

# Magnesium bioavailability after administration of sucrosomial® magnesium: results of an *ex-vivo* study and a comparative, double-blinded, cross-over study in healthy subjects

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**Abstract.** – **OBJECTIVE:** We conducted an *ex-vivo* analysis and a study in healthy subjects to compare magnesium bioavailability after administration of Sucrosomial® magnesium or commercially available preparations of magnesium citrate, magnesium oxide and magnesium bisglycinate.

**MATERIALS AND METHODS:** In the *ex-vivo* study we simulated magnesium intestinal absorption after digestion through sections of intestinal mucosa isolated from rats. We compared the absorption of magnesium oxide and Sucrosomial® magnesium at two different concentrations: 32.9 mg/ml and 329 mg/ml.

The human study was a single day double-blinded repeated crossover study in healthy subjects. Each subject was administered 350 mg magnesium in different formulations (Sucrosomial® magnesium, magnesium citrate, magnesium oxide or magnesium bisglycinate) after 1 week of washout. We collected blood and urine samples to measure magnesium concentration in blood, urine and red blood cells.

**RESULTS:** The *ex-vivo* evaluation showed that magnesium absorption after administration of Sucrosomial® magnesium was faster and with higher rates compared to a standard formulation of magnesium oxide. This finding was further confirmed by the results of the study in healthy subjects, that showed a more evident increase in magnesium concentration after administration of Sucrosomial® magnesium compared to the other formulations. In particular, the increase in magnesium concentration from baseline to 24 h was statistically higher in blood and in urine for Sucrosomial® magnesium compared to magnesium oxide, while in red blood cells Sucrosomial® magnesium had a statistically significant advantage compared to magnesium bisglycinate.

**CONCLUSIONS:** Our findings suggest that Sucrosomial® magnesium leads to an increased

bioavailability of magnesium compared to other formulations. Further studies are needed to investigate if this advantage turns into more evident clinical efficacy.

*Key Words:*

Sucrosomial®, Magnesium, Bioavailability, Healthy subjects.

## Introduction

Magnesium is a key regulator of human health. It is an essential co-factor for hundreds of enzymes and it plays a crucial role in a number of biological reactions and physiological pathways, including energy production, nucleic acid and protein synthesis, ion transport and cell signaling<sup>1,2</sup>. Therefore, magnesium is involved in maintaining correct homeostasis; the deficit of this ion has been associated with several pathological conditions, including diabetes mellitus, metabolic syndrome, osteoarthritis, chronic kidney disease, bone loss, cystic fibrosis, depression and anxiety<sup>2-9</sup>. Moreover, hypomagnesaemia can be the result of the use of several drugs, such as diuretics, epidermal growth factor receptor inhibitors, calcineurin inhibitors, and proton pump inhibitors<sup>10</sup>. Although quality of evidence is mixed, there is an overall consensus on the potential efficacy of magnesium supplementation in these conditions, and also for general well-being<sup>10</sup>. However, conventional oral magnesium supplementation exhibits poor absorption and bioavailability, likely due to the interference of dietary compounds or digestive factors<sup>11</sup>. Of note, enhanced bioavail-

ability would likely result in more effective supplementation of magnesium<sup>11</sup>.

*In vitro* studies have shown that sucrose esters in non-toxic concentrations increase permeability of the monolayer barrier in intestine<sup>12</sup>. On these basis, a Sucrosomial<sup>®</sup> magnesium formulation [ULTRAMAG<sup>®</sup> (Sucrosomial<sup>®</sup> magnesium) Pharnutra S.p.A., Pisa, Italy] in a sustained release matrix has been developed in order to enhance magnesium bioavailability. This oral formulation is an innovative preparation of magnesium oxide, covered by a phospholipids plus sucrose ester matrix and can be used as an alternative to common magnesium salts to improve magnesium supplementation effectiveness. Thanks to the encapsulation of magnesium ions within a Sucrosomial membrane, the ions can pass through the gastric and intestinal environment without any interaction with the intestinal mucosa and then reach the blood stream. However, evidence on bioavailability of this product is still poor.

