Relationship of Intracellular Magnesium of Cord Blood Platelets to Birth Weight

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Magnesium (Mg^{2+}) has an important role in insulin action, and insulin stimulates Mg^{2+} uptake in insulin-sensitive tissues. Impaired biologic responses to insulin are referred to as insulin resistance. Diabetic patients and obese subjects are reported to have intracellular magnesium ($[Mg^{2+}]_i$) deficiency. Many epidemiologic studies have disclosed that restricted fetal growth has been associated with increased risk of insulin resistance in adult life. We studied the relationship of $[Mg^{2+}]_i$ in cord blood platelets to birth weight. The subjects were 19 infants who were small for gestational age (SGA) and 45 who were appropriate for gestational age (AGA). By using a fluorescent probe, mag-fura-2, we examined the basal and insulin-stimulated $[Mg^{2+}]_i$ of platelets in the cord blood. Cord plasma insulin-like growth factor-1 (IGF-1)and leptin levels were determined with the use of enzyme-linked immunosorbent assay (ELISA). Birth weight was correlated with cord plasma IGF-1 ($P < .001$) and leptin ($P < .005$). Mean basal $[Mg^{2+}]_i$, but not plasma Mg^{2+}, was lower in the SGA than in the AGA group (291 ± 149 μmol/L *v* 468 ± 132 μmol/L, $P < .001$). The basal $[Mg^{2+}]_i$ was significantly correlated with the birth weight ($P < .001$) as well as birth length ($P < .001$). At 60 seconds after stimulation with insulin, there was no significant difference in stimulated $[Mg^{2+}]_i$ between the SGA and AGA groups. Although the SGA group had low $[Mg^{2+}]_i$, the platelets had good potentiality to compensate for low $[Mg^{2+}]_i$. $[Mg^{2+}]_i$ reflects the extent of fetal growth. Decreased $[Mg^{2+}]_i$ in SGA might underlie the initial pathophysiologic events leading to insulin resistance.

MAGNESIUM (Mg^{2+}), the second most abundant intracellular divalent cation, is a cofactor of many enzymes involved in glucose metabolism.[1] Mg^{2+} has an important role in insulin action, and insulin stimulates Mg^{2+} uptake in insulin-sensitive tissues.[2-4] Mg^{2+} deficiency occurs in patients with diabetes and vascular diseases.[5,6] We and other investigators reported that insulin could modulate $[Mg^{2+}]_i$ in platelets.[3,4] Recently, we reported that $[Mg^{2+}]_i$ is lower in children with diabetes mellitus (DM) and obesity.[7] It thus appears that alterations in cellular Mg^{2+} concentration contribute to the diminished cellular activities of insulin. Platelets are often used in the study of cellular cation metabolism in diseases,[8] because they are readily available for study and are thought to share a number of features with vascular smooth muscle cells. Human platelets have been shown to have insulin receptors with similar characteristics as those in other cells.[9]

Mg^{2+} supplementation during pregnancy reduces the rate of small for gestational age (SGA).[10] There is no doubt that low birth weight is associated with adult disorders characterized by insulin resistance, such as type 2 diabetes, hypertension, dyslipidemia, and coronary heart disease.[11,12] It has been proposed that this association results from fetal programming in response to the intrauterine environment.[13] Taken together, these experimental and epidemiologic results suggest that the correlation between $[Mg^{2+}]_i$ and birth weight are important determinants of insulin resistance.

Intracellular Mg^{2+} and its role in the pathogenesis of insulin resistance are not known. Our study aimed to test the hypothesis that low $[Mg^{2+}]_i$ may be an intrinsic abnormality present in infants with low birth weight.

MATERIALS AND METHODS

Subjects

The study group consisted of 64 subjects with gestational ages ranging from 36 to 41 weeks and birth weights ranging from 1,332 to 3,822 g. Gestational age was measured by dating the last menstrual period at the time of registration. No subject was treated with any medications, including Mg^{2+}, and did not show any evidence of endocrine malfunction or recent use of drugs that might potentially alter electrolyte balance. All the mothers were Japanese with no remarkable past medical histories, and they manifested no abnormal findings during pregnancy, such as pre-eclampsia. Cord blood of diabetic mothers with pre-existing diabetes and gestational DM were excluded. Infants were excluded if they had neural tube defect, chromosomal anomaly, or other severe congenital diseases.

