Diabetes Ther (2010) 1(1):25-31.
DOI 10.1007/s13300-010-0003-7

ORIGINAL RESEARCH

Intracellular Magnesium of Obese and Type 2 Diabetes Mellitus Children

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Received: May 28, 2010 / Published online: October 26, 2010

ABSTRACT

Introduction: Magnesium is a critical cofactor in numerous enzymatic reactions. Diabetic patients and obese subjects are often reported to have intracellular magnesium ($[Mg^{2+}]_i$) deficiency. We studied the change of $[Mg^{2+}]_i$ in obese children and children with type 2 diabetes mellitus (DM2) after educational intervention or treatment. ***Methods:*** A total of 25 subjects were included: 13 with simple obesity (10 male, 3 female; mean age 16±8 years, intervention period 1.0±0.6 years), 12 with DM2 (8 male, 4 female; mean age 15±3 years, medication period 1.1±0.7 years), and 16 controls (8 male, 8 female; mean age 17±7 years). By using a fluorescent probe, mag-fura-2, we examined the basal and insulin-stimulated $[Mg^{2+}]_i$ of platelets in the blood. Plasma leptin, ghrelin, adiponectin, and resistin levels were determined with the use of enzyme-linked immunosorbent assay (ELISA). ***Results:*** Mean basal $[Mg^{2+}]_i$ was lower in the obesity (160±65 μmol/L) and DM2 groups (140±30 μmol/L) compared with the control group (330±28 μmol/L). The elevated $[Mg^{2+}]_i$ after insulin stimulation was also lower in these two groups (420±140 μmol/L, and 330±70 μmol/L, respectively) compared with the control group (690±270 μmol/L). In the DM2 group, the basal $[Mg^{2+}]_i$was significantly increased after treatment, while in the obesity group, stimulated $[Mg^{2+}]_i$ was increased after intervention. ***Conclusion:*** Platelet $[Mg^{2+}]_i$ increased after intervention in children with obesity or DM2.

Keywords: child; education; magnesium; obesity

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INTRODUCTION

Magnesium deficiency occurs in patients with diabetes and vascular diseases.[1,2] We and other investigators have reported that insulin can mediate intracellular magnesium ($[Mg^{2+}]_i$) in platelets.[3,4] Platelets are often used in the study of cellular cation metabolism in diseases[5] 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 characteristics similar to those in other cells.[6]

The association between magnesium deficiency and insulin resistance in children has been reported previously.[7] However, there are few papers that report $[Mg^{2+}]_i$ and its role in the pathogenesis of insulin resistance.[8]

Previously, we have reported that $[Mg^{2+}]_i$ is lower in children with diabetes mellitus and obese children.[9] We hypothesized that $[Mg^{2+}]_i$ increases in accordance with improvement of diabetic control in obese children and children with type 2 diabetes mellitus (DM2).

METHODS

Subjects

These study groups consisted of children with simple obesity or DM2. Diabetes was defined using the criteria of the American Diabetes Association.[10] Consequently, if the diabetes criteria were met, the patient was considered to have DM2 regardless of body mass index (BMI). Height was measured to an accuracy of 0.1 cm and weight to 0.1 kg. Blood pressure was measured using a portable sphygmomanometer after the subject had rested in the sitting position for at least 5 minutes. A child was diagnosed as obese if his or her BMI was at least in the 95th percentile for age and sex or was over 25 kg/m^2. The percentage overweight definition was based on the percentage of a reference weight that is based on age, sex, and height.[11] The control group consisted of subjects with normal blood pressure and a negative family history of diabetes mellitus. Samples from the control group were obtained once for the $[Mg^{2+}]_i$ measurements.

Ethical Considerations

This study was in compliance with the Declaration of Helsinki. The study protocol was approved by the ethics committee of the Kansai Medical University. Written informed parental consent was obtained before recruitment.

