Magnesium Research 2005; **18** (4): 215-23 ORIGINAL ARTICLE

Study of magnesium bioavailability from ten organic and inorganic Mg salts in Mg-depleted rats using a stable isotope approach

C. Coudray, M. Rambeau, C. Feillet-Coudray, E. Gueux, J.C. Tressol, A. Mazur, Y. Rayssiguier

Centre de Recherche en Nutrition Humaine d'Auvergne, Laboratoire des Maladies Métaboliques et Micronutriments, INRA de Theix/Clermont-Ferrand, 63122 Saint-Genès-Champanelle, France

Correspondence: C. Coudray. UMR 866 Différenciation Cellulaire et Croissance, INRA, Centre de Recherche de Montpellier, 2 place Pierre-Viala, 34060 Montpellier Cedex 1, France. Tel: (+00 33) 4 99 61 26 87. Fax: (+00 33) 4 67 54 56 94. <coudray@ensam.inra.fr>

Abstract. Literature data on the bioavailability of various Mg forms provide scarce information on the best Mg salt to be used in animal and human supplementation. This study aimed to investigate the bioavailability of different forms of Mg in rats using Mg stable isotopes. Eighty male Wistar rats aged 6 weeks were fed a semi-purified Mg-depleted diet for three weeks. The rats were then randomised into ten groups and received, for two more weeks, the same diet repleted with Mg (550 mg Mg/kg) as: oxide, chloride, sulphate, carbonate, acetate, pidolate, citrate, gluconate, lactate or aspartate. After 10 days of Mg-repleted diet, the rats received orally 1.8 mg of an enriched ^{26}Mg. Faeces and urine were then collected for 4 consecutive days. Isotope ratios in faeces and urine were determined. The Mg absorption values obtained varied from 50% to 67%. Organic Mg salts were slightly more available than inorganic Mg salts. Mg gluconate exhibited the highest Mg bioavailability of the ten Mg salts studied. Urinary ^{26}Mg excretion varied from 0.20 mg to 0.33 mg, and feeding with the organic pidolate, citrate, gluconate and aspartate salts resulted in higher urinary ^{26}Mg excretion than with inorganic salts. Ultimately, ^{26}Mg retention was higher in the rats receiving the organic salts such as gluconate, lactate and aspartate than in those receiving the inorganic salts. Taken together, these results indicate that ^{26}Mg is sufficiently bioavailable from the ten different Mg salts studied in the present experiment, although Mg gluconate exhibited the highest bioavailability under these experimental conditions.

Key words: magnesium, intestinal absorption, supplementation, stable isotopes, rats

Magnesium deficit may occur because of a decrease in intake or absorption, internal redistribution, or increased loss of this element through either renal or non-renal routes. Magnesium deficiency is not uncommon among the general population: its intake has decreased over the years especially in the western world [1, 2]. As Mg plays an essential role in a wide range of fundamental biological processes, it is not surprising that Mg deficiency may lead to serious biochemical and symptomatic changes [3-5]. Increasing the consumption of vegetables and cereal products contributes to improved Mg intake but Mg

supplementation may be indicated when a specific health problem or condition causes an excessive loss of Mg or limits Mg absorption [6, 7].

Net Mg absorption results from dietary Mg absorption and Mg secretion into the intestinal tract via bile, gastric and pancreatic juices. In the healthy adult, 30 to 50% of dietary Mg is absorbed [8-10]. The secreted Mg is efficiently reabsorbed and endogenous faecal losses are only 20 to 50 mg/d. Mg absorption occurs along the entire intestinal tract but the distal small intestine (jejunum and ileum) are the primary sites. It is essentially a passive intercellular process mediated by electrochemical gradients and solvent drag, and active transport occurs only for extremely low dietary Mg intake and its regulation is unknown [11]. Mg uptake in the brush border may be mediated by a Mg/anion complex and Mg efflux across the basolateral membrane may involve Na/Mg antiport systems [12]. A gene implicated in Mg deficit in humans has been identified. It is expressed in the intestine and kidneys and appears to encode for a protein that combines Ca- and Mg-permeable channel properties with protein kinase activity [13]. This gene might be implicated in Mg absorption. Because of the importance of the passive process, the quantity of Mg in the digestive tract is the major factor controlling the amount of Mg absorbed.

Literature data on the bioavailability of various Mg forms provide scarce information on the best Mg salt to be used in animal and human Mg supplementation. Some human studies have attempted to investigate the bioavailability of different Mg salts, but these studies are based on two or three Mg preparations and were therefore unable to clearly establish which Mg salt possesses the best bioavailability. Moreover, these studies did not directly measure Mg absorption but often simply measured 24-hour urinary Mg excretion or plasma Mg as a marker of Mg bioavailability [14-16]. Measurement of intestinal Mg absorption is the only direct approach for comparing bioavailability between different Mg salts. The conventional balance study suffers from the methodological problems inherent to all balance studies [17], such as imprecision in Mg intake, environmental contamination and endogenous Mg excretion. The development of inductively coupled plasma mass spectrometry (ICP-MS) has provided the possibility of a new and reliable method for conducting Mg bioavailability studies [18-21]. This technique can be usefully applied to compare intestinal Mg absorption between a large number of Mg salts. Accordingly, we investigated the bioavailability of Mg from different organic and inorganic Mg salts using a single-labelling technique with Mg stable isotope in rats.

