

## Magnesium levels in plasma and erythrocytes before and after histamine challenge

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*Magnesium levels in plasma and erythrocytes before and after histamine challenge. E. Zervas, S. Loukides, G. Papatheodorou, K. Psathakis, K. Tsindiris, P. Panagou, N. Kalogeropoulos. ©ERS Journals Ltd 2000.*

**ABSTRACT:** Previous studies have assessed the protective effect of nebulized magnesium sulphate on bronchial hyperreactivity. This study investigated the effect of histamine challenge on intracellular (erythrocytes) and extracellular (plasma) levels of magnesium and the possible relationship between degree of bronchial hyperreactivity and levels of Mg in plasma and erythrocytes.

The authors studied 42 mildly asthmatic patients (10 on inhaled steroids) and 20 healthy subjects. Histamine challenge was performed by the dosimeter method and provocative dose causing a 20% fall in forced expiratory volume in one second (PD<sub>20</sub>) (FEV<sub>1</sub>) was calculated. Mg levels were measured with a calmagite colourimetric assay, both at baseline and when FEV<sub>1</sub> had fallen by 20%.

The results showed that Mg levels in plasma did not significantly change after histamine challenge (from 2.06±0.02 mg·dL<sup>-1</sup> to 2.08±0.02 mg·dL<sup>-1</sup> respectively, p=0.14). Conversely there was a statistically significant decrease in Mg levels in erythrocytes between these two time points (from 1.84±0.02 fmmol·cell to 1.78±0.02 fmmol·cell p<0.0001). Similar results were observed when the subgroups were studied separately. There was no significant correlation between PD<sub>20</sub>, the difference in both magnesium concentrations (baseline-PD<sub>20</sub> time) or the initial values of Mg levels in erythrocytes and plasma.

To conclude, histamine challenge reduces magnesium levels in erythrocytes while plasma levels remain unchanged. This histamine-induced decrease in magnesium levels occurs regardless of the diagnosis of asthma, and it is not correlated with the degree of bronchial hyperreactivity.

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Magnesium, the second most abundant intracellular cation (second only to potassium) [1], has shown to be an *in vitro* relaxant on bronchial smooth muscle [2]. The first description of the bronchodilator effect of Mg in acute asthma was carried out in 1936 by ROSSELLO and PLA [3]. Previous studies have demonstrated the role of intravenous or nebulized magnesium sulphate (MgSO<sub>4</sub>) as a helpful agent to reverse bronchospasm in acute asthma [4, 5].

Inhaled Mg attenuates methacholine and histamine-induced bronchoconstriction in patients with asthma in a dose-dependent way [6, 7]. There is also some evidence that hypomagnesemia and low Mg intake are related to airway hyperresponsiveness, whereas dietary supplementation may result in improvement in asthmatic patients [8, 9]. These earlier observations lead to the plausible explanation that Mg levels may interfere with bronchial reactivity. However, the role of Mg in asthma is still controversial as other studies failed to demonstrate any benefit from inhaled or intravenous Mg in the treatment of acute and stable asthma [10, 11] or to correlate Mg levels to airway reactivity or protection against bronchoconstriction challenge [12, 13].

The aim of this study was to investigate the effect of bronchial challenge with histamine on intracellular (erythrocytes) and extracellular (plasma) levels of magnesium and examine the possible relationship between degree of bronchial hyperreactivity and levels of magnesium in plasma and erythrocytes.

### Methods

#### Patients

Four different populations were tested: asthmatic patients (steroid treated or steroids-naive), healthy volunteers and a control group allowing the assessment of the analytical and biological variation of the magnesium assay. Forty-two subjects (all male mean±SD, forced expiratory volume in one second (FEV<sub>1</sub>) 93±1% of predicted (pred), mean age 22±0.4 yrs) were randomly selected from the outpatient clinic (Athens Army General Hospital). All were asthmatic according to the American Thoracic Society criteria [14]. Ten were receiving inhaled steroids and thirty two were steroid naive. Twenty healthy subjects,

