Fractional magnesium absorption is significantly lower in human subjects from a meal served with an oxalate-rich vegetable, spinach, as compared with a meal served with kale, a vegetable with a low oxalate content

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The aim of the present study was to evaluate Mg absorption from a test meal served with an oxalate-rich vegetable, spinach, as compared with a test meal served with a vegetable with a low oxalate content, kale. Mg absorption was measured by a stable-isotope technique based on extrinsic labelling of the test meals and faecal monitoring of the excreted isotope labels. Nine healthy adults participated in the study. The test meals were based on 100 g phytate-free white bread, served with 300 g spinach (6.6 mmol oxalate; 0.7 mmol $^{25}$Mg label added, 5.0 mmol total Mg) or 300 g kale (0.1 mmol oxalate; 1.2 mmol $^{26}$Mg label added, 4.8 mmol total Mg). The test meals were served on days 1 and 3, at breakfast and lunch, using a cross-over design. The results from the present study demonstrated that apparent Mg absorption was significantly lower from the meal served with spinach (26.7 (SD 10.4) %) than the meal served with kale (36.5 (SD 11.8) %) ($P=0.01$). However, the lower fractional apparent Mg absorption from the test meal served with spinach can be assumed to be, at least partly, counterbalanced by the higher native Mg content of spinach as compared with kale. Although based on indirect evidence, i.e. not based on an evaluation of added (or removed) oxalic acid, the difference in Mg absorption observed in the present study is attributed to the difference in oxalic acid content between the two vegetables.

Magnesium absorption: Spinach: Oxalate: Stable isotopes: Faecal monitoring

Oxalic acid and its salts are ubiquitous in plant cells and relatively large amounts are found in leafy vegetables such as spinach (Tabekhia, 1980), and also in fruits, grains, nuts, tea, coffee, and cocoa (Zarembski & Hodgkinson, 1962; Souci et al. 1994). Oxalate intakes vary with dietary habits and have, for example, been reported to be in the range of 70–150 mg/d in the UK (Zarembski & Hodgkinson, 1962; Anderson et al. 1971; Hodgkinson 1977a).

Although oxalic acid forms insoluble complexes at physiological pH with divalent cations such as Ca$^{2+}$, Zn$^{2+}$, and Mg$^{2+}$ (Weast, 1989), the influence of oxalate or foods rich in oxalate on mineral and trace element absorption in man has not been evaluated systematically. However, Ca absorption from a vegetable rich in oxalate, spinach, has been reported to be significantly lower than from kale (a vegetable with a low oxalate content) in adults (Heaney et al. 1988; Heaney & Weaver, 1990). In addition, Schwartz et al. (1984) reported significantly lower net (apparent) Mg absorption from bran muffins with added spinach as compared with added collard greens, a vegetable botanically similar to kale, but no difference as compared with added lettuce or turnip greens. However, these results are difficult to interpret as the quantity of spinach and the content of oxalate in the test meal are not reported and the study only included four subjects.

An inhibitory effect of oxalic acid-rich vegetables on Mg and Zn absorption in man is indicated by the observation that spinach added to the diet resulted in negative Mg and Zn balances (Kelsay & Prather, 1983). Furthermore, Mg has been shown to inhibit oxalate absorption in man (Berg et al. 1986; Hanson et al. 1989), suggesting that Mg forms insoluble, non-absorbable complexes with oxalic acid in the gastrointestinal tract.

The aim of the present study was to evaluate Mg absorption from test meals served with vegetables with high or low oxalate content (spinach or kale) in healthy adults. Mg absorption was measured by a stable-isotope technique based on extrinsic labelling of the meals and faecal monitoring of the excreted isotope label.

Subjects and methods

Subjects

Apparently healthy, free-living, i.e. non-hospitalised, subjects (ten adult men and women) were recruited. Lactating
and pregnant women were excluded from the study. No medication was allowed during the study except for oral contraceptives. The intake of mineral and vitamin supplements was not allowed from 2 weeks before the start of the study and during the entire study. The participants were asked not to change their dietary habits or lifestyle during the study. Information about the aims and the procedures of the study was given orally and in writing. Written informed consent was obtained from all participants. The study protocol was reviewed and approved by the ethical committee at the Swiss Federal Institute of Technology, Zurich.

Isotopic labels

Highly enriched $^{25}\text{MgO}$ (1·0–1·6 g), wheat bread (0·25 g), kale and spinach (1 g) as solid, $^{26}\text{MgO}$ (600 ml) with the added rare-earth elements was heated in a microwave oven before serving. Water (18 MΩ water; 600 ml) with the added rare-earth elements was served as a drink with test meals A and B. The test meals were divided into two identical portions served at breakfast (07.30–08.30 hours) and at lunch (12.00–13.00 hours).

