

## Measurement of magnesium absorption and retention in type 2 diabetic patients with the use of stable isotopes<sup>1-3</sup>

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### ABSTRACT

**Background:** Magnesium deficiency has been associated with type 2 diabetes and may reduce insulin sensitivity and impair glucose tolerance. The etiology of magnesium depletion in diabetes is unclear. Animal studies suggest that diabetes may impair magnesium absorption; however, there are no published data on magnesium absorption in humans with diabetes.

**Objective:** Magnesium absorption from a test meal and the excretion and retention of magnesium were compared between patients with type 2 diabetes and healthy control subjects.

**Design:** A meal labeled with 10 mg <sup>26</sup>Mg isotopic label was administered, and stool and urine samples were collected for 10 and 6 d, respectively. Apparent absorption was calculated as the difference between the oral dose of <sup>26</sup>Mg isotopic label and the total amount of the isotopic label excreted in the feces. Magnesium retention was calculated from the apparent absorption and urinary excretion of <sup>26</sup>Mg isotopic label in the 6 d after administration.

**Results:** Mean ( $\pm$ SD) values for fractional magnesium absorption in the diabetic patients and the control subjects were  $59.3 \pm 7.0\%$  and  $57.6 \pm 8.5\%$ , respectively (NS). Mean ( $\pm$ SD) urinary magnesium excretion values in the diabetic patients and the control subjects were  $11.2 \pm 2.6\%$  and  $11.7 \pm 3.8\%$ , respectively (NS); retention values were  $54.2 \pm 7.1\%$  and  $51.4 \pm 6.1\%$ , respectively (NS).

**Conclusion:** Dietary magnesium absorption and retention are not impaired in patients with reasonably well-controlled type 2 diabetes. *Am J Clin Nutr* 2003;78:448–53.

**KEY WORDS** Magnesium, absorption, excretion, retention, diabetes, stable isotopes, fecal monitoring

### INTRODUCTION

Magnesium deficiency has been reported in type 2 diabetes mellitus. In the United States, 25–39% of diabetic outpatients have low concentrations of serum magnesium (1). Low serum magnesium concentrations in patients with type 2 diabetes have also been reported in several European countries, eg, Austria, Germany, Italy, France, and Sweden (2–6). Similarly, we found significantly lower serum magnesium concentrations in type 2 diabetics than in healthy control subjects in a Swiss population (M Wälti, unpublished observations, 2002). Magnesium depletion has a negative effect on glucose homeostasis and insulin sensitivity in persons with type 2 diabetes (7, 8) and on the evolution of complications such as retinopathy (9), thrombosis (8), and hypertension (8).

The reasons why magnesium deficiency occurs in diabetes are not clear but may include higher urinary losses, lower dietary

intakes, or impaired absorption of magnesium compared with healthy persons. We recently reported that a low dietary intake of magnesium does not appear to contribute to impaired magnesium status in diabetic patients in Switzerland (10). Several studies reported increased urinary magnesium excretion in type 1 and 2 diabetes (6, 11–15), whereas other studies found no significant differences in magnesium excretion between diabetic patients and healthy control subjects (16, 17). Several authors have suggested that impaired intestinal absorption might contribute to the low magnesium status in diabetic patients (7, 18, 19). Fractional magnesium absorption is lower in alloxan-induced diabetic rats than in controls (20). Using in situ perfusion of intestinal segments, Miller and Schedl (21) reported significantly lower magnesium absorption in streptozocin-induced diabetic rats than in controls. However, there are no published data on magnesium absorption in humans with diabetes.

Magnesium has 3 stable isotopes, 2 of which (<sup>25</sup>Mg and <sup>26</sup>Mg) have low enough abundances (10.0% and 11.01%, respectively) to be used as isotopically enriched labels (22, 23). The objective of the present study was to compare magnesium absorption and retention between patients with type 2 diabetes and healthy control subjects with the use of stable isotopes.

### SUBJECTS AND METHODS

#### Subjects

Diabetic patients were recruited from the outpatient diabetes clinic at the University Hospital of Zurich and through advertisements in a local newspaper and a diabetes newsletter. All of the patients had a history ( $\geq 3$  y) of type 2 diabetes, were in reasonable metabolic control [mean glycated hemoglobin (Hb A<sub>1c</sub>): 7.3%], and had no history of nephropathy. Of the 12 diabetic patients enrolled, 3 were taking insulin, 7 were taking oral antidiabetic

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Received November 5, 2002.

Accepted for publication March 20, 2003.

