

NOTE

Plasma and Erythrocyte Magnesium Levels Are Correlated with Oxygen Uptake in Patients with Non-Insulin Dependent Diabetes Mellitus

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Abstract. The relationship between magnesium levels and oxygen uptake in patients with non-insulin dependent diabetes mellitus (NIDDM) without apparent visceral dysfunction was studied. Magnesium levels in plasma, erythrocytes and urine as well as oxygen uptake parameters were determined in NIDDM patients and controls. Oxygen uptake parameters were measured with an exercise ergometer and expired gas analysis according to Wasserman *et al.* Low oxygen uptake in NIDDM patients was correlated significantly with plasma and erythrocyte magnesium levels, but not with urinary magnesium excretion. In NIDDM patients, higher correlation coefficients were seen between oxygen uptake parameters at peak in men and at the anaerobic threshold in women and plasma or erythrocyte magnesium levels. These results confirm previous results indicating that plasma and erythrocyte magnesium levels in NIDDM patients are decreased, and further demonstrate that the decreased magnesium levels are positively correlated with oxygen uptake. Although the mechanism remains to be established, it is possible that the magnesium deficiency in NIDDM patients due to environmental or genetic factors may result in low oxygen uptake and decreased work capacity.

Key words: Oxygen uptake, Magnesium, Non-insulin dependent diabetes mellitus (NIDDM)

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DIABETES mellitus is a heterogeneous syndrome characterized by an absolute or relative deficiency of insulin and/or insulin resistance. A previous study in subjects with non-insulin dependent diabetes mellitus (NIDDM) has shown that plasma magnesium is inversely correlated with the degree of glycemic control [1]. A recent report suggested that insulin resistance in subjects with NIDDM impaired the ability of insulin to stimulate magnesium as well as glucose uptake [2].

On the other hand, insulin sensitivity is largely determined by physical exercise. In healthy

humans, a strong correlation between whole-body insulin-stimulated glucose uptake and maximal oxygen uptake has been reported [3, 4]. Furthermore, acute physical exercise improved sensitivity to insulin and responsiveness of glucose uptake [5, 6]. High-intensity exercise also increased glucose metabolism in obese subjects with insulin resistance [4]. An association between a low level of physical activity and an increased occurrence of NIDDM was also demonstrated in two large studies [7, 8].

Since magnesium, the second most abundant intracellular cation, is an essential factor in the activity of more than 300 enzymes, in particular those involved in cellular energy expenditure [9], the elucidation of a direct relationship between magnesium metabolism and oxygen uptake in NIDDM is of interest. In this paper, a positive

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correlation between plasma or erythrocyte magnesium levels and oxygen uptake in NIDDM is reported.

Subjects and Methods

Subjects

Eighty-nine patients (48 men and 41 women) with NIDDM and 31 normal subjects (13 men and 18 women) were enrolled in this study. All subjects were evaluated for glucose tolerance and physical fitness at our hospital. No subject showed apparent visceral dysfunction. All tests were conducted while patients were on a restricted diet prescribed individually and while controls followed their usual diet. No subject received diuretics or cathartics containing magnesium. Laboratory findings on serum levels of albumin, blood urea nitrogen and creatinine were within reference intervals, indicating no renal or chronic gastro-intestinal dysfunction.

Determination of magnesium

Heparinized whole blood drawn 3 to 4 h after the subject's most recent meal was divided into 2 portions, one for plasma studies after centrifugation and the other for studies of whole blood. After deproteinization with 0.2% hydrochloric acid and 3% trichloroacetic acid, magnesium concentrations in whole blood and plasma were determined with a graphite-furnace atomic-absorption spectrophotometer with Zeeman background correction (Hitachi Z-6100, Hitachi Co., Tokyo, Japan). Magnesium concentrations in erythrocytes were calculated by means of the following formula which considers the hematocrit value [10].

$$\begin{aligned} \text{Erythrocyte Mg (mg/dl in packed cells)} = \\ (\text{Whole blood Mg}/(\text{Ht}-2)) \times 100 - \text{plasma Mg} \\ \times (100 - (\text{Ht}-2))/(\text{Ht}-2) \end{aligned}$$

(Mg: magnesium, Ht: hematocrit)

Urine samples were collected at the same time as blood was drawn. The urinary magnesium concentration was determined by a colorimetric method (xylidyl blue reaction) [11]. The coefficient of correlation between the colorimetric method and the atomic-absorption method was 0.97. Results were expressed in terms of mg/gram creatinine

(mg/g-Cr).

