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Intracellular Magnesium and Adipokines in Umbilical Cord Plasma and Infant Birth Size

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ABSTRACT: Many epidemiologic studies have disclosed that restricted fetal growth has been associated with an increased risk of insulin resistance in adulthood. We studied the relationship of intracellular magnesium ($[Mg^{2+}]_i$) in cord blood platelets to adipocytokine and birth size. The subjects were 20 infants with small for gestational age (SGA) and 45 infants with appropriate for gestational age (AGA). By using a fluorescent probe, we examined $[Mg^{2+}]_i$ of platelets in the cord blood. Cord plasma insulin, IGF-I, ghrelin, adiponectin, plasminogen activator inhibitor-1 (PAI-1), and leptin levels were determined with the use of ELISA. Mean $[Mg^{2+}]_i$ was lower in the SGA than in the AGA groups ($p < 0.001$). Adiponectin and IGF-I were also lower in the SGA than in the AGA, whereas PAI-1 was higher in the SGA. $[Mg^{2+}]_i$ was significantly correlated with birth weight, birth length, and adiponectin. Birth weight was also correlated with cord plasma IGF-I, adiponectin, and leptin. Quantitative insulin sensitivity check index (QUICKI) was lower in the SGA group than in the AGA group. $[Mg^{2+}]_i$ and adiponectin were correlated with QUICKI in all subjects. $[Mg^{2+}]_i$, as well as leptin and IGF-I, reflect the extent of fetal growth. Decreased $[Mg^{2+}]_i$ may be involved in the underlying processes to insulin resistance. **(*Pediatr Res* 62: 700–703, 2007)**

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 (1,2). It has been proposed that this association results from fetal programming in response to the intrauterine environment (3). Intracellular magnesium $(Mg^{2+})_i$ deficiency occurs in patients with diabetes and vascular diseases (4–6). Taken together, these experimental and epidemiologic results suggest that the correlation between Mg^{2+} and birth weight are important determinants of insulin resistance. We and other investigators reported that insulin could regulate intracellular Mg^{2+} ($[Mg^{2+}]_i$) in platelets (7,8). Platelets are often used in the study of cellular cation metabolism in diseases (9), 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 (10).

Mg^{2+} is a critical cofactor in numerous enzymatic reactions. However, the role of $[Mg^{2+}]_i$ in the pathogenesis of insulin resistance is not known. Moreover, the association between $[Mg^{2+}]_i$ and adipokines, *i.e.* adipocyte-secreted hormones, has not yet been studied. We previously reported the correlation with $[Mg^{2+}]_i$ and birth weight (11). As the next step, we hypothesized that $[Mg^{2+}]_i$ may affect metabolic hormones and insulin resistance in infants. We studied the correlation among $[Mg^{2+}]_i$, adipokines in cord blood and insulin resistance index.

SUBJECTS AND METHODS

The study group consisted of 65 singleton subjects with gestational ages ranging from 33 to 41 wk, and birth weights ranging from 1332 to 4030 g. Most of these subjects had been previously recruited for an observational study of $[Mg^{2+}]_i$ (11). Gestational age was measured by dating the last menstrual period at the time of registration. None of the subjects were treated with medications, including magnesium, 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 preeclampsia. Cord blood of diabetic mothers, such as preexisting diabetes and gestational diabetes mellitus, were excluded. Infants were excluded if they had multiple gestation, neural tube defect, chromosomal abnormality, or other severe congenital diseases.

Definition of small and appropriate for gestational age (SGA and AGA). SGA was defined as birth weight below –1.5 standard deviation (SD) of the Japanese standard birth weight curve (Japanese Ministry of Health and Welfare Research Group 1983, revised in 1994) (12). 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 (13). Approximately 10 mL of cord blood was drawn into 3.8% (wt/vol) acid citrate buffer (10:1, vol/vol) and was centrifuged at 200 *g* for 10 min at room temperature. The platelet-rich plasma was decanted, further centrifuged at 1000 *g* for 10 min, and the cells were washed three times in HEPES buffer solution (HBS) containing 140 mM NaCl, 5 mM KCl, 25 mM glucose, 1 mM $MgCl_2$, 1 mM NaH_2PO_4, 25 mM HEPES (pH 7.2), and 0.2 mM EGTA. EGTA was omitted from the third washing, and 0.1% fatty-acid free BSA was added. Platelets were counted in a Celltac counter (Nihon Kohden, Tokyo, Japan). Unless otherwise indicated, platelets were suspended in HBS at a concentration of 2–3 $\times$ 10^7 platelets/mL. Platelets were studied within 4 h after blood drawing.

