Cellular-Free Magnesium Depletion in Brain and Muscle of Normal and Preeclamptic Pregnancy
A Nuclear Magnetic Resonance Spectroscopic Study

Lawrence M. Resnick, Mario Barbagallo, Mordechai Bardicef, Orit Bardicef, Yoram Sorokin, Jeffrey Evelhoch, Ligia J. Dominguez, Brian A. Mason, David B. Cotton

Abstract—Preeclampsia is a pregnancy disorder of unknown origin, characterized by vasospasm, elevated blood pressure, and increased neuromuscular irritability, features common to syndromes of magnesium deficiency. Evidence of serum and ionized magnesium metabolism disturbances have been observed in women with preeclampsia. This and the therapeutic utility of magnesium in preeclampsia led us to investigate the extent to which an endogenous tissue magnesium deficiency might be present in and contribute to its pathophysiology. We used $^{31}$P nuclear magnetic resonance spectroscopy to noninvasively measure in situ intracellular-free magnesium levels in brain and skeletal muscle of fasting nonpregnant women ($n=12$), and of third trimester women with uncomplicated pregnancies ($n=11$) and preeclampsia ($n=7$). Compared with nonpregnant controls (brain $519\pm 59 \mu$mol/L; muscle $604\pm 34 \mu$mol/L), brain and skeletal muscle intracellular magnesium levels were significantly lower in both normal pregnant (brain $342\pm 23 \mu$mol/L; muscle $482\pm 40 \mu$mol/L; $P=0.05$ for both tissues) and preeclamptic women (brain $229\pm 17 \mu$mol/L; muscle $433\pm 46 \mu$mol/L; $P=0.05$ for both tissues). Brain intracellular magnesium was further reduced in preeclampsics compared with normal pregnant subjects ($P=0.05$). For all pregnant subjects, blood pressure was significantly and inversely related to the concomitantly measured intracellular magnesium level in brain (systolic, $r=-0.59$, $P=0.01$; diastolic, $r=-0.52$, $P=0.02$) but not in muscle. Cellular magnesium depletion is characteristic of normal pregnancy and may be one factor contributing to the pathophysiology of preeclampsia. Furthermore, the influence of central nervous system factors on blood pressure may be mediated, at least in part, by ambient intracellular magnesium levels. (Hypertension. 2004;44:322-326.)

Key Words: preeclampsia ■ magnesium ■ metabolism ■ ions ■ pregnancy

Hypertension is the most common medical disorder during pregnancy. The exact incidence of gestational hypertension–preeclampsia in the United States is unknown. Estimates indicate that 5% to 8% of all pregnant women will have preeclampsia, defined as hypertension and proteinuria beginning during the second half of gestation. Preeclampsia may also be associated with increased neuromuscular irritability and seizures. Interestingly neuromuscular excitability, vasoconstriction, elevated blood pressure (BP), and increased vascular sensitivity to pressor agents are also characteristic of magnesium (Mg) depletion.

The therapeutic use of intravenous Mg sulfate is universal, at least in the United States, for women with mild preeclampsia to prevent eclampsia seizures, and its effectiveness has been confirmed in a recent metaanalysis showing that parental Mg more than halves the risk of eclampsia. However, a clear role of Mg deficiency in the pathophysiology of preeclampsia has not been clearly established, and dietary Mg supplementation does not seem to prevent the subsequent incidence of preeclampsia.

Our group has developed the use of $^{31}$P nuclear magnetic resonance (NMR) spectroscopic techniques to noninvasively measure intracellular-free magnesium (Mgi) content in a variety of clinical disease states, such as hypertension, where Mg levels were closely and inversely related to the height of BP. We have extended these NMR techniques to include the analysis of Mgi in situ in intact tissues such as brain and skeletal muscle tissues, where brain Mg was also closely related to BP.

Therefore, to investigate cellular Mg metabolism in hypertensive disorders of pregnancy, we measured brain and skeletal muscle Mg concentrations in situ in nonpregnant and pregnant women with and without the diagnosis of preeclampsia. Our present results document that tissue Mg...
depletion is a characteristic feature of normal pregnancy, especially those complicated by preeclampsia. Furthermore, the quantitative relation of cellular-free Mg content to concomitant BP levels also suggests a role for cellular Mg deficiency in the pathophysiology of preeclampsia.