Definition of SGA

SGA was defined as birth weight below the 10th percentile of birth-weight-for-gestational-age using the Japanese standard birth-weight curve (Japanese Ministry of Health and Welfare Research Group 1983, revised in 1994).[14] Ponderal index (birth weight [in kilograms] divided by birth length [in meters] cubed) was used as a measure of relative birth weight. Appropriate for gestational age (AGA) was defined as birth weight, birth length, and ponderal index ≥10th percentile and ≤10th percentile of the respective mean for the gestational age.

Platelet Preparation

Cord blood samples were collected by the labor ward staff and kept at 4°C. Platelets were isolated as previously described.[15] Approximately 10 mL cord blood was drawn into 3.8%(wt/vol) acid citrate buffer (10:1, vol/vol) and was centrifuged at 200 × *g* for 10 minutes at room temperature. The platelet-rich plasma was decanted, further centrifuged at 1,000 × *g* for 10 minutes, and the cells were washed 3 times in Hepes buffer solution (HBS) containing (mmol/L) NaCl 140, KCl 5, glucose 25, $MgCl_2$ 1, NaH_2PO_4 1, Hepes 25 (pH 7.2), and EGTA 0.2. EGTA was omitted from the third washing, and 0.1% fatty acid–free bovine serum albumin was added. Platelets were counted in a Celltac counter (Nihon Kohden, Tokyo, Japan). Unless otherwise indicated, platelets were sus-

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pended in HBS at a concentration of 2 to 3 × 10^7 platelets/mL. Platelets were studied within 4 hours after blood drawing. Plasma was separated immediately, stored at −80°C, and thawed only once before analysis.

The study protocol was approved by the ethics committee of the Kansai Medical University. Written informed parental consent was obtained before recruitment.

Measurements of Intracellular Mg^{2+} Concentrations

Intracellular ionic $[Mg^{2+}]_i$ concentrations were measured with a Hitachi F-2000 fluorescence spectrophotometer (Hitachi Instruments, Tokyo, Japan) by using a Mg-specific fura-2 probe as described by Raju et al.[16] A 2-μmol/L quantity of mag-fura-2/acetoxymethyl dye was added to the platelet suspension and incubated at 37°C for 30 minutes. After loading of the dyes, the platelets were washed twice with HBS, the fura dyes were removed by centrifugation, and the platelets were resuspended in HBS. The excitation wavelengths were set at 335/370 nm, and the emission wavelength was 510 nm. Each intracellular ionic concentration was calculated as described[16,17] by using dissociation constant (Kd) = 1,500 (μmol/L). The maximum intensities were determined by disrupting the cells with 0.1% Triton in the presence of 30 mmol/L $MgCl_2$. The minimum intensities were the values determined in the presence of 60 mmol/L EDTA. $MnCl_2$ (0.05 mmol/L) was used to quench the fluorescence from extracellular dye according to the methods of Ng et al.[18] Insulin was dissolved in deionized water, and 25 μL insulin was added to 2.5 mL platelet suspension.

Enzyme-Linked Immunosorbent Assay

Cord plasma glucose was measured by using a standard assay. Cord plasma leptin levels were determined with the use of a commercially available enzyme-linked immunosorbent assay (ELISA) (Immuno-Biological Laboratories, Gunma, Japan) with a detection limit of 195 ng/L [intra-assay and interassay coefficient of variations (CVs) of 6.9% and 7.7%, respectively]. Plasma insulin-like growth factor-1 (IGF-1) assay was performed by using a commercially available ELISA (R&D Systems, Minneapolis, MN) with a detection limit of 7 pg/L (intra-assay and interassay CVs of 4.3% and 8.1%, respectively). Plasma insulin concentrations were determined with the use of a commercially available ELISA (BIOSOURCE EUROPE S.A., Nivelles, Belgium) with a detection limit of 1.08 pmol/L (intra-assay and interassay CVs of 5.3% and 9.8%, respectively).

Chemicals

All chemicals were purchased from Sigma Chemical (St Louis, MO) unless stated otherwise. Mag-fura-2/acetoxymethyl was from Molecular Probes (Eugene, OR).