who were nonsmokers, with no history of atopy (negative skin prick tests to common allergens) were also included. All subjects were in a clinically stable condition with a baseline FEV<sub>1</sub> in excess of 80% of the predicted value and had not suffered from any respiratory tract infection for the last 6 weeks before the study. None of the subjects had risk factors for Mg depletion, received diuretics or any other Mg-wasting drugs [1]. In order to assess the analytical and biological variation of the Mg assay, 20 of the randomly selected subjects characterized as control group (mean±SD age 22±0.8 yrs, FEV<sub>1</sub> 91±2% of pred 6 asthmatics on inhaled steroids, 7 asthmatics steroid naive and 7 normal subjects), were asked to come on a different day, where Mg in erythrocytes and plasma were measured with a time difference of 15 min (the established average time for an histamine challenge procedure).

### Histamine challenge

Bronchial hyperreactivity was measured by a rapid histamine inhalation test for the determination of PD<sub>20</sub> [15]. Histamine 1% in 0.5% phenol (Hal, Lab BV, Harlem, the Netherlands) was inhaled in doubling doses starting from 100 µg until FEV<sub>1</sub> had fallen ≥20% compared with FEV<sub>1</sub> after an initial saline (0.9%) inhalation. The bronchial aerosol provocation system (APS Jaeger, Würzburg, Germany) with the Sandoz nebulizer (Jaeger) was used in this procedure. The Sandoz nebulizer was calibrated to nebulize 5 µL per nebulization and was triggered by inspiration on tidal ventilation. Each nebulization lasted for 0.5 seconds. Lung function was measured 1 min after each inhaled dose. PD<sub>20</sub> was determined by linear interpolation on a semi-logarithmic scale. Normal reactions were considered those >800 µg for PD<sub>20</sub>. In all subjects, antihistamines had been withdrawn for at least one week before the bronchial challenge. Asthmatic subjects stopped short or long-acting adrenergic bronchodilator for at least 12 h before the bronchial challenge.

### Magnesium concentration

Venous blood samples were obtained without tourniquet from all the subjects in sitting position before and after bronchial challenge. In the control group, a time difference of 15 min was used. The aspirated blood was transferred to a metal free test tube containing sodium heparin and a complete blood count (CBC) was measured immediately. Plasma was separated from the erythrocytes within 30 min (650 × g for 10 min). Of the isolated erythrocytes 100 µL was aspirated and was lysed with 1.5 mL double distilled water. In the first 20 samples Mg in erythrocytes was determined both after washing (3 times with caesium chloride) (CsCl) 145 mmol, pH: 7.40; osmolarity: 290–293 mOsm/K H<sub>2</sub>O) and without washing. No differences were detected (paired t-test>0.5) between the two methods. Thus, the remaining samples were analysed without washing. Samples of plasma with haemolysis or lipaemia were excluded from the study. The Mg concentration in plasma and in red blood cells was determined by a colourimetric assay using calmagite, a metallochromic dye [16]. In plasma the measurements were carried out without deproteinization and in red blood cells after deproteinization using

sodium tungstate (Na<sub>2</sub>WO<sub>4</sub>) at an acid pH (the lysed erythrocytes were reacted with 200 µL 0.3M Na<sub>2</sub>WO<sub>4</sub> and 200 µL 0.35 M hydrogen sulphate (H<sub>2</sub>SO<sub>4</sub>)). Briefly 1 mL of working solution (0.08 g·L<sup>-1</sup> calmagite, 0.04 g·L<sup>-1</sup> ethylene-glycol-bis-(β-aminoethylether)-N',N',N',N'-tetraacetic acid (EGTA), 0.28Mm KCN, 0.34M potassium chloride (KCl), 0.98 g·L<sup>-1</sup> Bion Ne-9 and 0.091 g·L<sup>-1</sup> Bion PVP) was reacted with 20 µL of the plasma or 200 µL of the supernatant from the erythrocytes pellet deproteinization for 2 min at room temperature. The reaction product, a pink magnesium-calmagite complex, was quantitated at 532 nm using a double beam spectrophotometer (Unicon 940, Kontron Instruments, Tegimenta AG, Zurich, Switzerland). The investigator (GP) who performed the magnesium measurements was not aware of the clinical and functional status of the subjects.