In order to comprehensively investigate magnesium bioavailability after administration of Sucrosomial<sup>®</sup> magnesium, we have performed a permeation study using an *ex-vivo* model. We also conducted a study in healthy subjects comparing magnesium bioavailability after administration of Sucrosomial<sup>®</sup> magnesium or commercially available preparations of magnesium citrate, magnesium oxide and magnesium bisglycinate.

## Materials and Methods

### Ex-vivo Study

We conducted an *ex-vivo* study in a rat model. This investigation was conducted in compliance with the regulations for animal studies applied in EU. After sacrifice, intestinal mucosa of male Wistar rats (weight 250-300 g, n=3) was isolated. The first 20 cm of the jejunum were removed and cut into 1.5×1.5 cm large samples. For each sample, the content of the lumen was then removed and transferred to an Ussing chamber (surface 0.78 cm<sup>2</sup>) without removing muscle stratum. Phosphate-buffered saline (PBS) (1 ml, 0.13 M, pH 6.8) was added at the apical side (donor medium), while another isotonic buffer solution (3 ml, 0.13 M, pH 7.4) was added at the basolateral side (acceptor medium). Experiments were carried out at 37°C by using of external water clamp system with controlled oxygenation (95%) and stirring. After 20 min, donor medium was replaced with a solution of either magnesium oxide or Sucro-

somial<sup>®</sup> magnesium (8 ml each) with a known quantity of magnesium and a definite concentration determined by spectrophotometry analysis<sup>13</sup>. The experiment was conducted with two different magnesium amounts (32.9 mg or 329 mg) of both tested samples. Each solution had been treated with pepsin (0.2 g in a HCl 0.1 M solution) and then stored for 60 min at pH 2 in order to simulate gastric digestion. 1 ml of sample from the acceptor medium was taken every 30 min, for a total period of 240 min, and immediately replaced with the same volume of fresh medium. Mg<sup>2+</sup> concentration was then measured by adding FURA-2 (an indicator) and EGTA (a ion chelator) to the sample. The spectrophotometer was calibrated using reference solution with known concentration of Mg<sup>2+</sup> ( $r^2=0.9999$ )<sup>13</sup>.

### Permeation Data Treatment

Permeation data were treated as previously described<sup>14</sup>, assuming that the transport of Mg<sup>2+</sup> across excised intestine occurred by passive diffusion. Accordingly, for each permeation run a value of apparent permeability coefficient,  $P_{app}$  for permeant across the excised rat intestinal mucosa was calculated from the following equation:  $P_{app} = dM/dt \cdot 1/(A \cdot C_0)$  (1) where  $dM/dt \cdot 1/A$ , the permeation flux, is the slope of the linear portion of the cumulative amount permeated per unit surface area vs. time plot, and  $C_0$  is the iron concentration introduced into the donor phase, supposed to be completely dissolved and constant over the time interval of linear permeation. For each plot, the linear regression analysis was extended to the set of data points that gave the best fit, as judged from the  $r^2$ -value. This, in all of the cases investigated, was greater than 0.9. The single  $P_{app}$  values were averaged to calculate the mean apparent permeability,  $P_{app}$  (n≥6). The significance of the difference between two  $P_{app}$  values was assessed by the Student's *t*-test ( $p < 0.05$ ). For the samples that produced a significant  $P_{app}$  increase, this was measured by the enhancement ratio (ER), defined as the ratio between the  $P_{app}$  values obtained with the formulation under test and the reference magnesium oxide.

### Human Study

#### Setting

This was a single-day, double-blinded, repeated crossover study in healthy subjects using 350 mg (elemental dose) of Sucrosomial<sup>®</sup> magnesium, magnesium citrate, magnesium oxide or magne-

sium bisglycinate. The study was conducted at the Maze Lab (Purchase, NY, USA) between December 2016 and March 2017. The protocol was approved by the local Ethical Committee and all participants signed an informed consent before inclusion.

Between each trial day, participants underwent a 1-week washout period. Subjects were switched either from one experimental to another experimental treatment on a double-blind basis. All formulations of magnesium were identical.

