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ORIGINAL CONTRIBUTION

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Effects of sulphate- and bicarbonate-rich mineral waters on net and fractional intestinal absorption and urinary excretion of magnesium in rats

Summary Magnesium (Mg) intake is below the recommended daily allowances in many developed countries. Mg-rich mineral waters can provide significant amounts of energy-free Mg and thus help to meet Mg requirements. We assessed the effects of different Mg-rich mineral waters on overall intestinal Mg absorption and urinary Mg excretion in 40 rats split into four groups: one received distilled water, another a solution of $MgCl_2$ and the others two different mineral waters, sulphated water (Hépar) and carbonated water (Badoit) mixed with the diet and as drinking water, for four weeks. The rats were given 3 mg of ^{26}Mg orally and 0.5 mg of ^{25}Mg intravenously. They were placed in metabolic cages, and diet consumption, and faeces and urine excretion were monitored during the last four days of the experiment. The rats were then sacrificed and blood was sampled. Mg levels in the diet, faeces, urine and biological samples were measured by atomic absorption spectrometry. Mg stable isotope measurements were performed by ICP/MS. Mg-rich mineral waters significantly increased net intestinal absorption of Mg by more than 30 %, but the proportions of both apparent and true intestinal absorption of Mg were similar in all four groups. Thus, net and fractional retention of Mg were similar in the three Mg-supplemented groups. In conclusion, both types of Mg-rich mineral waters studied similarly increased both absorption and urinary excretion of Mg with no positive effect on the overall retention of Mg, probably because the Mg status of the rats was already satisfactory.

Key words magnesium – absorption – retention – mineral water – stable isotopes – acid-base balance – rats

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Abbreviations

Ca Calcium
Mg Magnesium

Introduction

Magnesium (Mg) is an essential dietary element that is biologically important, acting as a cofactor of many enzymes [1, 2]. Mg is therefore critical for a great number of cellular functions including oxidative phosphorylation, glycolysis, DNA transcription and protein synthesis [3]. Current research continues to support the importance of Mg in human health and diseases [4, 5]. A significant number of people in industrialised countries consumes less than most international recommended dietary intakes and there is emerging evidence that usually low intakes of Mg are associated with aetiological factors in various metabolic diseases [4, 6]. In a recent French study [7], Mg intake was assessed in 5 448 subjects and it was found that 23 % of the women and 18 % of the men consumed less than two-thirds of the recommended dietary allowance [8]. Consequently, an adequate dietary intake of Mg with good bioavailability to ensure optimal Mg status is most important. Low Mg intake in industrialised countries is linked particularly to

food purification processes and people's desire to reduce dietary energy intake [9]. Grains are a very important source of Mg but they are usually subjected to various types of processing, such as milling, heat extraction, and cooking, which result in significant losses of fibre, vitamins and minerals, in particular Mg. It is thus desirable to be able to increase Mg intake while keeping the same dietary energy intake. Mineral waters represent a good source of Mg with very high nutritional density given the absence of energy intake. There are various types of mineral waters naturally rich or enriched in Mg. The Mg contents of such waters can vary from 10 to more than 100 mg/L. Mg in the mineral waters occurs in a soluble ionised form that is very readily absorbed by the body [10, 11]. However, the other ions in the mineral waters such as Ca, Na, sulphate and carbonate may influence the intestinal absorption of Mg [12–14]. Very little is known about the influence of these ions on the absorption of Mg. The presence of sulphate or carbonate can have an effect on intestinal Mg absorption, by modifying its solubility in the intestine [15], and on its urinary elimination, by modifying the acid-basic balance in blood [16, 17]. The objective of this work was to assess the effects of different Mg-rich mineral waters on overall intestinal Mg absorption and urinary Mg excretion in adult rats using Mg stable isotopes.

