Severe hypomagnesemia and low-grade inflammation in metabolic syndrome

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Abstract. To evaluate the association between severe hypomagnesemia and the low-grade inflammatory response in subjects with metabolic syndrome (MetS), ninety-eight individuals with new diagnosis of MetS were enrolled in a cross-sectional study. Pregnancy, smoking, alcohol intake, renal damage, hepatic disorders, infectious or chronic inflammatory diseases, malignancy, use of diuretics, statins, calcium antagonist, antioxidants, vitamins, anti-inflammatory drugs, or previous oral magnesium supplementation were exclusion criteria. According serum magnesium levels, participants were assigned to the following groups: 1) severe hypomagnesemia ($\leq$ 1.2 mg/dL); 2) hypomagnesemia ($>1.2 \leq 1.8$ mg/dL); 3) Normal serum magnesium levels ($>1.8$ mg/dL). The low-grade inflammatory response was defined by elevation of serum levels of (hsCRP $>1.0 \leq 10.0$ mg/L) or TNF-alpha (TNF-$\alpha >3.5$ pg/mL).

Severe hypomagnesemia, hypomagnesemia, and normomagnesemia were identified in 21 (21.4%), 38 (38.8%), and 39 (39.8%) individuals. The ORs, adjusted by WC, showed that severe hypomagnesemia (OR: 8.1; CI 95%: 3.6-19.4 and OR: 3.7; CI 95%: 1.1-12.1), but not hypomagnesemia (OR: 1.8; CI 95%: 0.9-15.5 and OR: 1.6; CI 95%: 0.7-3.6), was strongly associated with elevated hsCRP and TNF-$\alpha$ levels, and that normomagnesemia exhibited a protective role (OR: 0.32; CI 95%: 0.1-0.7 and OR: 0.28; CI 95%: 0.1-0.6) for elevation of CRP and TNF-$\alpha$.

Results of this study show that, in subjects with MetS, severe hypomagnesemia, but not hypomagnesemia, is associated with elevated concentrations of CRP and TNF-$\alpha$.

Key words: hypomagnesemia, inflammatory response, metabolic syndrome, CRP, TNF-$\alpha$

Genetic and environmental factors contribute to the development of the Metabolic Syndrome (MetS), a cluster of cardiovascular risk factors interrelated for cardiovascular disease and type 2 diabetes [1]. Among the environmental factors, it has been reported that obesity is associated with MetS [2-4]. Results from several clinical studies show that the increase in the synthesis and release of proinflammatory cytokines and the triggering of low grade chronic inflammation seem to be the link between obesity and MetS [5-7] and one of the pathophysiological mechanisms underlying the development of MetS [8, 9].

Furthermore, it has been proposed that hypomagnesemia is related to the triggering of low-grade chronic inflammation [7, 10-12] and that magnesium deficiency is associated with MetS [13-15]. These findings support the hypothesis that hypomagnesemia plays a key role in the pathophysiology of MetS, and that the triggering of inflammatory responses, induced by magnesium deficiency, could be the link between hypomagnesemia and MetS [10, 14, 16-18]. In this regard, an inverse association between serum magnesium levels and C-reactive protein (CRP), the most sensitive indicator of systemic
inflammation [19], has been consistently reported in clinical and epidemiological studies [20-24].

Given the hypothesis that MetS is an inflammatory condition and magnesium deficiency plays an important role in its development, in this study we evaluated the association between severe hypomagnesemia and the low-grade inflammatory response in subjects with MetS.

Material and methods

With the approval of the protocol by the Mexican Social Security Institute Research Committee and after obtaining the subjects’ informed consent, a cross-sectional study was carried out.

Outpatients who receive medical care in the Biomedical Research Unit at the Mexican Social Security Institute at Durango, a city in northern Mexico, were invited to participate in the study.