TABLE 1

Overview of the standardized diets provided on days 0 and 1 of the study

	Day 0	Day 1
Breakfast	Free choice	1 Wheat bread roll (60 g) and 300 g isotopically labeled ultrapure water
Lunch	Vegetable lasagna (480 g)	Vegetables and potato meal (600 g)
Snack	1 Apple and 1 cereal bar (17 g)	1 Apple and 1 cereal bar (17 g)
Dinner	4 Pancakes filled with mushrooms (240 g) and carrot salad (160 g)	Rice (140 g) and ratatouille (160 g)
Drinks	Only tap water	Only ultrapure water

agents, and 2 were not taking any antidiabetic medication. Non-diabetic subjects were recruited from the local community and from the staff of the Swiss Federal Institute of Technology Zurich. All subjects in both the diabetic and control groups were white. At screening, none of the subjects had detectable urinary glucose, protein, or ketone bodies in their urine (Keto-Diastix/Albustix; Bayer Corporation, Tarrytown NY). None of the subjects were regularly taking diuretics, laxatives, or other medications known to directly affect urinary or bowel function. Inclusion criteria included a body mass index (in kg/m<sup>2</sup>)  $\geq 20$  and  $\leq 29.9$ .

To ensure enrollment of subjects with a normal magnesium status, a venous blood sample was drawn 2–3 wk before the study began, and only those with normal plasma magnesium concentrations [reference range: 0.65–1.05 mmol/L (24)] were included in the study. Initially, 13 diabetic patients and 12 control subjects were enrolled. Two subjects (one in each test group) dropped out of the study after the test meal because of gastrointestinal discomfort, and one subject in the control group did not make a complete stool collection. Thus, 12 type 2 diabetic patients and 10 healthy nondiabetic subjects completed the study. Sample size allowed detection of an absolute difference of 12% in magnesium absorption between groups with a significance level of 0.05 and a power of 90%. Written informed consent was obtained from each subject, and the study protocol was approved by the Ethical Committee of the Swiss Federal Institute of Technology Zurich.

### Study protocol

The study protocol was based on the methods described by Turnlund and Keyes (25) and by Bohn (26). The subjects were instructed to take no vitamin-mineral supplements in the 2 wk before or during the study. The day before isotope administration, a fecal sample and a urine spot sample were collected in polyethylene containers for the determination of baseline magnesium isotope ratios. The weight and height of each subject were measured. The most recent Hb A<sub>1c</sub> concentration was recorded from the medical record of the diabetic subjects. For all subjects but one, this value was obtained within 2 mo of the study period. A standardized vegetarian diet was provided for the whole day (Table 1). At 2000 on the evening before the test meal was provided, 100 mg Brilliant Blue, a fecal marker, was given orally in a gelatin capsule to indicate the start of the fecal collection period.

After the subjects fasted overnight, they were served a standardized test meal consisting of a wheat-bread roll and 300 g isotopically labeled ultrapure water for breakfast. The water was labeled with 10 mg <sup>26</sup>Mg isotopic label and 5 mg Yb immediately before being administered. Ytterbium was used as a nonabsorbable marker to control for complete stool collection (27). The exact amounts of the ingested isotopic label and fecal marker were determined by weighing the water beaker before and after the test

meal was administered. The bread roll and the drink were ingested simultaneously. No food or drink was allowed for 3 h after ingestion of the test meal. A standardized diet and 2 L ultrapure water were provided for the remainder of day 1 (Table 1). From day 2 onward, the diet was unrestricted.

After isotope administration, the subjects collected all urine and stool in preweighed polyethylene containers. The containers used for urine collection contained 5 mL concentrated HCl/L urine to reduce precipitation. Six days after the isotopic label was ingested, 100 mg Brilliant Blue was again consumed orally, and stool was collected in individual portions until the excretion of the second fecal marker was complete. Urine was collected until the evening of day 6. Stool and urine samples were weighed and then stored at  $-25^{\circ}\text{C}$  until analyzed.

### Materials

#### Reagents

All chemicals used were analytic grade. Nitric acid and hydrochloric acid were further purified by subboiling distillation. All water used for the analytic procedures and as drinking water on day 1 was purified by ion exchange and reverse osmosis (18 M $\Omega$ , RD2000, Renggli AG, Rotkreuz, Switzerland; Nanopure Cartridge System, Skan AG, Basel, Switzerland). Acid-washed laboratory ware was used for stool and urine sample analyses.

#### Bread rolls

To reduce the phytic acid content of the wheat-bread rolls, commercial low-extraction wheat flour was used, and the fresh dough was allowed to ferment for 5 h. The dough was separated into identical portions of 74.0 g before being baked. The baked bread rolls were kept frozen until used.