Materials and methods

Reagents and equipment

Highly enriched Mg stable isotopes ^{26}Mg 99.4% and ^{25}Mg 99.39% in their oxide form were obtained from Trace Science International (Ontario, Canada). Suprapure HNO_3, suprapure H_2O_2, Suprapure HCl, lanthanum oxide and standard solutions of Mg (1 g/L) were obtained from Merck (Darmstadt, Germany). All other chemicals were of the highest quality available, and demineralised water (Milli Q) was used throughout. The Mg isotope ratio measurements were made using an ICP/MS instrument (Plasma Quad-II system, Fisons Instruments, Manchester, UK), equipped with a Meinhard nebuliser. A Perkin Elmer 560 atomic absorption spectrometer (Perkin Elmer, St-Quentin-en-Yvelines, France) was used for total Mg measurements.

Animals and diets

Forty male Wistar rats, from the laboratory animal colony of the National Institute of Agronomic Research (INRA), Clermont-Ferrand/Theix, France, were fed a commercial pellet diet (U. A.R, Villemoisson-sur-Orge, France) until their body weights reached ~200 g (age seven weeks). The animals were then fed a basal semi-purified diet whose the composition is indicated in Table 1. The planned chemical composition of this diet was as follows (mg/kg): calcium 5 000, phosphorous: 5 000; Mg: 600; zinc: 35; iron: 35. Mg content of this basal semi-purified diet is adequate in ordre to maintain the daily Mg requirement of the animals [18]. Four groups of 10 rats each were formed and fed the purified diets mixed with an equal weight of distilled water (control group), or $MgCl_2$ solution ($MgCl_2$ group), or Hépar water, *Vittel source, France* (sulphated water group) or Badoit water, *Badoit source, Saint Galmier, France* (carbonated water group). The chemical composition of the tested mineral waters is given in Table 2. The four rat groups also received as drinking water: distilled water, $MgCl_2$ solution, sulphated mineral water or carbonated mineral water, respectively. The animals were given fresh food and drink daily, available *ad libitum*. The whole experiment lasted 28 days. Food and water consumption and body weight were recorded weekly. During the experiment, the rats were housed two per cage (wire-bottomed to limit coprophagy) and maintained in a temperature-controlled room (22 °C) with the dark period from 08:00 pm to 08:00 am. On the morning of day 24, each rat was given 2 mL of ^{26}Mg stable isotope solution (about 3 mg ^{26}Mg) orally and 0.5 mL of ^{25}Mg stable isotope solution (about 0.5 mg ^{25}Mg) intravenously and placed individually in metabolic cages fitted with

Table 1 Composition of experimental diets[1]

Diet ingredients (g/kg)	Control	$MgCl_2$	Sulphated water	Carbonated water
Casein	200	200	200	200
Wheat starch	650	650	650	650
Corn oil	50	50	50	50
Alphacel, (cellulose)	50	50	50	50
DL-methionine	3	3	3	3
Choline bitartrate	2	2	2	2
AIN salt mix[2]	35	35	35	35
AIN vitamin mix[3]	10	10	10	10
Drinking water	distilled water	$MgCl_2$	Hépar	Badoit

[1] Powdered diet was daily mixed with equal weight of distilled water (control group), of $MgCl_2$ solution ($MgCl_2$ group), of Hépar water (sulphated water group) or of Badoit water (carbonated water group) to form a semi-liquid food prepared on site.

[2] AIN salt mixture, expressed in g/kg of mixture: Calcium phosphate dibasic: 500; sodium chloride: 74; potassium citrate monohydrate: 220; potassium sulfate: 52; magnesium oxide: 24; manganese carbonate: (43–48 % Mn) 3.5; ferric sulfate (16–17 % Fe): 6; zinc carbonate (70 % ZnO): 1.6; copper carbonate (53–55 % Cu): 0.3; potassium iodate: 0.01; sodium selenite: 0.01; chromium potassium sulfate: 0.55; sucrose, finely powdered: 118.