Individuals aged 40 to 75 years, with a new diagnosis of MetS, were candidates for inclusion in the study. Pregnancy, smoking, alcohol intake, renal damage, hepatic disorders, infectious or chronic inflammatory diseases, malignancy, use of diuretics, statins, calcium antagonist, antioxidants, vitamins, anti-inflammatory drugs, or previous oral magnesium supplementation were exclusion criteria.

According to their serum magnesium levels, participants were assigned to the following groups: 1) severe hypomagnesemia; 2) hypomagnesemia; 3) normal serum magnesium levels.

Criteria diagnosis

According the NCEP-Adult Treatment Panel III (ATPIII) definition [25] diagnosis of MetS was ascertained by the co-occurrence of at least three of the following risk factors: abdominal obesity [waist circumference (WC) >102 cm in men and >88 cm in women], high blood pressure (≥130/85 mmHg), hyperglycemia (fasting plasma glucose ≥100 mg/mL), hypertriglyceridemia (triglycerides ≥150 mg/dL), and low HDL-cholesterol (<40 mg/dL in men and <50 mg/dL in women). The diagnosis of MetS was made within the three 3 months before inclusion in the study.

The low-grade inflammatory response was defined by elevation of serum levels of hsCRP(≥1.0 ≤10.0 mg/L) [26] or TNF-alpha (TNF-α ≥3.5 pg/mL) [27].

Severe hypomagnesemia, hypomagnesemia and normal serum magnesium levels were diagnosed by serum magnesium concentrations <1.2 mg/dL, ≥1.2 ≤1.8 mg/dL, and >1.8 mg/dL, respectively [20, 28].

The HOMA-IR index was estimated using the formula Fasting insulin (U/mL) x Fasting glucose (mmol/L) / 22.5 [29].

Measurements

The WC was measured using standard protocols with the subjects in standing position, fasting conditions, light clothing, and without shoes; the WC was measured to the nearest centimeter with a flexible steel tape. The anatomical landmarks used were the midway between lowest portion of rib cage and superior border of iliac crest.

Brachial artery blood pressure was measured in seated participants after they had rested for five minutes using a baumanometer (Micro-life AG, Heerbrugg Switzerland) and stethoscope (3M Littman Classic II, Neuss, Germany). The technique of BP measurement and stages of hypertension were defined by criteria of the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure [30].

Assays

Venous whole blood samples were drawn after 8-10 hours overnight fasting. Plasma was separated from blood cells by centrifugation and stored in fraction of 0.5 mL at -70°C until analysis. Serum glucose was measured by the glucose-oxidase method; its intra- and inter-assay coefficients of variations (cv) were 2.1% and 3.4%. Triglycerides were measured enzymatically. HDL-cholesterol fraction was obtained after precipitation by phosphotungstic reagent. The intra- and inter-assay cv were 1.9% and 3.7% for triglycerides, and 1.5% and 3.1% for HDL-cholesterol.

Serum magnesium concentrations were measured by colorimetric methods, the intra- and inter-assay cv were 1.0% and 2.5%. The hsCRP was measured by an ultra-sensitive competitive immunoassay (Dade Behring, Darmstadt, Germany) with an inter-assay cv of 8.9%. TNF-α
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was measured by chemiluminescent immunometric assay (immulite TNF, EURO/DPC, USA), with an intra- and inter-assay cv of 1.8% and 3.5%.

Sample size estimation was based on a statistical power of 80% with a 0.05 alpha and an expected prevalence of severe hypomagnesemia of 5%. Based on a proportion 2:1 between the groups with severe hypomagnesemia and those with hypo- and normo-magnesemia, the sample size was of 20 subjects in the group with severe hypomagnesemia and 40 in the control groups.

Statistical analysis

Differences between the groups were estimated using unpaired Student t test (or Mann-Whitney U test for skewed data) for numerical variables, and chi-squared test for categorical variables.

Differences between multiple groups were established using the one-way ANOVA test with post-hoc analysis of Bonferroni. All the skewed numerical data were Logn transformed to normalize their distribution.

