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Intra-uterine growth retardation is associated with increased levels of magnesium in amniotic fluid

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Summary

Magnesium (Mg) and zinc (Zn) levels were measured in amniotic fluid of 30 normal pregnancies between the 16th and the 42nd week of amenorrhea and in 15 cases of intra-uterine growth retardation (IUGR) at 30–38th week of pregnancy. While Zn levels were stable throughout physiological pregnancy, the Mg concentration decreased progressively from 1.09 ± 0.31 mg/100 ml (mean $\pm$ SD) at the end of the second trimester to 0.75 ± 0.20 ($p < 0.02$) at term. In the case of IUGR, Mg levels (1.22 ± 0.28) were higher than in controls (0.89 ± 0.27, $p < 0.01$) matched for gestational age. No differences in Zn levels were found between the two groups. Although the mechanisms leading to Mg accumulation in the amniotic fluid of growth-retarded fetuses remain unknown, these data support the role played by Mg in fetal growth.

Amniotic fluid; Magnesium; Zinc; Fetal growth

Introduction

In recent years, attention has been paid to divalent cations, mainly magnesium (Mg) and zinc (Zn), as possible regulators of fetal growth. Both ions are cofactors of several enzymatic reactions, including nucleic acid and protein synthesis, oxygen transport and oxidative phosphorylation [1,2], thus suggesting that their deficiency could be associated with intra-uterine growth retardation (IUGR). Experimental data support this theory, since pregnant rats following a diet deficient in Mg or Zn

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showed embryo resorption, fetal malformations and fetal (but not maternal) anemia [3,4]. Moreover, in pregnant ewes fed with half the required Mg dose, the weights of the lambs were significantly lower than in controls, but were restored to normal upon Mg supplementation [5].

In humans, Mg and Zn levels circulating in the fetus at birth are higher than those in the mother, and Mg accumulates in fetal tissues with increasing gestational age [6,7]. Mg and Zn are detectable also in amniotic fluid (AF), and Mg levels show a decrease throughout pregnancy [8], whereas Zn levels seem to increase near term [9].

On these grounds, we measured Mg and Zn AF levels in normal pregnancy and in cases of IUGR, to assess whether Mg and/or Zn could be involved in the pathophysiology of fetal growth and might represent markers of this disorder.

Material and methods

Subjects

Thirty AF samples were collected from healthy pregnant women (between 16 and 42 weeks of pregnancy) undergoing amniocentesis for genetic purposes (16 cases) or for the assessment of fetal lung maturity (14 cases). No genetic defects were observed and lung maturation was ascertained in every case. These AF samples represented the control group, since the mothers delivered at term (five by elective Cesarean section) and the birth weights ranged between 2920 and 3970 g, all being above the 20th centile.

Fifteen additional samples were collected between the 30th and 38th week of pregnancy in patients affected by pregnancy-induced hypertension (8 cases) and/or intra-uterine growth retardation (IUGR). The diagnosis of IUGR was based on ultrasound evaluation of abdominal circumference and confirmed by birth weights below the 10th centile (range 700–2050 g). No alcohol abusers were included; six in the control group and four IUGR cases smoked during pregnancy (less than 10 cigarettes/day). Deliveries occurred between the 31st and 40th week of pregnancy (6 cases by Cesarean section). Placentas showed signs of infarction and localized calcifications.

Methods

The AF samples were obtained by transabdominal amniocentesis, using local anesthesia, and were stored in metal-free polyethylene tubes, previously cleaned in 0.5% nitric acid solution and rinsed with double-distilled water. After centrifugation to discard cells, the fluids were diluted with distilled water, 100- and 2-fold for Mg and Zn, respectively.

For technical reasons, the Zn levels could not be evaluated in seven of the control and four of the IUGR samples.

The two cations were determined by absorption spectrophotometry (Perkin Elmer, mod. 5000), using an air/acethylene flame, and a triple slot burner. The light source was a double element (Mg/Zn) hollow cathode lamp. Sample aspiration, flame composition, and the position of the burner head were adjusted to give

maximum sensitivity. Zinc absorption was measured at 214 nm and Mg absorption at 285 nm.

