No positive effect of oral magnesium supplementation in the decreases of inflammation in subjects with prediabetes: A pilot study

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Abstract. To determine whether oral magnesium supplementation modifies serum levels of high-sensitivity C-reactive protein (hsCRP), TNF-alpha, IL-6, and IL-10 in subjects with prediabetes, inflammation, and hypomagnesemia, a total of 26 subjects men and non-pregnant women were included and randomly allocated to receive 30 ml of MgCl2 5% solution (equivalent to 382 mg of magnesium) or placebo, daily during three months. At baseline conditions, there were not significant statistical differences between the groups. At end of the study, hsCRP levels were significantly lower in the intervention group (3.3 ± 2.5 vs 8.0 ± 5.9 mg/L, p = 0.03), as compared with the control group. However, the intra-group analysis of the individuals who received magnesium, did not shows significant statistical differences between baseline and final conditions (4.1 ± 3.0 and 3.3 ± 2.5, p = 0.45). In addition, TNF-alpha (1.2 ± 0.3 vs 1.1 ± 0.3 pg/mL, p = 0.69), IL-6 (0.3 ± 0.3 vs 5.0 ± 7.7 pg/mL, p = 0.08), and IL-10 (1.8 ± 0.4 vs 1.8 ± 0.5 pg/mL, p = 0.89) serum levels were not significantly different between the groups. Our results do not show a beneficial effect of oral magnesium supplementation on hsCRP, IL-6, TNF-alpha, and IL-10 levels in prediabetic subjects with hypomagnesemia and inflammation. Further studies with large sample sizes and longer time of follow-up are necessaries to verify the results of our pilot study.

Key words: Magnesium, inflammation, prediabetes

Magnesium is an essential cofactor of many physiological and biochemical processes including high-energy phosphate and enzymatic pathways implicated in the energetic metabolism, synthesis of protein, and several enzymes involved in glucose and insulin metabolism [1-4]. Magnesium deficiency may reduce tissue glucose uptake by interfering with insulin signaling pathways and promoting insulin resistance at peripheral insulin-sensitive tissues. Therefore, magnesium deficiency might plays an important pathophysiological role in the development of insulin resistance, diabetes, and hypertension [3, 5-7]. On this regard, it has been shown that induction of magnesium deficiency reduces insulin sensitivity in individuals without diabetes, and that magnesium supplementation improves metabolism of glucose in non-diabetic elderly individuals [8, 9]. Experimental models consistently show that animals with magnesium deficiency exhibit elevated circulating cytokine levels suggesting a generalized inflammatory state [10, 11]. An increasing body of evidence shows that magnesium...
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deficiency is involved in the synthesis and release of pro-inflammatory cytokines and acute phase reactants, in the impairment of peripheral insulin action, and in the development of glucose metabolism disturbances [3, 7, 12-16].

Furthermore, it has been shown that IL-10 exerts inhibitory effects on the synthesis of proinflammatory cytokines such as tumour necrosis factor (TNF)-alpha and interleukin-6 (IL-6) [17]. Thus, synthesis of IL-10 constitutes a feedback mechanism of the inflammatory response, in the sense that neutralization of IL-10 exacerbates inflammatory process [18].

It has been reported the improvement in insulin sensitivity and glucose control among prediabetic and diabetic subjects receiving magnesium supplementation [11, 19]. Experimental studies [20-22] show that low magnesium status is linked to the increase of glycemia and the etiology of chronic diabetic complications. These findings support the important role that magnesium plays on the insulin-mediated glucose uptake. On this regard, the objective of this study was to determine whether oral magnesium supplementation modifies serum levels of high-sensitivity C-reactive protein (hsCRP), TNF-alpha, IL-6, and interleukin-10 (IL-10) in subjects with prediabetes and hypomagnesemia.

Material and Methods

With the protocol approval by the Mexican Social Security Institute Research Committee and after obtaining the written informed consent, a clinical randomized double-blind placebo-controlled trial was carried out.

The sampling strategy was based on advertising strategies to general population of Durango, city in northern Mexico, to invite apparently healthy subjects, men and non-pregnant women aged 20 to 65 years, to participate in the study. Before their inclusion at study, all subjects were clinically evaluated and a standardized oral glucose tolerance test was performed. Individuals with newly diagnosis of prediabetes, inflammation, and hypomagnesemia were enrolled and randomly allocated to receive either magnesium supplementation or placebo daily for three months. In fasting conditions, subjects included in the intervention group received 30 ml of MgCl₂ 5% solution (equivalent to 382 mg of magnesium) daily. Subjects in the control group received 30 ml of placebo solution daily. In addition, subjects in both groups were advised to consume a diet comprising 45% carbohydrates, 28% lipids, and 27% proteins. Also, all the subjects were advised to perform physical activity at least 30 minutes three times per week.