Multivariate logistic regression analysis to compute the odds ratios (OR) between serum magnesium (independent variable) and hsCRP and TNF-α levels (dependent variables) was performed.

A confidence interval of 95% (CI95%) was considered, and a p value <0.05 defined the level of statistical significance. Data were analyzed using the statistical package SPSS 15.0 (SPSS Inc., Illinois USA 1998).

Results

A total of 98 subjects, 51 (52%) women and 47 (48%) men with average age of 43.6±15.3 years were enrolled.

Severe hypomagnesemia, hypomagnesemia, and normomagnesemia were identified in 21 (21.4%), 38 (38.8%), and 39 (39.8%) individuals.

Table 1 shows the characteristics of subjects in the overall group and by sex. There were no significant differences in the variables studied between men and women.

Table 2 shows the anthropometric and clinical characteristics of subjects with MetS; subjects with severe hypomagnesemia and hypomagnesemia exhibited higher WC and lower HDL-cholesterol levels than the individuals with normomagnesemia; however, there were no significant differences for WC and HDL-cholesterol between the subjects with severe hypomagnesemia and hypomagnesemia. Furthermore, subjects with severe hypomagnesemia showed higher serum levels of hsCRP and TNF-α than the subjects with hypomagnesemia and normomagnesemia; interestingly, there were no significant differences of hsCRP and TNF-α between the hypomagnesemic and normomagnesemic subjects.

A total of 52 (53.1%) and 58 (59.2%) subjects showed elevated hsCRP and TNF-α levels. The proportion of subjects with elevation of hsCRP was significantly higher in the group with severe hypomagnesemia as compared with the group with hypomagnesemia (95.2% and 81.0%, p=0.0006) and normomagnesemia (95.2% and 35.9%, p<0.0005); between the groups with hypomagnesia and normomagnesia there were no statistical significant differences (p=0.42).

On the other hand, the proportion of subjects with elevated TNF-α levels showed no significant differences between the groups with severe hypomagnesemia and hypomagnesemia (81.0% and 65.8%, p=0.31) and was significantly higher in the subjects with severe hypomagnesemia as compared with the subjects with normomagnesemia (81.0% and 41.0%, p<0.007); between the groups with hypomagnesia and normomagnesia there were no statistical significant differences (p=0.0.51).

In the overall population there was a significant negative correlation between serum magnesium and hsCRP (r=-0.489, p=0.02) and TNF-α (r=-0.455, p<0.05). The correlation between serum magnesium levels and hsCRP was -0.602 (p<0.01) for the subjects with severe hypomagnesemia, -0.228 (p=0.01), for the subjects with hypomagnesemia, and 0.062 (p=0.71) for those with normomagnesemia. Regarding TNF-α levels, the correlation with serum magnesium was -0.332 (p<0.05) for the subjects with severe hypomagnesemia, -0.228 (p=0.01), for the subjects with hypomagnesemia, and 0.062 (p=0.71) for those with normomagnesemia. Regarding TNF-α levels, the correlation with serum magnesium was -0.332 (p<0.05) for the subjects with severe hypomagnesemia, -0.193 (p=0.57) for the subjects with hypomagnesemia, and 0.113 (p=0.49) for the subjects with normomagnesemia.

Figure 1 shows the distriubition of the components of MetS according serum magnesium status. The proportion of subjects with 4 components of the syndrome, although it does not exhibit a statistically significant difference, was higher in the group with severe hypomagnesemia as compared
### Table 1. Characteristics of the target population.

