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Extracellular and intracellular magnesium depletion in pregnancy and gestational diabetes

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OBJECTIVE: Our purpose was to investigate a possible ionic basis linking pregnancy and gestational diabetes with the vascular disorders associated with them.
STUDY DESIGN: We used phosphorus 31 nuclear magnetic resonance spectroscopy and magnesium- and calcium-specific ion electrodes to measure erythrocyte intracellular free magnesium, plasma ionized magnesium, and ionized calcium in fasting nonpregnant (n = 26), normal pregnant (n = 20), and diet-controlled (class A1) gestational diabetic women (n = 13).
RESULTS: Compared with nonpregnant controls (total magnesium 0.91 ± 0.07 mmol/L, ionized magnesium 0.51 ± 0.03 mmol/L), total and ionized magnesium were significantly lower in both normal pregnant (total magnesium 0.72 ± 0.07 mmol/L, ionized magnesium 0.46 ± 0.02 mmol/L, significance < 0.0001) and gestational diabetic (total magnesium 0.74 ± 0.05 mmol/L, ionized magnesium 0.46 ± 0.02 mmol/L, significance < 0.0001) subjects. Gestational diabetic women had significantly lower intracellular free magnesium values compared with nonpregnant and normal pregnant individuals (140 ± 20 μmol/L vs 169 ± 27 μmol/L, significance = 0.007). Ionized calcium values were similar in all groups, resulting in significant elevation (significance < 0.0001) of ionized calcium/ionized magnesium ratios in both pregnant groups.
CONCLUSIONS: These results support the presence of magnesium depletion in pregnancy itself and to a greater extent in gestational diabetes. We suggest that magnesium depletion, or relative calcium excess, may predispose to vascular complications of pregnancy. (Am J Obstet Gynecol 1995;172:1009-13.)

Key words: Nuclear magnetic resonance spectroscopy, pregnancy, magnesium metabolism, gestational diabetes, ion-specific electrodes

Recently great pathophysiologic significance has been placed on the association of essential hypertension with such metabolic abnormalities as hyperinsulinemia, insulin resistance, and type 2 non-insulin-dependent diabetes mellitus,[1, 2] although the specific underlying pathophysiologic mechanisms of this syndrome complex remains poorly defined. To better understand these phenomena at the cellular level, we focused on the role of altered mineral ion metabolism in these disease states. We developed nuclear magnetic resonance spectroscopic techniques to noninvasively assess intracellular ion content and selective magnesium electrodes to assess extracellular serum ionized magnesium. By means of these techniques we have identified abnormalities of cellular calcium and magnesium in hypertensive and insulin resistance states[3-8] that may at least partially help to explain their frequent clinical coexistence.[9]

Pregnancy is also a state of insulin resistance. This

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Table I. Clinical characteristics of nonpregnant, pregnant, and gestational diabetic subjects

	Nonpregnant (n = 26)	*Control pregnant (n = 20)*	*Gestational diabetic (n = 13)*
Age (yr)	30.0 ± 7.1	26.8 ± 6.2	31.8 ± 5.5
Black/white	5/21*	12/8	10/3
Week of gestation	–	33.4 ± 4.5	31.9 ± 2.6
Gravidity	–	3.4 ± 2.2	4.4 ± 2.4
Parity	–	1.3 ± 1.2	1.8 ± 1.7
Body mass index (kg/m^2)	23.2 ± 2.5	30.9 ± 4.6	36.8 ± 9.3†
Systolic blood pressure (mm Hg)	112 ± 10	108 ± 11	104 ± 12
Diastolic blood pressure (mm Hg)	69 ± 8	70 ± 7	65 ± 10

*$p < 0.005$, versus both pregnant groups.
†$p < 0.05$, gestational diabetics versus control pregnant women.