[3] AIN vitamin mixture, expressed in mg/kg of mixture: thiamine hydrochloride: 600; riboflavin: 600; pyridoxine hydrochloride: 700; nicotinic acid: 3000; D-calcium pantothenate: 1600; folic acid: 200; D-biotin: 20; cyanocobalamine (vitamin B12): 1; retinyl palmitate (vitamin A) pre-mix (250 IU/mg): 1600; DL-a-tocopherol acetate (0.25 IU/mg): 20,000; cholecalciferol (vitamin D3) (400 IU/mg): 250; menaquinone (vitamin K2): 50; and sucrose, finely powdered

Table 2 Characteristics of studied sulphate- and bicarbonate-rich mineral waters

	Control[1]	$MgCl_2$	Sulphated water[2]	Carbonated water[2]
Dry residue, mg/L	0	432	2580	1200
pH	5–7	4.77	7.0	6.0
Osmolarity, mOs	0	18.2	76.3	51.5
Magnesium, mg/L	0	109.1	111.2	112.6
Calcium, mg/L	0	0	555	190
Sodium, mg/L	0	0	14	150
Bicarbonate, mg/L	0	0	403	1300
Sulphate, mg/L	0	0	1479	40

[1] Milli Q water
[2] According to the manufacturer

urine/faeces separators. Food intake was controlled for four successive days by weighing the offered meal and the leftover food for each rat. Whole faeces and urine for each rat were collected during the four-day balance period to determine apparent and true intestinal absorption of Mg. All the procedures complied with the Institute's guide for the care and use of laboratory animals.

Preparation of stable isotope solutions

200 mg of enriched ^{26}Mg stable isotope (^{26}Mg, 99.4 % ^{24}Mg 0.51 %, ^{25}Mg 0.09 %,) was moistened with 1 mL of distilled water, and 1 mL of 12 M HCl (ultrapure) was added gradually to convert the oxide into soluble Mg chloride. The solution was then diluted with 18 mL of distilled water and adjusted to pH 6 with powdered $NaHCO_3$ (10 mg ^{26}Mg/mL). A solution of 1.5 mg ^{26}Mg/mL was then prepared in distilled water for use in this study. Each rat received 2 mL of this solution orally (3 mg ^{26}Mg/rat).

200 mg of enriched ^{25}Mg stable isotope (^{25}Mg 99.39 %, ^{24}Mg 0.33 %, ^{26}Mg 0.30 %) was moistened with 1 mL of distilled water, and 1 mL of 12 M HCl (ultrapure) was added gradually to convert the oxide into soluble Mg chloride. The solution was then diluted with 18 mL of distilled water and adjusted to pH 6 with powdered $NaHCO_3$ (10 mg ^{25}Mg/mL) the solution was rendered isotonic by adding 180 mg of NaCl. A solution of 1 mg ^{25}Mg/mL was then prepared in 0.9 % NaCl solution for use in this study. Each rat received 0.5 ml of this solution intravenously (0.5 mg ^{25}Mg/rat).

Sampling procedures

At the end of the experiment, the rats were anaesthetised (40 mg/kg of sodium pentobarbital) and sacrificed just after the dark period (between 08:00 am and 10:00 am). Blood was sampled via the abdominal aorta, placed in microfuge tubes containing heparin, and centrifuged at 5 000 *g* for 10 minutes. Plasma and red blood cell samples were stored at –20 °C until Mg analysis. After blood sampling, the tibia was also removed for body Mg assay.

After stable isotope administration, five individual urine spots from each rat were collected at the following times: 0–6 h, 6–12 h, 12–24 h, 24–48 h and 48–96 hours. Urinary pH has been measured on the 24–48 h urine spot using Sentron pH-system 1001, Ac Roden, The Netherlands. Each urine sport was weighed and a subsample was acidified with 14 M HNO_3 (final acid concentration: 0.14 M) and kept at –20 °C until Mg analysis. The total faeces of each rat were weighed, freeze-dried, and re-weighed. To obtain homogenous faecal samples, the dried faeces were powdered for 60 s in an Electronic Spex Industrie grinder equipped with tubs and balls made of stainless steel. The powdered faeces were then kept at room temperature for total Mg and Mg stable isotope analysis.

