British Journal of Nutrition (2004), **91**, 113–120
DOI: 10.1079/BJN20031023

Comparison of urinary monitoring, faecal monitoring and erythrocyte analysis of stable isotope labels to determine magnesium absorption in human subjects

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(Received 5 May 2003 – Revised 2 September 2003 – Accepted 12 September 2003)

We have evaluated urinary monitoring and erythrocyte analysis to determine Mg absorption in human subjects as alternatives to the conventional technique of faecal monitoring by stable-isotope techniques. Ten healthy adults received 2·2 mmol ^{25}Mg in water, together with wheat bread, followed 15 min later by intravenous injection of 0·6 mmol ^{26}Mg (day 1). Brilliant blue and Yb (given on day 0 and day 1 respectively) served as qualitative and quantitative faecal markers. Urine was collected for 6 d after test meal intake. Complete collections of faeces were made until excretion of the second brilliant blue marker (given on day 7). Mg isotope ratios were determined by thermal ionisation-MS in urine and faeces and by inductively coupled plasma-MS in erythrocytes. Absorption was determined based on: (1) 6 d urine pools; (2) 24 h urine pools (collected 22–46 h after test meal intake); (3) erythrocytes from a blood sample drawn on day 14; (4) complete 6 d faecal pools; (5) faecal pools based on the first three consecutive stools after excretion of the first brilliant blue marker. Differences in mean Mg absorption (42 44 %) were statistically insignificant between techniques, except when based on 6 d urine pools for which the value was significantly lower (33 (SD 7) %, P=0·0003, ANOVA). The results indicate that Mg absorption can be determined from 24 h urine pools or erythrocytes obtained 14 d after test meal intake, an alternative method to the more time-consuming and labour-intense faecal monitoring. The choice of technique depends on practical and financial considerations.

Magnesium absorption: Stable isotopes: Faecal monitoring: Urinary monitoring: Erythrocytes

Inadequate intake of Mg and low serum Mg levels have been discussed in relation to major public health problems such as osteoporosis (Abraham, 1991), coronary artery disease (Karppanen, 1981) and non-insulin-dependent diabetes mellitus (Kao *et al.* 1999). Although information about dietary intake of Mg is available in many industrialised countries, very little is known about Mg bioavailability and the influence of diet composition on Mg absorption. The lack of information is at least partly due to the lack of suitable methodologies to investigate Mg metabolism in human subjects. The chemical-balance technique is not a useful tool, as absorption from single meals cannot be determined; although radioactive isotopes have been shown to be useful to study the absorption of minerals such as Ca (DeGrazia *et al.* 1965) and Zn (Arvidsson *et al.* 1978), Mg radioisotopes have half-lives that are too short (<21·3 h) to be useful.

Stable-isotopes techniques, however, can be used as Mg has three stable isotopes, two of them with low enough natural abundances to be employed as enriched labels: ^{25}Mg and ^{26}Mg (10·0 and 11·0 % natural abundance respectively; Catanzaro, 1966). The technique most often used to determine Mg absorption is based on faecal monitoring of non-absorbed stable isotope label. This technique has been applied to measure Mg absorption in human subjects in several studies (Schwartz *et al.* 1978, 1984; Schuette *et al.* 1993; Tahiri *et al.* 2001; Sabatier *et al.* 2003). A second isotope label can be injected intravenously so as to estimate the absorbed and re-excreted isotope label during the faecal collection period. By correcting apparent absorption (AA) of Mg via excretion of the intravenous label in faeces, true absorption (TA) of Mg as the fraction of isotopic label absorbed by the intestine can be calculated. A major drawback of faecal monitoring, however, is the need for complete faecal collections over several consecutive days. Non-absorbable rare earth elements as quantitative faecal markers have therefore been introduced, allowing the possibility

Abbreviations: AA, apparent absorption; ICP, inductively coupled plasma; TA, true absorption.

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of a reduced collection period (Schuette *et al.* 1993; Fairweather-Tait *et al.* 1997).

Urinary monitoring of an oral administered Mg isotopic label and a second intravenously injected Mg isotopic label was been introduced by Schwartz and colleagues as an alternative technique to determine the TA of Mg in human subjects (Schwartz *et al.* 1978, 1984). The first applications were limited by the precision in isotopic analysis achievable by neutron activation analysis and GC–MS. With the wider availability of thermal ionisation MS and inductively coupled plasma (ICP)-MS for high-precision Mg isotope-ratio measurements, urinary monitoring has been shown recently to be a potentially useful technique (Abrams & Wen, 1999; Sabatier *et al.* 2003).