<table>
<thead>
<tr>
<th></th>
<th>All n=98</th>
<th>Men n=47</th>
<th>Women n=51</th>
<th>P-value #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>43.6±15.3</td>
<td>42.8±15.9</td>
<td>44.4±14.8</td>
<td>0.60</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>105.5±13.7</td>
<td>106.0±12.8</td>
<td>105.1±14.5</td>
<td>0.73</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>123.4±20.6</td>
<td>125.2±19.5</td>
<td>121.8±21.5</td>
<td>0.72</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>75.3±11.8</td>
<td>76.3±11.1</td>
<td>74.4±12.5</td>
<td>0.41</td>
</tr>
<tr>
<td>Fasting glucose (mg/dL)</td>
<td>114.3±25.1</td>
<td>112.2±28.0</td>
<td>116.3±22.2</td>
<td>0.43</td>
</tr>
<tr>
<td>HOMA-IR index</td>
<td>3.2±1.9</td>
<td>3.3±2.0</td>
<td>3.1±1.8</td>
<td>0.78</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dL)</td>
<td>39.6±12.2</td>
<td>36.4±10.8</td>
<td>42.7±12.8</td>
<td>0.01</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>214.9±139.3</td>
<td>184.8±120.9</td>
<td>242.7±150.1</td>
<td>0.057*</td>
</tr>
<tr>
<td>hsCRP (mg/L)</td>
<td>3.7±3.5</td>
<td>3.5±3.6</td>
<td>3.8±3.4</td>
<td>0.53*</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>5.6±3.9</td>
<td>6.0±5.1</td>
<td>5.2±2.4</td>
<td>0.94*</td>
</tr>
<tr>
<td>Serum magnesium (mg/dL)</td>
<td>1.8±0.5</td>
<td>1.8±0.5</td>
<td>1.7±0.5</td>
<td>0.36</td>
</tr>
</tbody>
</table>

Data are mean±SD; # p-value between men and women groups; * p-value estimated using Mann Whitney U test.

### Table 2. Characteristics of the target population (n=98) according the serum magnesium levels.

<table>
<thead>
<tr>
<th></th>
<th>Severe hypomagnesemia n=21</th>
<th>Hypomagnesemia n=38</th>
<th>Normomagnesemia n=39</th>
<th>F</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>44.0±14.4</td>
<td>43.9±13.6</td>
<td>43.1±17.4</td>
<td>0.04</td>
<td>0.96</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>109.8±16.7</td>
<td>108.8±13.1</td>
<td>100.0±10.6</td>
<td>5.8</td>
<td>0.004* #</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>127.8±20.8</td>
<td>121.6±19.1</td>
<td>122.8±21.9</td>
<td>0.6</td>
<td>0.52</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>75.7±12.9</td>
<td>77.7±11.5</td>
<td>72.7±11.3</td>
<td>1.7</td>
<td>0.18</td>
</tr>
<tr>
<td>Fasting glucose (mg/dL)</td>
<td>116.8±16.5</td>
<td>114.9±30.0</td>
<td>112.9±24.4</td>
<td>0.2</td>
<td>0.84</td>
</tr>
<tr>
<td>HOMA-IR index</td>
<td>3.5±1.9</td>
<td>3.2±1.8</td>
<td>2.8±1.9</td>
<td>1.2</td>
<td>0.29</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dL)</td>
<td>36.0±8.8</td>
<td>37.5±11.4</td>
<td>43.5±13.5</td>
<td>3.5</td>
<td>0.03</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>202.6±87.4</td>
<td>212.3±159.5</td>
<td>224.1±139.3</td>
<td>0.2</td>
<td>0.84</td>
</tr>
<tr>
<td>hsCRP (mg/L)</td>
<td>7.2±3.4</td>
<td>3.4±3.1</td>
<td>2.0±2.1</td>
<td>20.5</td>
<td>&lt;0.0005#</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>9.3±6.3</td>
<td>5.3±2.5</td>
<td>3.9±1.3</td>
<td>17.0</td>
<td>&lt;0.0005#</td>
</tr>
<tr>
<td>Serum magnesium (mg/dL)</td>
<td>1.09±0.10</td>
<td>1.64±0.14</td>
<td>2.25±0.30</td>
<td>202.4</td>
<td>&lt;0.0005#</td>
</tr>
</tbody>
</table>

Severe hypomagnesemia, serum magnesium levels <1.2 mg/dL; Hypomagnesemia, serum magnesium levels 1.2≤1.8 mg/dL; Normomagnesemia, serum magnesium levels >1.8 mg/dL.