### *Population*

Adult ( $\geq 18$  years) subjects of either gender were eligible. Other inclusion criteria were as follows: no current smoking habit; Body Mass Index (BMI) 18-30 kg/m<sup>2</sup>; consent to consume standard meals and not to consume any foods and drinks with high magnesium level. Patients were excluded if they had history of bone diseases, diabetes mellitus, chronic fatigue syndrome, premenstrual symptoms, peptic ulcer, intestinal resection, inflammatory disease of the gastrointestinal tract, malabsorption/maldigestion, hypertension, gall bladder disease or any other relevant medical condition; current chronic medication intake; history or current abuse of drugs, medication or alcohol, or intake  $>2$  alcoholic beverages/day; known hypersensitivity to study product or any ingredient in the preparation; pregnant or lactating status, or recent ( $<6$  months) delivery; participation to other clinical trials within the last four week before enrolment.

### *Procedures*

Participants were instructed not to consume any magnesium rich foods or supplement for at least 1 week before the study.

On Day 1, following overnight fasting blood samples, participants took the magnesium dose assigned for that day. Blood and urine samples were then taken for baseline magnesium concentrations ( $T_0$ ). A standard breakfast was then offered (approximately 500 kcal, 15% protein, 30% fat and 55% carbohydrate), and participants were instructed not to consume any magnesium rich foods, drinks or supplement for each meal during the trial day (a food diary was also filled). Further blood samples and urine samples were then taken at 2, 4, 8, and 24 h. Magnesium content was then measured in three compartments: blood, red blood cells (RBCs), and urines. Magnesium absolute and relative concentrations were measured at baseline and then at each subsequent time-points

(0, 2, 4, 8 and 24 h after administration of the supplement).

### *Blood Sampling*

During each trial day, venous blood samples were drawn at 0, 2, 4, 8, and 24 h time points after the administration of the magnesium supplement. A 10-min window was permitted. Blood samples were collected into BD whole blood tubes with 7.5% EDTA Solution (Lavender top tube; Becton Dickinson, Franklin Lakes, NJ, USA) for RBCs sample collection, and in a serum separator tube (Vacutainer® – SST™, Gel and Clot Activator; Becton Dickinson, Franklin Lakes, NJ, USA) for serum sample collection. Both whole blood samples were stored on ice protected from light.

Sample processing was performed in specialized laboratories. The whole blood samples in Lavender Top tube were centrifuged at 1500-2000 rpm for 10-15 min at room temperature to isolate RBC. For serum collection, the serum separator tube was inverted five times gently, and blood was allowed to clot in an upright position for 30-60 min at room temperature. The whole blood samples in serum separator tube were then centrifuged at 3000 rpm for 10 min. The serum was immediately collected and analyzed. Magnesium content in RBCs was measured by atomic absorption spectrophotometry (AAS) with the emission mode at 285.2 nm using air-acetylene flame.

### *Urine Sampling*

Participants were provided with a kit containing a 16 Oz urine sample-collecting bottle. The collected urine samples at the pre-specified time-points were refrigerated and analyzed as soon as possible. Urine samples were analyzed for magnesium concentration by Monarch centrifugal analyzer, equipped with an appropriate magnesium assay kit.

### *Statistical Analysis*

10 patients were planned to participate, in line with the sample size of previous similar studies<sup>15,16</sup>. Magnesium concentrations were evaluated, for each compartment, both as absolute values and as percentage variation from  $T_0$  to  $T_{24}$ . According to their distribution, as assessed by Kolmogorov-Smirnov test, absolute values were reported as mean  $\pm$  standard deviation, percentage variations as median and interquartile range. Comparisons between last observation and baseline value of magnesium were performed with two-

tailed paired *t*-test; comparisons across different formulations were done with one way analysis of the variance (ANOVA), followed by a Tukey's post-hoc test. In each physiological compartment, the percent changes were studied with the Friedman test for non-parametric repeated-measurements one way ANOVA. Subsequently, post-hoc pairwise comparisons were performed, using a non-parametric Wilcoxon test with Tukey's correction. A *p*-value <0.05 was considered statistically significant. All the analyses were performed using the R statistical software<sup>17</sup>.