Techniques based on erythrocyte incorporation of isotope labels are routinely used to measure the TA of Fe either with radioisotope labels (Cook *et al.* 1972) or stable isotope labels (Kastenmayer *et al.* 1994), as most of newly absorbed Fe is incorporated into this tissue. Although erythrocytes are not a specific target tissue for Mg, their Mg concentration is relatively high, approximately 2·5 mmol/l (Durlach, 1988). Coudray *et al.* (1997) reported erythrocyte analysis after administration of stable Mg isotope labels to be a useful method to determine Mg absorption in rats.

The aim of the present study was to compare the TA of Mg in healthy adult human subjects as determined by different Mg double-stable-isotope techniques. Measurements were based on isotope ratios in 6 d and 24 h urine pools and erythrocytes after oral and intravenous administration of two different isotope labels (^{25}Mg and ^{26}Mg). Faecal monitoring as the established technique to assess the TA of Mg in human subjects has been chosen as the reference technique for comparison. TA has been determined by faecal monitoring based on 6 d pools as the conventional approach and based on three-stool pools corrected for the recovery of Yb (a non-absorbable faecal marker) to evaluate whether the stool collection period can be shortened.

Materials and methods

Subjects

Ten apparently healthy, free-living subjects (five men, five women; BMI 21·2 (SD 3·7) kg/m^2, age 35·0 (SD 10·0) years) were recruited for the study. No lactating or pregnant women were included and no medication was allowed, except for oral contraceptives. Intake of mineral and/or vitamin supplements was not permitted 2 weeks before and during the study. The subjects were informed orally and in written form about the aims and the procedures of the study and written informed consent was obtained. The study protocol was reviewed and approved by the Ethical Committee of the Swiss Federal Institute of Technology, Zurich.

Isotope labels

Highly enriched ^{25}MgO (^{24}Mg 1·04 (SD 0·01), ^{25}Mg 98·73 (SD 0·01), ^{26}Mg 0·23 (SD 0·01) %) and ^{26}MgO (^{24}Mg 0·39 (SD 0·01), ^{25}Mg 0·11 (SD 0·01), ^{26}Mg 99·51 (SD 0·01) %) were purchased from Chemgas, Paris, France. ^{25}MgO (29·3 mmol) was dissolved in 2·5 ml 4 M-HCl and diluted to 100 ml with water to be served as an orally ingested label. The pH of the solution was adjusted to 4–5 by addition of an aqueous $NaHCO_3$ (Merck, Darmstadt, Germany) solution.

Doses for intravenous administration were prepared at the Cantonal Pharmacy, Zurich, Switzerland. Sterile water and sterile materials were used for their preparation. ^{26}MgO (9·5 mmol) was dissolved in 2·5 ml 4 M-HCl, diluted with water to 50 ml and adjusted to pH 6 as described earlier. The sterile filtered solution was divided into individual doses of 3·2 ml (about 0·6 mmol ^{26}Mg), transferred into glass vials, capped and sealed. The Mg concentration of the ^{25}Mg and ^{26}Mg isotope label in solution was determined by isotope dilution using thermal ionisation-MS against a commercial Mg standard of natural isotopic composition (Titrisol; Merck).

Unless otherwise noted, all chemicals were of analytical grade, and all acids were further purified by surface distillation. Only 18 MΩ water (Milli Q water system; Millipore, Zurich, Switzerland) was used for laboratory work and test meal preparation.

Test meal

The standardised breakfast consisted of wheat bread rolls, prepared from 75 g white flour, using a standard recipe. A relatively long fermentation (5 h) was used to degrade phytic acid, a potential inhibitor of Mg absorption. Bread rolls were prepared in bulk and stored frozen. Water (200 g) was served as a drink, to which 2·20 (range 2·03–2·31) mmol ^{25}Mg and 25·4 (range 25·3–26·5) μmol Yb (as $YbCl_3.6H_2O$; Aldrich, Buchs, Switzerland) were added in solution. The amount of ingested isotope labels and Yb was determined by weighing.