A standardized interview, clinical examination, and laboratory tests were performed to determine the presence of smoking, alcohol intake, acute or chronic inflammatory disease, acute or chronic infection, glomerulopathies, renal disease, malignancy, diabetes, hypertension, and cardiovascular disease, as well as the intake of statins, anti-inflammatory drugs, or magnesium supplementation, which were exclusion criteria.

Definitions

Prediabetes was defined by the presence of impaired fasting glucose (IFG) (fasting plasma glucose levels ≥5.5 mmol/L < 6.9 mmol/L), or IGT (plasma glucose levels 2-h post-load ≥ 7.7 mmol/L < 11 mmol/L [23]. Subjects with IFG+IGT were included into the IGT group.

Sample size was estimated based on a statistical power of 80%, alpha value 0.05, and an expected difference in hs-CRP levels, between the subjects who received magnesium and subjects in the control group, of 0.60 (δ value). The required sample size to detect a treatment effect was of 10 subjects per group [24].

Low-grade chronic inflammation was defined by hsCRP levels > 1.0 mg/L [25].

Hypomagnesemia was defined by serum magnesium concentration ≤ 0.74 mmol/L (1.8 mg/dL).

Measurements

In the standing position and fasting conditions, waist circumference (WC), weight, and height were measured with the subjects in light clothing and without shoes. Weight and height were measured using a fixed scale with stadimeter (Tanita TBF-215, Tokyo, Japan); the BMI was calculated as weight (kilograms) divided by height (meters) squared. The WC was measured to the nearest centimeter with a flexible steel tape; the anatomical landmarks used were midway between the lowest portion of the rib cage and the superior border of the iliac crest [26].

The technique for measurement of blood pressure was the recommended in the Seventh Report
Assays

Whole blood sample was collected from antecubital vein 8-10 h after overnight fasting. Serum glucose was measured using the glucose-oxidase method; the intra- and inter-assay coefficients of variation (CV) were 1.1 and 1.5%. Serum magnesium concentration was measured by colorimetric method; the intra- and inter-assay CV were 1.0% and 2.5%, respectively. The hsCRP was measured by ultra-sensitive competitive immunoassay (Dade Behring, Darmstadt, Germany) with inter- and intra-assay CV of 8.9% and 5.1%. Serum levels of TNF-alpha, IL-6, and IL-10 were measured by Quantikine high-sensitive ELISA Human TNF-alpha Immunoassay, Quantikine high-sensitive ELISA Human IL-6 Immunoassay, and Quantikine high-sensitive ELISA Human IL-10 Immunoassay according recommendations of supplier (R&D Systems Inc. Minneapolis MN). It was used the microplate iMark reader, with Microplate Manager software (Bio-Rad Laboratories Inc. Mexico DF). The intra- and inter-assays CV were 1.5% and 3.1%, 2.1% and 3.9%, and 3.1%, and 4.5%, respectively. All laboratory measurements were performed at baseline and end of study using an Express 500 clinical chemistry autoanalyser (Ciba Corning, Diagnostic Corp., Overling, Ohio).

Statistical analysis

Differences between the groups were assessed using Mann-Whitney U test (for quantitative variables), and Fisher Exact Test (for qualitative variables).

A 95% confidence interval (CI95%) and p value <0.05 defined statistical significance. All data were processed and analyzed using the statistical package SPSS for windows, version 15.0 (SPSS, Chicago, IL).

Results

Screening was performed in 253 subjects, 192 (75.9%) women and 61 (24.1%) men; 227 (89.7%) individuals were excluded of the study because they did not fulfill the inclusion criteria or by the presence of exclusion criteria. A total of 153 (60.4%) individuals exhibited normomagnesemia, 28 (11.0%) hypomagnesemic subjects had not prediabetes, 25 (9.8%) with diagnoses of hypertension, 18 (7.1%) individuals with new diagnosis of type 2 diabetes, and three (1.2%) refused to participate. Thus, a total of 26 subjects with hypomagnesemia, inflammation and prediabetes were enrolled and randomly allocated; each group was integrated by 13 subjects.

Two subjects in each group dropped-out; in the intervention group, one of them by lack of adherence to treatment and one by adverse effects (mild diarrhea); in the control group, both dropped-out by lack of adherence to treatment. Finally, 22 subjects successfully completed the follow-up period and were included for analysis.