Methods

Three groups of patients were studied: (1) nonpregnant women of reproductive age (n = 12), (2) unmedicated third trimester women with uncomplicated pregnancies (n = 11), and (3) unmedicated third trimester pregnant women with preeclampsia (n = 7). Diagnosis of preeclampsia was based on the following criteria: increased BP accompanied by proteinuria, edema, or both. Hypertension was defined as a diastolic BP ≥ 90 mm Hg, a systolic BP ≥ 140 mm Hg, a rise in the former of ≥ 15 mm Hg, or in the latter of 30 mm Hg. These altered BP readings were obtained on at least 2 separate occasions 6 hours or more apart. Patients taking medication, with other existing medical problems, or both were excluded, as were all patients with any contraindication for MR. The study was approved by the Human Investigation Committee of Wayne State University. Written informed consent was obtained from all patients, and the procedures followed were in accordance with institutional guidelines. All women were clinically evaluated in the morning after an overnight fast. BP measurements were taken with the patient relaxed in a sitting position in a quiet room at a comfortable temperature after a short period of rest, and, if stable, patients were transferred to the MR center for brain and skeletal muscle NMR spectroscopy determination.

NMR Evaluation

31P NMR spectra were obtained from the brain and gastrocnemius muscle using 1D chemical shift imaging (CSI) (Figure 1). For brain CSI, subjects were placed in the magnet on their side with a 9-cm surface coil placed over the temporal parietal region. For muscle CSI, subjects were placed in the magnet on their side with a 9-cm surface coil. 31P NMR spectra were acquired with a repetition time of 3 seconds, 60(roughly the optimum flip angle for the ATP peaks), 0.5-ms triangular phase encoding gradients, 0.5-ms acquisition delay, 1024 data points with a 512-ms acquisition time (4000 Hz spectral width), and 12 (brain) or 6 (muscle) acquisitions for each of 32-phase encoding steps (total acquisition time was 19.6 minutes for brain and 10.0 minutes for muscle). This provided 31P NMR spectra from contiguous 1.25-cm-thick 8- or 9-cm-diameter slices within the sensitive volume of the surface coil. 31P CSI data set was processed on the Siemens VAX 4000. A 1 to 5 Hz Lorentzian filter was applied and the CSI data were Fourier transformed in 2 dimensions (1 spatial and 1 chemical shift). For further analysis, a single spectrum was selected from each CSI data set on the basis of resolution, sensitivity, and, in the case of brain, phosphocreatinine and phosphomonoesters levels consistent with brain 31P spectra. The baseline roll caused by the acquisition delay was removed by fitting and subtracting a cubic spline function to each spectrum. Peak positions were estimated from the spectra of interest using Siemens software.

Calculation of Mg, and pH

For the selected brain and muscle spectra, Mg, levels were calculated from the observed difference between the chemical shift difference of the α- and β-phosphoryl resonances of ATP, as previously described in detail. 11 pH values were also calculated from the same 31P NMR spectra, as previously described. 13

Statistical Analysis

Analysis of the data was performed using statistical software on a Macintosh personal computer (Stat View 4.01 and Super Anova, Abacus Concepts). To compare differences in variables among the diagnostic groups, 1-factor ANOVA was used with post-hoc testing (Bonferroni) for significance. Comparison between muscle and brain values for both Mg, and pH, in each group used paired Student t test analysis. Relationships between BP and Mg, levels used linear regression analysis with Pearson correlation coefficients. All values are reported as mean ± SEM.

Results

The clinical characteristics of all patients are presented in Table 1. All 3 patient groups were equivalent in age, and among pregnant subjects, in gestational age at the time of NMR-based intracellular ion measurements. Systolic and diastolic blood pressures were significantly higher in the preeclamptic patients compared with both nonpregnant and normal pregnant subjects (P < 0.0001 and P = 0.0007, respectively). Four preeclamptic women had mild preeclampsia, 2 had severe preeclampsia, and 1 had preeclampsia superimposed on prior ongoing chronic hypertension. All preeclamptic women were induced, and 5 of them delivered prema-

| TABLE 1. Clinical Characteristics of Nonpregnant, Pregnant, and Preeclamptic Women |
|---------------------------------|-----------------|-----------------|-----------------|------------------|
| Study Group | Age (y) | Systolic Blood Pressure (mm Hg) | Diastolic Blood Pressure (mm Hg) | Gestational Age (wk) |
| Nonpregnant (n=12) | 28.2 ±1.2 | 118 ±3 | 71 ±3 |
| Pregnant (n=11) | 23.8 ±1.8 | 110 ±4 | 71 ±3 | 35.2 ±0.8 |
| Preeclampsia (n=7) | 24.8 ±2 | 150 ±6* | 91 ±4* | 34.1 ±1.2 |

*P < 0.001 vs both nonpregnant and normal pregnant subjects.
turely. The gestational age at the time of delivery (35.8±1.1 versus 39.4±0.5 weeks, P=0.008) and the birth weights (2480±323 versus 3243±177 g, P=0.04) were lower among the preeclamptic group versus controls. Two preeclamptic women were delivered by caesarean section (1 for fetal distress and 1 for intrauterine growth retardation and fetal distress).