Study design

Urine and faecal spot samples were collected on the day before the test meal administration to determine baseline Mg isotopic ratios. A gelatine capsule containing about 100 mg brilliant blue (Warner Jenkinson Europe, King's Lynn, Norfolk, UK) was administered orally to mark the start of the faecal collection period. Venous blood samples (10 ml) were drawn into heparinised glass tubes (Vacutainer Systems, Plymouth, Devon, UK) for plasma Mg analysis. The test meal was served after an overnight fast. Subjects were instructed to eat half of the bread before drinking the labelled water in order to slow down gastrointestinal passage of the isotope label. Fifteen minutes after test meal intake, 0·60 (range 0·57–0·65) mmol ^{26}Mg was administered intravenously. A sterile injection system consisting of a two-way catheter and a septum injection port was used. The isotope label solution was transferred quantitatively by flushing the system with physiological saline (10 ml, 9 g NaCl/l). The amount of injected isotope label was determined by weighing the syringe before and after injection. No food or drink was allowed for 3 h following breakfast. Standardised meals were provided for lunch

(lasagne) and dinner (pizza) on day 1. Water (2 litres) was provided as the only beverage for day 1. No additional food was allowed on day 1. From day 2 onwards, diet was unrestricted. To standardise faecal collection, a second brilliant blue capsule (Warner Jenkinson Europe) was administered on day 7 to mark the endpoint of the faecal collection period. All stool samples were collected separately, starting immediately after test meal administration. Faeces were collected until the second brilliant blue marker appeared. Urine was collected in parallel in 24 h pools until the end of day 6. On day 14, a venous blood sample (10 ml) was drawn into a heparinised glass tube for Mg isotopic analysis of erythrocytes. Plasma was not analysed for its isotopic composition, as kinetic studies suggested a relatively low half-life of Mg in plasma, and therefore an isotopic enrichment too low for isotopic analysis at day 14 (Avioli & Berman, 1966; Feillet-Coudray *et al.* 2002).

Urine, stool and blood sampling

Urine samples were collected in pre-weighed polyethylene containers (Semadeni, Ostermundingen, Switzerland). Each 24 h pool was weighed, a 50 g portion was removed and acidified with 0·5 g 10 M-HCl before storage (−25°C). Urine pools (6 d) were prepared by combining 1 % of each 24 h pool by weight.

Stools were collected in pre-weighed polypropylene containers (Semadeni), freeze-dried (Modulyo, Edwards, North Bergen, NJ, USA), weighed and ground in a mortar. Two stool pools were prepared:

(1) 6 d stool pools: all stools collected from the appearance of the first brilliant blue marker until, but not including the stool dyed by the second brilliant blue marker (given on day 7). For preparation of the pools, 5 % of each stool were taken by weight, combined and thoroughly mixed.
(2) three-stool pools: the first three consecutive stools after the appearance of the first brilliant blue marker, including the first dyed stool, were pooled by combining 5 % of each stool by weight.

Venous blood samples (10 ml) were centrifuged at 20°C at 500 g (Omnifuge 2.0 RS; Heraeus, Zurich, Switzerland) to separate plasma from blood cells. Blood cell fractions were washed three times with small portions (2–3 ml) of physiological saline (9 g NaCl/l). Washed blood cell fractions, containing almost exclusively erythrocytes, were stored in acid-washed polyethylene vials (−25°C) for later analysis.

Sample digestion

Freeze-dried bread (1 g), acidified urine (3 ml), blood plasma (1 ml) and erythrocytes (1 ml) were digested with a mixture of 14 M-HNO_3 and 8·8 M-H_2O_2 (Merck), using a microwave (MLS 1200; MLS GmbH, Leutkirch, Germany). Faecal samples were dry-ashed in covered Pyrex glass beakers in a muffle furnace (M 110; Heraeus) at 550°C for 12 h after addition of 5 ml 14 M-HNO_3. The ashes were dissolved in 10 ml 5 M-HCl. Beakers were discarded after use.

Sample preparation for isotopic analysis

All samples were analysed in duplicate. Mg was separated from the digested samples by cation-exchange chromatography using a strongly acidic ion-exchange resin (AG 50W X-8, 200–400 mesh; Bio-Rad, Hercules, CA, USA). Portions of the digested stool, urine and erythrocyte samples, containing approximately 8·2 μmol (faecal samples) or 2·5 μmol (urinary and erythrocyte samples) Mg, were evaporated to dryness and redissolved in 1 ml 0·7 M-HCl and transferred onto the top of a column (Bio-Rad, 10 mm inner diameter), filled with the ion-exchange resin to a height of 70 mm. The column was rinsed with 56 ml 0·7 M-HCl, followed by 24 ml 0·9 M-HCl to elute Na and K. Mg was eluted with 12 ml 1·4 M-HCl. The solution was evaporated to dryness and redissolved in 50 μl water. Mg recovery was evaluated with a diluted Mg standard solution (Titrisol; Merck). At a recovery of 94·8 (SD 1·8) % (n 10), potential Mg isotopic fractionation on the column as a possible source of systematic bias is negligible. Resins were regenerated with 30 ml 6 M-HCl and renewed after the fifth run. Isotopic and elemental analysis was performed under blank control. Only acid-washed Teflon and polyethylene laboratory ware was used for sample processing. Portions of the ^{26}Mg isotope label were processed in parallel with each batch for blank monitoring from ion-exchange chromatography onwards. Sample contamination due to natural Mg was found to be 10·3 (SD 4·1) nmol (n 6) for combined sample preparation and filament loading, which was $<0{\cdot}5$ % of the Mg separated.