Mg determination by atomic absorption spectrometry

Mg levels were determined in the plasma, red blood cells and urine after simple appropriate dilution in 0.1 % lanthanum. For solid samples, sub-samples of diet, faeces (0.25 g) or one tibia were dried and mineralised for 10 h at 500 °C. The residue was then taken up in concentrated HNO_3 (14 M) and hydrogen peroxide (30 %) on a heating plate until complete decoloration. The mineralisates were then appropriately diluted in 0.1 % lanthanum. Mg concentration was measured using a flame atomic absorption spectrophotometer at 285 nm. Proper quality control and external calibration were ensured for each series of measurements. Within and between run percentage residual standard deviations for total Mg measurement were as follows: on standard solutions of Mg: 1.21 and 1.86, on plasma samples: 2.76 and 4.67, on red blood cell samples: 3.11 and 4.78, on urine samples: 3.22 and 5.55, and on faecal mineralisate solutions: 2.38 and 4.16.

Mg stable isotope determination

Sub-samples of faeces (0.25 g) were dry-ashed at 500 °C for 10 h. The ash was dissolved in 0.2 mL of concentrated HNO_3 and 9.8 mL of distilled water. In each case, an appropriate dilution with diluted HNO_3 (0.14 M) was then performed before the ICP/MS analysis to ensure a total Mg concentration of about 50 µg/L. Urine samples were simply appropriately diluted with 0.14 M HNO_3 to obtain Mg concentrations of about 50 µg/L. Stable isotope solutions were also appropriately diluted with 0.14 M

HNO_3 and checked by ICP/MS. Mg stable isotope concentrations were determined by ICP/MS under the following conditions. The mass spectrometer settings and plasma conditions were optimised with a solution of 10 µg/L of indium, and the instrument operating conditions were radio frequency generator 27.12 MHz, forward RF power 1 350 W, reflected RF power < 3W, outer argon flow rate 14 L/min, intermediate argon flow rate 0.7 L/min, nebuliser argon flow rate 0.76 L/min, mass resolution 0.9 a. m. u. at 10 % of peak height. Data collection parameters were as follows: total replicates per integration 5, signal integration time per replicate 40 s, dwell time per sweep 20.4 ms, scanning mode peak hopping, five points per peak, and sample uptake rate 0.6 mL/min. Within and between run percentage residual standard deviations for $^{25}Mg/^{26}Mg$ ratio were: on standard solutions of Mg: 0.31 and 0.65, on urine dilution: 0.54 and 0.72 and on faecal mineralisate solution: 0.43 and 0.67.

Calculations

Fractional apparent absorption (%) was determined as follows: 100 x ((Mg intake – faecal Mg)/(Mg intake)), and net apparent absorption (mg/d) as (Mg intake – faecal Mg).

Net Mg balance (mg/d) was calculated as follows: Mg intake – (faecal Mg + urine Mg).

Fractional Mg balance (%) was calculated as follows: 100 x [Mg intake – (faecal Mg + urine Mg)/Mg intake)].

Fractional 'true' intestinal absorption of Mg was calculated from the isotope faecal monitored technique as follows: 100 x ((administered ^{26}Mg – non-absorbed ^{26}Mg excreted in the faeces)/(administered ^{26}Mg)),

The unabsorbed ^{26}Mg isotope present in the faecal sample 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 – $R^{26}Mg/^{24}Mg$ baseline)), where total faecal Mg (mg) was determined by atomic absorption spectrometry, IR is the isotopic ratio, and 1.267 is 1/0.789, to convert ^{24}Mg faecal quantity to total faecal Mg [19].

True intestinal absorption of Mg (%) was measured in the urine samples and calculated from the isotope double labelling technique according to the formula of Yergey [20]: 100 x [(^{26}Mg nat x i. v. ^{25}Mg dose x enrich ^{26}Mg)/(^{25}Mg nat x oral ^{26}Mg dose x enrich ^{25}Mg)], where nat is the natural abundance of the two isotopes ^{25}Mg and ^{26}Mg, i. v. ^{25}Mg and oral ^{26}Mg refer to the exact doses given orally or intravenously, and enrich ^{25}Mg or ^{26}Mg represents isotopic enrichment percentage at the stated urine time points, e. g., 100 x [(0.11 x 0.5 mg x 75 %)/(0.10 x 3 mg x 20 %)] = 68 %.