Table II. Extracellular and intracellular divalent cations in nonpregnant, pregnant, and gestational diabetic subjects

	Nonpregnant (n = 26)	*Control pregnant (n = 20)*	*Gestational diabetic (n = 13)*
Plasma total magnesium (mmol/L)	0.91 ± 0.07	0.72 ± 0.07*	0.74 ± 0.04*
Plasma ionized magnesium (mmol/L)	0.51 ± 0.03	0.46 ± 0.02*	0.46 ± 0.02*
Intracellular free magnesium (μmol/L)	169 ± 27	160 ± 28	140 ± 20†
Plasma ionized calcium (mmol/L)	1.13 ± 0.04	1.13 ± 0.03	1.14 ± 0.03
Plasma ionized calcium/plasma ionized magnesium	2.22 ± 0.16	2.44 ± 0.15*	2.47 ± 0.19*
Plasma ionized magnesium/plasma total magnesium (%)	56.3 ± 5.9	64.2 ± 5.2*	62.7 ± 4.8*

*Significance < 0.0001, versus nonpregnant women.
†Significance = 0.007, versus control pregnant and nonpregnant women.

insulin resistance is exacerbated in gestational diabetes, which may also predispose patients to the various hypertensive syndromes of pregnancy.[10-13] Furthermore, although predictive of future diabetes, gestational diabetes most often resolves with the completion of pregnancy, implying the critical contribution of pregnancy-related factors to the diabetic state. In light of the above, we wondered whether altered mineral metabolism may also contribute to the mechanisms underlying this association of diabetes with pregnancy itself and with hypertensive disorders of pregnancy, often consequent to it. Therefore we compared extracellular ionized magnesium, ionized calcium, and intracellular free magnesium levels in normal pregnancy and gestational diabetes. We report our preliminary observations that alterations of both intracellular and extracellular magnesium content are characteristics of both normal pregnancy and gestational diabetes.

Material and methods

All subjects were evaluated at the Hutzel Hospital–Detroit Medical Center in the morning after an overnight fast, and 10 ml of venous blood was drawn with the patient in a seated position. None of the subjects were receiving medication for any current medical condition or had been on antidiabetic therapy. The class A1 diabetes mellitus group ($n = 13$) included those patients who were diet controlled after abnormal carbohydrate intolerance was demonstrated during a 100 gm, 3-hour oral glucose tolerance test (two or more abnormal blood glucose values). Patients with uncomplicated pregnancies ($n = 20$) were recruited from the low-risk antenatal clinics. All pregnant patients, diabetics and nondiabetics, were evaluated in the third trimester. Nonpregnant women ($n = 26$) of similar reproductive age served as a control group.

Blood samples obtained from the women were analyzed for serum total magnesium, serum ionized magnesium, and erythrocyte intracellular free magnesium. Blood was drawn into a heparinized tube and centrifuged at 2000 revolutions/min for 10 minutes, and the plasma was separated and frozen in 2 ml plastic vials at −20° C. The plasma was analyzed for total magnesium by standard techniques and for ionized calcium with a calcium-specific ion electrode module included as part of the ionized magnesium apparatus (see below). Plasma ionized magnesium was determined with a magnesium-specific ion electrode apparatus containing a neutral carrier-based membrane. This new apparatus for ionized magnesium has been recently characterized, and intracellular free magnesium values obtained by this technique from whole blood, serum, and plasma are virtually identical.[6]

Intracellular free magnesium was measured in the

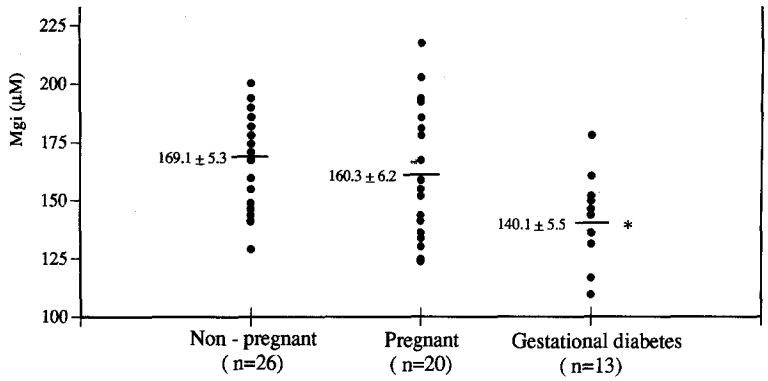

Fig. 1. Intracellular free magnesium levels in erythrocytes of nonpregnant, third-trimester pregnant and gestational diabetic women. *Mgi,* Intracellular free magnesium. Significance *(sig)* was determined by analysis of covariance.