Exercise load test and expired gas analysis

The exercise load test was performed with a cycle ergometer (CORIVAL 400, Lode BV, Groningen, The Netherlands) and an expired gas analyzer (Aeromoniter 280S, Minato Medical Science Co., Osaka, Japan) under electrocardiogram (CARDIO-PRO FX-4211, Fukuda Electric Co., Tokyo, Japan) monitoring. After the subject rested for 3 min, a constant work load of 20 watts/min for 10 min was performed with subsequent 20 watts/min increases in men and 10 watts/min in women until the subjects reached their individual peak exercise levels. This was followed by a 3 min recovery period with no loading. In accordance with the method of Wasserman *et al.* [12–14], various oxygen uptake parameters were measured: an anaerobic threshold (AT) point by the V-slope method, peak VO_2 , VO_2 /body weight (BW) at AT and peak, and VO_2 /heart rate (HR) at AT and peak. These parameters were determined by a computer program (AT system disk, version ASTS, Minato Medical Science Co.) in the gas analyzer. Since the exercise load test was performed for the assessment of physical work capacity for each individual including NIDDM patients as well as controls, data on NIDDM patients were shown according to the current type of therapy.

Statistical analysis

Analysis of variance followed by Duncan's multiple comparison and regression analysis were used for the statistical analysis. When the *P* value was less than 0.05, the difference or correlation was considered as significant.

Results

Clinical background of the subjects enrolled in the study

The clinical background of NIDDM patients and control subjects enrolled in this study is shown in Table 1 (men) and Table 2 (women). NIDDM patients were further divided into three groups according to their current therapy: dieting only

Table 1. Clinical background (men)

	Controls	NIDDM patients with current therapy of		
		dieting	sulfonylurea	insulin
Age (year)	51.8 ± 3.1 (13) ^{a)}	51.6 ± 2.4 (10)	57.0 ± 1.8 (25)	41.2 ± 3.5 (13) ^{b)}
Height (cm)	165 ± 7 (13)	164 ± 1 (10)	164 ± 1 (25)	167 ± 2 (13)
Weight (kg)	60.6 ± 6.2 (13)	65.8 ± 3.3 (10)	64.9 ± 2.0 (25)	59.4 ± 1.8 (13)
BMI	22.5 ± 2.5 (13)	24.3 ± 1.1 (10)	24.0 ± 0.4 (25)	21.3 ± 0.8 (13)
HbA1c (%)	5.6 ± 0.1 (13)	6.7 ± 0.5 (10)	8.4 ± 0.3 (25) ^{b)c)}	8.9 ± 0.7 (13) ^{b)c)}
Urinary C-peptide (μg/g·Cr)	63.5 ± 6.7 (7)	60.1 ± 16.8 (7)	87.4 ± 17.7 (13) ^{b)c)}	44.6 ± 16.5 (9) ^{b)c)}

a): mean ± SEM (n), b): $P < 0.01$ (vs. controls), c): $P < 0.01$ (vs. diet). BMI, body mass index; HbA1c, hemoglobin A1c.

Table 2. Clinical background (women)

	Controls	NIDDM patients with current therapy of		
		dieting	sulfonylurea	insulin
Age (year)	55.6 ± 2.2 (18) ^{a)}	61.8 ± 2.9 (8)	60.3 ± 2.3 (18)	53.2 ± 4.2 (15)
Height (cm)	152 ± 6 (18)	152 ± 2 (8)	151 ± 1 (18)	152 ± 2 (15)
Weight (kg)	58.0 ± 7.4 (18)	56.3 ± 2.3 (8)	57.5 ± 1.7 (18)	57.7 ± 2.0 (15)
BMI	25.0 ± 0.8 (18)	24.5 ± 0.9 (8)	25.1 ± 0.7 (18)	25.0 ± 0.9 (15)
HbA1c (%)	5.5 ± 0.2 (18)	6.3 ± 0.4 (8)	8.2 ± 0.3 (18) ^{b)c)}	9.4 ± 0.4 (15) ^{b)c)}
Urinary C-peptide (μg/g·Cr)	91.4 ± 15.2 (6)	111.8 ± 16.4 (8)	99.7 ± 16.9 (15)	44.6 ± 1.6 (9) ^{b)c)}

a): mean ± SEM (n), b): $P < 0.01$ (vs. controls), c): $P < 0.01$ (vs. diet). BMI, body mass index; HbA1c, hemoglobin A1c.