Stock standard solutions were purchased from Fisher Scientific Comp., and working solutions were prepared daily using a standard ranging from zero to 0.5 ppm for both elements. Accuracy of the analytical technique in AF was tested by the method of standard addition. A mean recovery of 98 and 101% of Zn and Mg, respectively, was observed throughout the range of values (0–0.5 ppm), and the coefficients of variation of repeated dilutions of AG were below 3%. Dilution of the samples with both lanthanium oxide solution in hydrochloric acid and nitric acid did not improve the absorbance.

The data were analyzed by one-way analysis of variance, and correlation coefficients were calculated by the least-squares method.

Results

The Mg AF levels in pregnancies associated with normal-weight infants decreased consistently toward term (Fig. 1). Subdividing the gestational age into three periods of 9 weeks length, Mg levels decreased significantly from the 16th to 25th week (1.09 ± 0.31 mg/100 ml, mean $\pm$ SD), to the 26th–33rd week (1.01 ± 0.31) to term (0.75 ± 0.20) ($F = 4.28$, $p < 0.02$). A significant linear correlation between Mg levels in AF and gestational age was found ($y = 1.61 - 0.022x$; $r = 0.56$, $p < 0.01$).

Mg levels in pregnancies carrying a IUGR fetus (1.22 ± 0.28) were significantly higher than in controls (0.89 ± 0.27, $F = 9.45$, $p < 0.01$), matched for gestational age (Fig. 1).

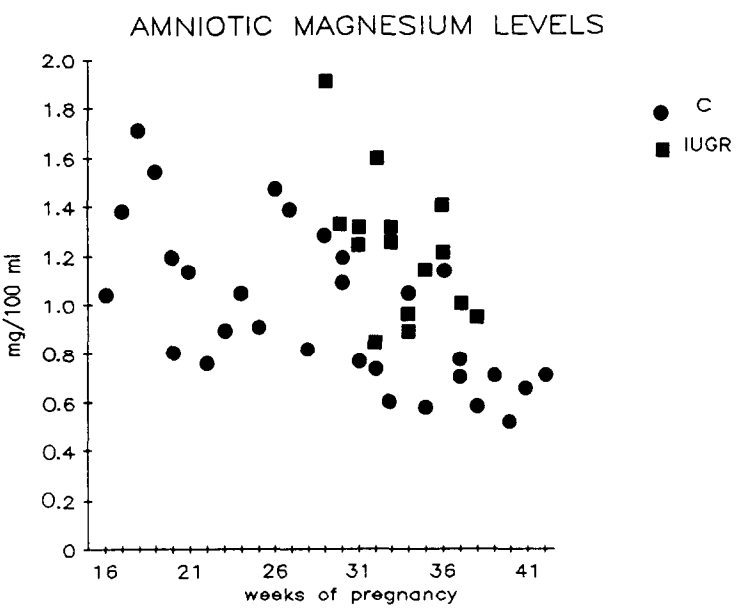

Fig. 1. Individual concentrations of magnesium in amniotic fluid of normal (C, circles) and pathological pregnancies (IUGR, squares).