## Results

### Ex-vivo Study

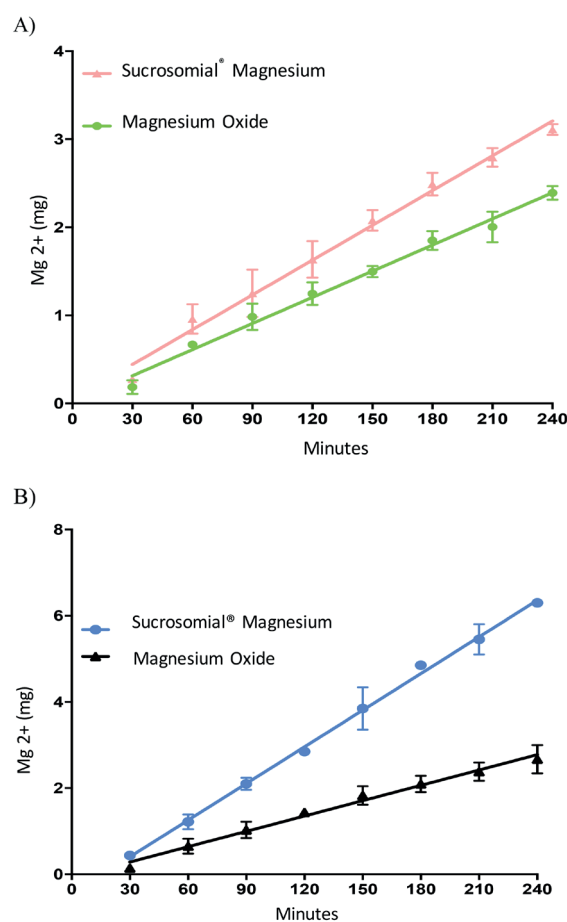
In order to understand permeation features of Sucrosomial<sup>®</sup> magnesium, tissues were treated with two different magnesium amounts (32.9 mg and 329 mg of elemental Mg) and observed ionic magnesium released from apical to basolateral side of the Ussing chamber.

Treatment of rat isolated intestine with Sucrosomial<sup>®</sup> magnesium leads to an increase of Mg<sup>2+</sup> concentration in the basal compartment of the Ussing chamber over time compared to magnesium oxide (Figure 1a). Similar findings were reported in the second test (Figure 1b). The overall enhancement ratio (ER) was 2.4 for the higher amount of Mg<sup>2+</sup>, with a 19±3% increase in permeability.

### Clinical Study

10 subjects participated to the study (4 males and 6 females with a mean age 34±14 years [range: 21-66]; mean BMI 25.5±2.5 kg/m<sup>2</sup> [range 21.5-29.3]).

The values of magnesium concentration, in all compartments and for the four treatments, are reported in Table I, while Table II displays % changes at 24 h, as compared with baseline (Figures S1-S3). All formulations significantly increased magnesium concentration in all compartments (*p*=0.041, for blood; *p*=0.011 for RBCs; *p*=0.008 for urine). Overall, Sucrosomial<sup>®</sup> magnesium resulted in a higher magnesium bioavailability compared with the other formulations, reaching a statistically-significant advantage over magnesium oxide in terms of magnesium content in blood and RBC (Table II). In urines, Sucrosomial<sup>®</sup> magnesium was superior to magnesium bisglycinate (Table II). No adverse events were reported during the study period.



**Figure 1.** Ex vivo study: Mg<sup>2+</sup> quantity that passes intestinal barrier over time (Panel A: administered amount 32.9 mg/ml; Panel B: 329 mg/ml).