Statistical analyses

Values are given as means ± SD and where appropriate the significance of differences between means was determined by ANOVA and multiple range comparisons by Fisher's least significant difference procedures. ANOVA assumes that the data are sampled from a population that follows a normal distribution. This assumption was tested using the method of Kolmogorov and Smirnov. ANOVA assumes that the data are sampled from a population with identical SDs. This assumption was tested using the method of Bartlett. Values of $p < 0.05$ were considered significant.

Results

Diet and water consumption

Administration of different types of mineral waters in the diet and as drinking water brings the whole intake of Mg from mineral waters to about 3.5 mg/d. This represents 25–30 % of the total consumed Mg. This mineral water consumption had no effect on body weight evolution (4 to 6 g/d) or diet consumption (from 19 to 22 g/d).

Intestinal Mg absorption (Table 3)

Total Mg intake was higher in the three groups consuming $MgCl_2$ or Mg-rich mineral waters ($p < 0.05$). The faecal Mg excretion was significantly higher in the sulphated water group than in the control rats, the values ranging between 6.7 and 8.9 mg/d. The net apparent absorption of Mg (mg/d) was thus significantly higher in the three groups receiving $MgCl_2$ or Mg-rich mineral waters than in the control rats. However, the fractional apparent absorption of Mg (%) was similar in the four groups, ranging between 37 and 40 %.

With regard to the isotope measurement, the faecal isotope ^{26}Mg excretion was not statistically different among the four groups and ranged between 1.16 and 1.45 mg. Consequently, both net (mg/d) and fractional (%) intestinal absorption of ^{26}Mg isotope were not different among the four groups. Fractional intestinal absorption of ^{26}Mg isotope ranged between 50 and 60 %.

True absorption of Mg was also measured on urine samples using the double labelling method. The isotopic measurements performed on different urine spots (6–12 h, 12–24 h, 24–48 h and 48–96 h) showed clearly that the consumption of mineral waters or $MgCl_2$-containing water did not modify true intestinal Mg absorption percent (Fig. 1). Only at the first urine spot (0–6 h) did we observe a significantly higher absorption value with carbonated water compared with the control rats ($p = 0.0101$).

Table 3 Effects of sulphate- and bicarbonate-rich mineral waters on true intestinal and apparent magnesium absorption in rats[1, 2]

	Control	$MgCl_2$	Sulphated water	Carbonated water
Apparent absorption				
Water Mg intake, mg/J	0.00a	3.19±0.73b	3.75±0.61b	3.53±0.72b
Total Mg intake, mg/d	10.7±0.7a	12.5±1.2b	13.7±1.2b	13.6±2.0b
Fecal Mg excretion, mg/d	6.74±0.84a	7.84±0.85a, b	8.88±1.92b	8.16±1.78ab
Mg absorption, mg/d	3.95±0.76a	4.68±0.61b	5.16±0.84b	5.43±1.32b
Mg absorption, %	37.0±6.5a	37.4±3.3a	38.1±7.4a	40.1±8.2a
True absorption				
^{26}Mg gavage, mg	2.86±0.02a	2.88±0.04ab	2.91±0.04b	2.88±0.03ab
Fecal ^{26}Mg excretion, mg	1.38±0.16a	1.16±0.16a	1.45±0.33a	1.29±0.27a
^{26}Mg absorption, mg	1.48±0.17a	1.72±0.18a	1.46±0.31a	1.60±0.26a
^{26}Mg absorption, %	51.9±5.8a	59.7±5.8a	50.1±10.6a	55.4±8.9a

[1] Four groups, of 10 rats each, were fed a purified semi-synthetic diet mixed with an equal weight of distilled water (control group), or $MgCl_2$ solution ($MgCl_2$ group), or Hépar water (sulphated water group) or Badoit water (carbonated water group). The four rat groups also received as drinking water: distilled water, $MgCl_2$ solution, sulphated mineral water or carbonated mineral water, respectively. The whole experiment lasted 28 days. Food intake was monitored and faeces were collected during the last four days for Mg absorption determination