Isotopic analysis by thermal ionisation-MS

Faecal and urine samples were analysed for isotopic composition by loading about 20 nmol separated Mg onto the metal surface of the evaporation filament of a double-Re filament ion source. Mg was coated with 5–10 μg silicagel 100, 0·8 μmol boric acid and 30 nmol Al as $AlCl_3$ (all chemicals from Merck). Compounds were loaded in aqueous solution and dried at 0·8 A after each step. Finally, the evaporation filament was heated to dull red heat (1·6 A) for 30 s. The ionisation filament remained unloaded. Isotopic ratios were determined with a single-focusing magnetic sector field instrument (MAT 262; Finnigan MAT, Bremen, Germany), equipped with a Faraday cup multicollector device for simultaneous ion-beam detection. The evaporation filament was heated to 1230°C using a standardised procedure. The ionisation filament was heated gradually to 1250–1350°C until a stable Mg^+ ion beam of $1-2\times10^{-11}$A was obtained. Each measurement consisted of thirty consecutive isotopic ratio measurements.

Reproducibility (five independent runs) was 0·2 % (relative SD) for the ^{24}Mg:^{25}Mg isotopic ratio and 0·4 % (relative SD) for the ^{24}Mg:^{26}Mg isotopic ratio.

Isotopic analysis by inductively coupled plasma-MS

Isotopic enrichment of erythrocytes was found to be too low for isotopic analysis by thermal ionisation-MS. Erythrocyte Mg was, therefore, analysed by multicollector ICP-MS at higher precision. All ICP-MS measurements

were carried out using a magnetic sector ICP-MS (IsoProbe; Micromass, Manchester, UK) equipped with a multi-collector system of nine Faraday cups. The sample introduction system consisted of a Micromist nebuliser and a Cinnabar spray chamber (both Glass Expansion, Romainmotier, Switzerland). The instrument utilises a hexapole collision cell for collisional focusing and interference reduction. The collision gases were H (1·0 ml/min) and He (7·5 ml/min).

Further studies using the high-resolution mode of the instrument revealed a small interference at mass 26 due to the formation of atomic or molecular ions in the ion source. This interference remained stable even with NaCl (100 μg/g) added to the measurement solution. No matrix effects on the measured isotopic ratios were observed. The interference on mass 26 was therefore corrected by the external blank subtraction.

The measurement sequence for each enriched sample was: NBS980 (standard reference material 980; National Institute of Standards and Technology, Gaithersburg, MD, USA), baseline sample, enriched sample, baseline sample and finally again NBS980. Drifts in mass discrimination were corrected by assuming a linear drift between the two measurements of the isotope reference material. Reproducibility (six independent runs) was 0·01 % (relative SD) for the ^{24}Mg:^{25}Mg isotopic ratio and 0·02 % (relative SD) for the ^{24}Mg:^{26}Mg isotopic ratio. More details about the ICP-MS procedure applied can be obtained from Klingbeil *et al.* (2001).

Magnesium and ytterbium

Total Mg content of stool pools, plasma and bread rolls was determined after dilution of the digested samples by flame atomic absorption spectroscopy (SpectrAA 400; Varian, Mulgrave, Victoria, Australia) using standard procedures. A commercial Mg standard (Titrisol; Merck) was used for internal calibration (standard addition technique) to minimise matrix effects. Certified reference materials (Wheat Flour 1567a, National Bureau of Standards, Gaithersburg, MD, USA; Seronorm Trace Elements Serum, Nycomed, Oslo, Norway) were analysed in parallel for quality control. Yb was measured in the digested and diluted faecal samples by electrothermal atomic absorption spectroscopy, using an external calibration technique against a Yb standard solution (Titrisol; Merck).