The values of Mg, and pH, in brain and skeletal muscle are displayed in Table 2. Both pregnant and preeclamptic individuals had significantly lower brain and muscle Mg levels than nonpregnant patients (ANOVA; brain, P=0.0005; muscle, P=0.01; P=0.05 for both versus nonpregnant). In addition, brain Mg levels in women with preeclampsia were significantly further suppressed compared with pregnant patients themselves (P=0.05; Figure 2).

For all pregnant subjects, a significant inverse relation was observed between both systolic and diastolic BP and the concomitant measured cellular-free Mg, levels in brain (systolic blood pressure, r=−0.59, P=0.01; diastolic blood pressure, r=−0.52, P=0.02; Figure 3). No significant relations were observed between BP and muscle Mg levels. No relation was also present between the length of pregnancy and the occurrence of low brain Mg concentrations.

Pregnancy was associated with an altered relationship between brain and muscle Mg values. In nonpregnant controls, brain and muscle Mg levels were equivalent. However, in both pregnant groups, brain Mg levels were significantly lower than levels in muscle (pregnant controls, P=0.01; preeclampsia, P=0.004) because of the greater fall in brain Mg values during pregnancy (Figure 4). Altogether, for all subjects, despite these effects related to pregnancy, brain and muscle Mg, values were significantly and positively related (r=0.55, P<0.002). pH, values in brain and muscle did not differ significantly among any of the diagnostic groups (Table 2).

### Discussion

Applying NMR spectroscopic techniques to noninvasively measure Mg, levels in pregnancy, we have observed in this study: (1) that pregnancy itself is characterized by lower Mg, values both in brain and muscle tissue; (2) that brain Mg, levels are further suppressed in preeclamptic compared with normal pregnant and nonpregnant women; (3) that both systolic and diastolic blood pressures are quantitatively and inversely related to brain Mg, values; and lastly, (4) Mg, depletion in pregnancy appears to be differentially expressed in brain vis a vis muscle, Mg concentrations being equivalent in the nonpregnant state, but, with pregnancy, decreasing in brain to a greater extent than in muscle.

Mg functions intracellularly as a necessary cofactor of >300 enzyme systems, and a decrease in cellular Mg would result in partial membrane depolarization and decreased repolarization in association with cellular calcium accumulation and potentiated calcium-dependent cell actions including, in smooth muscle, vasoconstriction14–17; in neural tissue, enhanced sympathetic activity18,19; and in skeletal muscle and fat tissue, insulin resistance.20,21 These alterations have indeed been reported in cellular Mg-deficient states, such as essential hypertension4 and noninsulin-dependent diabetes mellitus.16 Furthermore, these same defects can be induced

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**Table 2. Brain and Skeletal Muscle Intracellular-Free Magnesium and pH Levels in Nonpregnant, Pregnant, and Preeclamptic Subjects**

<table>
<thead>
<tr>
<th>Study Group</th>
<th>Brain Mg (µmol/L)</th>
<th>Muscle Mg (µmol/L)</th>
<th>Brain pH TD</th>
<th>Muscle pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonpregnant (n=12)</td>
<td>519±59</td>
<td>604±34</td>
<td>7.03±0.017</td>
<td>7.08±0.005</td>
</tr>
<tr>
<td>Pregnant (n=11)</td>
<td>342±23*</td>
<td>483±41*</td>
<td>7.07±0.012</td>
<td>7.09±0.008</td>
</tr>
<tr>
<td>Preeclampsia (n=7)</td>
<td>229±17*†</td>
<td>433±46*</td>
<td>7.06±0.012</td>
<td>7.10±0.011</td>
</tr>
</tbody>
</table>

Mg indicates intracellular-free magnesium; pH, intracellular pH.

*P=0.0005 (ANOVA), P=0.05 vs nonpregnant.
†P=0.01 (ANOVA), P=0.05 vs pregnant.

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**Figure 2.** Mg, in nonpregnant, pregnant, and preeclamptic women. NP indicates nonpregnant; P, pregnant; PE, preeclampsia.

**Figure 3.** Relation of brain Mg, levels and blood pressure in pregnancy. SBP indicates systolic blood pressure; DBP, diastolic blood pressure.
experimentally by dietary Mg depletion, directly causing vasoconstriction or vascular spasm in various vascular beds including cerebral, coronary, and placental vessels as well as elevated BP, increased neuromuscular irritability, and frank tetany.