Data are mean±SD; * Statistically significant difference between the groups with hypomagnesemia and normomagnesemia; # Statistically significant difference between the groups with severe hypomagnesemia and normomagnesemia; † Statistically significant difference between the groups with severe hypomagnesemia and hypomagnesemia.

with individuals in the groups with hypomagnesemia and normal serum magnesium. In the same way, the proportion of subjects with 3 components of the syndrome was lower in the individuals with severe hypomagnesemia than in the groups with hypomagnesemia and normal serum magnesium levels. Finally, the proportion of subjects with 4 and 3 components of the syndrome was similar in the groups with hypomagnesemia and normal magnesium levels.
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Figure 1. Distribution of the components of the Metabolic syndrome according to serum magnesium status. Five components of metabolic syndrome (black bar); four components (white bar); three components (gray Bar). Nonetheless, subjects in the group with severe hypomagnesemia showed a lower proportion of three components and higher proportion of four components of metabolic syndrome, as compared with the individuals in the hypomagnesemic and normomagnesemic groups, there were no statistically significant differences between the groups.

Table 3 shows the crude and adjusted ORs that computes the relationship between serum magnesium levels and elevation of hsCRP and TNF-α levels. In both models, subjects with severe hypomagnesemia showed a significant association with elevation of CRP and TNF-α levels. In the unadjusted model, the subjects with hypomagnesemia showed a significant association between serum magnesium and both, the elevated CRP and TNF-α levels; however, in the adjusted model, the associations were not statistically significant. Finally, in both models, normomagnesemia showed a protective role for inflammation.

Discussion

In the middle-aged subjects with a new diagnosis of MetS in this study we found a significant association between severe hypomagnesemia and elevation of both hsCRP and TNF-α. In addition, an unexpected finding was the absence of significant differences in CRP and TNF-α levels between the subjects with hypomagnesemia and normomagnesemia.

Discrepancies between the results of our study and others [20, 21, 24] could be explained on the

<table>
<thead>
<tr>
<th></th>
<th>Crude Odds Ratio</th>
<th>Odds Ratio adjusted by waist circumference</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>hsCRP</td>
<td>OR</td>
</tr>
<tr>
<td>Severe hypomagnesemia</td>
<td>14.1</td>
<td>2.1-23.2</td>
</tr>
<tr>
<td>Hypomagnesemia</td>
<td>2.1</td>
<td>1.2-14.7</td>
</tr>
<tr>
<td>Normomagnesemia</td>
<td>0.5</td>
<td>0.2-0.8</td>
</tr>
</tbody>
</table>
basis that in previous reports severe hypomagnesemia and hypomagnesemia were not differentiated; thus, it is probable that, in previous studies, the groups with low serum magnesium levels included an elevated proportion of subjects with severe hypomagnesemia, which could be responsible for the association between serum magnesium and inflammation. In addition, is necessary to consider that hypomagnesemia (serum magnesium ≤1.8 mg/dL) could have a high proportion of false negative tests for identifying magnesium deficiency.

Epidemiological studies have shown that magnesium intake and serum magnesium are inversely associated with serum concentration of CRP [20, 22, 31], suggesting that decreased serum magnesium levels could lead to the triggering of low-grade chronic inflammation [20]. As magnesium deficiency is involved in the triggering of the inflammatory response, and inflammation contributes to pro-atherogenic changes in lipoprotein metabolism, endothelial dysfunction, hypertension, and hyperglycemia, it is biologically plausible to consider that magnesium deficiency is not only associated with development of MetS, but it could also aggravate the components of the syndrome. In this regard, it has recently been reported that oral magnesium supplementation improves endothelial function [32], insulin resistance [33, 34], lipid profile [34], glucose levels [35, 36], high blood pressure and cardiovascular disease [37-40], all components of the MetS. Our results, which show that severe hypomagnesemia is strongly associated with inflammation, whereas normomagnesemia exhibits a protective role in subjects with MetS, support the abovementioned statement.