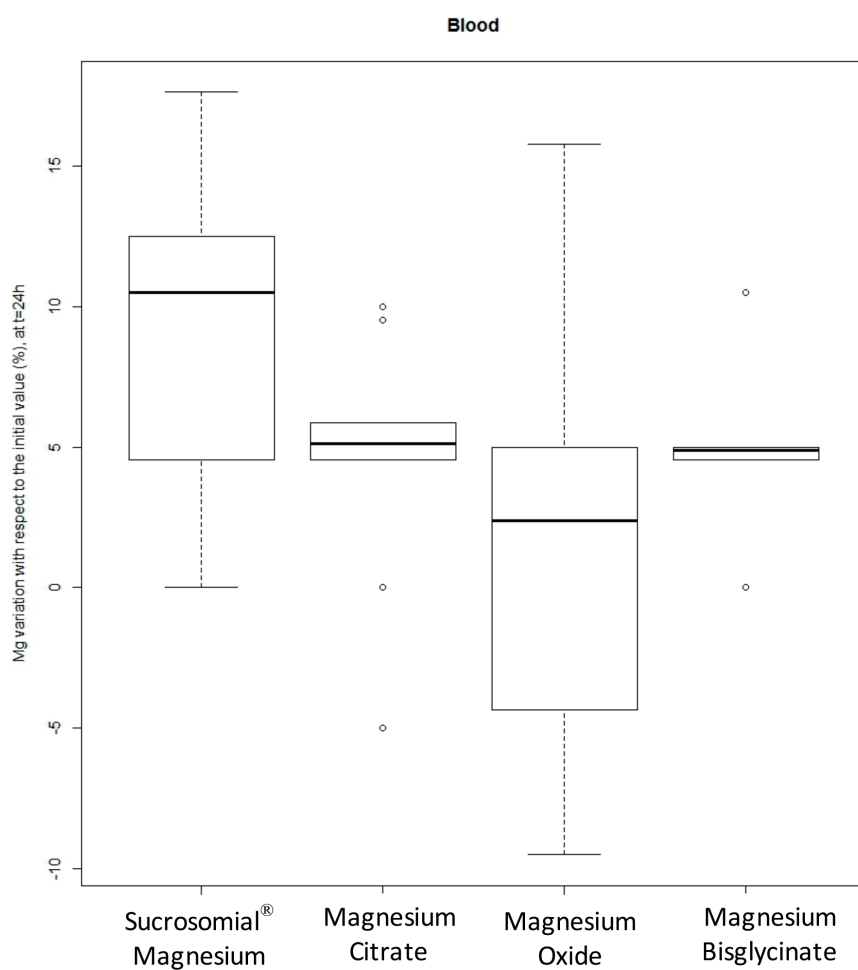
## Discussion

Magnesium is a key element for human health and well-being, and its deficit has been associated with several diseases<sup>2-9</sup>. Magnesium supplementation appears an attractive and overall safe strategy to help prevent these conditions and increase well-being<sup>10</sup>. Several studies are currently focusing on magnesium supplementation in different diseases<sup>18-21</sup>. Conventional oral magnesium supplementation presents, however, a very poor intestinal adsorption, which in turn results in modest bioavailability and limits its efficacy<sup>11</sup>.

