# Magnesium Deficiency in IDDM Related to Level of Glycosylated Hemoglobin

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**SUMMARY**

**Magnesium and potassium were analyzed in plasma, erythrocytes, and urine collected during 24 h and in muscle biopsies from 25 subjects with insulin-dependent, type I diabetes mellitus (IDDM). Magnesium was also measured in mononuclear cells. The results were compared with those of 28 healthy controls, and were also correlated with the degree of diabetic control as estimated by analysis of the level of glycosylated hemoglobin ($HbA_{1c}$). Subjects with IDDM had significantly lower muscle ($P < 0.01$) and plasma ($P < 0.001$) concentrations of magnesium compared with those of healthy controls. The $HbA_{1c}$ levels correlated significantly with the concentrations of magnesium in muscle ($r = -0.62$, $P < 0.001$), plasma ($r = -0.62$, $P < 0.001$), and mononuclear cells ($r = -0.47$, $P < 0.05$). The results indicate that some patients with IDDM have lowered contents of magnesium in striated muscular and/or plasma, and that those parameters are dependent on the degree of diabetic control. DIABETES 1986; 35:459–63.**

Magnesium is essential for humans and acts as a cofactor in several enzyme systems, i.e., ATPase-dependent reactions.[1–3] The concentration of magnesium ions may be related to carbohydrate metabolism in several ways. For instance, insulin increases uptake of magnesium in muscle,[4,5] and the release of glucagon from cultured pancreatic cells is stimulated under magnesium-deficient conditions;[6] furthermore, glucose ingestion inhibits the renal tubular reabsorption of magnesium.[7]

Subjects with diabetes mellitus have been found to have decreased concentrations of magnesium in plasma and serum,[8–15] but normal[16] and even high levels[17] have been reported, depending on how the clinical materials have been selected and subdivided. Urinary excretion of magnesium is often raised in patients with diabetes mellitus.[9,13,15]

Magnesium is mainly an intracellular ion[18] and, consequently, analysis of intracellular magnesium may be essential to confirm a suspected deficiency. A good estimate of the intracellular concentration of magnesium is achieved by analysis of magnesium in striated muscle.[19] Studies based on analyses of magnesium in muscle biopsies from patients with diabetes mellitus, however, have given conflicting results and the levels of magnesium have been reported to be low[9] or normal.[8] Normal concentrations of magnesium have been found in the erythrocytes[8,15] and leukocytes[8] of diabetic individuals.

In the present study, analysis of magnesium in plasma, erythrocytes, mononuclear cells, urine, and muscle tissue was performed in 25 patients with insulin-dependent diabetes mellitus (type I, IDDM) and in 28 healthy controls. Potassium was also analyzed, as its metabolism is closely related to that of magnesium.[19–23] In the diabetic patients, these findings were correlated with concentrations of glycosylated hemoglobin ($HbA_{1c}$). The purpose of this study was thus to gather further information about the relation between magnesium status and the degree of control of the disease in IDDM.

## MATERIALS AND METHODS

**Subjects.** Twenty-five subjects with IDDM were included in the study (Table 1). All subjects presented with onset of disease before the age of 30 yr and/or a tendency to ketonuria. The mean duration of the disease was 19 yr (range 2–52 yr) and all were treated with insulin. The subjects all had normal blood pressure and only one had a raised level of serum creatinine (>115 μmol/L). None of the subjects was treated with drugs known to disturb the balance of electrolytes in the body. Magnesium and potassium were measured in plasma, erythrocytes, and urine collected during 24 h and in muscle biopsies; magnesium was also analyzed in mononuclear cells. The muscle biopsy and all blood samples, including

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TABLE 1
Patient characteristics in diabetic (IDDM) and healthy control subjects

| | N | Sex | Age | | Body mass index |
|---|---|---|---|---|---|
| | | | Mean | Range | |
| IDDM | 25 | 17 M, 8 F | 39 | 19–64 | 23.1 (0.50) |
| Healthy controls | 28 | 19 M, 9 F | 35 | 17–69 | 23.1 (0.46) |

N = number of subjects, M = male, F = female. and age = yr. The values for body mass index (wt/ht$^2$) are given ε means with SD in parentheses.

that for $HbA_{1c}$ analysis, were obtained fror each subject at the same time after an overnight fast. Urii ɜ collection was also ended at the same time in the morning ε ter the overnight fast. The results were compared with thos.ɹ of 28 matched (in respect to sex, age, and body mass index) and healthy control subjects (Table 1).

**Muscle biopsy analysis.** The muscle biopsy was taken from the lateral portion of the quadriceps muscle by the method described by Bergström.[20] The biopsy was dissected free from blood, visible fat, and connective tissue on a piece of quartz glass and then attached to a preweighed hook made of platinum wire. The hook with the muscle biopsy attached was placed in an oven (100–110°C) overnight. Neutral fat was extracted with 3.0 ml petroleum ether during 3 h in a tube made of quartz glass. After another 3 h at 100–110°C, fat-free dry solid (FFDS) weight was calculated. Electrolytes were extracted from the muscle biopsy with 0.5 ml of 1 M nitric acid over 18 h in a quartz glass tube. Samples and standards were diluted with a solution consisting of $LaCl_3$ (10,000 ppm) and CsCl (1000 ppm) in 1% nitric acid. All magnesium and potassium analyses were performed by atomic absorption spectroscopy (Varian AA6).