Materials and methods

Materials and reagents

Enriched Mg isotopes (^{26}Mg) as MgO were obtained from Chemgas, (Boulogne, France). HNO_3 (ultrapure), Mg and beryllium standard solutions (1 g/L) were obtained from Merck (Darmstadt, Germany). All other chemicals were of the highest quality available. Distilled water was used throughout. A Perkin-Elmer 6100DRC system (Perkin-Elmer Instruments, Courteboeuf, France) with a Meinhard nebulizer was used for isotopic measurement, and a Perkin Elmer 560 (Perkin Elmer Instruments, Courteboeuf, France) was used for total Mg measurement.

Animals and diet

Eighty male Wistar rats aged about 6 weeks and weighing 150 g were used in the present study. They were derived from the colony of laboratory animals of the National Institute of Agronomic Research (INRA) of Clermont-Ferrand/Theix, France. The rats were housed under constant temperature conditions (20-22° C), constant humidity (45-50%) and a standard dark cycle (20:00 to 08:00). They received human care in compliance with European Community guidelines for the use of experimental animals (L358-86/609/EEC). At the beginning of the experiment (d0), blood samples were obtained from 20 animals by orbital sinus puncture to evaluate baseline Mg status in these rats. The animals received *ad libitum* a semi-purified diet that contained (g/kg) [22]: casein, 200; starch, 650; corn oil, 50; fibre, 50; AIN-93 mineral mixture (1993) 35; including magnesium oxide (MgO) as necessary, AIN-93A vitamin mixture (1993), 10; DL-methionine, 3; and choline bitartrate, 2. In a first three-week stage, all 80 animals received this diet containing only 150 mg Mg/kg, to induce Mg deficiency. Then, blood samples were again taken from 20 animals by orbital vein puncture to evaluate Mg status after the period of insufficient Mg intake. The rats were then randomised into 10 groups (8 rats/group) and for two weeks received a semi-purified diet containing sufficient Mg levels but in different salt forms: group 1 received Mg oxide, group 2 received Mg chloride; group 3 received Mg sulphate, group 4 received Mg carbonate, group 5 received Mg acetate, group 6 received Mg pidolate, group 7 received Mg citrate, group 8 received Mg gluconate, group 9 received Mg lactate, and group 10 received Mg aspartate. The target Mg level in these diets was 600 mg Mg/Kg diet. Powder diet (100 g) was made up with 100 mL of distilled water to form a kind of semi-liquid food prepared on site.

Stable isotope administration and biological sample collection

Two hundred mg of the enriched ^{26}Mg (in oxide form = 323 mg) were first moistened with two mL of distilled water, and then two mL of 12 N HCl (ultrapure) were added to transform the oxide into the soluble chloride of Mg. The solution was then diluted with 120 mL of distilled water and adjusted to pH6 with powdered sodium bicarbonate. The isotopic analysis of ^{26}Mg solution yielded the following atomic percentages: ^{24}Mg = 2.33%, ^{25}Mg = 1.28%, ^{26}Mg = 96.38%, and gave ^{26}Mg concentration as 1.51 mg/ml. Animals received by gavage 1.2 mL of this solution one week after the consumption of the different Mg salts (about 1.8 mg of ^{26}Mg per rat). The urine and faeces of each rat were quantitatively collected for four consecutive days. Urine volume was determined and 5 mL urine were sampled, acidified with 50 µl of concentrated HNO_3 (12 N) and frozen until analysis. The faeces were freeze-dried, powdered and kept at room temperature until analysis. The rats were sacrificed at the end of the experiment and the blood and femur were sampled for Mg analysis. The gastrointestinal tract was also sampled and divided into three parts corresponding to the small, large intestine and to the cecum. Red blood cells were separated from plasma by centrifugation, washed twice with saline solution and then lysed into ten volumes of distilled water.

Total Mg and isotopic ^{26}Mg analysis

Lysed red blood, faeces, femur and intestine contents were dried and ashed at 500°C for 10 hours and the ash was dissolved in 0.2 mL of concentrated HNO_3 and 9.2 mL of distilled water. For total Mg determination, samples were diluted appropriately with 0.1% lanthanum chloride and Mg level was determined by flame atomic absorption spectrometry (Perkin Elmer 560, Courteboeuf, France). For isotopic Mg determination, samples were appropriately diluted before analysis using 1% HNO_3. Mg concentration and isotope ratios were determined by ICP-MS (Perkin-Elmer 6100DRC system) using Mg and beryllium as external and internal standards, respectively. The following instrument operating conditions were set after optimization with a solution of 1µg indium/l (RF Power: 1050 W, Nebulizer Ar flow rate: 0.79 L/min, Auxiliary Ar flow rate: 1.2 L/min, Outer Ar flow rate: 15 L/min). Data acquisition conditions were as follows (Sweeps/reading: 50, Readings/replicate: 1, Number of replicates: 3, Dwell time: 100ms, Scanning mode: peak hopping).

Calculations

Mg has three stable isotopes having the following percentage natural abundance: ^{24}Mg (78.90%), ^{25}Mg (10.00%) and ^{26}Mg (11.10%) [23]. Isotopic enrichment percentages were obtained from the following equation = 100 x (($^{26}Mg/^{24}Mg$ measured ratio - $^{26}Mg/^{24}Mg$ baseline ratio)/ ($^{26}Mg/^{24}Mg$ baseline ratio)), where isotopic natural baseline ratio was calculated as follows: $^{26}Mg/^{24}Mg$ = 0.1407.