#### Isotopic label

For preparation of the isotopic label, highly enriched <sup>26</sup>MgO (96.8%) was purchased from AMT Ltd (St Kiryat Ono, Israel) and converted into <sup>26</sup>MgCl<sub>2</sub> by dissolution in 4 mol HCl/L. The <sup>24</sup>Mg/<sup>25</sup>Mg isotope ratio was adjusted close to the natural <sup>24</sup>Mg/<sup>25</sup>Mg isotope ratio of 7.899 (22, 23) by using a <sup>25</sup>MgCl<sub>2</sub> solution prepared from <sup>25</sup>MgO (enrichment 97.86%; AMT Ltd) according to the same procedure as described for <sup>26</sup>MgCl<sub>2</sub>. An adjustment of the ratio of <sup>24</sup>Mg to <sup>25</sup>Mg in the isotopic label was necessary to allow for internal normalization of the data. Normalization of the isotope ratios increases reproducibility provided that the natural ratio of <sup>24</sup>Mg to <sup>25</sup>Mg is not altered significantly by addition of the label. After gravimetric preparation of the mixture, the isotopic composition was verified by thermal ionization mass spectrometry (TIMS; see below) against a commercial standard of natural isotopic composition (Titrisol; Merck, Darmstadt, Germany). The normalized

$^{24}\text{Mg}/^{25}\text{Mg}$  isotope ratio (3 independent runs) of the isotopic label was  $8.029 \pm 0.014$ .

#### Fecal markers

Ytterbium chloride hexahydrate (99.998% purity; Aldrich Chemical Co, Milwaukee) was used as a quantitative fecal marker and added to the drink as aqueous solution. Gelatin capsules containing 100 mg Brilliant Blue per capsule were prepared by using Brilliant Blue powder (food quality; Travosa AG, Goldach, Switzerland) and mannitol as a diluent (pharmaceutical quality; Häseler AG, Herisau, Switzerland).

#### Sample preparation and analysis

Venous blood samples were drawn into heparin-containing tubes (10 mL) for the measurement of plasma magnesium concentrations at recruitment. Plasma was separated from blood cells by centrifugation at  $1550 \times g$  for 15 min at  $20^\circ\text{C}$  (Omnifuge 2.0 RS; Heraeus GmbH, Hanau, Switzerland) and stored in plastic vials at  $-25^\circ\text{C}$  until analyzed. Frozen stool samples were freeze-dried (Lyolab B; LSL Secfroid SA, Aclens-Lausanne, Switzerland) in the containers used for collection. Freeze-dried feces were ground with a pestle and dried in an incubator at  $65^\circ\text{C}$  for 20 h (WTB-binder, Tuttlingen, Germany). The dried samples (including the first fecal sample dyed by Brilliant Blue and including all samples before the second appearance of Brilliant Blue) were milled with the use of a centrifugal mill (1.0 mm mesh, Retsch Type ZM1; Retsch GmbH, Haan, Germany) in the order of collection. The samples from each subject were combined and mixed mechanically by overhead rotation at  $\approx 60$  rpm for 2 h.

All urine samples were mechanically shaken for  $\geq 1$  h before being pooled; 6-d urine pools were prepared by combining 1% of each sample (by wt) and were stored at  $-25^\circ\text{C}$ . Aliquots of stool pools (1.6 g) and urine pools (3 mL) were mineralized in duplicate by using a mixture of concentrated nitric acid and hydrogen peroxide (30%, suprapur quality; Merck) in a microwave digestion system (MLS 1200; MLS GmbH, Leutkirch, Germany).

The total magnesium content of the mineralized samples was determined by flame atomic absorption spectrometry (SpectrAA 400; Varian, Mulgrave, Australia) at 285.2 nm with the use of the parameters recommended by the manufacturer (28). A commercial magnesium standard (CertiPUR; Merck) was used for internal calibration by standard addition to minimize matrix effects. Magnesium concentrations of the final diluted solutions ranged from 0.1 to 0.4  $\mu\text{g}/\text{mL}$ . Lanthanum nitrate (Fluka Chemie GmbH, Buchs, Switzerland) was added as a matrix modifier (5 mg La/mL in the final solutions). All samples were analyzed in duplicate and repeated if the difference between individual values relative to the mean was  $> 5\%$ .

Quantitative analysis of magnesium in plasma was done by flame atomic absorption spectrometry by standard addition technique after 200-fold dilution of the plasma samples and the addition of lanthanum nitrate (5 mg La/mL). The accuracy of the method was verified by analyzing a serum control sample for magnesium (Seronorm Trace Elements Serum, Nycomed, Oslo). Samples were analyzed in triplicate and repeated if the SD relative to the mean was  $> 10\%$ . Bread rolls were freeze-dried and mineralized and analyzed for their magnesium content by using the same atomic absorption spectrometric procedure as described for the stool and urine samples. Ytterbium in the fecal pools was quantified by electrothermal atomic absorption spectrometry (SpectrAA 400 and GTA-96 graphite furnace atomiser; Varian) at 398.8 nm

with the use of the parameters recommended by the manufacturer (29). A 5-point external calibration curve was established by using a commercial ytterbium standard (Merck). Samples were analyzed in duplicate and repeated if the difference between individual values relative to the mean was  $> 5\%$ .