Plasma was separated immediately, stored at –80°C, and thawed only once before analysis.

Measurements of $[Mg^{2+}]_i$ concentrations. $[Mg^{2+}]_i$ 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.* (14). Briefly, a 2 μM quantity of mag-fura-2/acetoxymethyl dye was added to the platelet suspension and incubated at 37°C for 30 min. After loading the dye, the platelets were washed twice with HBS, the fura dye was

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Abbreviations: AGA, appropriate for gestational age; **Mg^{2+},** magnesium; **$[Mg^{2+}]_i$,** intracellular magnesium; **PAI-1,** plasminogen activator inhibitor-1; **QUICKI,** quantitative insulin sensitivity check index; **SGA,** small for gestational age

Table 1. *Clinical and analytical characteristics in SGA and AGA*

	SGA (n = 20)	AGA (n = 45)
Gender (male/female)	9/11	22/23
Gestational age (wk)	37.3 ± 0.6*	39.3 ± 0.2
Birth weight (g)	2110 ± 79*	3110 ± 41
Length (cm)	5.6 ± 0.5*	0.1 ± 0.2
Ponderal index (kg/m^3)	4.0 ± 0.8	4.6 ± 1.4
Glucose (mmol/L)	3.25 ± 0.38	3.01 ± 0.21
Insulin (pmol/L)	69 ± 28	55 ± 15
QUICKI	0.35 ± 0.02**	0.41 ± 0.01

* $p < 0.001$, ** $p < 0.01$.

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 (14,15) by using dissociation constant (Kd) = 1500 (μM). The maximum intensities were determined by disrupting the cells with 0.1% Triton in the presence of 30 mM $MgCl_2$. The minimum intensities were the values determined in the presence of 60 mM EDTA. $MnCl_2$ (0.05 mM) was used to quench the fluorescence from extracellular dye according to the methods of Ng *et al.* (16).

ELISA assay. Cord plasma glucose was measured using a standard assay. Cord plasma leptin levels were determined with the use of a commercially available ELISA (Immuno-Biologic Laboratories Co., Ltd., Gunma, Japan) with a detection limit of 195pg/mL [intra- and interassay coefficients of variation (CV) of 6.9% and 7.7%, respectively]. Plasma IGF-I, and adiponectin assays were performed using commercially available ELISA (R & D Systems, Minneapolis, MN) with a detection limit of 7 pg/L and 0.80 ng/mL (intra- and interassay CV of 4.3–5.3% and 8.1–9.8%, respectively). Plasma insulin concentrations were determined with the use of a commercially available ELISA (Linco Research, Inc., St. Charles, MO) with a detection limit of 1.08 pmol/L (intra- and interassay CV of 5.3% and 9.8%, respectively). Plasma plasminogen activator inhibitor-1 (PAI-1) assay was performed using a commercially available ELISA (Hyphen Bio Med., Neuville-sur-Oise, France) with a detection limit of 0.5 ng/mL (intra- and interassay CV of 5% and 7.5%, respectively). Plasma desacyl ghrelin assay was performed using a commercially available ELISA (SCETI Co., Ltd., Tokyo, Japan) with a detection limit of 2.5 fmol/mL (intra- and interassay CV of 5% and 10%, respectively).

Quantitative insulin sensitivity check index (QUICKI). QUICKI was calculated from the cord insulin and glucose levels instead of their fasting concentrations using the following formula: QUICKI = 1/[log plasma insulin (μU/mL) + log fasting plasma glucose (mg/dL)].