Non-absorbed ^{26}Mg isotope present in the faecal sample (coming only from the ^{26}Mg isotope label) was calculated as follows = (total faecal Mg X ($IR^{26}Mg/^{24}Mg$ sample - $IR^{26}Mg/^{24}Mg$ baseline)) / (1.267 + ($IR^{26}Mg/^{24}Mg$ sample - $IR^{26}Mg/^{24}Mg$ baseline)), where IR was isotopic ratio, total faecal Mg (mg) was determined by atomic absorption spectrometry, and 1.267 is the sum of 1/0.789 converting ^{24}Mg faecal quantity to total faecal Mg. The calculation could also be made directly from ICP-MS data. The two modes of calculation give the same results when the ICP-MS quantitative procedure is used [24].

Statistical analysis

Results were expressed as means (SD). Statistical analysis were based on one-way ANOVA followed by a Student-Newman-Keuls test for parametric variables and a Kruskal-Wallis test for non-parametric variables. The limit of statistical significance was set at $p < 0.05$. Statistical analyses were performed using GraphPad software (V3.00, GraphPad Software, San Diego, CA).

Results

Effect of Mg-deficient diet on Mg status

Mg status was evaluated at the beginning of the experiment and after 3 weeks on the Mg-deficient diet. The results showed normal Mg status at the beginning of the experiment with plasma Mg level at 16.3 ± 1.0 mg/L and Mg red blood cell level at 45.9 ± 3.5 mg/L. Blood Mg levels (n = 20) after three weeks of Mg-deficient diet decreased significantly to 7.8 ± 1.2 mg/L in plasma ($p < 0.0001$) and to 42.5 ± 3.2 mg/L in red blood cells ($p < 0.05$). The inter- and intra-variability of total Mg and Mg isotope measurements have been previously reported [24].

Effect of intake of different Mg salts on rat body weight

Rat body weight was monitored twice per week throughout the duration of the study. There was no

significant difference in the body weight evolution between the ten different groups at any point in the experiment (*table 1*).

Effect of intake of different Mg salts on intestinal Mg solubility

The pH content of different fragments of intestine and caecum increased gradually from the proximal intestine (around pH 5.8) to the distal intestine (around pH 6.5), and reached around 6.8 in the caecum in the different groups. There was no significant difference in intestine and caecum pH between the different groups (*table 2*). In contrast, Mg solubility decreased gradually from the proximal intestine (85%) to the distal intestine (50%) down to about 40% in the caecum in all the groups. There was no significant between-group difference in intestinal or caecal Mg solubility (*table 2*).

Intake effects of different Mg salts on intestinal Mg absorption (*tables 3 and 4*)

With both classical and isotopic approaches, the intestinal Mg absorption from the different tested salts seemed adequate and exceeded 35% of ingested Mg. However, the results from the classical approach showed that Mg absorption seemed better from the organic Mg salts (+ 13%) than from the inorganic Mg salts and in particular from gluconate salt. This last salt exhibited the best bioavailability with an intestinal Mg absorption of 56% accompanied with very high urinary Mg excretion, proving the higher intestinal absorption of Mg from this organic salt.

With regard to the isotopic approach, faecal excretion of ^{26}Mg varied between 0.62 and 0.89 mg, *i.e.* between 35 and 50% of ^{26}Mg given per group. The highest excretion of ^{26}Mg was observed in the rats receiving $MgSO_4$ and $MgCO_3$, whereas the lowest excretion of ^{26}Mg was observed in the rats receiving Mg gluconate and Mg aspartate. There was a significant difference in ^{26}Mg excretion between rats receiving the Mg gluconate and rats receiving MgO, $MgCl_2$, $MgSO_4$, $MgCO_3$ and Mg acetate. Thus, ^{26}Mg absorption varied between 0.92 and 1.22 mg. The lowest absorption of ^{26}Mg was observed in the rats receiving MgSO4 and MgCO3, whereas the highest absorption of ^{26}Mg was observed in the rats receiving the organic Mg salts, in particular Mg gluconate. There was a significant difference in ^{26}Mg absorption between rats receiving the Mg gluconate and rats receiving MgO, $MgCl_2$, $MgSO_4$, $MgCO_3$ and Mg acetate. Urinary ^{26}Mg excretion values confirmed the absorption data. Urinary ^{26}Mg varied between 0.20 and 0.33 mg. The lowest urinary ^{26}Mg excretion was observed in the rats receiving the four inorganic Mg salts, Mg aspartate and Mg lactate, whereas the highest urinary ^{26}Mg excretion was observed in the rats receiving the organic Mg salts, in particular Mg gluconate and Mg pidolate. There was a significant difference in urinary ^{26}Mg excretion in the rats receiving Mg gluconate compared to all the other rats. Finally, ^{26}Mg retention varied between 0.71 and 0.90 mg. The lowest ^{26}Mg retention was observed in the rats receiving $MgSO_4$ and $MgCO_3$, whereas the highest ^{26}Mg retention was observed in the rats receiving the organic Mg salts, in particular Mg gluconate and Mg lactate. ^{26}Mg retention in the rats receiving Mg gluconate and Mg lactate only differed significantly from ^{26}Mg excretion in the rats receiving $MgSO_4$.

Effect of intake of different Mg salts on Mg status

Mg status was monitored at the end of the study by measuring Mg levels in plasma, red blood cells and bone (femur). There was no significant difference in the three Mg status parameters between the different groups studied in this experiment (*table 5*).