(diet), oral hypoglycemic agents (sulfonylureas) (drug), and insulin preparation group (Novolin 30R and/or Novolin R (Novo Nordisk Pharma Co., Tokyo, Japan) (insulin). Differences in age, height, weight and body mass index (BMI) among the four groups were not significant, except for the age of men in the insulin group (41.2 ± 3.5). Although patients in the insulin group were younger than in the other groups, their clinical course and laboratory findings did not indicate insulin dependent diabetes mellitus (IDDM). HbA1c levels in drug and insulin groups were significantly higher than in control and diet groups. Urinary C-peptide excretion levels (μg/g·Cr) were higher only in the drug group in men and lower in the insulin group for both sexes as compared with the control and diet groups.

Oxygen uptake parameters and magnesium levels

Results of the various oxygen uptake parameters and the magnesium levels in the controls and NIDDM groups are shown in Table 3 (men) and Table 4 (women). Among men in all 3 groups

with NIDDM, peak VO_2 in drug and insulin groups and peak VO_2/BW decreased significantly as compared with controls. On the other hand, in women, VO_2 at AT point, peak VO_2 , and VO_2/BW at AT in NIDDM with drug or insulin therapy decreased significantly as compared with both controls and those in the diet group.

Plasma and erythrocyte magnesium levels showed a tendency to be decreased in both sexes, although a significant reduction was seen only in female NIDDM patients receiving drug or insulin therapy as compared with the control or diet groups.

The urinary magnesium excretion in NIDDM patients showed a tendency to be decreased in both sexes as compared with controls, although the differences were not statistically significant.

Correlation between oxygen uptake parameters and magnesium levels

Table 5 shows the coefficients of correlation between oxygen uptake parameters and magnesium concentrations in plasma, erythrocytes

Table 3. Oxygen uptake parameters and magnesium levels in controls and diabetics (men)

	Controls	NIDDM patients with current therapy of		
		dieting	sulfonylurea	insulin
VO ₂ (AT)(ml/min)	1039 ± 80 (13) ^{a)}	1095 ± 56 (10)	939 ± 62 (25)	917 ± 111 (13)
VO ₂ (peak)(ml/min)	1970 ± 116 (13)	1722 ± 107 (10)	1457 ± 109 (25) ^{b)}	1515 ± 174 (13) ^{b)}
VO ₂ /BW (AT)(ml/min/kg)	17.1 ± 1.3 (13)	17.0 ± 1.1 (10)	14.4 ± 0.8 (25)	15.3 ± 1.7 (13)
VO ₂ /BW (peak)(ml/min/kg)	32.6 ± 2.1 (13)	26.6 ± 1.7 (10) ^{b)}	22.2 ± 1.4 (25) ^{b)}	25.2 ± 2.6 (13) ^{b)}
VO ₂ /HR (AT)(ml/beat)	8.8 ± 1.4 (13)	9.4 ± 0.3 (10)	8.3 ± 0.5 (25)	7.8 ± 0.8 (13)
VO ₂ /HR (peak)(ml/beat)	11.5 ± 2.2 (13)	11.7 ± 0.5 (10)	10.3 ± 0.7 (25)	9.9 ± 1.1 (13)
Plasma Mg (mg/dl)	2.18 ± 0.41 (12)	2.12 ± 0.05 (9)	2.10 ± 0.02 (24)	2.10 ± 0.19 (13)
Erythrocyte Mg (mg/dl)	6.98 ± 0.19 (7)	7.03 ± 0.70 (8)	6.36 ± 0.38 (13)	5.88 ± 0.53 (6)
Urinary Mg (mg/g-Cr)	84.6 ± 8.0 (6)	60.4 ± 7.9 (9)	62.7 ± 6.0 (15)	66.3 ± 5.5 (11)

a): mean ± SEM (n), b): $P < 0.01$ (vs. controls). BW, body weight; HR, heart rate; AT, anaerobic threshold; Mg, magnesium.

Table 4. Oxygen uptake parameters and magnesium levels in controls and diabetics (women)