### Lung function

FEV<sub>1</sub> was measured using a dry bellows spirometer (Vitalograph, 44000 series, Buckingham, UK). The best value of three measurements was expressed as a percentage of the predicted value.

### Statistics

The study population was divided into four groups: (a) asthmatics steroid naive; (b) asthmatics on inhaled steroids; (c) healthy subjects; and finally (d) control group. The Mg levels in plasma and erythrocytes were equally compared not only in the whole study population, but also in each subgroup separately before and after bronchial challenge with histamine. In the control group the comparison was done between two measurements performed with a 15 min time difference. When the whole study population PD<sub>20</sub> measurements were analysed they were not normally distributed, while Mg measurements showed a near normal distribution. Within each subgroup both Mg and PD<sub>20</sub> measurements were normally distributed. Differences of Mg measurements within each subgroup were tested by a paired t-test, while differences between subgroups were tested with one way analysis of variance (ANOVA) with appropriated *post hoc* test (Bonferroni) for multiple group comparison. The relationship between PD<sub>20</sub> and both magnesium concentrations (plasma and erythrocytes) were tested in the whole study population by Spearman's Rank test and in subgroups by the Pearson's test. Results are expressed as mean±SEM. A p-value of <0.05 was considered significant. The study was approved by the Hospital Ethics Committee.

## Results

The general characteristics of all the subjects and of each subgroup separately, as well as baseline Mg levels in plasma and erythrocytes and PD<sub>20</sub> are summarized in table 1. The initial Mg concentration in plasma and in erythrocytes did not differ significantly between normal subjects, asthmatics on inhaled steroids and those who were steroid-naive (p=0.9, p=0.8 respectively). There was no significant difference between Mg concentration in plasma

Table 1. – Subjects general characteristics

	Age yrs	FEV1 % pred	Mg plasma mgr·dL <sup>-1</sup>	Mg erythrocytes fmmol·cell <sup>-1</sup>	PD20 mg
All subjects n=62	22±0.4	93.0±1.0	2.06±0.02	1.84±0.02	0.56±0.05
Asthmatics steroid naive n=32	21±0.4	90.5±1.5	2.06±0.03	1.84±0.03	0.23±0.02
Asthmatics on inhaled steroids n=10	22±0.1	94.0±3.5	2.04±0.04	1.86±0.06	0.49±0.2
Normal subjects n=20	23±0.8	97.5±2.0	2.10±0.03	1.84±0.03	1.20±0.30
Control group n=20	22±0.8	91.0±2.0	2.07±0.02	1.84±0.03	

Data are presented as mean±SD. FEV1: forced expiratory volume in one second; Mg: Magnesium; PD20: provocative dose causing a 20% fall.

before and after bronchial challenge with histamine (from 2.08±0.02 mg·dL<sup>-1</sup>, to 2.06±0.02 mg·dL<sup>-1</sup>, p=0.14). In contrast, Mg concentration in erythrocytes decreased significantly compared to the baseline values (from 1.84±0.02 fmmol·cell<sup>-1</sup> to 1.78±0.02 fmmol·cell<sup>-1</sup> p=0.0001) (fig. 1a). Similar results were observed when the 4 subgroups were studied separately (asthmatics: 1.84±0.03 fmmol·cell<sup>-1</sup>–1.78±0.03 fmmol·cell<sup>-1</sup>, p<0.0001; asthmatics steroid naive: 1.86±0.06 fmmol·cell<sup>-1</sup>–1.81±0.06 fmmol·cell<sup>-1</sup>, p<0.001, asthmatics on inhaled steroids: 1.84±0.03 fmmol·cell<sup>-1</sup>–1.77±0.03 fmmol·cell<sup>-1</sup>, p<0.0001, in normal subjects) (fig. 1b), except the control group in which no difference was observed for both Mg concentrations (2.07±0.02 mg·dL<sup>-1</sup>–2.09±0.02 mg·dL<sup>-1</sup>, p=0.18 in plasma, 1.84±0.03 fmmol·cell<sup>-1</sup>–1.82±0.03 fmmol·cell<sup>-1</sup> p=0.16 in erythrocytes) (fig. 1b).