Calculations

Mg absorption was calculated by five different techniques based on the molar amounts of the ^{25}Mg and ^{26}Mg isotope label in the samples, which were calculated based on double-isotope dilution principles (Walczyk *et al.* 1997; Sabatier *et al.* 2002). Molar amounts of isotope labels were calculated from measured isotope ratios, as the stable isotope labels used were not mono-isotopic. The ratio of the molar amounts of both isotope labels in the sample is referred to as their molar amount ratio in the following. Techniques involving quantification of both isotope labels (urinary monitoring, erythrocyte analysis and faecal monitoring after correction for re-excreted isotopic label) give the TA of Mg as the amount of oral isotope label absorbed by the intestine. Faecal monitoring based solely on the recovery of the oral isotope label in faeces delivers the AA of Mg. The AA of Mg is generally lower than the TA, as it does not take into account the fraction of oral isotope label that has been absorbed and re-excreted into faeces during the faecal collection time.

Technique 1: true absorption via urinary monitoring of both isotope labels. TA (% oral isotope label) was calculated from the molar amount ratio of oral and intravenous isotope label n_{25}:n_{26} in 6 d pools (technique 1a) and 24 h pools collected 22–46 h after test meal intake (technique 1b) respectively:

$$\text{TA } (\%) = \frac{n_{25}}{n_{26}} \times \frac{D_{iv}}{D_0} \times 100, \quad (1)$$

where D_{iv} is the amount isotope label injected (μmol) and D_0 the amount oral isotope label ingested (μmol).

Technique 2: true absorption measured via isotopic analysis of erythrocytes. TA of the oral isotope label (%) was calculated from the molar amount oral:intravenous isotope label ratio in erythrocytes drawn 14 d after isotope label administration. Calculations followed equation 1 using the amount ratio of both isotope labels n_{25}:n_{26} in erythrocytes instead of those in urine.

Technique 3: apparent absorption via recovery of oral isotope label. AA (%) was calculated as the difference between the amount of oral isotope label given (D_0) and the amount excreted in faeces (F_0) in μmol for the 6 d faecal pools assuming a complete recovery of non-absorbed oral isotope label (technique 3a).

$$\text{AA } (\%) = 100 \times \frac{D_0 - F_0}{D_0}. \quad (2)$$

For a shortened faecal collection period (technique 3b), apparent Mg absorption was calculated using equation 3. Calculations were based on the first three consecutive stools after appearance of the first brilliant blue marker, including the first dyed stool. Incomplete recovery of the oral isotope label was corrected for by using the fraction of rare earth element recovered in faeces (% REE):

$$\text{AA } (\%) = 100 \times \left(1 - \frac{D_0 - F_0}{D_0}\right) \times \frac{100}{\%\ \text{REE}}. \quad (3)$$

Technique 4: true absorption via faecal monitoring of oral and intravenous isotope label. TA (%) was derived from AA by correction for the molar amount of oral isotope label that was absorbed and excreted within the faecal collection period. The molar amount of intravenous label (F_{iv}) recovered in the 6 d pools was used for correction (technique 4a).

$$\text{TA } (\%) = 100 \times \left(\frac{(D_0 - F_0)/D_0}{1 - F_{iv}/D_{iv}}\right). \quad (4)$$

For a shortened faecal collection period covering three consecutive stools including the first dyed stool (technique 4b), incomplete recovery of the oral label was corrected via the recovery of the administered rare earth element in the

faecal pool as described for technique 3b.

$$\text{TA } (\%) = 100 \times \left(\frac{(D_0 - F_0)/D_0}{1 - F_{iv}/D_{iv}} \right) \times \frac{100}{\%\ \text{REE}}. \quad (5)$$

Statistics

Calculations were performed using commercial spreadsheet software (Excel 97, Microsoft and SPSS 10.0; SPSS Inc., Chicago, IL, USA). ANOVA was made using a general linear model, followed by either a Bonferroni test or Student's paired two-tailed *t* test to determine differences between methods. Statistical significance was considered for $P<0{\cdot}05$. Correlation between methods was evaluated by Pearson correlation coefficients. Normal distribution of absorption values was verified by skewness and the Kolmogorov–Smirnoff test. Homogeneity of variance between the methods was verified with Levene's test. Absorption values are presented as arithmetic means and standard deviations.

Results

One subject was excluded from the three-stool pool evaluation due to gastrointestinal problems, and one subject was excluded from the double-isotope evaluations due to incomplete intravenous injection. Plasma concentrations of Mg were 1·02 (range 0·92–1·22) (SD 0·09) mmol/l (*n* 10; reported normal range 0·75–0·96 mmol/l; Lowenstein & Stanton, 1986). The native Mg content of the wheat bread was 1·91 mmol per serving.