	Controls	NIDDM patients with current therapy of		
		dieting	sulfonylurea	insulin
VO ₂ (AT)(ml/min)	889 ± 58 (18) ^{a)}	896 ± 65 (8)	702 ± 44 (18) ^{b)c)}	730 ± 50 (15) ^{b)c)}
VO ₂ (peak)(ml/min)	1172 ± 68 (18)	1118 ± 63 (8)	1023 ± 83 (18) ^{b)c)}	1025 ± 70 (15) ^{b)c)}
VO ₂ /BW (AT)(ml/min/kg)	15.6 ± 1.0 (18)	15.9 ± 1.1 (8)	12.3 ± 0.7 (18) ^{b)c)}	12.6 ± 0.7 (15) ^{b)c)}
VO ₂ /BW (peak)(ml/min/kg)	20.5 ± 1.2 (18)	20.0 ± 1.1 (8)	17.7 ± 1.2 (18)	17.7 ± 0.9 (15)
VO ₂ /HR (AT)(ml/beat)	7.2 ± 1.3 (18)	6.9 ± 0.4 (8)	5.8 ± 0.4 (18)	7.1 ± 0.4 (15)
VO ₂ /HR (peak)(ml/beat)	8.1 ± 1.6 (18)	7.6 ± 0.4 (8)	7.3 ± 0.5 (18)	8.1 ± 0.4 (15)
Plasma Mg (mg/dl)	2.16 ± 0.05 (17)	2.18 ± 0.07 (8)	1.92 ± 0.05 (18) ^{b)c)}	1.97 ± 0.02 (14) ^{b)c)}
Erythrocyte Mg (mg/dl)	6.48 ± 0.36 (11)	7.27 ± 0.36 (5)	6.10 ± 0.60 (7)	5.76 ± 0.53 (6)
Urinary Mg (mg/g-Cr)	103.2 ± 7.6 (10)	101.4 ± 13.2 (6)	95.4 ± 11.8 (13)	92.5 ± 8.5 (15)

a): mean ± SEM (n), b): $P < 0.01$ (vs. controls), c): $P < 0.01$ (vs. diet). BW, body weight; HR, heart rate; AT, anaerobic threshold; Mg, magnesium.

Table 5. Correlation between oxygen uptake parameters and magnesium levels

		Plasma Mg		Erythrocyte Mg		Urinary Mg	
		men	women	men	women	men	women
VO ₂ (AT)	NIDDM	0.326 (44) [#]	0.400 (40) [*]	0.255 (25)	0.594 (19) ^{**}	-0.060 (33)	-0.010 (34)
	Control	0.478 (12)	0.187 (17)	0.544 (7)	0.648 (14) [*]	-0.179 (5)	-0.139 (11)
VO ₂ (Peak)	NIDDM	0.402 (44) ^{**}	0.147 (40)	0.412 (25) [*]	0.370 (19)	-0.199 (33)	-0.011 (34)
	Control	0.481 (12)	0.258 (17)	0.496 (7)	0.543 (14) [*]	-0.096 (5)	-0.079 (11)
VO ₂ /BW (AT)	NIDDM	0.260 (44)	0.506 (40) ^{**}	0.226 (25)	0.487 (19) [*]	-0.021 (33)	-0.043 (34)
	Control	0.567 (12)	0.260 (17)	0.324 (7)	0.373 (14)	-0.194 (5)	-0.023 (11)
VO ₂ /BW (Peak)	NIDDM	0.341 (44) [*]	0.268 (40)	0.403 (25) [*]	0.253 (19)	-0.216 (33)	-0.030 (34)
	Control	0.498 (12)	0.379 (17)	0.129 (7)	0.374 (14)	-0.186 (5)	-0.179 (11)
VO ₂ /HR (AT)	NIDDM	0.412 (44) ^{**}	0.140 (40)	0.621 (25) ^{**}	0.415 (19)	-0.179 (33)	-0.051 (34)
	Control	0.593 (10)	0.253 (17)	0.765 (7)	0.394 (14)	-0.123 (5)	-0.033 (11)
VO ₂ /HR (Peak)	NIDDM	0.463 (44) ^{**}	0.040 (40)	0.661 (25) ^{**}	0.308 (19)	-0.298 (33)	-0.102 (34)
	Control	0.293 (10) [*]	0.379 (17)	0.298 (6)	0.438 (14)	-0.049 (5)	-0.129 (11)

#: Coefficients of correlation are calculated in NIDDM patients and controls (number of cases in parenthesis) only whose data are available. *: $P < 0.05$, **: $P < 0.01$. BW, body weight; HR, heart rate.

and urine in NIDDM patients and control only whose data were available. There was no statistically significant difference in age of each group between NIDDM and controls, and among plasma, erythrocyte and urinary magnesium. A statistically significant correlation was observed between oxygen uptake parameters and plasma or erythrocyte magnesium levels in NIDDM men, except VO_2/BW at AT in plasma and VO_2 and VO_2/BW at AT in erythrocytes. In NIDDM women, a significant correlation was only observed in both VO_2 and VO_2/BW at AT. In controls, a similar correlation between oxygen uptake parameters and plasma or erythrocyte magnesium levels was observed, although several discordances were noticed due to differences in the number of cases.

None of the oxygen parameters correlated significantly with urinary magnesium levels.