Educational Intervention and Treatment

We educated the children and parents in a formal program of intensive lifestyle modification through anticipatory guidance about healthy dietary and activity habits. According to information from the American Academy of Pediatrics,[12] we prescribed and supported healthy eating habits such as avoiding the consumption of calorie-dense, nutrient-poor foods (for example, sweetened beverages, sports drinks, fruit drinks and juices, most "fast food" types, and calorie-dense snacks). We prescribed eating timely, regular meals, particularly breakfast. We prescribed 30 minutes of daily moderate physical activity, such as walking briskly or riding a bicycle. We supported a decrease in time spent in sedentary activities, such as watching television, playing video games, or using computers for recreation. Screen time was advised to be limited to 1-2 hours per day. No obesity subject was treated with any medication, including magnesium, and none showed any evidence of endocrine malfunction or recent use of drugs that might potentially alter electrolyte balance. The patients with DM2 were treated with metformin (250-750 mg orally twice daily), glybencramide 2.5 mg twice daily, and subcutaneous insulin 0.2-0.4 units/kg body weight twice daily, in combination with lifestyle modification.

Platelet Preparation

Blood samples were collected with citrate acid buffer and kept at 4°C. Platelets were isolated as previously described.[13] Approximately 5 mL of blood was taken and added to 3.8% (w/v) acid citrate buffer (10:1, v/v) then centrifuged at 200 *g* for 10 minutes at room temperature.

The platelet-rich plasma was decanted, further centrifuged at 1000 *g* for 10 minutes, and the cells were washed three times in Hepes buffer solution (HBS) containing NaCl 140 mM, KCl 5 mM, glucose 25 mM, $MgCl_2$ 1 mM, NaH_2PO_4 1 mM, 2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonic acid (HEPES) 25 mM (pH 7.2), and ethylene glycol-bis(2-aminoethylether)-*N*,*N*,*N*',*N*'-tetraacetic acid (EGTA) 0.2 mM. 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 suspended in HBS at a concentration of $2\text{-}3\times10^7$ platelets/mL. Platelets were studied within 4 hours after blood drawing.

Measurements of Intracellular $[Mg^{2+}]_i$ Concentrations

Ionic $[Mg^{2+}]_i$ concentrations were measured with a Hitachi F-2000 fluorescence spectrophotometer (Hitachi Instruments, Tokyo, Japan) by using an Mg-specific fura-2 probe as described by Raju et al.[14] A 2 µmol/L quantity of mag-fura-2/acetoxymethyl dye (fura 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 previously,[14,15] by using the dissociation constant K_d=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. Manganese (II) chloride (0.05 mM) was used to quench the fluorescence from extracellular dye according to the methods of Ng et al.[16] Insulin was dissolved in deionized water. A total of 25 µL of insulin was added to 2.5 mL of platelet suspension. The final concentration of insulin was 0.72 nmol/L.

Enzyme-Linked Immunosorbent Assay

Plasma for enzyme-linked immunosorbent assay (ELISA) was separated immediately, stored at –80°C, and thawed only once before analysis. Plasma glucose levels were measured by the glucose oxidase methods. Plasma leptin levels were determined with the use of a commercially available ELISA kit (Immuno-Biological Laboratories Co., Ltd., 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). A plasma adiponectin assay was performed using a commercially available ELISA kit (R&D System Inc. Minneapolis, MN, USA) with a detection limit of 0.246 ng/mL (intra-assay and interassay CVs of 3.4% and 6.8%, respectively). Plasma resistin assay was performed using a commercially available ELISA kit (R&D System Inc. Minneapolis, MN, USA) with a detection limit of 0.026 ng/mL (intra-assay and interassay CVs of 5.3% and 8.2%, respectively). Plasma desacyl ghrelin concentrations were determined with the use of a commercially available ELISA kit (Mitsubishi Kagaku Iatrons, Inc., Tokyo, Japan) with a detection limit of 1.08 fmol/mL (intra-assay and interassay CVs of 5.3% and 9.8%, respectively).

Insulin concentrations were measured by means of a chemiluminescence immunological assay (Chemilumi Insulin; Kyowa Medics, Tokyo, Japan).

Chemicals

All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA), unless

stated otherwise. Mag-fura-2/acetoxymethyl was purchased from Molecular Probes (Eugene, OR, USA).

Statistical Analysis

Data were expressed as the mean±SD. Statistical significance was assessed using analysis of variance (ANOVA). Outcome variables were compared between the subgroups (obesity, DM2, and control) using *t* tests. A value of $P<0.05$ was considered significant. All statistical analyses were performed using StatView software (SAS Institute Inc., Cary, NC, USA).

RESULTS

Subjects

The obesity study group consisted of 13 subjects with simple obesity (10 male, 3 female; mean age 16±8 years, intervention period 1.0±0.6 years). The DM2 group consisted of 12 subjects with DM2 (8 male, 4 female; mean age 15±3 years, medication period 1.1±0.7 years). Five patients were treated with metformin, one with glybencramide, and two with insulin in combination with lifestyle modification. The control group consisted of 16 subjects (8 male, 8 female; mean age 17±7 years).