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

Statistical analysis. Data were expressed as the mean ± SD. Statistical significance was assessed using ANOVA, followed by Tukey-Kramer honestly significant difference test. Outcome variables were compared between the subgroups (AGA and SGA) using t tests. The correlations between cord blood $[Mg^{2+}]_i$ levels and birth size, IGF-I, and insulin, respectively were examined by linear regression and Spearman rank correlation coefficient analyses. A value of $p < 0.05$ was considered significant. All statistical analyses were performed using StatView software (SAS Institute Inc., Cary, NC).

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

RESULTS

Profile of each group. Table 1 summarizes the clinical characteristics and anthropometric indices of SGA and AGA. No statistical differences between the groups were observed for plasma glucose, insulin levels, and ponderal index. QUICKI was lower in the SGA than in the AGA group. Gestational age in the SGA group was shorter than that of the AGA group. Each group did not significantly differ in terms of maternal age and parity. Table 2 shows the comparison of hormone concentrations between the groups. Plasma Mg^{2+} and leptin concentrations did not differ significantly between the SGA and AGA groups, whereas serum adiponectin, IGF-I and $[Mg^{2+}]_i$ were significantly lower in the SGA group. In contrast, PAI-1 was significantly higher in the SGA group than in the AGA group (Table 2). There was no difference of adipocytokines value between genders, including leptin value.

Table 2. *Comparison of the metabolic hormones between SGA and AGA*

	SGA (n = 20)	AGA (n = 45)
$[Mg^{2+}]i$ (μmol/L)	284 ± 33†	468 ± 132
Adiponectin (μg/mL)	11.4 ± 1.8**	17.1 ± 1.0
IGF-I (ng/mL)	14.3 ± 2.1**	30.3 ± 2.2
Leptin (pg/mL)	845 ± 215	1260 ± 137
Ghrelin (fmol/mL)	76.8 ± 11.0	53.7 ± 5.1
PAI-1 (ng/mL)	13.2 ± 2.5*	8.0 ± 0.9
Plasma Mg (mg/dL)	1.45 ± 0.07	1.48 ± 0.04

* $p < 0.05$, ** $p < 0.005$, † $p < 0.0001$.

Table 3. *Correlation between $[Mg^{2+}]i$ and anthropometric indices and other metabolic hormones*

Parameters	r	p Value
Gestational age	0.485	<0.0001
Birth weight	0.618	<0.0001
Length	0.476	<0.0001
Ponderal index	0.053	0.691
Glucose	0.014	0.908
Insulin	−0.138	0.253
IGF-I	0.272	0.030
QUICKI	0.592	<0.0001
Leptin	0.073	0.547
Ghrelin	−0.227	0.064
Adiponectin	0.246	0.040
PAI-1	−0.180	0.138

Correlation and multiple regression analysis of plasma adipokines and $[Mg^{2+}]_i$. The relationship between $[Mg^{2+}]_i$ and metabolic hormones or anthropometric indices are summarized in Table 3. $[Mg^{2+}]_i$ did not correlate significantly with ghrelin, insulin, leptin, or PAI-1. $[Mg^{2+}]_i$ was significantly associated with gestational age, adiponectin ($r = 0.246$, $p = 0.04$) (Fig. 1*A*), IGF-I ($r = 0.272$, $p < 0.03$), QUICKI ($r = 0.592$, $p < 0.0001$) (Fig. 1*B*) and anthropometric indices including birth weight (Fig. 1*C*) and length (Fig. 1*D*). The birth weight was by far the most powerful determinant of $[Mg^{2+}]_i$ levels. Birth weight was positively correlated with adiponectin, leptin, and IGF-I as well as $[Mg^{2+}]_i$ (Table 4).

Correlation of insulin resistant parameter and anthropometric indices. QUICKI was positively correlated with gestational age ($p < 0.0001$), birth weight ($p < 0.0001$), length ($p < 0.0001$), head ($p < 0.01$) and chest circumstance ($p < 0.0005$), and adiponectin ($p < 0.0005$), as well as $[Mg^{2+}]_i$ ($p < 0.0001$). QUICKI was negatively correlated with ghrelin ($p < 0.005$).