No food or drink was allowed between breakfast and lunch on days 1 and 3 and for 3 h after lunch. Standardised dinners (pizza and white wheat crisp bread) were provided on days 1 and 3. Drinking water (18 MΩ water; 2 litres) was provided on days 1 and 3. No additional foods or drinks were allowed on days 1 and 3. Diet was unrestricted at all other times.

Pre Weighed polypropylene containers (Semadeni, Ostermundingen, Switzerland) were provided for stool collections. The subjects collected all stools separately, starting immediately after the intake of the first labelled test meal on day 1. On day 8, a brilliant blue capsule was again administered. The collections were continued until excretion of the second brilliant blue marker. Stool samples were stored frozen ($–25^\circ \text{C}$) until processed.

Preparation of faecal pools

Each individual stool sample was freeze-dried using a freeze dryer (Modulyo; Edwards, North Bergen, NJ, USA) and ground to a powder in a mortar. All stools, from the first stool dyed by brilliant blue until, but not including, the stools dyed by the second dose of brilliant blue, were included in the faecal pool. After a drying step (20 h at 65°C) in a drying chamber (Binder, Tuttingen, Germany) to standardise humidity, followed by cooling at room temperature (4 h), all individual stools were weighed and milled, starting with the first (most enriched) samples. A mill (ZM1; Retsch, Haan, Germany) equipped with a sieve of 1 mm pores was used for this purpose. Each milled stool was transferred back into its original container, dried again for 20 h at 65°C, cooled for 4 h at room temperature and re-weighed to determine losses. All milled stools included in a single pool were combined in a 2 litre polyethylene container (Semadeni, Ostermundingen, Switzerland) and mixed for 90 min using a rotator (UG 70/20; Micro Motor, Basel, Switzerland).

Wet ashing

Portions of the freeze-dried pooled stool samples (1·0–1·6 g), wheat bread (0·25 g), kale and spinach (1 g) as
well as plasma (1 g), were wet ashed in a microwave system (MLS 1200; MLS GmbH, Leutkirch, Germany) in a mixture of 14 M-HNO₃ and 8·8 M-H₂O₂ (Merck, Darmstadt, Germany). All samples were wet ashed in duplicate.

**Separation of magnesium**

Mg was separated from the wet-ashed stool samples by cation-exchange chromatography using a strongly acidic ion-exchange resin (AG 50W X-8, 200–400 mesh; Bio-Rad, Hercules, CA, USA). Samples containing about 30 µmol Mg were evaporated to dryness, re-dissolved in 0·7 M-HCl (1 ml) and transferred onto the top of the column (10 mm inner diameter; Bio-Rad, Hercules, CA, USA), filled with ion-exchange resin to a height of 70 mm. The column was rinsed with 0·7 M-HCl (56 ml), followed by 0·9 M-HCl (24 ml) to elute Na and K. Mg was eluted with 1·4 M-HCl (12 ml). The solution was evaporated to dryness and re-dissolved in 50 µl water. Mg recovery, evaluated with a diluted Mg standard solution (Titrisol; Merck, Darmstadt, Germany) was found to be 94·8 (sd 1·8) % (n 10). Resins were regenerated with 6 M-HCl (30 ml) and replaced after the fifth run. Only acid-washed Teflon and polyethylene laboratory ware were used for sample processing. Samples of the ²⁶Mg isotope label were processed in parallel with each batch for blank monitoring, starting at the ion-exchange chromatography step. Sample contamination due to natural Mg was found to be 10·8 (sd 7·0) nmol (n 9) for the combined sample preparation and filament loading, which was <0·4 % of the amount of Mg separated.

**Isotopic analysis by thermal ionisation mass spectrometry**

About 20 nmol separated Mg from faecal samples was loaded onto the metal surface of the evaporation filament of a double-isotope dilution principles (Walczyk et al. 1997; Sabatier et al. 2002). Fractional apparent Mg absorption (AA %) was based on the dose (µmol) of Mg stable isotope administered (D₀) and the amount of the isotopic label excreted in faeces (F₀).

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\text{AA(\%)} = \frac{D₀ - F₀}{D₀} \times 100.
\]

Recovery of the rare-earth elements Eu and Yb was used to evaluate the completeness of the stool collections.