[2] Each value is the mean ± SD, n = 10. Values in a row with different superscript letters differ, $p < 0.05$

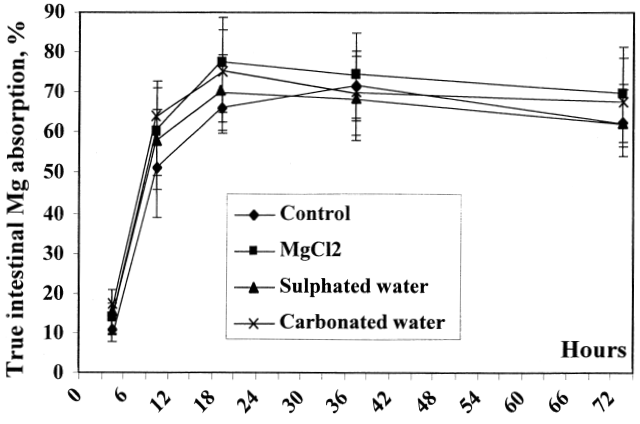

Fig. 1 Effects of sulphate- and bicarbonate-rich mineral waters on true intestinal magnesium absorption in rats[1, 2]

[1] Four groups, of 10 rats each, were fed a purified semi-synthetic diet mixed with an equal weight of distilled water (control group), or $MgCl_2$ solution ($MgCl_2$ group), or Hépar water (sulphated water group) or Badoit water (carbonated water group). The four rat groups also received as drinking water: distilled water, $MgCl_2$ solution, sulphated mineral water or carbonated mineral water, respectively. The whole experiment lasted 28 days. To determine true intestinal Mg absorption using the double isotopic labelling technique, each rat received orally 2 mL of a solution of 1.5 mg ^{26}Mg/mL (3 mg ^{26}Mg/rat). 30 minutes later, each rat received also intravenously 0.5 ml of a solution of 1 mg ^{25}Mg/mL (0.5 mg ^{25}Mg/rat), and urine samples were collected from 0 to 6 h, from 6 to 12 h, from 12 to 24 h, from 24 to 48 h and from 48 to 96 h.

[2] Each value is the mean ± SD, n = 10. Values in a raw with different superscript letters differ, $p < 0.05$

Urinary Mg excretion (Table 4)

Urinary Mg excretion was significantly higher in the three groups receiving $MgCl_2$ or mineral waters than in controls ($p = 0.0043$). There was no difference amongst the groups receiving $MgCl_2$ or mineral waters. This is in line with the higher Mg intake and Mg absorption in these three groups compared with the controls. Consequently, there was no difference in the Mg retention amongst the four experimental groups. The isotopic results showed that urinary excretion of both ^{25}Mg and ^{26}Mg stable isotopes was significantly higher in the three groups receiving $MgCl_2$ or mineral waters than in controls ($p = 0.0108$, $p = 0.0006$ for both ^{25}Mg and ^{26}Mg, respectively). However, there was no difference among the three groups receiving $MgCl_2$ or mineral waters. The urinary pH values were not significantly different among the four studied groups.

Mg status indices (Table 5)

Administration of Mg-rich mineral waters for four successive weeks did not significantly alter any Mg status indices. Plasma Mg levels ranged from 17.3 mg/L in the $MgCl_2$ group to 18.1 mg/L in the sulphated water group ($p = 0.8117$). Erythrocyte Mg levels ranged from 60.6 mg/L in the $MgCl_2$ group to 63.1 mg/L in the control group (0.4771). Tibia Mg levels ranged from 4.48 mg/g dry weight in the control group to 4.94 mg/L in the $MgCl_2$ group (0.0682).