Table 1. Clinical Characteristics

	IUGR (n = 19)	AGA (n = 45)	*P* Value
Gender (male/female)	8/11	22/23	
Gestational age (wk)	37.3 ± 2.5	39.3 ± 1.1	.001
Birth weight (g)	2,120 ± 307	3,110 ± 276	<.001
Birth length (cm)	45.6 ± 1.9	50.1 ± 1.2	<.001
Ponderal index (kg/m^3)	24.0 ± 2.8	24.6 ± 1.4	.230
Glucose (mmol/L)	3.36 ± 1.64	3.02 ± 1.41	.470
Leptin (ng/L)	841 ± 249	1,260 ± 926	.038
IGF-1 (μg/L)	13.8 ± 7.8	30.3 ± 15.3	<.001
Insulin (pmol/L)	13.0 ± 54	99 ± 38	.710
Plasma magnesium (mmol/L)	0.60 ± 0.10	0.61 ± 0.10	.730

Table 2. Intracellular Magnesium of Each Group

	SGA (n = 19)	AGA (n = 45)	*P* Value
Basal (μmol/L)	291 ± 149	468 ± 132	<.001
Insulin stimulated (μmol/L)	579 ± 291	712 ± 245	.068

Statistical Analysis

Data are expressed as the mean ± SD. Statistical significance was assessed by using analysis of variance (ANOVA). Outcome variables were compared between the subgroups (AGA and SGA) by using *t* tests. The correlation between cord blood $[Mg^{2+}]_i$ levels and birth size, IGF-1, and insulin were examined by linear regression and Pearson product moment correlation analyses. Leptin had a skewed distribution, and therefore the factors were expressed as the median value (interquartile range), and log-transformed when appropriate for the statistical analyses. A value of $P < .05$ was considered significant. All statistical analyses were performed using StatView software (SAS, Cary, NC).

RESULTS

Profile of Each Group

Clinical characteristics of the study subjects are shown in Table 1. No statistical differences among the groups were observed for plasma Mg^{2+}, glucose, and insulin levels. Gestational age in the SGA group was shorter than that of the AGA group. Each group did not differ significantly in terms of maternal age and parity. Leptin and IGF-1 in the SGA group were lower than those in the AGA group. There was no difference in leptin and IGF-1 values between gender. Birth weight was correlated with cord plasma IGF-1($P < .001$) and leptin ($P < .005$), but not insulin ($P = .264$).

Insulin-Stimulated Intracellular Mg^{2+}

In the SGA group, basal $[Mg^{2+}]_i$ was significantly lower than in the AGA group (291 ± 149 μmol/L *v* 468 ± 132 μmol/L, respectively, $P < .001$, Table 2). In all subjects, the basal $[Mg^{2+}]_i$ was significantly correlated with birth weight ($P < .001$, $r = 0.60$) (Fig 1) and birth length ($P < .001$, $r = 0.48$). To examine the response of platelets to insulin, we studied $[Mg^{2+}]_i$ after insulin stimulation. At 60 seconds after stimulation with 0.72 nmol/L insulin, there was no significant difference in stimulated $[Mg^{2+}]_i$ between the SGA and AGA groups (579 ± 291 μmol/L *v* 712 ± 245 μmol/L, $P = .07$; Table 2).

DISCUSSION

We found that $[Mg^{2+}]_i$ measured in umbilical cord platelets correlated significantly with infant birth weight and birth length. As $[Mg^{2+}]_i$ plays a promotive role in fetal growth, low $[Mg^{2+}]_i$ may partly be responsible for SGA. On the other hand, the fact that the prenatal environment can modify adult diseases is now firmly established and is supported by both epidemiologic data[11,12] and experimental studies.[19] Mg^{2+} deficiency occurs in adult patients with DM and vascular diseases.[5,6] However, the processes that explain the link between reduced fetal growth and insulin resistance or glucose intolerance in adult life are not understood. Previ-

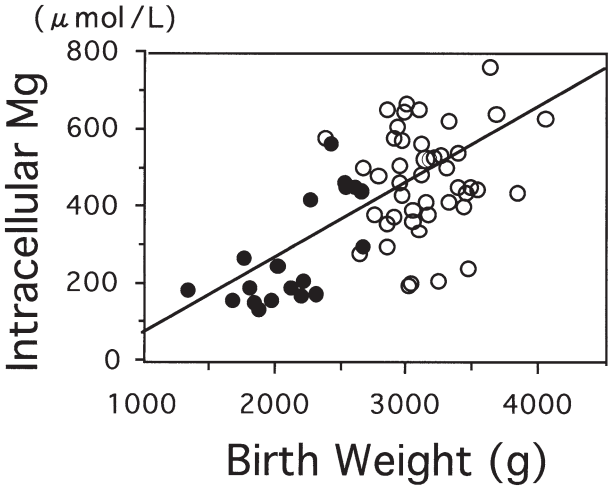

Fig 1. The correlation of intracellular Mg^{2+} and birth weight. The basal level of intracellular Mg^{2+} ($[Mg^{2+}]_i$) of cord platelets is significantly correlated with birth weight ($P < .001$, $r = 0.60$). ○, AGA; ●, SGA.