Mean Mg absorption from the wheat bread consumed with water varied from 32·9 to 44·2 % (Table 1) based on the different techniques. AA values differed significantly from TA values (6 d stool pools $P=0{\cdot}001$, three-stool pools $P=0{\cdot}01$). TA based on the 6 d urine pools was significantly lower than TA calculated using the other techniques ($P=0{\cdot}0003$, ANOVA). There were no significant differences between TA values based on the 24 h urine pools (22–46 h), erythrocyte analysis and the 6 d faecal pools or three-stool pools.

Brilliant blue appeared in faeces on day 1 or 2 in all subjects. Completeness of faecal collection was evaluated by the recovery of Yb; 98·7 (range 91·3–107·9) (SD 5·6) % (*n* 10) was recovered in the 6 d stool pools. Mean duration of faeces collection using brilliant blue for marking the beginning and end of faeces collection was 6·3 (range 5–7) (SD 0·7) d for the 6 d faecal pools. Excretion of the ^{25}Mg label and of Yb measured in the three-stool pools were significantly correlated (Fig. 1; $P<0{\cdot}01$). Yb recovery in the three-stool pools, excreted within 3·0 (SD 0·9) d after intake of the isotope labels, was 80·5 (range 55·2–101·0) (SD 18·5) % (*n* 9). AA and TA determined by faecal monitoring based on the 6 d stool pools and the three-stool pools were significantly correlated (AA *r* 0·76, $P<0{\cdot}05$; TA *r* 0·83, $P<0{\cdot}05$) as was TA based on 6 d and 24 h urine pools (*r* 0·73, $P<0{\cdot}05$). Mg absorption based on erythrocyte analysis was significantly correlated only to absorption based on the 24 h urine pools (*r* 0·78, $P<0{\cdot}05$).

A difference in isotopic composition between erythrocytes and Mg of non-biological origin was observed. Baseline Mg isotopic ratios in erythrocytes of ten subjects were greater by 2·1 (SD 0·3) and 3·6 (SD 0·8) ‰ (^{25}Mg:^{24}Mg and ^{26}Mg:^{24}Mg respectively) than the certified isotope

Table 1. Fractional magnesium absorption (%) based on different stable isotope techniques*†

Method...	Urinary monitoring		Erythrocyte analysis	Faecal monitoring			
Information...	True absorption		True absorption	Apparent absorption		True absorption	
Sample...	6 d pool	24 h pool	day 14	6 d pool	three stool pool	6 d pool	three stool pool
Technique...	1a	1b‡	2	3a	3b	4a	4b
Subject 1	26·2	37·9	34·7	39·7	34·2	51·1	42·8
Subject 2	43·8	50·0	51·4	52·7	47·1	63·6	52·8
Subject 3	43·3	55·5	49·4	44·4	43·8	47·8	50·1
Subject 4	28·3	34·0	38·8	36·3	36·0	40·5	37·2
Subject 5	24·5	45·0	45·8	29·9	36·7	32·4	39·0
Subject 6	35·2	51·2	48·7	36·9	41·0	48·5	41·6
Subject 7	29·9	39·0	30·0	32·5	39·3	38·9	40·2
Subject 8	ND	ND	ND	30·1	29·2	ND	ND
Subject 9	33·4	39·1	44·9	38·1	ND	48·8	ND
Subject 10	31·8	36·9	37·9	22·8	34·3	25·7	36·7
Mean	32·9[a]	43·2[b]	42·4[b]	36·3[c]	38·0[c]	44·2[b]	42·6[b]
SD of mean	6·9	7·6	7·4	8·4	5·5	11·2	5·9
Δ(^{24}Mg : ^{25}Mg)§	5·6	6·4	1·7	15·2	28·2	15·2	28·2
SD of mean	1·0	1·1	0·2	3·1	12·4	3·1	3·1
Δ(^{24}Mg : ^{26}Mg)§	4·4	3·9	1·0	0·8	0·9	0·8	0·9
SD of mean	0·5	0·5	0·2	0·5	0·5	0·5	0·5

ND, not determined.
[a,b,c] Mean absorption values within a row with unlike superscript letters were significantly different ($P<0{\cdot}05$).
* For details of procedures, see p. 116.
† Isotope ratio measurements were determined by thermal ionisation-MS in urine and faeces and by inductively coupled plasma–MS in erythrocytes obtained 14 d after isotope administration.
‡ Based on urine collected 22–46 h after test meal intake.
§ Mean isotopic enrichments (%) over baseline values in the sample material are given for the ^{24}Mg:^{25}Mg [Δ(^{24}Mg:^{25}Mg)] and the ^{24}Mg:^{26}Mg [Δ(^{24}Mg:^{26}Mg)] isotope ratio, respectively.

