water and about 1 volume NaOH (5 g/l) to maintain pH (approximately 8.5). The slurry then passed through a Fryma colloid mill, a heat treatment (90–92° for 200 s), followed by rapid cooling to 62° and deaeration. The slurry next passed through a centrifugal decanter and the protein-rich fraction was cooled to 20°. This fraction, the milk base, was brought to pH 6.5 before sterilizing under UHT and packing into 250-ml tetrabriks.

The soya-bean-milk base was prepared from whole soya beans, *Glycine max*, Canadian No. 1 Yellow, after soaking in water and then following the procedure for the lupin-milk base.

Lupin kernel flour was prepared by stone-milling dehulled lupin seed, and passing the product through a 420 μm screen to remove any grits.

Lupin-protein isolate was prepared from lupin flour by solubilizing protein at pH 9.0, using 2 M-KOH, and then precipitating the protein fraction from the solubles by adjusting the pH to 4.2 with 1 M-HCl. After centrifugation the isolated protein was brought to pH 6.5 and then freeze-dried.

Mineral analyses were as described by Sandström *et al.* (1987). NBS Liver No. 1577a was used for the reference standards. The coefficients of variation (%) were: Ca 7.3, Fe 5.3, Mg 9.6, Zn 5.3, P 4.2. N analysis was by a micro-Kjeldahl technique (Technicon Instruments Co. Ltd., London). Phytate was by the method of Davies & Reid (1979). Details of the meals used are shown in Tables 1 and 2.

RESULTS

The concentrations of N, minerals and phytic acid in the test meals for the two experiments are shown in Table 1, together with the absorption of Zn from each of the meals, and the phytate:Zn and (phytate \times Ca): Zn ratios. A comparison between the absorption of Zn from these meals and similar soya-bean preparations, determined in earlier studies, is shown in Table 2.

The absorption of Zn from the lupin milk and bread made with lupin flour was similar to that from comparable soya-bean products. However, the absorption from the flour 'sauce' meal, 28.2%, and the isolate 'sauce' meal, 32.7%, were both significantly higher than the earlier values obtained for similar soya-bean preparations, 14.0 and 20.9% respectively.

The absorption of Zn from the lupin-milk base, 26.3%, was unaffected by adding Ca (26.5%). Similarly the absorption of Zn from the soya-bean-milk base, 17.6%, was not affected by adding Ca (15.1%).

DISCUSSION

The fractional absorption of Zn from lupin flour and isolate 'sauces' was higher than from comparable soya-bean products, even though the phytate:Zn molar ratios were similar. A phytate:Zn ratio higher than 10 would be expected to reduce absorption to below 20% for

Table 1. *Chemical composition of, and zinc absorption from, test meals containing lupin (Lupinus angustifolius) or soya-bean protein**
(Values for zinc absorption are means and standard deviations for eight subjects)

Meal†	Protein source	N (g)	Zn (μmol)	Ca (mmol)	Phytate (μmol)	P (mmol)	Mg (mmol)	Fe (μmol)	Phytate		Zn absorption (%)		Zn absorption (μmol) Mean
									Zn	Ca × Phytate Zn	Mean	SD	
1	Lupin milk	1.7	17.9	8.4	190	2.7	2.5	47.9	10.6	89.2	16.2	4.8	2.89
2	Lupin, wheat, cow's milk	2.2	22.5	4.2	50	10.0	2.2	70.6	2.2	9.3	27.0	5.9	6.08
3	Lupin, rice	4.3	43.5	2.5	730	11.4	4.8	91.3	16.8	42.0	28.2	8.3	12.27
4	Lupin-protein isolate, rice	4.1	2.3	1.8	565	9.8	2.1	89.8	24.1	43.5	32.7	1.6	7.65
5	Lupin-milk base	1.3	7.9	0.6	58	1.3	1.0	25.5	7.3	4.4	26.3	6.2	2.08
6	Lupin-milk base (+ Ca)	1.3	7.9	4.7	58	1.3	1.0	25.5	7.3	34.2	26.5	3.9	2.09
7	Soya-bean-milk base	1.5	16.9	1.3	330	3.5	2.1	10.1	19.5	25.4	17.6	5.5	2.97
8	Soya-bean-milk base (+ Ca)	1.5	16.9	4.7	330	3.5	2.1	10.1	19.5	91.0	15.1	5.2	2.55

* For details of procedures, see pp. 866-867.

† The meals comprised: (1) 450 ml lupin milk (100 g solids/l); (2) 87 g bread (230 g lupin flour/kg) + 10 g butter + 200 ml milk (5 g fat/l); (3) 55 g lupin flour, 24 g tomato paste, 70 g onion, 30 g butter, salt, pepper, 40 g rice + 275 ml water; (4) 20 g lupin-protein isolate, 28 g tomato paste, 82 g onion, 35 g butter, salt, pepper, 40 g rice + 288 ml water; (5) 250 ml lupin-milk base (90 g solids/l); (6) meal 5 supplemented with calcium; (7) 250 ml soya-bean-milk base (90 g solids/l); (8) meal 7 supplemented with calcium.