Discussion

Mg absorption and bioavailability depend on a variety of factors, including the Mg salt form. Literature data on the bioavailability of various Mg forms provide scarce information on the best Mg salt to be used in animal and human supplementation. Indeed, in the few human studies published, only two or three Mg salts were compared in terms of their bioavailability. None of these studies directly measured intestinal Mg absorption of these salts, but simply measured 24-hour urinary Mg excretion or plasma Mg levels [14-16, 25]. However, given the variety of Mg preparations examined, the available studies do not clearly identify the best Mg preparation to be used for supplementation. This is understandable because it is very difficult to examine all these salts in humans in one single study. Moreover, surprisingly, there are very few, if any, animal studies investigating the bioavailability of different Mg salts. Cook [26] investigated in 1973 the availability of various inorganic magnesium salts in rats using balance studies. These included carbonate, chloride, oxide, phosphate, sulfate and silicate. They concluded that Mg from chloride and carbonate was slightly more available than from the other four salts. We, therefore, conducted the present study to investigate the bio-

Table 1. Effects of a two-week intake of organic or inorganic Mg salts on rat growth rate.

	Inorganic Mg salts				Organic Mg salts						*p*
	MgO	**MgCl2**	**MgSO4**	**MgCO3**	**Acetate**	**Pidolate**	**Citrate**	**Gluconate**	**Lactate**	**Aspartate**	
Body weight (g)											
At D 0	146 ± 5	148 ± 7	150 ± 5	152 ± 6	150 ± 11	149 ± 7	149 ± 11	146 ± 8	147 ± 9	147 ± 7	NS
At D 4	164 ± 6	167 ± 10	170 ± 6	169 ± 8	169 ± 11	165 ± 8	167 ± 10	167 ± 9	168 ± 11	165 ± 7	NS
At D 11	221 ± 11	219 ± 14	222 ± 9	221 ± 13	228 ± 15	219 ± 12	219 ± 14	217 ± 14	225 ± 14	214 ± 11	NS
At D 18	271 ± 10	268 ± 19	271 ± 12	270 ± 18	278 ± 15	268 ± 17	267 ± 22	265 ± 18	276 ± 18	259 ± 14	NS
At D 25	311 ± 12	312 ± 23	313 ± 14	308 ± 21	320 ± 19	308 ± 21	310 ± 29	308 ± 22	321 ± 21	297 ± 18	NS
At D 28	320 ± 14	317 ± 29	323 ± 13	319 ± 24	331 ± 22	321 ± 22	319 ± 28	318 ± 25	332 ± 20	310 ± 19	NS
At D 32	334 ± 18	335 ± 28	340 ± 15	333 ± 26	345 ± 23	334 ± 23	334 ± 29	338 ± 28	350 ± 22	324 ± 21	NS
At D 36	355 ± 21	360 ± 32	365 ± 18	359 ± 30	371 ± 26	360 ± 28	357 ± 32	357 ± 30	377 ± 26	345 ± 23	NS

Animals received an Mg-depleted diet (150 mg Mg/kg) for three weeks and were then divided into ten groups receiving this diet for two more weeks but repleted with one of the above Mg salts (600 mg Mg/kg). Results are expressed as means (SD). Statistical analysis were based on one-way ANOVA followed by a Student-Newman-Keuls test for parametric variables and a Kruskal-Wallis test for non-parametric variables. Statistical significance was set at $p < 0.05$.

Table 2. Effects of two-week intake of organic or inorganic Mg salts on intestinal pH and intestinal Mg solubility in rats.

	Inorganic Mg salts				Organic Mg salts						*p*
	MgO	**MgCl2**	**MgSO4**	**MgCO3**	**Acetate**	**Pidolate**	**Citrate**	**Gluconate**	**Lactate**	**Aspartate**	
Small intestine											
pH	5.78 ± 0.25	5.72 ± 0.11	6.00 ± 0.26	5.68 ± 0.23	5.81 ± 0.24	5.70 ± 0.14	5.82 ± 0.27	6.00 ± 0.38	5.70 ± 0.24	5.92 ± 0.44	NS
Mg solubility, %	98.9 ± 6.1	86.8 ± 10.6	97.9 ± 19.1	87.5 ± 12.7	85.8 ± 11.8	89.9 ± 10.1	80.6 ± 10.5	89.4 ± 20.1	90.6 ± 10.7	83.0 ± 10.3	NS
Large intestine											
pH	6.60 ± 0.19	6.48 ± 0.19	6.58 ± 0.15	6.37 ± 0.51	6.44 ± 0.89	6.68 ± 0.17	6.72 ± 0.40	6.54 ± 0.32	6.54 ± 0.18	6.56 ± 0.34	NS
Mg solubility, %	61.5 ± 14.2	53.0 ± 17.9	46.7 ± 8.6	52.1 ± 15.5	56.2 ± 21.4	43.8 ± 7.2	48.7 ± 11.2	48.7 ± 17.9	52.2 ± 14.1	56.1 ± 12.3	NS
Caecum											
pH	6.89 ± 0.55	6.67 ± 0.54	6.99 ± 0.30	6.45 ± 0.16	6.55 ± 0.20	7.01 ± 0.27	6.72 ± 0.55	6.78 ± 0.54	6.80 ± 0.41	6.61 ± 0.27	NS
Mg solubility, %	42.3 ± 7.5	43.2 ± 9.8	39.2 ± 4.7	46.1 ± 13.2	52.0 ± 11.0	50.1 ± 13.0	47.5 ± 12.6	49.9 ± 13.1	43.9 ± 4.0	43.3 ± 9.9	NS

Animals received an Mg-depleted diet (150 mg Mg/kg) for three weeks and were then divided into ten groups receiving this diet for two more weeks but repleted with one of the above Mg salts (600 mg Mg/kg). The gastrointestinal tract was divided into three parts corresponding to the small, large intestine and to the cecum. Results are expressed as means (SD). Statistical analysis were based on one-way ANOVA followed by a Student-Newman-Keuls test for parametric variables and a Kruskal-Wallis test for non-parametric variables. Statistical significance was set at $p < 0.05$.