There was no correlation between PD20 and the difference of both magnesium concentrations (baseline PD20 timepoint) or the initial values of magnesium levels in erythrocytes and plasma (r=0.08 p=0.5, r=0.16, p=0.7 respectively).

## Discussion

The authors have shown that bronchial challenge with histamine did not change Mg concentration in plasma, but caused a statistically significant decrease in intracellular Mg concentration. This fall was attributed to the histamine-induced bronchoconstriction, since Mg concentrations did not change significantly over a period of 15 min in the control group.

The usual method to determine Mg homeostasis is to measure Mg levels in the plasma. However, studies have shown the superiority of measuring the intracellular content of Mg in determining the Mg status of patients [1]. The preferred method for the determination of Mg in biological specimens is atomic absorption spectrophotometry. However, the instrumentation required is not generally available in clinical laboratories. Additionally, the calmagite colourimetric method (which was used in this study) is fairly accurate and precise and widely used in clinical laboratories [17], with a rather small negative proportional bias [18] and a good correlation (r=0.986) [19] compared to atomic absorption spectrophotometry.

Mg has long been thought to be a vital ion for maintaining the homeostasis of the bronchial smooth muscle. It is not known whether magnesium works *via* pharmacological effects or repletion of body stores. Regarding pharmacological effects, several mechanisms have been proposed to explain the potential beneficial effect of magnesium in asthma. It has been shown to relax airway smooth

muscle *in vitro* [2], probably by the modulation of calcium ion movement within the cell [20] and through transmembrane calcium channels [21, 22], as well as to broncho-dilate asthmatic airways *in vivo* [4, 5]. It has been demonstrated that histamine and methacholine PD20 threshold dose is moved to the right when the patients were pretreated with aerosolized magnesium sulfate, with this effect considered to be similar than that observed with calcium channel blockers [6, 7]. All of the above data supports the hypothesis that Mg may exert its effect on the final common path of bronchoconstriction by interfering with calcium handling on bronchial smooth muscle cells.