Discussion

The main results of this work show clearly that the studied Mg-rich mineral waters increased the net intestinal

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Table 4 Effects of sulphate- and bicarbonate-rich mineral waters on urinary total Mg and Mg stable isotopes excretion in rats[1, 2]

	Control	$MgCl_2$	Sulphated water	Carbonated water
Total Mg				
Urinary Mg, mg/d	3.68±0.33a	4.53±0.72b	4.63±0.70b	4.60±0.67b
Retained Mg, mg/d	0.27±0.64a	0.15±0.67a	0.53±0.91a	0.82±0.88a
Retained Mg, %	2.5±6.0a	1.3±5.1a	4.0±6.8a	5.9±6.2a
Mg stable isotopes				
Administered ^{26}Mg, mg	2.86±0.02a	2.88±0.04ab	2.91±0.04b	2.88±0.03ab
Urinary ^{26}Mg, mg	0.33±0.04a	0.44±0.05b	0.46±0.10b	0.46±0.08b
Administered ^{25}Mg, µg	504±16a	502±12a	500±12a	495±24a
Urinary ^{25}Mg, µg	164±21a	184±26b	193±11b	189±18b
Urinary pH	5.87±0.15a	5.78±0.13a	5.80±0.08a	5.85±0.14a

[1] Four groups, of 10 rats each, were fed a purified semi-synthetic diet mixed with an equal weight of distilled water (control group), or $MgCl_2$ solution ($MgCl_2$ group), or Hépar water (sulphated water group) or Badoit water (carbonated water group). The four rat groups also received as drinking water: distilled water, $MgCl_2$ solution, sulphated mineral water or carbonated mineral water, respectively. The whole experiment lasted 28 days. Urines were collected during the last four days

[2] Each value is the mean ± SD, n = 10. Values in a row with different superscript letters differ, $p < 0.05$

Table 5 Effects of sulphate- and bicarbonate-rich mineral waters on magnesium status in rats[1]

	Control	$MgCl_2$	Sulphated water	Carbonated water
Plasma Mg, mg/L	17.9±1.8a	17.3±1.9a	18.1±2.3a	17.8±0.7a
Erythrocyte Mg, mg/L	63.1±3.2a	60.6±3.8a	60.9±5.8a	62.3±3.2a
Tibia Mg, mg/g dw.	4.48±0.4a	4.94±0.4a	4.64±0.4a	4.58±0.2a

[1] Each value is the mean ± SD, n = 10. Values in a raw with different superscript letters differ, $p < 0.05$

absorption of Mg (mg/d), but never modified the fractional intestinal absorption of Mg (%). On the other hand, the urinary excretion of Mg was significantly higher in all the rats receiving mineral water or water enriched in $MgCl_2$ compared to the control rats.

We chose two Mg-rich mineral waters, which we compared with two controls: in the first control, the rats did not receive Mg in their drinking water, while in the second control, the rats received Mg in the form of $MgCl_2$ in similar amounts to that supplied by the mineral waters. We made sure the intake of Mg from the mineral waters was nutritionally significant and could be extrapolated to plausible human consumption levels. The rats consumed these waters at the same time in their semi-fluid diet (1 part diet + 1 part water by weight) and as drinking water. Thus, about 30 % of ingested Mg was supplied by the mineral waters studied. This corresponds to the consumption of about 150 mg Mg from mineral waters in humans, i. e., approximately 1.5 L of mineral water, which is perfectly reasonable [21].

In this study, we applied different methods to determine the effects of two mineral waters on intestinal Mg absorption: chemical balance, isotope faecal monitoring and isotope double labelling. All clearly showed that neither of these mineral waters significantly altered the fractional apparent or true intestinal Mg absorption after four weeks of water administration. The apparent Mg absorption values obtained ranged between 37 % and 40 %, in close agreement with those reported in the literature [19, 22]. This apparent Mg absorption includes both true absorption and endogenous Mg excretion. One can speculate that the absence of effects of mineral waters on Mg absorption could be due to mutual masking of differences in true absorption and endogenous Mg excretion. We thus determined the true intestinal absorption of Mg using available stable Mg isotopes. The oral administration of an isotope and the measurement of the fraction that excreted in the faeces assess the 'true' absorption of Mg without interference from endogenous Mg excretion. Given the relatively high natural abundance of available stable Mg isotopes, a large amount of ^{26}Mg was orally administered (3 mg). Such a large dose was necessary to obtain faecal material with enough ^{26}Mg enrichment and also to obtain sufficient ^{26}Mg enrichment in urine samples up to four days. A four-day faeces collection period was necessary to ensure recovery of all the unabsorbed isotope [19]. The true Mg absorption values again showed that neither of the tested mineral waters significantly altered the true intestinal Mg absorption after four weeks of water ad-