Table 3. Effects of a two-week intake of organic or inorganic Mg salts on intestinal absorption, urinary excretion, and retention of Mg in rats.

	Inorganic Mg salts				Organic Mg salts						*p*
	MgO	**MgCl2**	**MgSO4**	**MgCO3**	**Acetate**	**Pidolate**	**Citrate**	**Gluconate**	**Lactate**	**Aspartate**	
Food intake, g/d	19.8 ± 2.1	21.1 ± 1.5	20.4 ± 1.8	20.8 ± 2.0	20.6 ± 2.5	20.7 ± 1.5	20.5 ± 1.8	21.4 ± 3.0	21.9 ± 1.6	20.4 ± 1.5	NS
Mg intake, mg/d	12.6 ± 1.3abc	14.1 ± 1.0c	12.0 ± 1.1ab	12.9 ± 1.3abc	13.1 ± 1.7bc	13.1 ± 0.9abc	12.7 ± 1.1abc	12.1 ± 1.7ab	11.5 ± 0.8a	12.9 ± 0.9abc	0.0015
Fecal Mg, mg/d	6.53 ± 1.11b	7.23 ± 1.28b	7.91 ± 1.88ab	7.25 ± 0.76b	6.92 ± 0.94b	6.80 ± 1.31ab	6.35 ± 1.15ab	5.27 ± 1.44a	6.02 ± 0.72ab	6.86 ± 0.65ab	0.0024
Mg absorption, mg/d	6.07 ± 0.65b	6.90 ± 1.31b	4.14 ± 1.20a	5.66 ± 0.73b	6.18 ± 1.09b	6.28 ± 1.25b	6.39 ± 1.20b	6.83 ± 1.19b	5.49 ± 0.35b	6.05 ± 0.95b	< 0.0001
Mg absorption,%	48.4 ± 4.5bc	48.8 ± 8.8bc	34.8 ± 10.8a	43.8 ± 3.2b	47.2 ± 4.3cb	48.0 ± 9.4bc	50.1 ± 8.2bc	56.8 ± 8.9c	47.9 ± 3.3bc	46.8 ± 5.3bc	< 0.0001
urinary Mg, mg/d	1.80 ± 0.86a	1.90 ± 0.33ab	1.92 ± 0.44ab	2.80 ± 0.53c	2.38 ± 0.24abc	2.09 ± 0.46abc	2.02 ± 0.68ab	2.48 ± 0.27abc	2.39 ± 0.27abc	2.64 ± 0.53bc	0.0007
Mg retention, mg/d	4.27 ± 0.85bc	5.00 ± 1.26c	2.22 ± 1.02a	2.86 ± 0.93a	4.17 ± 1.01bc	4.19 ± 1.49bc	4.37 ± 1.56bc	4.35 ± 1.30c	3.11 ± 0.46ab	3.41 ± 0.93ab	< 0.0001
Mg retention,%	33.8 ± 5.6bc	35.3 ± 8.1c	18.6 ± 8.6a	21.9 ± 6.2ba	29.8 ± 5.1b	32.0 ± 11.7bc	33.9 ± 11.4bc	36.0 ± 9.6c	26.9 ± 3.0ab	26.2 ± 5.7ab	0.0001

Animals received an Mg-depleted diet (150 mg Mg/kg) for three weeks and were then divided into ten groups receiving this diet for two more weeks but repleted with one of the above Mg salts (600 mg Mg/kg). Results are expressed as means (SD). Statistical analysis were based on one-way ANOVA followed by a Student-Newman-Keuls test for parametric variables and a Kruskal-Wallis test for non-parametric variables. Statistical significance was set at $p < 0.05$.

Table 4. Effects of a two-week intake of organic or inorganic Mg salts on intestinal absorption, urinary excretion, and retention of ^{26}Mg in rats.

	Inorganic Mg salts				Organic Mg salts						*p*
	MgO	**MgCl2**	**MgSO4**	**MgCO3**	**Acetate**	**Pidolate**	**Citrate**	**Gluconate**	**Lactate**	**Aspartate**	
^{26}Mg gavaged, mg	1.81 ± 0.04	1.83 ± 0.03	1.81 ± 0.05	1.83 ± 0.02	1.81 ± 0.04	1.85 ± 0.10	1.82 ± 0.02	1.84 ± 0.03	1.86 ± 0.11	1.81 ± 0.04	NS
^{26}Mg faecal mg	0.78 ± 0.13ab	0.83 ± 0.13b	0.89 ± 0.14b	0.88 ± 0.08b	0.81 ± 0.06b	0.74 ± 0.10ab	0.77 ± 0.14ab	0.62 ± 0.13a	0.76 ± 0.12ab	0.72 ± 0.13ab	0.0009
^{26}Mg absorbed, mg	1.03 ± 0.12a	1.00 ± 0.13a	0.92 ± 0.14a	0.95 ± 0.09a	1.00 ± 0.09a	1.10 ± 0.12ab	1.05 ± 0.15ab	1.22 ± 0.13b	1.10 ± 0.13ab	1.09 ± 0.14ab	0.0007
^{26}Mg absorbed,%	57.0 ± 7.0a	54.8 ± 7.1a	51.0 ± 7.5a	52.0 ± 4.6a	55.1 ± 4.1a	59.7 ± 7.7ab	57.6 ± 7.7ab	66.5 ± 7.1b	59.0 ± 6.1ab	60.4 ± 7.5ab	0.0006
^{26}Mg urine, mg	0.22 ± 0.05a	0.22 ± 0.06a	0.21 ± 0.05a	0.20 ± 0.03a	0.22 ± 0.04a	0.27 ± 0.06a	0.24 ± 0.07a	0.33 ± 0.05b	0.20 ± 0.04a	0.25 ± 0.06a	< 0.0001
^{26}Mg retention, mg	0.81 ± 0.10ab	0.78 ± 0.09ab	0.71 ± 0.12a	0.75 ± 0.09ab	0.78 ± 0.09ab	0.83 ± 0.14ab	0.81 ± 0.11ab	0.89 ± 0.10b	0.90 ± 0.13b	0.85 ± 0.11ab	0.0239
^{26}Mg retention,%	44.8 ± 5.9ab	42.8 ± 0.51ab	39.5 ± 6.8a	41.0 ± 4.6ab	42.9 ± 4.0ab	45.0 ± 5.8ab	44.7 ± 5.6ab	48.7 ± 5.4b	48.0 ± 5.5b	46.9 ± 5.8ab	0.0240