Clinical and biochemical characteristics of participants are summarized in Table 1. In basal conditions, anthropometric and biochemical variables were similarly distributed in both groups.

Serum magnesium levels significantly increased in the intervention group and remained unchanged in the control group. At the end of follow-up, there were not significant statistical differences between the groups for anthropometric variables in study. Subjects in the intervention group showed higher serum magnesium levels and lower hsCRP levels as compared with the control group. In addition TNF-alpha (1.2 ± 0.3 vs 1.1 ± 0.3 pg/mL, p = 0.69), IL-6 (0.3 ± 0.3 vs 5.0 ± 7.7 pg/mL, p = 0.08), and IL-10 (1.8 ± 0.4 vs 1.8 ± 0.5 pg/mL, p = 0.89) serum levels were not significantly different between the groups, Table 1.

Figure 1A shows hsCRP levels at baseline and final conditions; at final of study there were a significant decrease in the intervention group as compared with the control group. However, the intra-group analysis of the individuals who received magnesium, did not shows significant statistical differences between baseline and final conditions (4.1 ± 3.0 and 3.3 ± 2.5, p = 0.45). The figure 1B shows TNF-alpha levels, which remained without significant changes through follow-up in both groups. The IL-6 levels increased in the control group and slightly decreased in the intervention, group without significant statistical differences, figure 1C. Finally, regarding IL-10 levels, baseline and final conditions were similar in both groups, figure 1D.
### Table 1. Clinical and biochemical characteristics of the target population at baseline and final conditions

<table>
<thead>
<tr>
<th></th>
<th>Screening</th>
<th>Baseline</th>
<th>MgCl₂</th>
<th>Control</th>
<th>Final</th>
<th>MgCl₂</th>
<th>Control</th>
<th>Final</th>
<th>p value*</th>
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<tr>
<td><strong>n</strong></td>
<td>253</td>
<td>11</td>
<td>11</td>
<td>253</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td>11</td>
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<tr>
<td><strong>Age, years</strong></td>
<td>42.9 ± 11.1</td>
<td>44.2 ± 10.8</td>
<td>43.2 ± 7.8</td>
<td>0.78</td>
<td>–</td>
<td>–</td>
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<tr>
<td><strong>Women, n (%)</strong></td>
<td>192 (75.8%)</td>
<td>7 (63.6%)</td>
<td>7 (63.6%)</td>
<td>1.00</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td><strong>Body mass index, kg/m²</strong></td>
<td>31.8 ± 8.2</td>
<td>30.5 ± 4.4</td>
<td>35.1 ± 7.0</td>
<td>0.18</td>
<td>29.8 ± 3.8</td>
<td>34.4 ± 6.7</td>
<td>0.06</td>
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<td><strong>Waist circumference, cm</strong></td>
<td>99.1 ± 14.7</td>
<td>97.2 ± 11.1</td>
<td>101.7 ± 9.4</td>
<td>0.46</td>
<td>94.8 ± 9.8</td>
<td>103.3 ± 9.4</td>
<td>0.15</td>
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<td><strong>Total body fat, %</strong></td>
<td>38.7 ± 8.6</td>
<td>36.6 ± 9.4</td>
<td>40.0 ± 7.7</td>
<td>0.44</td>
<td>35.5 ± 9.8</td>
<td>38.2 ± 8.9</td>
<td>0.54</td>
<td></td>
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<tr>
<td><strong>Systolic blood pressure, mmHg</strong></td>
<td>120.3 ± 16.5</td>
<td>116.9 ± 7.6</td>
<td>118.4 ± 8.2</td>
<td>0.84</td>
<td>115.5 ± 18.0</td>
<td>114.0 ± 9.7</td>
<td>0.84</td>
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<tr>
<td><strong>Diastolic blood pressure, mmHg</strong></td>
<td>70.7 ± 10.2</td>
<td>66.7 ± 6.9</td>
<td>71.8 ± 7.5</td>
<td>0.24</td>
<td>65.6 ± 10.4</td>
<td>66.6 ± 8.7</td>
<td>0.72</td>
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<td><strong>Fasting glucose, mmol/L</strong></td>
<td>5.8 ± 2.1</td>
<td>5.7 ± 1.2</td>
<td>6.3 ± 1.3</td>
<td>0.21</td>
<td>6.2 ± 2.2</td>
<td>5.2 ± 1.1</td>
<td>0.25</td>
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<td><strong>Post-load glucose, mmol/L</strong></td>
<td>7.5 ± 3.8</td>
<td>8.5 ± 1.4</td>
<td>7.6 ± 1.5</td>
<td>0.18</td>
<td>8.0 ± 3.9</td>
<td>7.4 ± 2.3</td>
<td>0.66</td>
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<tr>
<td><strong>Magnesium, mmol/L</strong></td>
<td>0.82 ± 0.12</td>
<td>0.74 ± 0.04</td>
<td>0.74 ± 0.04</td>
<td>0.78</td>
<td>0.86 ± 0.12</td>
<td>0.74 ± 0.08</td>
<td>0.03</td>
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<tr>
<td><strong>hsCRP, mg/L</strong></td>
<td>2.5 ± 6.6</td>
<td>4.1 ± 3.0</td>
<td>6.6 ± 4.9</td>
<td>0.08</td>
<td>3.3 ± 2.5</td>
<td>8.0 ± 5.9</td>
<td>0.03</td>
<td></td>
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<tr>
<td><strong>TNF-alpha, pg/mL</strong></td>
<td>–</td>
<td>1.1 ± 0.4</td>
<td>1.2 ± 0.2</td>
<td>0.46</td>
<td>1.2 ± 0.3</td>
<td>1.1 ± 0.3</td>
<td>0.69</td>
<td></td>
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<tr>
<td><strong>IL-6, pg/mL</strong></td>
<td>–</td>
<td>0.6 ± 1.1</td>
<td>1.8 ± 5.3</td>
<td>1.00</td>
<td>0.3 ± 0.3</td>
<td>5.0 ± 7.7</td>
<td>0.08</td>
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<tr>
<td><strong>IL-10, pg/mL</strong></td>
<td>–</td>
<td>1.8 ± 0.5</td>
<td>1.9 ± 0.6</td>
<td>0.71</td>
<td>1.8 ± 0.4</td>
<td>1.8 ± 0.5</td>
<td>0.89</td>
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Values are mean ± Standard Deviation  
* p value between MgCl₂ and Control group