ministration. The 'true' Mg absorption values obtained ranged between 58 % and 68 % and were close to those reported in the literature [10, 19].

We also measured true Mg absorption by the isotopic double labelling technique by administrating one isotope orally (^{26}Mg) and another intravenously (^{25}Mg). The measurement of these isotopes in blood or urine then yields the true Mg absorption. Because blood sampling is problematic in rats during the balance study, we used urine samples for this determination. As we have recently shown [19], the first two samples of urine are inadequate to determine Mg absorption because equilibrium is not yet reached. The values obtained from urine collected 12 h after isotope administration seem to be correct and ranged between 62 % and 78 % depending on the group tested and the time point measured. This elegant double labelling technique clearly showed that fractional true intestinal Mg absorption (%) did not vary in the four experimental groups at any time point except the first one (urine 0 to 6 h), where Mg absorption in the carbonated water group was significantly higher than in the control group. All three different approaches measuring apparent and true intestinal Mg absorption show that the fractional absorption of Mg (%) was similar in the rats receiving distilled water, $MgCl_2$-enriched water and the two mineral waters studied. This suggests that the fractional absorption of Mg from the diet is as good as that from the mineral waters under the experimental conditions of this study.

It is well established that diet and certain food components appreciably affect the body's acid-base balance [23–25]. It is known that typical Western diets may have an acidifying effect, whereas vegetable-based diets may have an alkalinising effect. Organic ions such as carbonate and citrate are responsible for the alkalinising effect, while sulphate may be responsible for the acidifying effect [26, 27]. The latter effect may result in higher urinary mineral losses, in particular of Ca and Mg [28, 29]. In this work, we compared the effect of two mineral waters (sulphated and carbonated) on urinary pH and urinary Mg excretion. Our results clearly show that chronic administration of 30 % of dietary Mg in the form of chloride, sulphate or carbonate had no effect on urinary pH in the groups receiving the additional Mg compared with the control group. The measured urinary pH ranged between 5.78 in the rats receiving $MgCl_2$ and 5.87 in the control rats. The urinary Mg excretion was also similar in the three groups receiving the additional Mg and ranged between 4.53 and 4.63 mg/day. The measurement of stable isotopes of Mg (^{25}Mg and ^{26}Mg) excreted in the urine also clearly showed that the three groups receiving the supplemental Mg had higher urinary Mg stable isotope excretion compared to the control group. This was possibly because adequate tissue Mg levels were more fully repleted in the groups concerned than in the controls, although this was not statistically observed. Consequently, we did not note any urine acidifying or alkalinising effects for the mineral waters studied. This may be because more than 70 % of remaining dietary Mg intake was in the form of oxide and may have a strong impact on the final urinary pH and thus on urinary Mg excretion in this study.

In conclusion, the studied mineral waters are a good source of bioavailable energy-free Mg and increase net intestinal absorption of Mg. However, although mineral water Mg occurs in soluble ionised form, it does not seem to be more bioavailable than dietary Mg in the oxide form. Excess absorbed Mg is rapidly excreted in the urine once the body has an adequate Mg status. Drinking acidifying sulphated water or alkalinising carbonated water did not lead to any significant difference in urinary excretion of Mg under the experimental conditions of this study. It would be interesting to investigate this effect in rats with a depleted dietary Mg intake and for a longer period. Mg-rich mineral waters may be indicated for people at risk of Mg deficiency and who wish to avoid a high energy intake. Further studies are still necessary to determine the effect of acidifying or alkalinising mineral water in different dietary Mg intake conditions and in different types of diets.

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