Animals received an Mg-depleted diet (150 mg Mg/kg) for three weeks and were then divided into ten groups receiving this diet for two more weeks but repleted with one of the above Mg salts (600 mg Mg/kg). Results are expressed as means (SD). Statistical analysis were based on one-way ANOVA followed by a Student-Newman-Keuls test for parametric variables and a Kruskal-Wallis test for non-parametric variables. Statistical significance was set at $p < 0.05$.

Table 5. Effects of a two-week intake of organic or inorganic Mg salts on Mg status parameters.

	Inorganic Mg salts				Organic Mg salts						*p*
	MgO	**MgCl2**	**MgSO4**	**MgCO3**	**Acetate**	**Pidolate**	**Citrate**	**Gluconate**	**Lactate**	**Aspartate**	
Plasma Mg, mg/L	18.0 ± 1.2	18.3 ± 0.7	17.9 ± 0.7	17.4 ± 1.2	17.2 ± 0.7	17.9 ± 0.4	17.2 ± 0.9	17.5 ± 0.8	17.1 ± 1.46	17.3 ± 0.9	NS
RBC Mg, mg/L	45.3 ± 4.0	45.0 ± 5.5	44.9 ± 4.0	45.5 ± 3.2	46.1 ± 5.8	44.3 ± 4.1	44.6 ± 3.0	46.7 ± 2.4	45.1 ± 6.7	43.4 ± 4.4	NS
Bone Mg, mg/g	3.11 ± 0.13	3.13 ± 0.31	3.14 ± 0.23	3.17 ± 0.24	3.04 ± 0.14	3.08 ± 0.14	3.06 ± 0.17	2.98 ± 0.13	2.96 ± 0.25	3.00 ± 0.28	NS

Animals received an Mg-depleted diet (150 mg Mg/kg) for three weeks and were then divided into ten groups receiving this diet for two more weeks but repleted with one of the above Mg salts (600 mg Mg/kg). Results are expressed as means (SD). Statistical analysis were based on one-way ANOVA followed by a Student-Newman-Keuls test for parametric variables and a Kruskal-Wallis test for non-parametric variables. Statistical significance was set at $p < 0.05$.

availability of ten different forms of organic and inorganic salts of Mg in rats using Mg stable isotope.

We applied a robust technique using Mg stable isotope to determine intestinal Mg absorption and urinary excretion. This approach allows much greater precision than in the conventional balance technique where many methodological problems have been reported [17]. In the isotopic approach, the amount of isotope administered in the whole animal is well controlled, with minimal repercussions resulting from any environmental contamination during sample collection and preparation or Mg measurement. Moreover, using stable isotope allowed us to work with "true" intestinal absorption because endogenous isotope excretion is considerably low in comparison to total Mg endogenous excretion in the faeces [27]. Our results clearly showed that Mg organic salts are better sources of Mg than Mg inorganic salts, and suggest that Mg gluconate is the best source of Mg because it exhibited the highest Mg absorption and Mg retention values in the ten studied groups (66.5% and 48.7%, respectively). It is important to stress that Mg inorganic salts remain a good source of Mg because the absorption and the retention of Mg observed with these salts were perfectly acceptable (more than 50% and 39%, respectively). Although human and rat have some differences in intestinal physiology, these results may be extrapolated to human Mg nutrition with the necessary precautions. These results are in agreement with a study in adult humans by Walker's team [14], who reported that Mg is more bioavailable from Mg citrate than from Mg oxide. Firoz & Graber [25] have determined Mg bioavailability in four commercial Mg preparations - Mg oxide, Mg chloride, Mg lactate and Mg aspartate - in human subjects using urinary Mg excretion. They concluded a relatively poor bioavailability of Mg oxide but greater and equivalent bioavailability of the other three Mg salts. Lindberg [28] compared Mg citrate and Mg oxide with respect to *in vitro* solubility and *in vivo* gastrointestinal absorbability in normal healthy volunteers. He concluded that Mg citrate had better solubility and bioavailability than Mg oxide. In an old study, based on 24-h urinary Mg excretion, Morris [15] reported that Mg was absorbed to a limited extent in healthy adults following administration of Mg sulfate. More recently, the influence of three different salts at different concentrations on Mg absorption in the rat small intestine using the area under the curve as end-point of Mg bioavailability has been studied [29], where Mg absorption was shown to be most efficient from gluconate compared to fumarate or chloride forms.