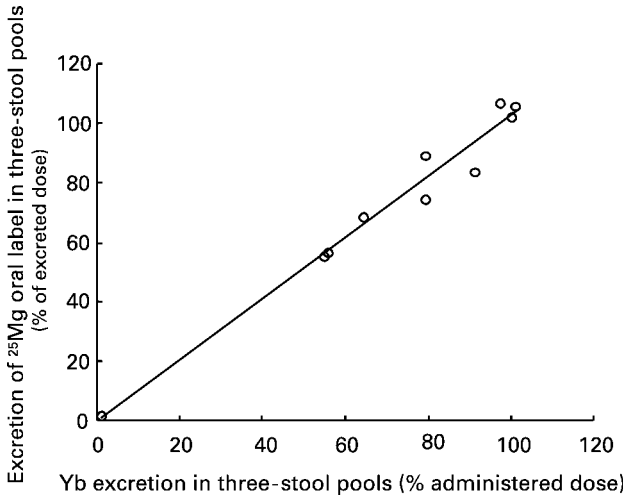

Fig. 1. Correlation between fractional excretion of the oral isotope label (^{25}Mg) and the quantitative faecal marker (ytterbium) in three-stool pools ($y = 1{\cdot}024x - 0{\cdot}241$; R 0·99, $P<0{\cdot}01$). Values are means for ten subjects.

reference material (NBS980; standard reference material 980; National Institute of Standards and Technology) ($P=0{\cdot}00001$, n 10).

Discussion

Our present results demonstrate for the first time that the molar amount ratio of orally ingested and intravenously administered Mg stable isotope labels in erythrocytes (technique 2) is a potentially useful technique to determine the TA of Mg in human subjects (see Table 1). The same is given for the urinary monitoring technique based on 24 h urine pools collected 22–46 h after test meal administration (technique 1b). For either technique, the TA of Mg from the test meal was not significantly different when compared with results obtained by the conventional double-isotope faecal-monitoring method (technique 4a). In addition, we have shown that the shorter three-stool pool method, corrected for incomplete faecal collections by excretion of Yb (techniques 3b and 4b), resulted in similar Mg absorption results as the 6 d faecal-monitoring method (techniques 3a and 4a respectively). It appears, therefore, that any of the methods to determine the TA of Mg will give similar results when used to monitor dietary factors influencing Mg absorption, at least in a healthy young study population. The only method that resulted in a significantly lower TA relative to faecal monitoring was that based on the 6 d urine pools. As expected, TA (techniques 4a and 4b respectively) was significantly higher than AA (technique 3a and 3b respectively).

Erythrocytes are easily accessible and, without the need for complete faecal or urine collections, erythrocyte analysis of isotope labels would greatly reduce the time and labour needed to determine Mg absorption. The method assumes that, after absorption, the isotope label added as an extrinsic tag to a meal is metabolised in an identical way to an isotope label injected intravenously. Although injecting the isotope label intravenously as a bolus 15 min after consumption of a simple test meal appeared satisfactory in our present studies, the timing and duration of the infusion needs further evaluation with more complex meals. Fe absorption is routinely determined using erythrocyte incorporation of a single orally administered isotope label, assuming a mean erythrocyte incorporation of 80 % after 14 d (Cook *et al.* 1972). In our present studies, about 1 % of the absorbed oral dose was recovered in erythrocytes. Although further studies are needed to validate erythrocyte analysis as a method for determination of Mg absorption, this approach is supported by an earlier study in rats: there was no significant difference in absorption based on erythrocytes, plasma and urine samples collected 48 h (erythrocytes and plasma) or 36–48 h (urine) after oral and intravenous isotope label administration (Coudray *et al.* 1997). Isotopic analysis of erythrocytes, however, is more demanding. Isotopic enrichments in erythrocytes are significantly lower when compared with faecal pools and require more precise isotope ratio measurements to generate meaningful data.

Measurement of low isotopic enrichment in biological samples might be associated with an additional methodological problem. Natural isotopic abundances of an element can be altered, in principle, by physiological processes (Galimov, 1985). Using thermal ionisation-MS, no such effects were observed for Mg in faecal and urine baseline samples relative to a Mg standard of non-biological origin (Titrisol; Merck), but the more precise multicollector ICP-MS measurements revealed differences in Mg isotopic composition between erythrocytes and an isotope reference material of non-biological origin. This problem was overcome by measuring the enriched sample together with the baseline sample for each subject.