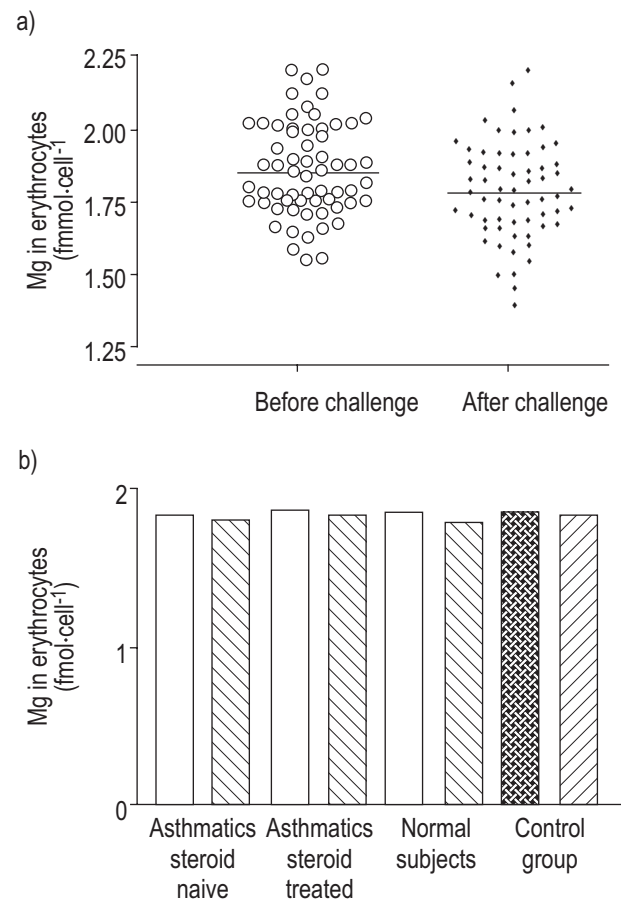


Fig. 1. – a) Magnesium concentration in erythrocytes before and after histamine challenge. p<0.0001. b) Magnesium concentration in erythrocytes before and after histamine challenge in the four study populations. □ : before challenge; ▨ : after challenge; ▩ : baseline; ▧ : 15 min later; asthmatics steroid naive: p<0.0001; asthmatics steroid treated: p<0.0001; normal subjects: p<0.0001.

The authors observed a significant decrease in the Mg concentration in erythrocytes after bronchial challenge with histamine in both asthmatics and healthy subjects, while at the same time plasma concentration remained stable. It is thus believed that the Mg ion moved from the intracellular space and was used as a natural calcium-channel blocker in order to relax airway smooth muscle and reverse bronchoconstriction. This hypothesis is partially supported by the protective role of magnesium in histamine induced bronchoconstriction [6], and by experimental data showing that Mg relaxes arterial smooth muscle, prestimulated with histamine by decreasing intracellular calcium [23].

The authors assume that when bronchoconstriction occurs during the histamine challenge test, free radicals such as hydrogen peroxide can be released through direct action of histamine in enzymatic reactions [24] and inflammatory cells [25] or indirect action *via* activation of C-fibres and tachycinins [26]. The hydrogen peroxide can act as an indirect trigger to the erythrocytes (destroying the Na<sup>+</sup>/Mg<sup>2+</sup> ATPase antiport) to poor out magnesium [27]. Inflammatory chemokines as interleukins might take part in this process but they cannot explain the fall of Mg in the erythrocytes of normal subjects. This significant fall of Mg concentration in erythrocytes seems to be irrespective of bronchial hyperresponsiveness severity as is shown by the lack of correlation between PD<sub>20</sub> to histamine and the percentage (%) fall of Mg concentration in erythrocytes. This confirms the main hypothesis that Mg ions move out from the intracellular space when bronchoconstriction occurs, independently of the dose of histamine (degree of bronchial hyperreactivity) required for this effect. The results of the control group also confirm that the observed fall of erythrocytes Mg after histamine challenge is due to challenge itself and not because of the biological and analytical variation of magnesium concentration in erythrocytes. This is in agreement with previous observations which showed good reproducibility of erythrocytes Mg measurements within the day (coefficient of variation (CV)=4%) [28].

In this study it was been shown that the concentrations of Mg in plasma and erythrocytes of asthmatic patients are within normal rates and did not differ significantly from those found in healthy subjects. This is in agreement with previous reports [12, 29, 30], but in contrast to two recent reports [31, 32] which demonstrated low magnesium concentrations in the erythrocytes of asthmatic patients. These controversial results can be attributed to the differences in magnesium intake between different countries. The "Greek diet" is rich in products with a high magnesium content (unprocessed cereals, vegetables and legumes) [1] leading to a rather small possibility of hypomagnesemia in the majority of the population. Another possible explanation for the above difference is that their studies included subjects with dissimilar age ranges compared to this study (only young males) which probably makes them incomparable, since intracellular magnesium varies substantially with age [33]. Another finding which was not confirmed by this study was the correlation between bronchial hyperreactivity and the initial values of Mg concentration in erythrocytes. Since the authors did not observe any difference in intracellular Mg concentration between asthmatic and healthy subjects it is reason-

able not to expect any correlation between bronchial hyperreactivity and Mg concentration in erythrocytes.

To conclude, this study shows that bronchial challenge with histamine caused a statistically significant fall in intracellular (erythrocytes) Mg with no effect in plasma concentration of this ion. This fall did not correlate with the degree of bronchial hyperreactivity to histamine. Further studies are needed to determine the role of intra- and extracellular concentrations of Mg in exacerbated asthma.

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