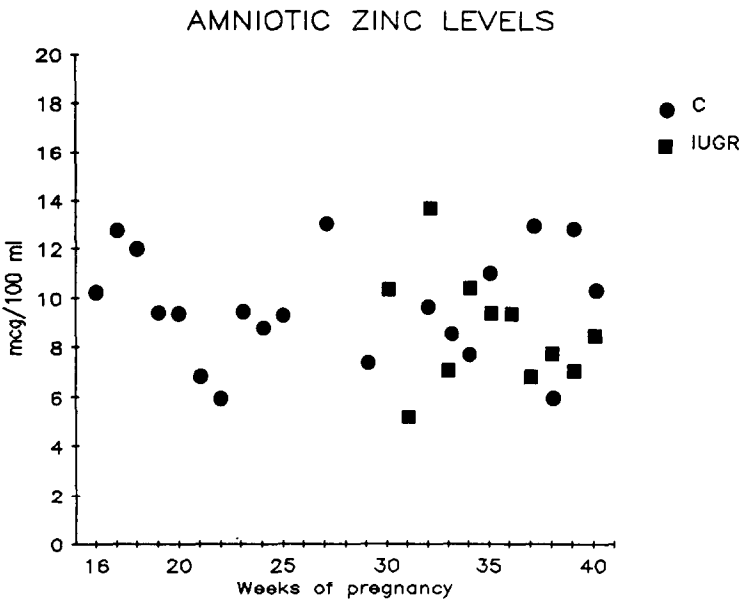

Fig. 2. Individual concentrations of zinc in amniotic fluid of normal (C, circles) and pathological pregnancies (IUGR, squares).

Fig. 2 illustrates AF Zn levels in both normal and abnormal pregnancies. In the three gestational periods we considered, the AF Zn concentrations of control patients remained substantially constant (9.41 ± 2.17 mcg/100 ml, 9.76 ± 2.03 and 10.02 ± 2.66, respectively) and no significant correlation was found with gestational age. In addition, Zn levels of pathological pregnancies did not show any significant difference when compared to controls.

No correlations were found between Mg and/or Zn levels in AF and birth weights.

Discussion

This study offers further evidence to support the concept that pregnancy is a condition characterized by a negative Mg balance. Confirming previous reports, a progressive fall in AF Mg levels takes place from the beginning of the second trimester to term [8,10,11]. Such a phenomenon could not be related to an aspecific alteration in membrane exchange of bivalent cations, since Zn levels in the same samples show more or less constant values throughout pregnancy [9]. The physiological reduction of AF Mg has been ascribed to a progressive increase of the uptake by fetal structures in view of animal experiments demonstrating the accumulation of Mg in fetal tissues [4].

The important issue of the present data is related to the high AF levels of Mg found in cases of IUGR, whether or not maternal PIH was present. Considering the main contribution of fetal urines to AF composition, this finding could be explained through an impairment of those fetal kidney mechanisms accounting for the

reduced excretion of the ion. Zn levels were unchanged in the same condition, thus indicating that the Mg excess could be regarded as specific in cases of pathological fetal growth. This is not surprising, since a mild Mg deficiency induced by diet in pregnant ewes was accompanied by increased blood pressure, hystopathological kidney lesions and a 50% reduction of the lambs birth weights: i.e., the same clinical findings of human PIH [5]. Interestingly, it was ascertained a long time ago that serum Mg levels in women with PIH were lower than normal [12], and a recent study found increased Mg plasma levels in mothers with a small for gestational age infant [13].

In our series, only 8/15 patients had PIH, while the remnant carried a IUGR fetus for unknown reason. However, it is well established that one of the main causes of IUGR is represented by a difficult oxygen and nutrient transport across the placenta to the fetus. The umbilical vein vasoconstriction is a pathogenetic factor in this respect and the 'prostaglandin theory' has been recently reviewed [14]. The elegant experiment by Altura et al. [15] in vitro indicated that Mg should also be taken into consideration. They indeed demonstrated that umbilical vessels are very sensitive to the Mg concentration in the medium: outside the physiological range, there was an increased tone and an increased sensitivity to known vasoconstrictor agents such as prostaglandin $F_{2\alpha}$ and angiotensin II.

A stronger association between Mg balance and fetal growth derives from studies where prophilactic treatments with Mg were carried out. Retrospective studies indicated that a Mg supplementation starting as early as possible was accompanied by an increased maintenance of pregnancy [16] and by a lower prevalence of IUGR, the birth weight being higher in Mg-supplemented than in the non-treated group [17]. More convincing data originate from a double-blind, prospective study of Spaetling and Spaetling [18] who compared the effects of magnesium aspartate vs. placebo. In the Mg-treated group, there were fewer preterm deliveries and higher birth weights than in the placebo-treated group. Moreover, Mg supplementation was accompanied by a reduction in maternal and neonatal morbidity.

In conclusion, these data demonstrate that AF levels of Mg are pathologically elevated in cases of IUGR, while Zn levels remain in the normal range. The mechanisms allowing this finding remained to be elucidated. Nevertheless, these data support the importance of magnesium in fetal growth.

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