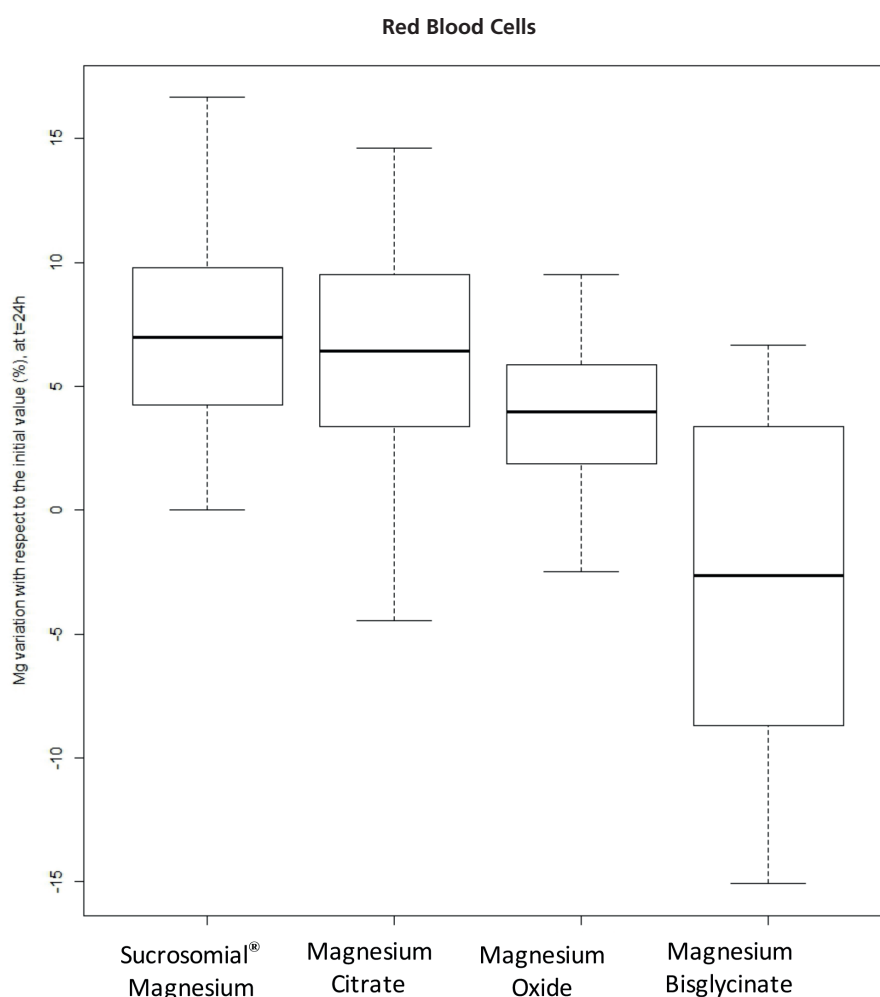
In line with previous studies, we have developed a Sucrosomial<sup>®</sup> magnesium formulation, based on a proprietary technology able to promote adsorption of Mg<sup>2+</sup> ions, with the aim to increase intestinal adsorption and, therefore, bioavailability. Moreover, we have conducted a comprehensive evaluation of magnesium bioavailability after

**Table I.** Magnesium concentrations (expressed as mg/dl) in the study samples.

Treatment	T = 0 h	T = 2 h	T = 4 h	T = 8 h	T = 24 h
<i>Blood</i>					
Sucrosomial® magnesium	1.93 ± 0.20	2.01 ± 0.17	2.06 ± 0.14	2.05 ± 0.16	2.09 ± 0.12
Magnesium citrate	1.99 ± 0.11	2.00 ± 0.19	2.09 ± 0.11	2.11 ± 0.18	2.08 ± 0.12
Magnesium oxide	2.05 ± 0.11	2.08 ± 0.11	2.10 ± 0.13	2.14 ± 0.13	2.09 ± 0.15
Magnesium bisglycinate	2.02 ± 0.13	2.10 ± 0.11	2.09 ± 0.12	2.11 ± 0.16	2.13 ± 0.14
<i>Red Blood Cells</i>					
Sucrosomial® magnesium	4.84 ± 0.49	4.95 ± 0.47	5.10 ± 0.60	5.00 ± 0.46	5.20 ± 0.67
Magnesium citrate	4.68 ± 0.57	4.51 ± 0.91	4.49 ± 0.48	4.60 ± 0.54	4.97 ± 0.54
Magnesium oxide	4.91 ± 0.59	4.97 ± 0.74	4.94 ± 0.71	5.00 ± 0.62	5.09 ± 0.66
Magnesium bisglycinate	5.09 ± 0.59	5.07 ± 0.62	5.08 ± 0.69	5.07 ± 0.64	4.94 ± 0.71
<i>Urines</i>					
Sucrosomial® magnesium	5.69 ± 2.39	7.33 ± 6.44	7.15 ± 5.48	3.99 ± 2.75	9.99 ± 6.24
Magnesium citrate	4.74 ± 3.07	7.15 ± 6.11	5.96 ± 5.81	2.81 ± 2.13	5.20 ± 3.34
Magnesium oxide	7.88 ± 3.41	9.11 ± 7.30	8.23 ± 4.10	5.78 ± 3.78	7.53 ± 4.88
Magnesium bisglycinate	8.04 ± 5.49	9.67 ± 4.95	8.91 ± 6.44	4.50 ± 4.22	7.60 ± 4.37


**Figure S1.** Blood. Boxplots for percent variation of magnesium concentration with respect to its initial value, at 24 h. The box represents the interquartile range, the thick line represents the median value, the whiskers represent the range of the distribution. The circles represent potential outliers.