Profile of Each Group

A profile of the study subjects is shown in Table 1. No statistical differences among the groups were observed for serum magnesium. No significant difference in plasma leptin, adiponectin, ghrelin, resistin, and insulin levels existed between the obesity and DM2 groups (Table 2). Each group did not differ significantly in terms of medication or prescription. There was no difference of leptin values between genders (data not shown).

The Effect of Intervention or Treatment

In the obesity group, body weight, BMI, percentage overweight, and blood pressure were decreased after educational prescription. Plasma leptin and resistin were decreased after intervention. Adiponectin and ghrelin were increased but not significantly. In the DM2 group, glycated hemoglobin (HbA_{1c}), mean glucose, and blood pressure were

Table 1. Characteristics of the study participants.

	Control	Obesity	Type 2 DM
n	16	13	12
Male/female	8/8	10/3	8/4
Age, years	17±7	16±8	15±3
Duration of therapy, years	–	1.0±0.6	1.1±0.7
BMI, kg/m^2	23±6	33±5*	29±7
Systolic blood pressure, mmHg	110±12	130±12	120±13
Diastolic blood pressure, mmHg	70±12	83±19	81±13
Basal $[Mg^{2+}]_i$, μmol/L	330±28	160±65*	140±30*
Stimulated $[Mg^{2+}]_i$, μmol/L	690±270	420±140*	330±70*
Serum Mg, mg/dL	2.0±0.1	2.0±0.1	1.9±0.1

BMI=body mass index; DM=diabetes mellitus; Mg=magnesium; $[Mg^{2+}]_i$=intracellular magnesium.
*$P<0.01$ vs. control.

decreased after treatment. But there was no significant difference in adipocytokine levels after treatment (Table 2).

$[Mg^{2+}]_i$

In the obesity and DM2 groups, basal $[Mg^{2+}]_i$ was significantly lower than in the control group (Table 1). 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 of insulin, stimulated $[Mg^{2+}]_i$ in the obesity and DM groups was lower than in the control group (Table 1).

Change in $[Mg^{2+}]_i$ After Intervention or Treatment

In the DM2 group, the basal $[Mg^{2+}]_i$ significantly increased after treatment. However, there was no difference between before and after stimulated $[Mg^{2+}]_i$ levels with insulin (Table 2). Stimulated $[Mg^{2+}]_i$ decreased after treatment in only two of the 12 subjects. However, in all DM2 subjects, basal $[Mg^{2+}]_i$ increased after treatment.

In the obesity group, stimulated $[Mg^{2+}]_i$ increased after treatment. However, there was no difference in basal $[Mg^{2+}]_i$ levels between groups before and after intervention (Table 2). Basal $[Mg^{2+}]_i$ decreased after treatment in only one subject. In all obese subjects, stimulated $[Mg^{2+}]_i$ increased after treatment.

DISCUSSION

Previously, we reported that children with diabetes and obesity have $[Mg^{2+}]_i$ deficiency.[9] We further tested whether $[Mg^{2+}]_i$ deficiency may improve after management. After a short educational program or treatment for obesity and DM2, we found that $[Mg^{2+}]_i$ increased in accordance with the improvement of diabetes

Table 2. Effect of intervention or treatment.

	Obesity		Type 2 DM	
	Before	**After**	**Before**	**After**
Body weight, kg	82±24	79±21	73±20	79±21
BMI, kg/m^2	33±5	32±4	29±7	30±8
HbA_{1c}, %	5.0±0.3	5.1±0.3	7.8±3.7	5.9±1.5
Basal $[Mg^{2+}]_i$, μmol/L	160±65	210±80	140±30	180±40†
Stimulated $[Mg^{2+}]_i$, μmol/L	420±140	540±280*	330±70	360±75
Serum Mg, mg/dL	2.0±0.1	2.0±0.1	1.9±0.1	2.0±0.2
Glucose, mg/dL	95±8	110±40	190±100	150±50
Immunoreactive insulin, μU/mL	52±32	96±130	64±40	68±62
Leptin, pg/mL	7100±4100	5800±2600	2600±3700	5700±4400
Adiponectin, μg/mL	1.88±0.34	4.06±2.61	2.61±1.21	2.60±1.16
Ghrelin, fmol/mL	45.3±35.0	49.0±29.0	33.1±18.6	33.8±9.2
Resistin, ng/mL	17.8±3.3	7.5±6.1	5.9±2.6	4.2±2.2

BMI=body mass index; DM=diabetes mellitus; HbA_{1c}=glycated hemoglobin; Mg=magnesium; $[Mg^{2+}]_i$=intracellular magnesium.