For the magnesium isotopic analysis, magnesium was separated from the mineralized samples by cation-exchange chromatography with a strongly acidic ion-exchange resin as described previously (26). Aliquots of the mineralized samples were evaporated to dryness in polytetrafluoroethylene vials, and the residue was dissolved in 1.0 mL of 0.7 mol HCl/L and loaded on commercial Pyrex columns (Econo column, 1.0 cm  $\times$  10 cm; Bio-Rad, Hercules, CA) filled with ion-exchange resin (AG 50 W X-8, 200–400 mesh; Bio-Rad). After sodium and potassium were eluted with 0.7 and 0.9 mol HCl/L, magnesium was eluted with 1.4 mol HCl/L. The magnesium fraction was evaporated to dryness in polytetrafluoroethylene vials, and the residue was dissolved in 0.5 mL concentrated HCl, transferred to Eppendorff tubes, evaporated to dryness in a furnace at  $70^\circ\text{C}$  (WTB-binder), and redissolved in water.

Isotope ratios were determined by TIMS with a single-focusing magnetic sector field instrument (MAT 262; Finnigan MAT, Bremen, Germany) equipped with a Faraday cup multiple collector device for simultaneous ion beam detection. About 20 nmol separated magnesium from fecal and urine samples was loaded onto the metal surface of the evaporation filament of a double-rhenium filament ion source. Magnesium was coated with 5–10  $\mu\text{g}$  silica gel, 0.8  $\mu\text{mol}$  boric acid, and 30 nmol Al as  $\text{AlCl}_3$  (all chemicals from Merck). Each measurement consisted of 50 consecutive isotope ratio measurements. Systematic isotope fractionation effects in the ion source were corrected by internal normalization techniques with the use of an exponential law (30). Reproducibility for the  $^{24}\text{Mg}/^{25}\text{Mg}$  isotope ratio (6 independent runs) was  $\pm 0.01\%$  (relative SD).

#### Data and statistical analysis

Apparent magnesium absorption and retention from the isotopically labeled test meals were calculated on the basis of the amount of ratio of isotopic label to natural magnesium in 6-d fecal pools and 6-d urinary pools and isotope dilution principles (26). The amount of  $^{26}\text{Mg}$  isotopic label in each fecal and urine sample was calculated from the amount of ratio of  $^{26}\text{Mg}$  isotopic label to natural magnesium as determined by TIMS analysis and the total magnesium amount in the feces and urine as determined by atomic absorption spectrometry. Apparent absorption was calculated as the difference between the orally administered dose and the total amount of isotope excreted in feces and expressed as the percentage of the administered dose. Retention of  $^{26}\text{Mg}$  was calculated by subtracting the total amount of  $^{26}\text{Mg}$  isotopic label recovered in the feces and urine from the administered dose. Complete collection of stool material was verified via ytterbium recovery. Subjects with an ytterbium recovery  $< 75\%$  were excluded. When the ytterbium recovery was between 75% and 90%, absorption was corrected by using the following formula (31):

$$\text{Corrected absorption (\%)} = [1 - (\% \text{ isotope dose excreted} / \% \text{ rare earth dose excreted})] \times 100 \quad (1)$$

An ytterbium recovery of 90–110% was considered to be in the range of analytic error of the method.

**TABLE 2**Baseline characteristics of the subjects in the type 2 diabetic and the healthy control group<sup>1</sup>

Characteristics	Diabetic group (n = 8 M, 4 F)	Control group (n = 7 M, 3 F)
Age (y)	65.0 ± 7.0	71.6 ± 5.5 <sup>2</sup>
Height (cm)	170.5 ± 7.2	170.5 ± 7.8
Weight (kg)	76.0 ± 6.0	73.1 ± 4.2
BMI (kg/m <sup>2</sup> )	26.2 ± 2.1	25.2 ± 1.7
Plasma magnesium (mmol/L)	0.83 ± 0.08	0.87 ± 0.07
Duration of diabetes (y)	7.5 (3–51) <sup>3</sup>	—
Glycated hemoglobin (%)	7.30 ± 0.89	— <sup>4</sup>

<sup>1</sup> $\bar{x} \pm$  SD unless indicated otherwise.<sup>2</sup>Significantly different from the diabetic group,  $P = 0.025$ .<sup>3</sup>Median; range in parentheses.<sup>4</sup>Not measured, but all subjects had a negative test result for urinary glucose.

EXCEL 97 (Microsoft, Seattle) and SPSS for WINDOWS 11.0 (SPSS Inc, Chicago) were used for data processing and statistical analysis. The normal distribution of data was verified by calculating the quotient of the skewness divided by its SE. A normal distribution is assumed when this quotient lies between  $-2.5$  and  $2.5$ . Data were expressed as arithmetic means  $\pm$  SDs. Differences between groups were evaluated by using unpaired Student's  $t$  test and were considered statistically significant at  $P < 0.05$ . Analysis of variance was used to test for associations with magnesium absorption, excretion, and retention as dependent variables.