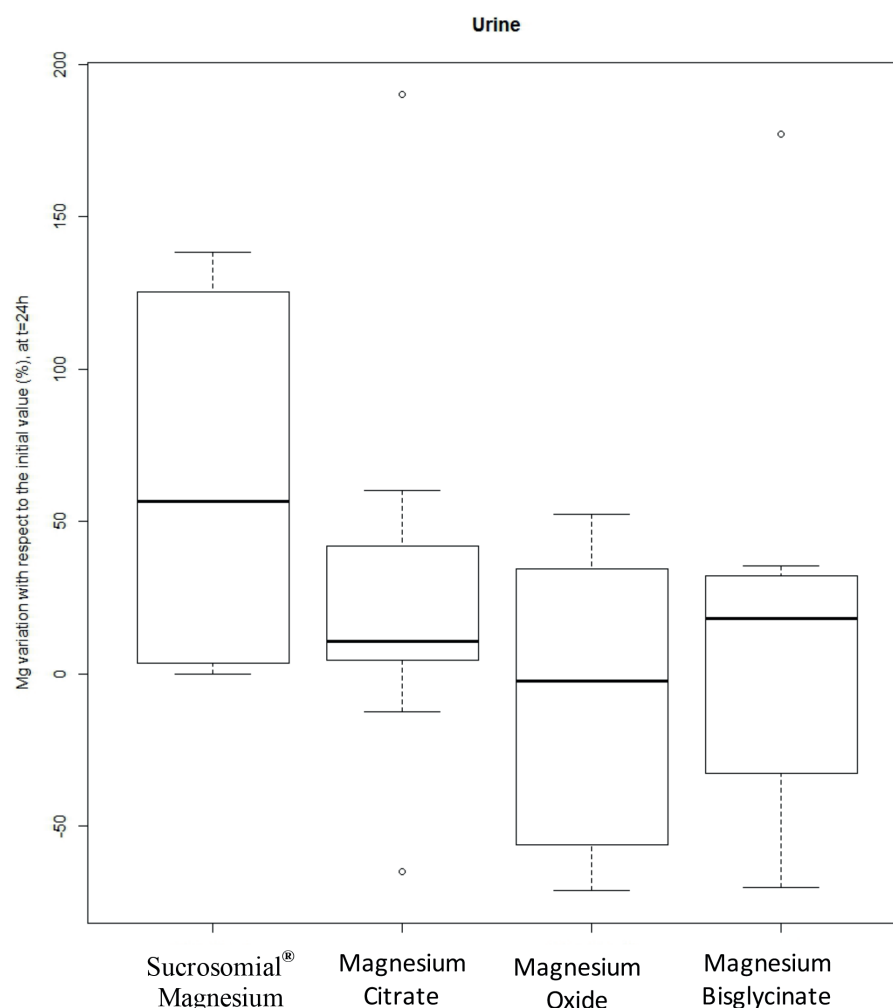


**Figure S2.** Red Blood Cells. Boxplots for percent variation of magnesium concentration with respect to its initial value, at 24 hours. The box represents the interquartile range, the thick line represents the median value, the whiskers represent the range of the distribution.

administration of sucrosomial® magnesium. First, we investigated this parameter under controlled and standardized conditions (*ex-vivo*). Then, we evaluated bioavailability in human subjects in order to investigate whether this formulation actually enhances intestinal absorption and bioavailability when compared with other standard and commercially-available magnesium salts. Some limitations inherent to any bioavailability study must, however, be acknowledged (e.g., overall small sample size and short follow-up period). We chose magnesium oxide as control because sucrosomial® magnesium is developed starting from magnesium oxide salt itself. Of note, magnesium oxide is the least bioavailable form of magnesium salt that contains the highest percentage of elemental magnesium. However, it is the most used salt as supplement. Sucrosomial® technology