**Statistics**

Calculations were made using commercial software (Excel 97; Microsoft, Chicago, IL, USA and SPSS 10.0; SPSS Inc., Chicago, IL, USA). Results are presented as arithmetic means and standard deviations. The normal distribution of absorption values was verified by the Kolmogorov–Smirnov test. Paired Student’s t tests (two-tailed) were used to compare Mg absorption from the two different test meals. P values below 0·05 are referred to as statistically significant.

**Results**

The mean age and mean BMI of the subjects were 23 (sd 1) years and 22·6 (sd 3·2) kg/m² (n 9). The mean plasma Mg
concentration was 0.80 (range 0.73–0.89) mmol/l. Two individuals had slightly lower plasma Mg concentration than the reported normal range of 0.75–0.96 mmol/l (Lowenstein & Stanton, 1986).

The Mg and oxalate contents of the test meals are presented in Table 1. The native Mg content of the bread, spinach, and kale was 0.96 (SD 0.03), 1.12 (SD 0.09), and 0.89 (SD 0.01) mmol/100 g, respectively (n 3). Of total oxalates, 87.5 (SD 2.8) % were found to be soluble in spinach (n 3) and 51.8 (SD 11.7) % in kale (n 3). The phytic acid content was below the detection limit in wheat bread (<0.5 μmol/100 g).

Mg absorption from the test meal served with spinach (26.7 (SD 10.4) %) was significantly lower (P<0.01) than from the test meal served with kale (36.5 (SD 11.8) %) as shown in Fig 1. The within-subject difference in Mg absorption between the two test meals was 9.8 (SD 7.2) %.

Absorption ratios (test meal served with spinach/test meals served with kale) were 0.73 (SD 0.19). One subject was excluded from the evaluation due to a low recovery of Yb (<85 %). For all other subjects, mean Yb recovery was 98.8 (range 90.6–115.4) % and mean Eu recovery was 95.0 (range 90.0–99.1) %.

Complete faecal collections were made during 8.9 (SD 0.6) days. The mean loss of faecal material during sample preparation, determined by weighing faecal pools before and after milling, was 1.1 (SD 0.6) %. The measured isotopic enrichment of the stool pools was 5.6 (SD 1.8) % (24Mg:25Mg range 2.5–9.3 %) and 8.1 (SD 2.4) % (24Mg:26Mg range 5.3–13.1 %) based on differences of the measured isotope ratios of faecal pools and natural isotope ratios of a standard (Titrisol; Merck, Darmstadt, Germany), divided by the measured isotope ratio of the standard.

Discussion

The present study is, to our knowledge, the first report clearly demonstrating an inhibitory effect of an oxalate-rich vegetable on Mg absorption in human subjects, based on the stable-isotope technique. The mean fractional apparent Mg absorption from the labelled test meal served with spinach was about 35 % lower than from the test meal served with kale in the present study. However, these results should not be interpreted as suggesting that spinach is a poor source of dietary Mg. The observed decrease in fractional apparent Mg absorption can be assumed to be, at least partly, counterbalanced by the approximately 30 % higher Mg content of spinach as compared with kale (Holland et al. 1994; Souci et al. 1994); native Mg content of spinach was 26 % higher than that of kale in the test meals evaluated in the present study.

In the present study, we evaluated Mg absorption from extrinsically labelled test meals based on phytate-free wheat bread, served with spinach or kale. Limited information is available on the validity of the extrinsic labelling technique for studies of Mg absorption in human subjects although an earlier study reported no significant differences in Mg absorption from intrinsically and extrinsically labelled leafy vegetables, including spinach and collards, in man (Schwartz et al. 1984). Similar findings have also been reported in rats (Schwartz et al. 1980); however, it should be stressed that the usefulness of animal models in these evaluations is not clear. Contrary to the data (which are rather limited) on the validity of extrinsic labelling of leafy vegetables with Mg isotopes, the extrinsic labelling technique has been demonstrated not to be valid for Ca absorption from spinach (Weaver & Heaney, 1991). Interestingly, the study by Weaver & Heaney (1991) reported Ca absorption from calcium oxalate to be twice as well absorbed as from spinach oxalate in healthy adult women. These data thus indicate different absorption mechanisms for, or different physical and chemical properties of, pure calcium oxalate and spinach Ca and highlight the limited usefulness of evaluating the influence of pure calcium oxalate on Ca absorption from complex food matrices such as spinach.