## RESULTS

The baseline characteristics of the 2 groups are shown in **Table 2**. There were no significant differences in the sex ratio, height, weight, body mass index, or plasma magnesium concentrations between the groups. The magnesium content of the wheat-bread rolls was  $13.5 \pm 0.7$  mg. The total magnesium content of the test meals (native magnesium plus isotopic label) was  $23.6 \pm 1.0$  mg.

The apparent absorption, urinary excretion, and retention of magnesium in the diabetic and control groups are shown in **Table 3**. There were no significant differences in mean fractional magnesium absorption, mean urinary magnesium excretion, or mean magnesium retention between the groups. Data from 1 subject in the control group were excluded from the analysis because of a low ytterbium recovery ( $<20\%$  of the dose). For 3 subjects (2 diabetic patients, 1 control subject), fecal magnesium excretion was corrected on the basis of ytterbium recovery (*see* Data and

statistical analysis). In one diabetic patient, magnesium absorption was calculated; however, magnesium retention was not because of incomplete urine collection. The analysis of variance showed that, sex, age, body mass index, baseline plasma magnesium, Hb A<sub>1c</sub> concentration, and duration of diabetes and diabetes medication use did not significantly predict the dependent variables magnesium absorption, excretion, and retention.

## DISCUSSION

In diabetic patients, enteric neuropathy and microvascular disease can alter the intestinal absorption of carbohydrates, amino acids, and minerals (32–34). The intestinal absorption of calcium and zinc is lower in diabetic rats and humans than in healthy control subjects (35–37). The results of studies in diabetic rats suggest that magnesium absorption may also be impaired. Schneider and Schedl (20) reported lower fractional magnesium absorption in alloxan-induced diabetic rats (18%) than in control animals (27%). Net magnesium absorption was not significantly different between groups because the diabetic rats were hyperphagic and their magnesium intake was 60% higher than that of the controls. Miller and Schedl (21) measured magnesium absorption by in situ perfusion of intestinal segments in streptozocin-induced diabetic rats. Magnesium absorption was significantly lower in the intestine of diabetic rats than of controls, and the authors attributed these findings in part to the abnormal vitamin D metabolism that was observed in the diabetic rats. These studies were performed in rat models for type 1 diabetes. In contrast with the findings of these animal studies, our data suggest that dietary magnesium absorption and retention are not lower in humans with reasonably well-controlled type 2 diabetes than in healthy control subjects. However, if we had enrolled subjects with advanced diabetes and established neuropathy, microangiopathy, or both, we might have detected a difference in absorption. In addition, the differences in results may have been due to differences in the methods used or to inherent differences between animal models of diabetes and human diseases.

In the present study, the mean apparent magnesium absorption from our test meal was between 57.6% and 59.3%. This value is higher than that previously showed in a balance study for whole diets, in which the magnesium absorption was 21–27% from self-selected diets with a mean daily magnesium content of 323 and 234 mg in men and in women, respectively (38). Fractional magnesium absorption ranged from 10% to 65% in balance studies that used different diets or supplements (39, 40). In stable-isotope studies in adults, apparent absorption varied from 20% to 60% (26, 41–43). Fractional magnesium absorption is highly dependent on the amount of magnesium in the meal; the larger the amount, the lower the fractional absorption (39). Moreover,

**TABLE 3**Comparison of magnesium absorption, retention, and excretion between the type 2 diabetic and the healthy control groups<sup>1</sup>

	Diabetic group (n = 11)	Control group (n = 10)
Apparent magnesium absorption (% of administered dose)	59.3 ± 7.0 <sup>2</sup>	57.6 ± 8.5
Urinary magnesium excretion (% of absorbed dose)	11.2 ± 2.6	11.7 ± 3.8
Magnesium retention (% of administered dose)	54.2 ± 7.1	51.4 ± 6.1
Total urinary magnesium in 6-d pool (mg)	497.6 ± 120.6	482.8 ± 184.3
Mean daily magnesium excretion (mg)	82.9 ± 20.1	80.5 ± 30.7

<sup>1</sup> $\bar{x} \pm$  SD. There were no significant differences between groups by unpaired Student's  $t$  test.<sup>2</sup> $n = 12$ .


magnesium absorption may be increased (protein and fructose) or inhibited (cellulose, phytic acid, and oxalic acid) by other food components (44). The high fractional magnesium absorption from our test meal was presumably due to the very low magnesium content (23.6 mg) of the meal, which contained no known inhibitors of magnesium absorption.

Use of an oral isotopic label alone does not allow determination of true magnesium absorption, only that of apparent absorption. Magnesium is reexcreted in the intestine in pancreatic, bile, and other intestinal excretions and by cell sloughing. Few data on endogenous intestinal losses of magnesium are available, with reports ranging from 2 to 38 mg/d (45–47). Measured apparent absorption is therefore lower than true absorption. As has been shown previously in stable-isotope studies, the absolute difference between apparent and true absorption is 2–10% in healthy subjects (26, 42, 48), which is not of major practical significance for the comparison of magnesium absorption.