*$P<0.05$ vs. before intervention or treatment.

†$P<0.01$ vs. before intervention or treatment.

control and obesity. In this study, neither magnesium supplementation nor the test for dietary magnesium intake were adopted.

Leptin, ghrelin, adiponectin, and resistin are thought to take part in the regulation of energy metabolism. These hormones have important roles in energy homeostasis, glucose, and lipid metabolism.[17] In this study, however, no significant change to levels of these hormones was observed after intervention.

In the DM2 group, in chronic diabetic states hyperglycemia may induce a decline of $[Mg^{2+}]_i$ levels. The $[Mg^{2+}]_i$ level was much lower in the DM2 group compared with the obesity group. Several factors are reported to have effects on $[Mg^{2+}]_i$. Hyperglycemia may also have an effect on magnesium transport and induce a decline of $[Mg^{2+}]_i$. However, there was no difference between before and after levels of stimulated $[Mg^{2+}]_i$ in the DM2 group. The in-vitro glucose-independent ionic effects of insulin are blunted in cells with a reduced basal $[Mg^{2+}]_i$ level.[18,19] Corsonello et al. reported that the decrease in serum magnesium concentration is correlated with fasting blood glucose, HbA_{1c}, albumin excretion, and duration of diabetes.[20] In our study, there was no correlation between plasma glucose and basal $[Mg^{2+}]_i$ levels. In addition, we did not find any correlation between HbA_{1c} levels and $[Mg^{2+}]_i$ levels. The reason for this discrepancy is considered to be the short study period. One of the study limitations is that the study group was fairly small, which might explain some of the negative findings. Further studies will be needed to consider this limitation.

In the obesity group, we did not find a significant difference in basal $[Mg^{2+}]_i$ levels before and after educational prescription. However, in the obesity group, $[Mg^{2+}]_i$ levels in response to insulin stimulation increased after treatment. The difference between the change of $[Mg^{2+}]_i$ levels in the two groups may be due to the following reasons: (1) the grade of deficiency in $[Mg^{2+}]_i$ levels was different in these groups ($[Mg^{2+}]_i$ levels in the DM2 group were much lower compared to that in the obesity group), (2) the duration of the study was short—longer treatment may have elevated basal $[Mg^{2+}]_i$ levels in the obesity group and/or stimulated $[Mg^{2+}]_i$ levels in the DM2 group.

Insulin has specific ionic effects that stimulate the transport of magnesium from extracellular to intracellular components, thus increasing $[Mg^{2+}]_i$.[3] The mechanism by which insulin acutely increases $[Mg^{2+}]_i$ is still unclear. Insulin regulates cellular Mg^{2+} metabolism in part via an increase in the affinity for Na^+ of the Na^+/Mg^{2+} exchange and phosphatidylinositol 3-kinase activation.[21] In turn, $[Mg^{2+}]_i$ is critical in the phosphorylation of the tyrosine kinase of the insulin receptor as well as all other protein kinases. Magnesium deficiency may result in disorders of tyrosine kinase activity on the insulin receptor and events related to the development of post-receptor insulin resistance.[22] The lower the basal $[Mg^{2+}]_i$, the greater the amount of insulin required to metabolize the same glucose load, indicating decreased insulin sensitivity.[23]

In obesity, platelet $[Mg^{2+}]_i$ is decreased before the poor insulin reactivity occurs in the platelets. The present study was conducted on the basis of the hypothesis that decreased $[Mg^{2+}]_i$ might underlie the initial pathophysiologic events leading to insulin resistance. In addition, $[Mg^{2+}]_i$ increased in accordance with the improvement of diabetic control and obesity.

CONCLUSION

Platelet $[Mg^{2+}]_i$ levels increased after intervention in children with obesity or DM2.

ACKNOWLEDGMENTS

This work was supported by the Danone Institute of Japan with the financial support

of the 2009 DIJ Research Grant and the Mami Mizutani Foundation.

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