packed cell fraction by means of phosphorus 31 nuclear magnetic resonance spectroscopic techniques, the details of which have been previously described.[3] Briefly, after the heparinized blood was centrifuged, the packed cells were decanted into 10 mm, thin-walled nuclear magnetic resonance tubes, and placed in the nuclear magnetic resonance spectrometer for analysis. All spectra were obtained on a 300 MHz General Electric nuclear magnetic resonance spectrometer operating at 37° C. Intracellular free magnesium levels (Mgi) were determined according to the formula

$$Mgi = K_d(MgATP)\ \{\phi^{-1} - 1\}$$

where K_d(MgATP) is the dissociation constant of adenosine triphosphate, which is taken as 38 mmol/L at 37° C, and where ϕ is the free, unbound fraction of adenosine triphosphate, calculated from the chemical shift differences of the α and β phosphoryl resonances of adenosine triphosphate on the ^{31}P nuclear magnetic resonance spectrum.[14]

Analysis of the data was performed with statistical software on a personal computer (Stat View 4.0 and Super Anova, Abacus Concepts, Berkeley, Calif.). The various magnesium values were compared between gestational diabetic, pregnant, and nonpregnant control subjects by analysis of covariance controlling for the potential confounding effects of race and body mass index. Clinical variables between diabetic and nondiabetic pregnant women were compared by Student unpaired *t* test. The χ^2 test was used to analyze the significance of the proportional racial representation among pregnant and nonpregnant groups. All values are reported as mean ± SD.

Results

Comparison of demographic and clinical characteristics among the three patient groups is shown in Table I. There were no significant differences in gestational age, gravidity, parity, and blood pressure between the two pregnant groups. Pregnant women with gestational diabetes had greater body mass index values compared with normal pregnant women ($p < 0.05$). Both pregnant groups had a greater proportional representation of black women compared with normal nonpregnant subjects ($\chi^2 = 16.8$, $p < 0.005$).

The values for plasma total magnesium, plasma ionized magnesium, erythrocyte intracellular free magnesium, and plasma ionized calcium among the three patient groups are displayed in Table II. Both measures of extracellular magnesium (total magnesium, ionized magnesium) were significantly lower in the two pregnant groups compared with normal nonpregnant subjects (significance < 0.0001). However, the percentages of ionized magnesium values were significantly higher in both pregnant groups (significance < 0.0001). These differences between pregnant and nonpregnant subjects persisted when only white subjects were included in the analysis. Because ionized calcium levels were similar in all three subject groups, the ionized calcium/ionized magnesium ratio was significantly increased in both pregnant groups compared with the nonpregnant subjects (significance < 0.0001).

Diabetic pregnant subjects differed both from nondiabetic pregnant and nonpregnant individuals in that the intracellular free magnesium values were modestly but significantly lower (significance = 0.007) (Fig. 1).

Analysis of covariance indicated that these differences remained significant when both race and body mass index were controlled for.

Comment

We have previously demonstrated abnormalities of cellular divalent cation content in hypertensive and other insulin-resistant states such as obesity and type II diabetes mellitus.[1-3] We hypothesized that these ionic

abnormalities may provide the cellular basis underlying the clinical linkage between hypertension, insulin resistance, and their cardiovascular consequences.[4, 5, 9] Because normal pregnancy and, to an even greater extent gestational diabetes, are both associated with an increased risk of hypertension and other vascular disorders,[10-13] we wondered whether similar ionic defects might exist in these clinical conditions as well. By utilizing the recently described nuclear magnetic resonance and selective magnesium ion electrode techniques to measure intracellular and extracellular free magnesium content, respectively, we observed that alterations in extracellular and intracellular magnesium levels do indeed exist in both normal pregnancy and in pregnancy with gestational diabetes. Plasma total and ionized magnesium levels were significantly lower in both pregnant groups compared with nonpregnant women. Magnesium status was further compromised in gestational diabetic subjects, in whom intracellular free magnesium levels were also significantly suppressed compared with both nonpregnant and normal nondiabetic pregnant women. Because no significant differences were observed in ionized calcium values among the three groups, ionized calcium/ionized magnesium ratios, which represent an index of tissue calcification potential,[15-17] were significantly increased in all pregnant subjects compared with nonpregnant controls. Altogether these data suggest (1) that pregnancy itself is a state of extracellular magnesium depletion, (2) that diabetes in pregnancy is associated with a further depletion of intracellular free magnesium, and (3) that these lower ambient magnesium levels, and the higher resultant ionized calcium/ionized magnesium ratios observed in both pregnant groups, may help to explain the predisposition to vascular abnormalities in pregnancy.