Discussion

This is the first study on magnesium metabolism in NIDDM patients with exercise intolerance. In these patients, oxygen uptake was decreased and the level of decrease correlated with plasma and erythrocyte magnesium concentrations, but not with the urinary magnesium excretion. Oxygen uptake, which represents physical work capacity, is known to decrease with aging [17, 18]. It has also been reported that results of oxygen uptake parameters were affected by sex, height or body weight [19, 20]. Among the study subjects, there were no significant differences in age with the exception of men in the insulin group (Tables 1, 2). Nevertheless, oxygen uptake in the insulin group showed a more significant reduction than in drug and control groups (Tables 3, 4). No differences were seen in the height, weight and body mass index (BMI) among controls and the 3 groups of NIDDM patients. Glycohemoglobin (HbA1c) levels were higher in the drug and insulin groups than in the control and diet therapy groups (Tables 1, 2). Urinary C-peptide excretion was higher in male NIDDM patients on drug therapy and lower in both sexes in the insulin group than in the other groups. This clinical background of the subjects that was obtained indicates that the observed data on oxygen uptake and magnesium levels can be reliably compared among the groups.

Magnesium concentrations in plasma and erythrocytes showed a tendency to be lower the NIDDM groups than in the control group, but significant decreases were seen only in NIDDM women in the drug and insulin groups (Tables 3, 4). These results agreed with previous reports stating that reduced magnesium plasma levels were frequently seen in NIDDM [1, 17, 18]. Although the cause of the low plasma magnesium concentration observed in diabetic individuals is not yet fully understood, it is highly probable that glucosuria contributes to magnesium deficiency by causing excessive urinary loss [21]. In the present study, urinary magnesium excretion in NIDDM patients did not show significant changes. On the contrary, a tendency toward lower urinary magnesium excretion was observed in the NIDDM group. This discrepancy may be explained by the extent of therapeutic intervention. In the present study, the blood and urine samples were taken in an outpatient clinic from the NIDDM patients who had already been treated for various periods. Severe hypomagnesemia and hypermagnesuria were only seen in patients with high blood glucose and glucosuria [1]. Although HbA1c in the current patients was higher than in the controls, their glucosuria was not severe enough to cause hypomagnesemia and hypermagnesuria (Tables 3, 4). On the other hand, a reduced supply of magnesium due to a restricted diet might be the cause of hypomagnesemia with the low plasma and erythrocyte magnesium and the unchanged urinary magnesium.

An association between a low level of physical activity and an increased occurrence of NIDDM has been demonstrated in two large prospective studies [7, 8]. Insulin resistance is considered to be a cardinal feature in the pathogenesis of NIDDM [22]. It has been shown that this condition correlates with both low oxygen uptake [23] and low plasma magnesium levels [2]. In the present study, a direct relationship between magnesium levels in plasma and erythrocytes and oxygen uptake is demonstrated. Although the erythrocyte is not classically recognized as insulin sensitive, magnesium levels in the erythrocyte are considered to represent the intracellular magnesium level [1, 17, 24, 25]. Hypomagnesemia in NIDDM has been reported to be accompanied by unchanged [24] or decreased [1] erythrocyte magnesium levels. The

results reported here support the latter. This evidence indicates that magnesium deficiency, both extracellular and intracellular, is a characteristic of chronic and stable NIDDM patients as a result of decreased sensitivity to insulin in erythrocytes.

There was no significant correlation between HbA1c levels and oxygen uptake parameters (data not shown). The insulin group showed the poorest control and the lowest oxygen uptake according to most of the parameters for the 4 groups (Tables 3, 4). This suggests that factors other than glycemic control, for example, genetic factors, magnesium intake or metabolism might be causes of the decreased oxygen uptake in NIDDM.

Data on the correlation between oxygen uptake parameters and magnesium levels indicate a clear sex difference (Table 5). Sex differences in physical activity have been generally recognized. A sex difference in oxygen uptake has been reported previously [19, 20]. The present data also indicate some aspects of sex difference in physical activity, although the precise mechanism remains to be clarified.

The mechanism of magnesium deficiency in NIDDM patients is also currently unclear. One explanation is urinary loss of magnesium due to decreased tubular reabsorption [21]. Another possibility is reduced dietary magnesium intake [26, 27]. Several reports suggested that routine supplementation of magnesium is beneficial in

improving insulin response and glycemic control in NIDDM patients [28, 29]. It would be of interest to study the effects of magnesium treatment on energy metabolism and physical work capacity in NIDDM patients.

In summary, the current study confirms previous results showing that plasma and erythrocyte magnesium levels were reduced in NIDDM, and further demonstrates that the reduced magnesium levels are correlated with oxygen uptake and physical work capacity. It appears that magnesium is an important factor in physical work capacity in NIDDM patients.

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