Several studies have shown elevated urinary magnesium excretion in both type 1 and 2 diabetic patients (6, 11–15), and elevated urinary magnesium excretion in diabetes is associated with elevated fasting blood glucose and Hb A<sub>1c</sub> concentrations (11). Studies in type 1 diabetic patients have shown that urinary magnesium excretion increases in response to a glucose infusion (49). Urinary magnesium excretion is also elevated in diabetic patients with osmotic diuresis secondary to glycosuria (11–13). However, other studies found no significant differences in urinary magnesium excretion between diabetic patients and healthy control subjects (16, 17). In the present study, mean urinary magnesium excretion in the diabetic group was not higher than that of the healthy control group. This may have been due to the fact that the diabetic patients were in reasonable metabolic control, and none had detectable nephropathy.

Our sample size allowed detection of an absolute difference in magnesium absorption of  $\geq 12\%$  between groups. A  $\beta$  error was possible because of the small sample size. However, we judged that a difference in absorption of  $\geq 12\%$  between groups would be of clinical relevance and could potentially contribute to a low magnesium status in diabetes. A potential source of error in our method was an incomplete fecal or urine collection. To correct for an incomplete collection of stool, ytterbium was administered together with the labeled test meal. Ytterbium is a rare earth element, the absorption of which is  $< 0.05\%$  when administered orally (50). Rare elements have been evaluated in studies of iron and magnesium (26, 27, 51) and have been shown to be useful tools to correct for incomplete stool collection. Bohn (26) showed that the excretion of ytterbium and the magnesium isotopic label was significantly correlated. Unfortunately, there is no suitable marker available to check for the completeness of urine collections. *p*-Aminobenzoic acid has been used as a quantitative urine control marker (52–54), but its safety has been questioned (55). The use of creatinine as a quantitative control marker has low sensitivity, particularly in older age groups (53, 56). Therefore, we judged the completeness of the urine collection on the basis of the total volume collected and on confirmation of the compliance of our subjects.

In summary, the results of the present study indicate that type 2 diabetic patients in reasonable metabolic control and without nephropathy absorb and retain dietary magnesium to an extent similar to that of healthy control subjects. Because nephropathy is common in patients with long-standing diabetes, and because good glycemic control is difficult to achieve, these findings may

not be generalizable to the diabetic population as a whole. It is possible that poor magnesium status is a problem only in poorly controlled diabetic patients, who have elevated urinary magnesium losses as a result of glycosuria. Additional studies in less well-controlled patients with type 2 diabetes and in patients with type 1 diabetes are needed to confirm our finding that impaired magnesium absorption does not contribute to poor magnesium status in diabetic patients. 

We thank the subjects for their participation in the study, C Zeder and S Renggli (Swiss Federal Institute of Technology Zurich) for the TIMS measurements, T Bohn (Swiss Federal Institute of Technology Zurich) for scientific advice during the study, and M Krähenbühl (University Hospital, Zurich) for the collection of blood samples.

All authors contributed to the study design, data collection and analysis, and writing and editing of the manuscript. None of the authors had a financial or personal conflict of interest.