DISCUSSION

We previously reported that $[Mg^{2+}]_i$ measured in umbilical cord platelets correlated significantly with infant birth weight and birth length (11) and further extended the study to show the correlation with $[Mg^{2+}]_i$, insulin sensitivity index, and adipokines. As $[Mg^{2+}]_i$ plays a promotive role in fetal growth, low $[Mg^{2+}]_i$ may be partly responsible for SGA. The fact that the prenatal environment can modify adult diseases is now

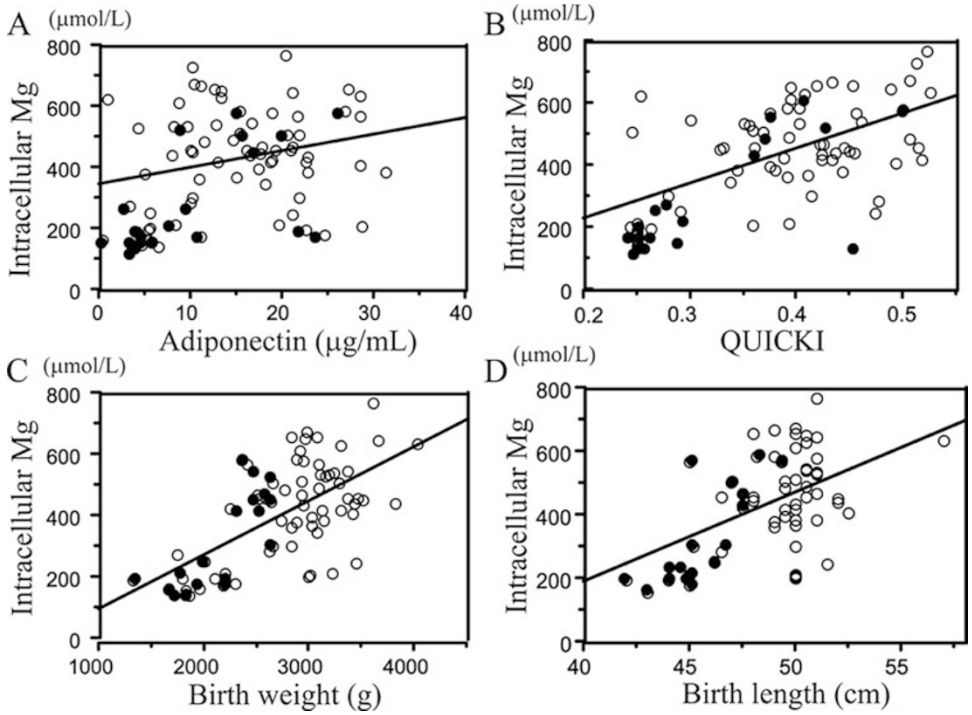

Figure 1. (*A*) The correlation between intracellular Mg^{2+} and adiponectin. The basal level of intracellular Mg^{2+} of cord blood platelets is significantly correlated with adiponectin ($p < 0.001$, $r = 0.59$). ○, AGA; ●, SGA. (*B*) The correlation between intracellular Mg^{2+} and QUICKI. The basal level of intracellular Mg^{2+} of cord blood platelets is significantly correlated with birth weight ($p < 0.001$, $r = 0.59$). ○, AGA; ●, SGA. (*C*) The correlation between intracellular Mg^{2+} and birth weight. The basal level of intracellular Mg^{2+} of cord blood platelets is significantly correlated with birth weight ($p < 0.001$, $r = 0.61$). ○, AGA; ●, SGA. Figure was adapted from (11), and another three subjects were added. (*D*) The correlation of intracellular Mg^{2+} and birth length. The basal level of intracellular Mg^{2+} of cord blood platelets is significantly correlated with birth weight ($p < 0.001$, $r = 0.48$). ○, AGA; ●, SGA.