(patent n°MI2013A001483) thanks to the presence of a phospholipid matrix, is able to increase minerals, in particular iron, absorption and bioavailability<sup>14</sup>. Because of it, Sucrosomial® technology could help to increase  $Mg^{2+}$  absorption; therefore, developing a formulation like Sucrosomial® magnesium would be an optimal nutritional target. The results of the *ex-vivo* evaluation, specifically conducted to simulate intestinal adsorption after gastric digestion, show higher adsorption of magnesium after administration of Sucrosomial® magnesium compared with a standard formulation of magnesium oxide. This enhanced intestinal adsorption was also confirmed under physiological conditions in healthy subjects, without any noticeable adverse event. Although all the tested preparations have increased magnesium concentration 24 h from administration, with re-

**Figure S3.** Urine. Boxplots for percent variation of magnesium concentration with respect to its initial value, at 24 hours. The box represents the interquartile range, the thick line represents the median value, the whiskers represent the range of the distribution. The circles represent potential outliers.



**Table II.** Percent changes in magnesium concentrations in the study samples at 24h versus baseline.

Treatment	Percent change vs. baseline [95% CI]
<i>Blood</i>	
Sucrosomial® magnesium	10.53 [4.60-12.32]*
Magnesium citrate	5.13 [4.66-5.73]
Magnesium oxide	2.38 [-3.26-5.00]
Magnesium bisglycinate	4.88 [4.60-5.00]
<i>Red Blood Cells</i>	
Sucrosomial® magnesium	6.99 [4.33-9.51] <sup>+</sup>
Magnesium citrate	6.44 [3.65-9.47] <sup>+</sup>
Magnesium oxide	3.98 [1.96-5.50] <sup>+</sup>
Magnesium bisglycinate	-2.63 [-7.91-2.45]
<i>Urines</i>	
Sucrosomial® magnesium	56.48 [13.21-122.20]*
Magnesium citrate	10.55 [5.16-36.90]
Magnesium oxide	-2.41 [-44.93-27.97]
Magnesium bisglycinate	18.18 [-32.65-32.04]

\* $p < 0.05$  vs. Magnesium Oxide; <sup>+</sup> $p < 0.05$  vs. Magnesium Bisglycinate.

spect to baseline, Sucrosomial® magnesium determined the more evident increase. This finding was evident in all the three compartments tested, namely blood, RBCs and urines, confirming the increased magnesium bioavailability obtained with Sucrosomial® magnesium. In particular, the percentage increase in magnesium concentration vs. baseline values with this preparation was significantly greater than the increase associated with magnesium oxide in blood and RBC and the increase seen with magnesium bisglycinate in urines. Noteworthy, the increase in urine concentration (+56%) was 3-4 times higher than what reported for the other formulations: this finding is of special interest since magnesium concentration in this compartment has been recently shown to accurately indicate overall bioavailability<sup>22</sup>.

It is important to consider that magnesium bioavailability and homeostasis are regulated by the activity of the intestine, bone and kidneys. Absorption occurs mainly in the intestine,

thanks to passive paracellular mechanisms that account for 80-90% of the whole intestinal uptake; adsorption rate is driven by high luminal magnesium concentrations. Of note, intestinal absorption is not directly proportional to  $Mg^{2+}$  intake but is mainly influenced by magnesium status, as we observed in our study<sup>23</sup>. Kidneys also play a crucial role by regulating magnesium excretion and re-absorption; in particular renal excretion with urines is the main determinant of magnesium serum concentrations<sup>18</sup>. Last of all magnesium concentrations are influenced by various hormones, such as 1,25-dihydroxyvitaminD, that stimulates  $Mg^{2+}$  absorption, and estrogen and parathyroid hormones, which are involved in  $Mg^{2+}$  excretion<sup>23</sup>.

## Conclusions

According to the above-described findings, we can speculate that our comprehensive analysis conducted both *ex-vivo* and in healthy subjects, provides robust evidence of increased magnesium bioavailability after administration of Sucrosomial® magnesium compared with other commercially available magnesium supplementations. This finding is particularly evident in urines, representing a well-grounded evidence of enhanced magnesium bioavailability with Sucrosomial® magnesium. Future studies will evaluate whether this advantage turns into more evident clinical efficacy.

## Acknowledgments

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## Conflict of Interest

Elisa Brilli and Germano Tarantino are PharmaNutra S.p.A. employees. The other Authors declare that they have no conflict of interests.

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