## REFERENCES

- Nadler JL, Rude RK. Disorders of magnesium metabolism. *Endocrinol Metab Clin North Am* 1995;24:623–41.
- Schnack C, Bauer I, Pregant P, Hopmeier P, Scherthner G. Hypomagnesaemia in type 2 (non-insulin-dependent) diabetes mellitus is not corrected by improvement of long-term metabolic control. *Diabetologia* 1992;35:77–9.
- De Lenardis M. Elektrolytstatus bei hypertonen Altersdiabetikern und Therapeutische Konsequenzen. (Mineral status in hypertensive type 2 diabetics and therapeutic consequences.) PhD thesis. University Hohenheim, Germany, 1999 (in German).
- Paolisso G, Sgambato S, Giugliano D, et al. Impaired insulin-induced erythrocyte magnesium accumulation is correlated to impaired insulin-mediated glucose disposal in type 2 (non-insulin-dependent) diabetic patients. *Diabetologia* 1988;31:910–5.
- Schlienger JL, Grunenberger F, Maier EA, Simon C, Chabrier G, Leroy MJF. Disturbances of plasma trace-elements in diabetes—relations with glycemic control. *Presse Med* 1988;17:1076–9.
- Sjogren A, Floren CH, Nilsson A. Magnesium, potassium and zinc deficiency in subjects with type II diabetes mellitus. *Acta Med Scand* 1988;224:461–6.
- Durlach J, Rayssiguier Y. Données nouvelles sur les relations entre magnésium et hydrates de carbone. (New data on the relationship between magnesium and carbohydrates.) *Magnesium* 1983;2:192–224 (in French).
- Nadler JL, Buchanan T, Natarajan R, Antonipillai I, Bergman R, Rude R. Magnesium deficiency produces insulin resistance and increased thromboxane synthesis. *Hypertension* 1993;21:1024–9.
- McNair P, Christiansen C, Madsbad S, et al. Hypomagnesemia, a risk factor in diabetic retinopathy. *Diabetes* 1978;27:1075–7.
- Wälti MK, Zimmermann MB, Spinass GA, Jacob S, Hurrell RF. Dietary magnesium intake in type 2 diabetes. *Eur J Clin Nutr* 2002;56:409–14.
- McNair P, Christensen MS, Christiansen C, Madsbad S, Transbol I. Renal hypomagnesaemia in human diabetes mellitus: its relation to glucose homeostasis. *Eur J Clin Invest* 1982;12:81–5.
- Fujii S, Takemura T, Wada M, Akai T, Okuda K. Magnesium levels in plasma, erythrocyte and urine in patients with diabetes mellitus. *Horm Metab Res* 1982;14:161–2.
- Johannsson G, Danielson BG, Ljunghall S, Wibell L. Evidence for a disturbed magnesium-metabolism in diabetes mellitus. *Magn Bull* 1981;2:178–80.
- Brown IR, McBain AM, Chalmers J, Campbell IW, Brown ER, Lewis MJ. Sex difference in the relationship of calcium and magnesium excretion to glycaemic control in type 1 diabetes mellitus. *Clin Chim Acta* 1999;283:119–28.