Oxalates in plants are present as water soluble, bound to Na or K or present as free oxalates, as well as water-insoluble compounds, i.e. calcium oxalate and magnesium

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**Table 1.** Test meal contents of total magnesium, stable-isotope labels (25Mg or 26Mg), oxalic acid (OA), oxalic acid: magnesium molar ratios and non-absorbable faecal markers, ytterbium and europium*  
(Mean values and standard deviations)

<table>
<thead>
<tr>
<th></th>
<th>Bread plus spinach</th>
<th>Bread plus kale</th>
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</thead>
<tbody>
<tr>
<td>Total Mg (mmol)</td>
<td>4.98 ± 0.01</td>
<td>4.82 ± 0.05</td>
</tr>
<tr>
<td>Added Mg label</td>
<td>0.66 ± 0.01</td>
<td>–</td>
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<tr>
<td>25Mg (mmol)</td>
<td>–</td>
<td>1.19 ± 0.02</td>
</tr>
<tr>
<td>26Mg (mmol)</td>
<td>6.6 ± 0.2</td>
<td>0.11 ± 0.01</td>
</tr>
<tr>
<td>OA (mmol)</td>
<td>1.33 ± 0.58</td>
<td>–</td>
</tr>
<tr>
<td>OA:Mg molar ratio</td>
<td>25Mg:25Mg range 2.5–9.3 %</td>
<td>8.1 (SD 2.4) %</td>
</tr>
<tr>
<td>Yb (nmol)</td>
<td>31.38 ± 0.58</td>
<td>–</td>
</tr>
<tr>
<td>Eu (nmol)</td>
<td>33.17 ± 0.26</td>
<td>–</td>
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* Test meals consisted of 100 g phytate-free white wheat bread served with 300 g spinach or kale.

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**Fig. 1.** Apparent magnesium absorption from a meal based on 100 g white wheat bread served with 300 g spinach (6.6 mmol oxalate; test meal A) or 300 g kale (0.11 mmol oxalate; test meal B). (△), Individual data (n 9); ( ), mean absorption values.
oxalate (Hodgkinson, 1977b; Souci et al. 1994). It is not clear to what extent insoluble oxalates dissolve in the gastric juice and it could be speculated that soluble oxalates would have a more pronounced negative impact on mineral and trace element absorption due to their ability to form complexes with minerals and trace elements in the gastrointestinal tract. In addition, calcium oxalate has been suggested to be absorbed intact (Hanes et al. 1994) and it could be hypothesised that the more soluble magnesium oxalate could also be absorbed intact. In the present study, a large proportion (88 %) of total oxalates was present as water-soluble oxalates in spinach. However, at the present time, there is no information about the relative importance of soluble and insoluble oxalates on mineral and trace element absorption in man. Published data on the proportion of soluble oxalates relative to total oxalates also differ considerably; for example data for spinach vary from 15–20 % (Toma & Tabekhia, 1979; Souci et al. 1994) up to 93 % (Hodgkinson, 1977b).

Although based on indirect evidence, the observed difference in fractional Mg absorption in the present study is attributed to the differences in oxalate content; 6.6 mmol in the meal served with spinach and 0.1 mmol in the meal served with kale. Based on present knowledge, it is improbable that other plant components present in green leafy vegetables such as fermentable fibre or phenolic compounds would have influenced the results. For example, kale contains about 2 % fibre as pentosans and hexosans, about double the amount found in spinach (Souci et al. 1994). These partly soluble hemicelluloses are non-digestible and could be expected to be fermented by bacteria in the large intestine, similarly to fructo-oligosaccharides, which were reported to increase fractional Mg absorption in human subjects significantly when consumed in amounts of 10 g/d (Tahiri et al. 2001). Recently, the enhancing effect of polyols (100 g/d) on apparent Mg absorption in man was also demonstrated (Coudray et al. 2003a). However, information about the effect of dietary fibre on Mg absorption is not conclusive, as several human studies have not demonstrated statistically significant differences in Mg absorption after an increased intake of fermentable fibre (for a review, see Coudray et al. 2003b). In addition, a study based on the chemical balance technique reported a significant negative effect on Mg absorption after the intake of a mixture of galactans, pentosans, and hexosans (14 g/d) by adolescent boys (Drews et al. 1979). Furthermore, phenolic compounds have been reported to have a strong negative effect on Fe absorption (Gillooly et al. 1983; Brune et al. 1989). However, although phenolic compounds have not been evaluated for their effect on Mg absorption, the amount of total phenolic compounds has been reported to be similar in spinach and kale (Lucarini et al. 2000).

In conclusion, the results from the present study demonstrate that the mean fractional apparent Mg absorption from a test meal served with spinach was about 35 % lower than from a test meal served with kale. It is suggested that this reduction in Mg absorption is due to the higher oxalate content of spinach. However, the significantly lower fractional apparent Mg absorption from spinach can be assumed to be, at least partly, counterbalanced by the higher native Mg content of spinach as compared with kale.

References