Measurements of total plasma magnesium may not adequately reflect physiologic magnesium stores because plasma contains $< 1\%$ of total body magnesium, which is stored predominantly intracellularly or in bone.[18] Also, few studies have measured total intracellular erythrocyte levels of magnesium in pregnancy by atomic absorption techniques, but this method also may poorly reflect the physiologic status of magnesium.[19] Thus the lack of a more routinely available, accurate, and reproducible measurement of physiologic, meaningful magnesium stores has long hampered progress in this area. The noninvasive measurement of free cytosolic magnesium has only recently been possible with the advent of nuclear magnetic resonance spectroscopic techniques,[3, 14] which provide values indistinguishable from levels assessed by direct intracellular electrode techniques and which closely reflect physiologic variables such as blood pressure and peripheral insulin resistance.[3, 4] Furthermore, in the extracellular space, although serum ionized calcium measurements have long been available, an ion-specific electrode apparatus for measuring serum ionized magnesium has only recently been described.[6] With this technique we have shown a significant suppression of circulating ionized magnesium in fasting mild diabetic subjects, even when no differences in total magnesium levels were observed.[8] Thus our application of these two methods have allowed us to analyze magnesium metabolism in pregnancy in a more precise manner than has previously been possible.

A greater proportion of pregnant subjects studied here were black compared with nonpregnant controls. However, although race may be a significant independent factor in magnesium metabolism,[20] the differences in magnesium values between pregnant and nonpregnant subjects remained significant when only white subjects were included in the analysis, indicating an independent effect of pregnancy on ionized magnesium values.

Similarly, we do not believe that these magnesium differences can be accounted for on the basis of the great disparities of body mass index among the groups, because obesity itself is not associated with significantly different magnesium values.[1] Indeed, when directly tested by analysis of covariance, no significant interactions between either race or body mass index and the various magnesium values were observed. Last, the lower ionized magnesium values in both pregnant groups cannot be attributed to alterations in protein binding, commonly found in pregnancy. Indeed, these differences were emphasized by the greater percentage of magnesium that was ionized among pregnant subjects, which somewhat compensated for the lower absolute total magnesium values found here and in the literature.[21]

What is the possible clinical significance of these observations? Numerous clinical in vivo and in vitro studies indicate that magnesium depletion has multiple effects on vascular tone, blood pressure, capillary lumen size, susceptibility of cells to oxidative stress, and thus the viability of cells in an ischemic environment.[22-24] Metabolic effects also include impaired insulin metabolism, hyperlipidemias, and accelerated atherosclerosis.[25-27] Furthermore, vasoconstricted high-renin states in nonpregnant hypertensive individuals is associated with significantly lower serum magnesium values.[28] Pregnancy is itself a high-renin state and together with gestational diabetes predisposes to vascular disease syndromes, including preeclampsia, which involves pathologic vasospasm, increased vascular resistance, and ischemic damage.[29, 30] Consistent with the above, we hypothesize that the magnesium depletion documented here may be at least one mechanism underlying the vascular complications of pregnancy.

This hypothesis is also supported by the literature. Indeed, several investigators have recently reported on the association of gestational glucose intolerance, or even one abnormal value in an oral glucose tolerance test, with pregnancy-induced hypertension and preeclampsia.[10-13] Preliminary data suggest that ionized magnesium levels are lower in umbilical vein cord blood of pregnant women with transient hypertension during labor compared with normotensive pregnant women.[31] Similarly, preeclampsia may be a magnesium-depleted state.[19] Our data thus extend to pregnancy per se and to gestational diabetes in particular, findings of magnesium depletion similar to those previously found in other states associated with hypertensive or vascular disease, such as essential hypertension, non-insulin-dependent diabetes mellitus,[1-4] and migraine.[32] Further studies will be needed to investigate the possibilities raised by these preliminary data. In particular, the diagnostic utility of more precise magnesium measurements during pregnancy and the possible therapeutic benefits of magnesium supplementation in at least some predisposed pregnant groups need to be defined.

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