Table 2. Percentage zinc absorption from test meals containing lupin (*Lupinus angustifolius*) or soya-bean protein, in human subjects

(Mean values and standard deviations)

Test meal base	Zn (μmol)	N (g)	Ca (mmol)	Phytate (μmol)	Zn absorption (%)	
					Mean	SD
Lupin milk*	18	1.7	8.4	190	16.2	4.8
Soya-bean formula†	26	1.4	6.2	200	14.0	1.0
Bread (lupin flour)*	23	2.2	4.2	50	27.0	5.9
Bread (soya-bean flour)‡	26	2.4	7.6	140	24.2	7.7
Lupin flour 'sauce'*	43	4.3	2.5	730	28.2	8.3
Soya-bean flour 'sauce'‡	37	3.2	4.3	880	14.0	2.8
Lupin isolate 'sauce'*	23	4.1	1.8	565	32.7	1.6
Soya-bean isolate 'sauce'‡	18	3.5	2.5	510	20.9	5.9

* Present paper.

† Values from Sandström *et al.* (1983).‡ Values from Sandström *et al.* (1987).

humans (Ellis *et al.* 1987; Sandström *et al.* 1987) and ratios higher than 15 reduce the absorption from soya-beans by rats (Lo *et al.* 1981). The present results for lupins suggest that phytate alone is not the factor limiting Zn absorption.

It has been shown for rats that Ca exacerbates the negative effect of phytate on Zn absorption from soya-bean-protein meals (Forbes *et al.* 1983). (Phytate \times Ca):Zn molar ratios above 91, adjusted for energy intake, appear to be of potential concern for humans (Gibson *et al.* 1991). This has, however, not been confirmed in human studies. In the present study we found no effect of adding Ca to either lupin or soya-bean milks on Zn absorption. This finding supports the earlier observation of Lönnerdal *et al.* (1984) who found that adding approximately 800 mg Ca/l soya-bean-protein isolate formula (phytate:Zn ratio 6:1) had no significant effect on Zn absorption. A positive effect on Zn absorption of milk, a high-Ca food, to a phytate-containing meal has earlier been reported (Sandström *et al.* 1980). The levels of Ca used in animal experiments are in general higher than in human diets so that might explain the different results. At lower levels Ca might block the phytate and render Zn free to be absorbed. Thus within the range of Ca intake that can be found in human diets the phytate-Zn-Ca interaction does not seem to be of nutritional significance.

The observed differences in fractional absorption of Zn from the lupin milk (16.2%) and the milk base (26.3%) cannot be explained in terms of phytate:Zn ratios as the fractional absorption was higher than could be expected from the rather small differences in ratio (10.6 *v.* 7.3). The (phytate \times Ca):Zn ratio does not seem to be a good predictor either, as similar absorption figures were observed from meals differing in this ratio from 4.4 to 42. Three factors could have contributed to the difference observed between the lupin milk and the base. First, and probably the most important one, there was about a twofold difference in the amount of Zn consumed. A recent review by Sandström (1992) showed that the fractional absorption of Zn decreases with increasing amount ingested. Second, the lupin milk was spray-dried and reconstituted, and this may have resulted in more stable complexes being formed between the divalent ions and protein and/or any fibre present. Third, the lupin milk was extracted at about neutral pH and the final pH adjusted to 6.0 whereas the lupin-milk base was prepared by an alkaline extraction followed by

neutralization to pH 6.5. Different protein profiles with different digestibility or mineral and phytate binding properties might have resulted from these procedures. A lower small-intestinal digestibility of soya-bean protein compared with animal protein has been observed in studies of human ileostomized subjects (Sandström *et al.* 1986). The binding of phytic acid to glycinin, the major protein of soya beans, is highly pH-dependent (Okubo *et al.* 1976) and it is reasonable to assume that this is also the case for lupin proteins.

The absorption of Zn from the lupin-milk base, 26.3 (SD 6.2)%, was similar to reported values for cow's milk, 28 (SD 15)%, and infant formulas in studies using the same radionuclide technique and the same amount of formula or test meal (Sandström *et al.* 1983) as in the present study. The values are also similar to those of Egan *et al.* (1991) using a cow's milk formula intrinsically labelled with stable Zn isotopes (26.7%). Studies in infants using stable isotopes have found 16–40% absorption from an infant formula, with the higher absorption at a low Zn intake (Ziegler *et al.* 1989). From a term formula given to very-low-birth-weight infants 23.6% was absorbed (Ehrenkrantz *et al.* 1989). This high absorption of Zn, high protein content together with the low allergenicity of lupin proteins (Gross, 1988) might make lupin milk an attractive alternative to soya-bean milk for infant formulas and in cases where intolerance of or allergy to cow's milk occurs. With the low phytate content of the lupin-milk base the absorption of other elements besides Zn and Ca can also be expected to be high. Addition of Zn to this base will most likely result in a larger amount of Zn being absorbed and hence further improve the nutritional value of the product.

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