Historically consistent with the above, it was the ability of Mg to suppress neural irritability that first led investigators more than 70 years ago to use Mg therapeutically in preeclamptic pregnancy, and Mg sulfate remains a standard therapeutic maneuver and the drug of choice to prevent convulsions in women with preeclampsia, although the exact mechanism of action of Mg remains unknown. Two recent randomized trials have documented that Mg sulfate is superior to a placebo for prevention of convulsions in women with severe preeclampsia. Among all women enrolled in the large Magpie trial, 1 of the largest randomized trials to date that enrolled 10,141 women with preeclampsia in 33 nations, the rate of eclampsia was significantly lower in those assigned to Mg sulfate (0.8% versus 1.9%; relative risk, 0.42; 95% confidence interval, 0.29, 0.60). A recent Cochrane systematic review has shown that Mg sulfate is superior to other regimens for preventing eclamptic seizures, more than halving the risk, and may reduce the risk of maternal death, although not improving the outcome for the baby. However, despite the long-standing therapeutic use of intravenous Mg sulfate in preeclampsia, oral magnesium supplementation does not seem to influence the incidence of preeclampsia.

Clinically, Mg deficiency and preeclampsia share many features, including placental vasospasm, elevated BP, and increased neuromuscular irritability. This and our previous findings of cellular Mg deficiency in essential hypertension, made it seem reasonable to investigate the possibility that an endogenous-tissue Mg depletion might underlie or predispose to at least some pathophysiological aspects of preeclampsia. Surprisingly, although this hypothesis has been suggested, it is virtually absent from discussions of the cause of preeclampsia in current textbooks of obstetrics. Our present findings suggest a role for cellular Mg depletion, especially in the central nervous system, in predisposing to or directly participating in the pathophysiology of preeclampsia. From our data we cannot determine the mechanism of the more pronounced suppression of Mg levels found in the brain tissue (Table 2) and whether these tissue specific alterations are related to the increased predisposition to neuromuscular irritability and seizures of preeclampsia as it is suggested. This should be the goal of future studies.

However, we believe the present observations add significantly to previous studies in pregnancy, which have been few, that have usually measured total rather than free Mg concentrations and have focused on circulating blood cells. Kisters et al observed significantly lower erythrocyte total cellular and membrane Mg content but not altered plasma total Mg levels in preeclampsia versus normal pregnant women, suggesting the presence of a disturbance in the transmembrane Mg distribution in preeclampsia. In contrast, Ryzen et al, measuring total Mg per mg cell protein in circulating mononuclear cells of preeclamptic versus normal pregnant subjects, did not detect lower total cellular Mg levels. Interestingly, compared with nonpregnant subjects, both normal pregnant and preeclamptic subjects had lower extracellular total Mg levels in this study. Using recently developed ion-specific Mg electrode techniques, we and others have also observed a deficit in biologically active ionized Mg in pregnant and preeclamptic women, which in itself could be responsible for the presently observed lower Mg values in brain and muscle tissue. Similar to our findings in muscle, circulating Mg levels in preeclamptic subjects were no further suppressed than those in normal pregnancies. Altogether, these previous studies may be limited by the fact that plasma, erythrocyte, and white blood cell total Mg content may poorly reflect total body Mg status, and that metabolically active cellular-free Mg activity represents only 10% to 20% of total cellular Mg.

Despite the suggestion emerging from this study of a role for Mg, especially in the central nervous system, in the regulation of BP in pregnancy and preeclampsia, certain caveats need to be considered. First, longitudinal data obtained serially during pregnancy are required to ascertain whether or not the development of Mg depletion necessarily corresponds with BP throughout pregnancy and thus in particular, whether preeclamptic pregnancies can be distinguished before the onset of clinical signs and symptoms. However, at least in experimental animals, Schooley et al have recently shown that a moderate dietary Mg deficiency during pregnancy in rats leads to significantly increased BP. Second, the mechanism by which cellular Mg depletion follows pregnancy, and how especially in the central nervous system this ionic lesion is linked to preeclampsia, has not been addressed in this study and is unknown. One would presume that if the suppression of Mg levels characteristic of normal and preeclamptic pregnancy were nutritional in origin, or the result of decreased extracellular availability of Mg to the cell, then oral Mg supplementation during pregnancy might significantly influence the subsequent incidence of preeclampsia. This has not been the case, however, in the few reported trials published thus far, although the numbers of subjects studied may not have been sufficiently large. Reversal of the cellular Mg deficit may therefore not be easily amenable to dietary maneuvers.

**Perspectives**

Our present results document that normal pregnancy is associated with a cellular Mg depletion both in brain and in...
magnesium loss is well established.16,17,20,21,24 Future studies are needed to support any relationship between insulin and insulin sensitivity and the alterations of magnesium metabolism in brain and muscle shown in this article. Future studies should also determine the mechanism of the more pronounced suppression of Mg levels found in the brain tissue and whether these tissue specific alterations are related to the increased predisposition to neuromuscular irritability and seizures of preeclampsia.

References

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