ously, we reported that children with diabetes and obesity also have $[Mg^{2+}]_i$ deficiency.[7] We further tested whether the origin of $[Mg^{2+}]_i$ deficiency may start from fetal life. Low $[Mg^{2+}]_i$ may be set in SGA by genetic factors or the intrauterine environment.

Although the SGA group has low $[Mg^{2+}]_i$, the platelets have good potentiality to compensate for low $[Mg^{2+}]_i$. We previously reported that platelets responded well to insulin in type 2 DM and obese groups in children.[7] Taken together, these findings may be integrated into the following concept that $[Mg^{2+}]_i$ is decreased before the poor reactivity to insulin occurs in platelets under insulin-resistant states. The increased percentage of insulin stimulation in the SGA group was significantly higher than in the AGA group (117% ± 97% *v* 54% ± 23%). These results were also conducted on the basis of the hypothesis that decreased $[Mg^{2+}]_i$ might underlie the initial pathophysiologic events leading to insulin resistance. High reactivity to insulin may be acquired in platelets of children with SGA to compensate for low $[Mg^{2+}]_i$.

In normal pregnancies, there is a direct correlation between the maternal blood glucose levels in the third trimester of pregnancy and the birth weight of the child.[20,21] Hattersley et al[22] postulated that fetal insulin-related growth reflects not only maternal glycemia, but also fetal genetic factors that regulate insulin secretion by the fetal pancreas and the sensitivity of fetal tissues to the effects of insulin. Insulin secretion by the fetal pancreas, or the action of insulin on insulin-dependent tissues, would all result in reduced fetal growth.[23] In our study, however, no correlation was observed between the cord plasma insulin levels and birth weight.

Serum IGF-1 is also reported to be associated with both fetal and postnatal growth.[24] Some studies demonstrated inverse relationships between IGF-1 in childhood and birth weight.[25,26] Another study reported that IGF-1 and leptin levels correlated with birth weight and length.[27] It has also been postulated that hypoxic conditions during pre-eclampsia affect cord leptin,[28] and that fetal insulin stimulates fetal adipocyte leptin production.[29] In our study, the cord plasma IGF-1 and leptin level correlated with the birth weight. IGF and leptin in utero, which may be regulated by several factors, can mediate fetal growth.

Low birth weight is a reflection of nutritional deprivation in utero. The "thrifty phenotype hypothesis" proposes that the epidemiologic associations between poor fetal growth and the subsequent development of type 2 DM and of metabolic syndrome result from the effects of poor nutrition in early life.[20] The poorly nourished mother essentially gives the fetus a forecast of the nutritional environment into which it will be born. Processes are set in motion, which lead to a postnatal metabolism adapted to survival under conditions of poor nutrition.[30] Maternal malnutrition may also have effects on $[Mg^{2+}]_I$ in the cord blood. Supplementation of Mg^{2+} will increase $[Mg^{2+}]_i$ and thereby might reduce the incidence of SGA.[10] In this study, however, none of the mothers manifested malnutritional findings during pregnancy. The gestational age of the SGA group was shorter than that of the AGA group, and $[Mg^{2+}]_i$ was correlated with gestational age ($P < .05$). But birth weight after adjustment for gestational age was much more significantly correlated with $[Mg^{2+}]_i$ ($P < .0001$), showing the independent contribution of gestational age to $[Mg^{2+}]_i$. Future research should assess the relative roles of genetics and fetal malnutrition in SGA.

Birth weight is only a crude index of early growth and indicates nothing about the success of a fetus in achieving its growth potential. $[Mg^{2+}]_i$ may be a marker of early growth restriction, which may be of future diagnostic use as an early predictor of adult diseases. Low $[Mg^{2+}]_i$, which may represent the prenatal programming of insulin resistance, has lifelong effects on metabolic regulation. Our results indicate that a biologic interpretation of the association between birth size and risk of insulin-resistant diseases should emphasize the possible underlying roles of $[Mg^{2+}]_i$.

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