TA was also determined in the present study based on molar amounts of oral:intravenous isotope label ratio in urine. TA of Mg based on 6 d urine pools was, however, significantly lower ($P<0{\cdot}05$) compared with all other techniques of TA, including the 24 h urine pools (Table 1). Recently Sabatier *et al.* (2003) reported a significantly lower TA based on 3 d (days 1–3) urine pools compared with 12 d faecal pools. It has been suggested that absorption is underestimated when based on urine collected during the first hours after isotope label administration, because oral and intravenous labels are metabolised differently and absorption of the oral isotope label might not yet be complete (Abrams & Wen, 1999; Sabatier *et al.* 2003). The inclusion of the first 24 h pools in our 6 d urine pools could thus explain the lower absorption value generated by the 6 d pools. In addition, a potential concern is the administration of the intravenous Mg label. Serum Mg levels are strictly regulated by the kidney (Elin, 1994). Thus, if the intravenous bolus results in a non-physiologically high level of free Mg in the blood, excess Mg may be rapidly excreted in urine, and the molar amounts of oral:injected isotope label ratio in urine will underestimate absorption in the first hours after isotope administration. To overcome this problem, Coudray *et al.* (1997) suggested that the intravenous dose should be infused over a long period of time or divided into several smaller doses. Our present findings indicate that a single intravenous bolus administered 15 min after the oral isotope label is suitable to determine Mg absorption, provided that urine collection is

initiated later than about 22 h after isotope label administration; this is in agreement with the findings of Sabatier *et al.* (2003). It can be assumed that after about 22 h, TA based on urinary monitoring of isotope labels corresponds to TA as determined by the conventional faecal-monitoring technique using an intravenous isotope label to correct for re-excreted oral isotope label.

The conventional method for measuring Mg absorption based on faecal monitoring of all non-absorbed isotope labels is time consuming and laborious. To determine TA by this technique, a second intravenous isotope label is necessary to estimate the amount of absorbed oral isotope label that is re-excreted during the faecal collection period. This makes the method as invasive and expensive as urinary monitoring or erythrocyte analysis of isotope labels. For paired comparisons, however, determination of AA of Mg by faecal monitoring remains the only option. Because both isotope labels are used for test meal labelling, no further isotope label is available for intravenous administration. TA of Mg is significantly underestimated by this method (Table 1), but it is less invasive and cheaper than other techniques as intravenous administration of a second isotope label is not required.

A major technical problem with faecal monitoring is the incomplete recovery of non-absorbed oral isotope label, either due to incomplete faecal collection by the subjects or due to prolonged gastrointestinal passage, resulting in an overestimate of absorption. This source of error was minimised in our present study by the use of brilliant blue to determine the starting point and the endpoint of the faecal collection period, and by using Yb as a non-absorbable quantitative faecal marker for stool collection. Our present results indicate that faecal collections could be shortened from 6 d to three consecutive stools after excretion of the first dose of brilliant blue by correcting for incomplete recovery of isotope label using the rare earth element Yb. In the three-stool pools, excretion of the oral Mg isotope label and Yb was significantly correlated (Fig. 1), and AA and TA determined by both techniques did not differ significantly (Table 1, techniques 3a, 3b and 4a, 4b respectively). Gastrointestinal passage time of the oral Mg isotope label and Yb were relatively fast and the minimum recovery was 55 % in the three-stool pools. Schuette *et al.* (1993) suggested that at least 40 % of the rare earth element should be excreted in order to correct for incomplete collection, using Dy as a faecal marker to correct for Mg absorption. Although it can be assumed that many of the rare earth elements exhibit similar excretion patterns (Fairweather-Tait *et al.* 1997), we chose Yb rather than Dy or Sm because it could be detected with higher sensitivity by electrothermal-atomic absorption spectroscopy. In order to apply a correction by using a non-absorbable faecal marker, excretion patterns of Mg and the rare earth elements have to be similar. Based on the results from the present study, the shorter stool collection technique is well suited for studies in young healthy adults.

In conclusion, our present study showed that erythrocyte analysis of stable isotope labels 14 d after administration of an oral and an intravenous isotope label as well as monitoring of both labels in urine collected 22–46 h after isotope administration could be useful alternative methods to the faecal-monitoring technique to determine Mg absorption from food. If isotopic ratios can be measured at high precision, erythrocyte analysis offers a relatively simple technique avoiding faeces and urine collections. In addition, in a young healthy study population, 6 d faecal monitoring can be shortened to three consecutive stool pools by including Yb as a non-absorbable faecal marker.

Acknowledgements

We would like to thank Guido Fisher at the Cantonal Pharmacy Zurich, Switzerland, for preparing the intravenous doses, Michael Zimmermann for administration of the intravenous doses and Marlies Krähenbühl for drawing blood samples.

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