15. Roffi M, Kanaka C, Mullis PE, Peheim E, Bianchetti MG. Hypermagnesiuria in children with newly diagnosed insulin-dependent diabetes mellitus. *Am J Nephrol* 1994;14:201–6.
16. Sjogren A, Floren CH, Nilsson A. Magnesium deficiency in IDDM related to level of glycosylated hemoglobin. *Diabetes* 1986;35:459–63.
17. el-Yazigi A, Hannan N, Raines DA. Effect of diabetic state and related disorders on the urinary excretion of magnesium and zinc in patients. *Diabetes Res* 1993;22:67–75.
18. Sheehan JP. Magnesium deficiency and diabetes mellitus. *Magn Trace Elem* 1991;10:215–9.
19. Tosiello L. Hypomagnesemia and diabetes mellitus. A review of clinical implications. *Arch Intern Med* 1996;156:1143–8.
20. Schneider LE, Schedl HP. Effects of alloxan diabetes on magnesium metabolism in the rat. *Proc Soc Exp Biol Med* 1974;147:494–7.
21. Miller DL, Schedl HP. Effects of diabetes on intestinal magnesium absorption in the rat. *Am J Physiol* 1976;231:1039–42.
22. Holden NE, Martin RL. Atomic weights of the elements 1981. *Pure Appl Chem* 1983;55:1101–18.
23. Holden NE, Martin RL, Barnes IL. Isotopic compositions of the elements 1981. *Pure Appl Chem* 1983;55:1119–36.
24. Ryan MF, Barbour H. Magnesium measurement in routine clinical practice. *Ann Clin Biochem* 1998;35:449–59.
25. Turnlund JR, Keyes WR. Automated-analysis of stable isotopes of zinc, copper, iron, calcium and magnesium by thermal ionization mass-spectrometry using double isotope-dilution for tracer studies in humans. *J Micronutr Anal* 1990;7:117–45.
26. Bohn T. Magnesium absorption in humans. PhD thesis (no. 14930.) Swiss Federal Institute of Technology Zurich, Switzerland, 2003.
27. Fairweather-Tait SJ, Minihane AM, Eagles J, Owen L, Crews HM. Rare earth elements as nonabsorbable fecal markers in studies of iron absorption. *Am J Clin Nutr* 1997;65:970–6.
28. Varian Techtron Pty Limited. Analytical methods for flame atomic absorption spectrometry. Mulgrave, Australia: Varian Techtron Pty Limited, 1989.
29. Beach L, Brodie K, Dodge P, et al. Analytical methods for graphite tube atomizers. Mulgrave, Australia: Varian Techtron Pty Limited, 1988.
30. Russell WA, Papanastassiou DA, Tombrello TA. Ca isotope fractionation on earth and other solar-system materials. *Geochim Cosmochim Acta* 1978;42:1075–90.
31. van Dokkum W, Fairweather-Tait SJ, Hurrell RF, Sandström B. Study techniques. In: Mellon FA, Sandström B, eds. *Stable isotopes in human nutrition*. London: Academic Press, 1996:23–42.
32. May RJ, Goyal RK. Effects of diabetes mellitus on the digestive system. In: Kahn CR, Weir GC, eds. *Joslin's diabetes mellitus*. 13th ed. Philadelphia: Lea & Febiger, 1994.
33. Casirola DM, Vinnakota RR, Ferraris RP. Intestinal amino acid transport in mice is modulated by diabetes and diet. *J Nutr* 1994;124:842–52.
34. Eliasson B, Bjornsson E, Urbanavicius V, et al. Hyperinsulinaemia impairs gastrointestinal motility and slows carbohydrate absorption. *Diabetologia* 1995;38:79–85.
35. Schneider LE, Schedl HP. Diabetes and intestinal calcium absorption in the rat. *Am J Physiol* 1972;223:1319–23.
36. Kiilerich S, Hvid-Jacobsen K, Vaag A, Sorensen SS. <sup>65</sup>Zinc absorption in patients with insulin-dependent diabetes mellitus assessed by whole-body counting technique. *Clin Chim Acta* 1990;189:13–8.
37. Verhaeghe J, van Herck E, Visser WJ, et al. Bone and mineral metabolism in BB rats with long-term diabetes. Decreased bone turnover and osteoporosis. *Diabetes* 1990;39:477–82.
38. Lakshmanan FL, Rao RB, Kim WW, Kelsay JL. Magnesium intakes, balances, and blood levels of adults consuming self-selected diets. *Am J Clin Nutr* 1984;40:1380–9.
39. Fine KD, Santa Ana CA, Porter JL, Fordtran JS. Intestinal absorption of magnesium from food and supplements. *J Clin Invest* 1991;88:396–402.
40. Spencer H, Lesniak M, Gatzka CA, Osis D, Lender M. Magnesium absorption and metabolism in patients with chronic renal failure and in patients with normal renal function. *Gastroenterology* 1980;79:26–34.
41. Schwartz R, Spencer H, Welsh JJ. Magnesium absorption in human subjects from leafy vegetables, intrinsically labeled with stable <sup>26</sup>Mg. *Am J Clin Nutr* 1984;39:571–6.
42. Schwartz R, Spencer H, Wentworth RA. Measurement of magnesium absorption in man using stable <sup>26</sup>Mg as a tracer. *Clin Chim Acta* 1978;87:265–73.
43. Sabatier M, Arnaud MJ, Kastenmayer P, Rytz A, Barclay DV. Meal effect on magnesium bioavailability from mineral water in healthy women. *Am J Clin Nutr* 2002;75:65–71.
44. Brink EJ, Beynen AC. Nutrition and magnesium absorption: a review. *Prog Food Nutr Sci* 1992;16:125–62.
45. Freeman JB, Wittine MF, Stegink LD, Mason ED. Effects of magnesium infusions on magnesium and nitrogen balance during parenteral nutrition. *Can J Surg* 1982;25:570–2, 574.
46. Avioli LV, Berman M. Mg<sup>28</sup> kinetics in man. *J Appl Physiol* 1966;21:1688–94.
47. Graham LA, Caesar JJ, Burgen ASV. Gastrointestinal absorption and excretion of Mg<sup>28</sup> in man. *Metabolism* 1960;9:646–59.
48. Sabatier M. Etude de l'absorption et du métabolisme du magnésium chez l'homme au moyen d'isotopes stables. (Study of magnesium absorption and metabolism in humans using stable isotopes.) PhD thesis. Université d'Auvergne, France, 2001 (in French).
49. Djurhuus MS, Skott P, Vaag A, et al. Hyperglycaemia enhances renal magnesium excretion in type 1 diabetic patients. *Scand J Clin Lab Invest* 2000;60:403–9.
50. Arvela P. Toxicity of rare earths. In: Grobecker H, Kahl GF, Klaus W, Schmidt H, van Zurieten PA, eds. *Progress in pharmacology*. Stuttgart, Germany: 1979:69–114.
51. Schuette SA, Janghorbani M, Young VR, Weaver CM. Dysprosium as a nonabsorbable marker for studies of mineral absorption with stable isotope tracers in human subjects. *J Am Coll Nutr* 1993;12:307–15.
52. Bingham S, Cummings JH. The use of 4-aminobenzoic acid as a marker to validate the completeness of 24 h urine collections in man. *Clin Sci (Lond)* 1983;64:629–35.
53. Bingham SA, Cummings JH. The use of creatinine output as a check on the completeness of 24-hour urine collections. *Hum Nutr Clin Nutr* 1985;39:343–53.
54. Williams DR, Bingham SA. Sodium and potassium intakes in a representative population sample: estimation from 24 h urine collections known to be complete in a Cambridgeshire village. *Br J Nutr* 1986;55:13–22.
55. Worobec S, LaChine A. Dangers of orally administered para-aminobenzoic acid. *JAMA* 1984;251:2348.
56. Leclercq C, Maiani G, Polito A, Ferro-Luzzi A. Use of PABA test to check completeness of 24-h urine collections in elderly subjects. *Nutrition* 1991;7:350–4.