Table 4. *Correlation between birth weight and parameters*

Parameters	*r*	*p* Value
$[Mg^{2+}]$i	0.618	<0.0001
Adiponectin	0.477	<0.0001
IGF-I	0.469	<0.0001
Leptin	0.361	0.003
Ghrelin	−0.341	0.006
PAI-1	−0.225	0.068

firmly established and is supported by both epidemiologic data (1,2) and experimental studies (17). However, the processes that explain the link between reduced fetal growth and insulin resistance or glucose intolerance in adulthood are not fully understood. Mg^{2+} deficiency occurs in adult patients with diabetes mellitus and vascular diseases (4,5). We also reported that children with diabetes and obesity have $[Mg^{2+}]_i$ deficiency (6). 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 (18) by genetic factors or intrauterine environment. Taken together, we hypothesized that decreased $[Mg^{2+}]_i$ might underlie the initial pathophysiologic events leading to insulin resistance.

A growing body of evidence suggests that adiponectin may also play a role in the development of insulin resistance (19). Adipokines are considered the group of adipose secreted proteins. It has direct actions on the liver, skeletal muscle, and vasculature, with prominent roles to improve hepatic insulin sensitivity, to increase fuel oxidation (*via* up-regulation of AMP-activated protein kinase activity) and to decrease vascular inflammation (20). In fact, it has been reported that circulating adiponectin in obese or diabetic rodents is associated with a reduction in endogenous glucose production and an increase in insulin sensitivity (19). In the present study, we have confirmed that plasma adiponectin levels of the cord blood were lower in the SGA group than in the AGA group and were correlated with $[Mg^{2+}]_i$. Lower adiponectin and $[Mg^{2+}]_i$ levels in the SGA group may suggest that the SGA group is less sensitive to insulin than in the AGA group. In addition, the higher levels of PAI-1 in the SGA group may suggest that the SGA subjects have potent tendencies to insulin resistance. However, there was no difference between the AGA and SGA groups in ponderal index, which is often used as a marker of infant fat mass. This may be due, at least in part, to the different distribution of neonatal adipose tissue, which, in contrast to adults, is mainly subcutaneous. Furthermore, birth weight was well correlated with IGF-I, leptin, and adiponectin, but not with insulin levels in the cord blood, which is in agreement with the findings of the previous report (21). Concerning fetal life, it has been postulated that not only nutritional and environmental factors during pregnancy, but also hormonal factors, are implicated in fetal growth (22–24) in addition to genetic predisposition.

Because measurement of glucose tolerance and insulin sensitivity is practically difficult and ethically not permissible in newborns, we instead quantified QUICKI calculated by cord plasma insulin and glucose levels to assess insulin resistance in fetuses. The ghrelin and adiponectin levels as well as $[Mg^{2+}]_i$ correlated positively with QUICKI, a marker of insulin resistance (25). Mean QUICKI levels in the SGA group were significantly lower than in the AGA group. Previously, surrogate indices such as homeostasis model assessment of insulin resistance or fasting glucose-to-insulin ratio in different situations have been reported be useful as parameter for insulin resistance in adults (25), while there are discussions on the validity of these markers in children (26). Although, we checked the homeostasis model assessment of insulin resistance ($p = 0.44$) and fasting glucose-to-insulin ratio ($p = 0.09$), still QUICKI showed a significantly better linear correlation with the $[Mg^{2+}]_i$. Log-transformation of data of in-

sulin and glucose values may improve the correlation of the parameters in cord blood. Nonetheless, surrogate indexes for insulin sensitivity, especially in cord blood, need to be studied further. In addition, the levels of $[Mg^{2+}]_i$ might depend on different population of platelets, which are isolated by each protocol. The study of platelet population and $[Mg^{2+}]_i$ needs to be clarified in future investigations.

In conclusion, $[Mg^{2+}]_i$ of the cord blood platelet as well as adiponectin may be a marker of early fetal growth. Low $[Mg^{2+}]_i$ may represent the prenatal programming of insulin resistance. Undernourished fetuses make metabolic adaptations that benefit them in the short term by increasing fuel availability, although these adaptations may become persistent throughout life, resulting in insulin resistance. Our results indicate a possible role of $[Mg^{2+}]_i$ in fetal life for future disorders characterized by insulin resistance.

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