The solubility of minerals in the intestinal tract is an essential factor in their absorption. In previous studies we showed that administration of fermentable fibers which decreased cecal pH content increase considerably Mg absorption in human and animals [30]. That is why we examined Mg solubility in the different segments of rat intestine. The values of pH and Mg solubility of intestinal contents confirmed increasing pH and decreasing Mg solubility throughout the intestine from the proximal part to the distal part of intestine and then to the caecum. However, the administration of different organic and inorganic Mg salts did not result in any significant differences in these intestinal and caecum parameters. This may explain why there is no major difference in intestinal Mg absorption between the different Mg salts investigated in this study. Given that the main intestinal site of Mg absorption is the ileum in humans and in particular the caecum in the rat, pH content and Mg solubility in the caecum are more significant indicators than in the proximal and the distal parts of intestine. Again, we observed higher caecal Mg solubility, even if not significantly so, in the rats receiving principally the Mg organic salts, in particular acetate, pidolate, citrate and gluconate. This may also partially explain why intestinal Mg absorption was slightly higher with the organic rather than the inorganic Mg salts in this study.

Mg levels in biological samples such as plasma, erythrocyte or urine have been used as markers of Mg bioavailability in several studies, although biological variations in Mg levels in these media in apparently healthy humans are very large [31]. So, we evaluated the efficiency of the ten Mg organic and inorganic salts in restoring Mg status. The rats presented a low Mg status at the beginning of Mg salt administration. Two weeks later, Mg status was efficiently restored in the different groups. Globally, mean plasma Mg levels in the rats receiving the four inorganic salts and the six organic salts were 17.9 mg/L and 17.4 mg/L, respectively, and mean erythrocyte Mg levels were 45.2 mg/L and 45.0 mg/L, respectively, whereas mean bone Mg levels were 3.14 mg/g dry weight and 3.02 mg/g dry weight of inorganic and organic Mg salts, respectively. Indeed, the statistical analysis failed to show any trend or significant difference between the ten groups for the three Mg status parameters measured in this study, i.e. plasma Mg, erythrocyte Mg and bone Mg levels. From many previous studies, it has been shown that intestinal Mg absorption was generally proportional to the dietary Mg intake [32]. This may explain why the various Mg forms studied all showed the same efficacy in overcoming marginal Mg deficiency in our model. In agreement with our results, Cook [26] showed that all of the inorganic Mg salts he studied were nearly equivalent in their ability to support growth, plasma Mg levels and kidney Mg concentrations. However, it was reported that Mg L-asparate was more bioavailable than Mg oxide in healthy volunteers [33]. White *et al.* [34] determined Mg bioavailability from Mg chloride solution, slow-release Mg chloride tablets and Mg gluconate tablets in humans. Urinary Mg excretion and Mg serum levels were not different between the three supplement forms. Borella *et al.* [35] reported that erythrocyte Mg level was a suitable marker for evaluating Mg retention after oral physiological supplementation in marginally Mg-deficient subjects. Wilimzig *et al.* [36] studied the bioavailability of different dosages of trimagnesium dicitrate in humans by measuring plasma Mg levels between 0 and 12 h of Mg salt administration.

In conclusion, the present study demonstrated that all ten organic and inorganic Mg salts were equally efficient in restoring blood Mg levels in plasma and red blood cells in rats. Because of the importance of the passive process, the quantity of Mg in the digestive tract is the major factor controlling the amount of Mg absorbed. However, the organic forms of Mg, in particular Mg gluconate, seem more absorbable than inorganic salts as assessed by intestinal absorption and urinary excretion.

Acknowledgements

The authors are gratefully to Claudine Lab, Séverine Thien and Lydia Jaffrelo for their enthusiastic cooperation.

References

1. Galan P, Preziosi P, Durlach V, Valeix P, Ribas L, Bouzid D, Favier A, Hercberg S. Dietary magnesium intake in a French adult population. *Magne. Res* 1997; 10: 321-8.
2. Schimatschek HF, Rempis R. Prevalence of hypomagnesemia in an unselected German population of 16,000 individuals. *Magnes Res* 2001; 14: 283-90.
3. Rayssiguier Y, Mazur A, Durlach J. *Advances in Magnesium Research, Nutrition and health.* London: John Libbey & Company Limited. England, 2001; (455 pp).
4. Vormann J. Magnesium: nutrition and metabolism. *Mol Aspects Med* 2003; 24: 27-37.
5. Feillet-Coudray C, Rayssiguier Y. Magnesium: physiology, dietary sources and requirements. Encycl. Nutr., 2nd Edition. Oxford, UK: Elsevier, 2005 : 191–5.
6. Levrat-Verny MA, Coudray C, Bellanger J, Lopez HW, Demigne C, Rayssiguier Y, Remesy C. Wholewheat flour

ensures higher mineral absorption and bioavailability than white wheat flour in rats. *Br J Nutr* 1999; 82: 17-21.