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**Figure 1.** Serum levels of hsCRP and cytokines in subjects with prediabetes, inflammation, and hypomagnesemia, who received oral magnesium supplementation (black squares) and placebo (white circles), during three months.  
**A)** hsCRP; **B)** TNF-alpha; **C)** IL-6; **D)** IL-10.
There were not significant statistical differences in the response to MgCl$_2$ between the subjects with serum magnesium of 0.74 mmol/L as compared with those who had serum magnesium <0.74 mmol/L, p = 0.09).

Discussion

Our results do not show a beneficial effect of oral magnesium supplementation on hsCRP, IL-6, TNF-alpha, and IL-10 levels in prediabetic subjects with hypomagnesemia and inflammation.

At end of follow-up, subjects in the intervention group increase serum magnesium and decrease hsCRP levels as compared with the control group; similar finding to those reported in critically ill patients with type 2 diabetes, admitted in the Intensive Care Unit, and among patients with heart failure, in which oral magnesium supplementation significantly decreased CRP levels [29, 30].

Although the sequence of early events that produces the acute-phase response is not entirely known, it has been proposed that magnesium deficiency may be the triggering of the exacerbated inflammatory response [31]. In addition, a consistent body of evidence shows that the mechanism responsible for the early trigger of the acute-phase response in the magnesium deficiency status, involve the release of neuropeptides, specifically of substance P.

Epidemiological studies [14, 15, 32] consistently show that low serum magnesium levels are strongly related with elevated TNF-alpha and CRP concentrations. In this study, we did not observe significant changes in TNF-alpha and IL-10 levels, which could be related with co-expression of these cytokines that occurs in several diseases and conditions. Regarding effects of magnesium intake on IL-6 and TNF-alpha serum levels are inconsistent, with some studies showing an inverse association [33] but other showing not association [34].

These finding may reflect the intrinsic biological properties of CRP as main downstream mediator of the acute phase response [35]. On this regard, our results support the hypothesis of a possible independent pathway induced by hypomagnesemia in the triggering of systemic inflammation, which may explain, at least in part, the findings of our study.

The main limitation this study was the small sample size; it is possible that the lack of effect of magnesium on cytokines is related with the lack of statistical power.

The strengths of this study include its design, which represents the most solid to determine causality; furthermore, we controlled the potential confounders related to the inflammatory process.

In conclusion, our results do not show a beneficial effect of oral magnesium supplementation on hsCRP, IL-6, TNF-alpha, and IL-10 levels in prediabetic subjects with hypomagnesemia and inflammation. Nonetheless, further studies with large sample sizes and longer time of follow-up are necessaries in the field to verify the results of our pilot study.

Disclosure

Financial support: This research was conducted with the financial support from the Mexican Social Security Institute Foundation, Civil Association. Conflict of interest: none.

References

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