Nonetheless, is necessary to keep in mind the controversy related to the use of MetS in the clinical setting. On the one hand, it has been established that making the diagnosis of MetS does not bring much understanding for the pathophysiology or for clinical utility, and that deciding that individuals do not have the syndrome because they fail to satisfy the arbitrarily chosen criteria, may result in withholding relevant therapeutic intervention [41, 42]. On the other hand, it has been reported that the best available evidence suggests that people with MetS are at an increased risk of cardiovascular events, a finding that can help clinicians when counseling patients to consider lifestyle interventions for the prevention of cardiovascular disease [43]. So, the notion of MetS should be considered as an approach for evaluating and planning policies focused on the primary prevention of cardiovascular disease and type 2 diabetes.

The use of serum magnesium appears to be the elective diagnostic test for use in the clinical setting; however, the cutoff point for diagnosing hypomagnesemia (serum magnesium ≤1.8 mg/dL) has low sensitivity. Our results, that show the absence of significant differences of CRP and TNF-α levels between the subjects in the groups with hypomagnesemia and normomagnesemia, and that severe hypomagnesemia, but not the hypomagnesemia, is associated with inflammation in individuals with MetS highlights the need for further studies in order to establish the most appropriate cutoff value of serum magnesium for use in the clinical setting. Furthermore, our finding could explain, at least in part, the inconsistency between epidemiological and clinical studies regards the correlation or association between low serum magnesium levels and biochemical markers.

Obesity is associated with low-grade chronic inflammation and MetS [44-48]; however, Hak et al. [44] showed that, although body mass index accounted for the relationship between CRP and other components of MetS, the association between measures of obesity and insulin resistance is not affected by adjustment for CRP, data in disagreement with the hypothesis that adipose-tissue-derived cytokines may mediate the relation between obesity and MetS. Nonetheless, the subjects with severe hypomagnesemia and hypomagnesemia in our study had similar WC; the CRP and TNF-α levels in the subjects with severe hypomagnesemia were significantly higher than in the subjects with hypomagnesemia. This finding suggests that severe hypomagnesemia could be the missing link to explain the triggering of the inflammatory response in subjects with MetS. In this way, the association between CRP and components of MetS may be mediated by magnesium deficiency, as we have previously hypothesized [49].

Finally, about the HOMA-IR index, although there was a progressive increase from the group with normomagnesemia to the group with severe hypomagnesemia (table 2), there were no significant differences between the groups. Given that all the individuals in our study had MetS, they exhibited high HOMA-IR indexes; so the absence of significant differences between the groups was
an expected finding. Further research, with the appropriate design and sample size, is mandatory for adding new knowledge in this field.

Some limitations of this study deserve to be mentioned. First, subjects were enrolled into groups in study according to their serum magnesium levels; however, given that magnesium is a predominant intracellular ion, its serum measurements could be not representative of the magnesium status. Reducing serum magnesium levels according the established cutoff points [28] increased the sensitivity of the diagnostic test based on serum magnesium levels, minimizing this limitation; however, further research is needed to establish the best cutoff point of serum magnesium levels as an indicator of magnesium deficiency. Second, because our study is based on a cross-sectional design, causation could be not inferred with certainty. Whether severe hypomagnesemia is a risk factor for the triggering of inflammatory response or merely an associated epiphenomenon of MetS can not be assured.

On the other hand, the main strength of our study was the inclusion of incident cases of MetS that minimize the possibility of analysis bias.

In conclusion, our results show that, in subjects with MetS, severe hypomagnesemia, but not hypomagnesemia, is associated with elevated levels of CRP and TNF-α.

Disclosure

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None of the authors has any conflict of interest to disclose.

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