7. Coudray C, Rayssiguier Y. Impact of vegetable products on intake, intestinal absorption and status of magnesium. In: Rayssiguier Y, Mazur A, Durlach J, eds. *Advances in magnesium research: nutrition and health*. London: John Libbey, 2001: 115-23.
8. Schweigel M, Martens H. Magnesium transport in the gastrointestinal tract. *Front Biosci* 2000; 5: D666-D677.
9. Tahiri M, Tressol JC, Arnaud J, Bornet F, Bouteloup-Demange C, Feillet-Coudray C, Ducros V, Pepin D, Brouns F, Rayssiguier Y, Roussel AM, Coudray C. Five-week intake of short-chain fructo-oligosaccharides increases intestinal absorption and status of magnesium in postmenopausal women. *J Bone Miner Res* 2001; 16: 2152-60.
10. Coudray C, Bellanger J, Vermorel M, Sinaud S, Wils D, Feillet-Coudray C, Brandolini M, Bouteloup-Demange C, Rayssiguier Y. Two polyol, low digestible carbohydrates improve the apparent absorption of magnesium but not of calcium in healthy young men. *J Nutr* 2003; 133: 90-3.
11. Sabatier M, Keyes WR, Pont F, Arnaud MJ, Turnlund JR. Comparison of stable-isotope-tracer methods for the determination of magnesium absorption in humans. *Am J Clin Nutr* 2003; 77: 1206-12.
12. Quamme GA. Renal magnesium handling: new insights in understanding old problems. *Kidney Int* 1997; 52: 1180-95.
13. Wolf FI. TRPM7: channeling the future of cellular magnesium homeostasis? *Sci STKE* 2004; 233: pe23.
14. Walker AF, Marakis G, Christie S, Byng M. Magnesium citrate found more bioavailable than other magnesium preparations in a randomised, double-blind study. *Magnes Res* 2003; 16: 183-91.
15. Morris ME. Absorption of magnesium from orally administered magnesium sulfate in man. *Clin Toxicol* 1987; 25: 371-82.
16. Bøhmer T. Bioavailability of oral Mg supplementation in female students evaluated from elimination of Mg in 24-hour urine. *Magnes Trace Elem* 1990; 9: 272-8.
17. Mertz W. Use and misuse of balance studies. *J Nutr* 1987; 117: 1811-3.
18. Schuette S, Vereault D, Ting BTG, Janghorbani M. Accurate measurement of stable isotopes of magnesium in biological materials with inductively coupled plasma mass spectrometry. *Analyst* 1988; 113: 1837-42.
19. Schuette S, Ziegler EE, Nelson SE, Janghorbani M. Feasibility of using the stable isotope ^{25}Mg to study Mg metabolism in infants. *Pediatr Res* 1990; 27: 36-40.
20. Abrams SA, Griffin IJ, Herman S. Using stable isotopes to assess the bioavailability of minerals in food fortification programs. *Food Nutr Bull* 2002; 23: S158-S165.
21. Feillet-Coudray C, Coudray C. The stable isotope use in the exploration of bioavailability and metabolism of magnesium. *Curr Nutr Food Sci* 2005; 1: 63-70.
22. Reeves PG, Nielsen FH, Fahey GC. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition Ad Hoc writting committee on the reformulation of the AIN-76A rodent diet. *J Nutr* 1993; 123: 1939-51.
23. De Bievre P, Taylor PDP. Table of isotopic composition of the elements. *Intern J Mass Spectrom Ion Process* 1993; 123: 149-66.
24. Coudray C, Pepin D, Tressol JC, Bellanger J, Rayssiguier Y. Study of magnesium bioavailability using stable isotopes and the inductively-coupled plasma mass spectrometry technique in the rat: single and double labelling approaches. *Br J Nutr* 1997; 77: 957-70.
25. Firoz M, Graber M. Bioavailability of US commercial magnesium preparations. *Magnes Res* 2001; 14: 257-62.
26. Cook DA. Availability of magnesium: balance studies in rats with various inorganic magnesium salts. *J Nutr* 1973; 103: 1365-70.
27. Abrams SA, Wen JP. Methodologies for using stable isotopes to assess magnesium absorption and secretion in children. *J Am Coll Nutr* 1999; 18: 30-5.
28. Lindberg JS. Mg bioavailability from Mg citrate and Mg oxide. *J Am Coll Nutr* 1990; 9: 48-55.
29. Dolinska B, Ryszka F. Influence of salt form and concentration on the absorption of magnesium in rat small intestine. *Boll Chim Farm* 2004; 143: 163-5.
30. Coudray C, Demigne C, Rayssiguier Y. Effects of dietary fibers on magnesium absorption in animals and humans. *J Nutr* 2003; 133: 1-4.
31. Djurhuus MS, Gram J, Petersen PH, Klitgaard NA, Bollerslev J, Beck-Nielsen H. Biological variation of serum and urinary magnesium in apparently healthy males. *Scand J Clin Lab Invest* 1995; 55: 549-58.
32. Coudray C, Feillet-Coudray C, Grizard D, Tressol JC, Gueux E, Rayssiguier Y. Fractional intestinal absorption of magnesium is directly proportional to dietary magnesium intake in rats. *J Nutr* 2002; 132: 2043-7.
33. Mühlbauer B. Mg-L-aspartate-HCl and Mg-oxide: bioavailability in healthy volunteers. *Eur J Clin Pharmacol* 1991; 40: 437-8.
34. White J, Massey L, Gales SK, Dittus K, Campbell K. Blood and urinary magnesium kinetics after oral magnesium supplements. *Clin Ther* 1992; 14: 678-87.
35. Borella P, Ambrosini G, Concari M, Bargellini A. Is magnesium content in erythrocytes suitable for evaluating cation retention after oral physiological supplementation in marginally magnesium-deficient subjects? *Magnes Res* 1993; 6: 149-53.
36. Wilimzig C, Latz R, Vierling W, Mutschler E, Trnovec T, Nyulassy S. Increase in magnesium plasma level after orally administered trimagnesium dicitrate. *Eur J Clin Pharmacol* 1996; 49: 317-23.