**Plasma analysis.** Peripheral venous blood (7–10 ml) was collected in heparinized vacutainer tubes (Becton-Dickinson, Rutherford, New Jersey) from an antecubital vein. Plasma was separated from red blood cells by centrifuging at 300 × *g* for 10 min.

**Mononuclear cell analysis.** Mononuclear cells were separated from peripheral venous whole blood using the Isopaque-Ficoll technique.[24] The cells were washed three times at 300 × *g* for 5 min with 13–14 ml of 0.9% NaCl at room temperature. A cell suspension consisting of 1–4 × $10^6$ cells/ml was obtained. Cell protein was measured from an aliquot of the same sample using the method described by Lowry and colleagues.[25] Magnesium concentrations in cell suspensions were determined after lysis in 3% nitric acid in 1% $LaCl_3$ for 0.5 h. Magnesium contents in mononuclear cells are expressed as nanomoles per milligram protein.

TABLE 2
Comparisons between mean level of magnesium in muscle (mmol/100 g FFDS), plasma (mmol/L), erythrocytes (mmol/L), mononuclear cells (nmol/mg protein), and mean urinary excretion of magnesium during 24 h (mmol/24 h) in subjects with IDDM (N = 25) and healthy control subjects (N = 28)

| | IDDM | Healthy controls | |
|---|---|---|---|
| Mg/muscle | 4.11 (0.22) | 4.27 (0.19) | $P < 0.01$ |
| Mg/plasma | 0.69 (0.08) | 0.78 (0.08) | $P < 0.001$ |
| Mg/erythrocytes | 2.28 (0.29) | 2.10 (0.39) | NS |
| Mg/mononuclear cells | 69.4 (16) | 73.0 (16) | NS |
| Mg/urine | 3.46 (1.41) | 3.31 (1.1) | NS |

Values are given as means with SD in parentheses.

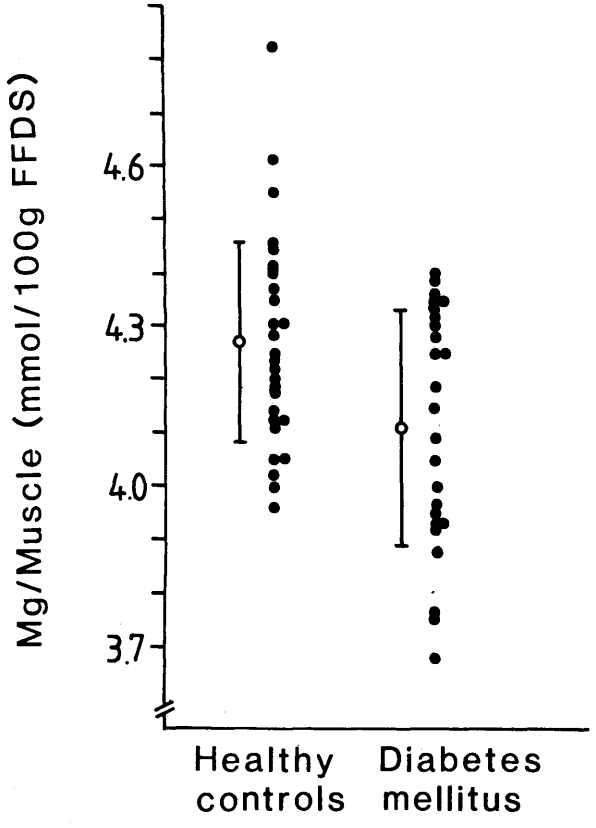


**FIGURE 1. The level of magnesium (mmol/100 g FFDS) in striated muscle from diabetic (N = 25) and healthy subjects (N = 28). The mean values (indicated in the figure) are significantly ($P < 0.01$) different from each other.**

**Erythrocyte analysis.** An indirect method was used for the determination of magnesium in erythrocytes.[26] One milliliter of whole blood was lysed by dilution with ion-free water up to a volume of 10 ml. After centrifugation at 1200 × *g* for 15 min, magnesium and potassium were determined in the supernatant. The hematocrit value was routinely obtained by the hospital laboratory and the plasma concentration was determined as described above; hence, the erythrocyte level of magnesium and potassium was estimated and is expressed as millimoles per liter.

**Urinary analysis.** Urine was collected during 24 h in acid-washed plastic bottles containing a small amount of concentrated nitric acid to avoid bacterial growth and to prevent the precipitation of magnesium. The sample was stored at −20°C until analyzed.

**$HbA_{1c}$ analysis.** The stable fraction of glycosylated hemoglobin ($HbA_{1c}$) was determined by ion-exchange chromatography using microcolumns (Bio-Rad, Richmond, California).

**Chemicals.** The following chemicals were used: nitric acid (Suprapure, Merck, Darmstadt, Federal Republic of Germany), petroleum ether (Burdick and Jackson, Muskegon, Michigan), CsCl (Suprapure, Merck), $LaCl_3$ (Puriss, Fluka, Buchs, Switzerland), and NaCl (Merck). Standard solutions of magnesium and potassium were prepared from $MgNO_3$

TABLE 3
Comparisons between mean level of potassium in muscle (mmol/100 g FFDS), plasma (mmol/L), erythrocytes (mmol/L), and mean urinary excretion of potassium during 24 h (mmol/24 h) in subjects with IDDM (N = 25) and healthy control subjects (N = 28)

| | IDDM | Healthy controls | |
|---|---|---|---|
| K/muscle | 43.7 (3.1) | 46.4 (3.6) | $P < 0.01$ |
| K/plasma | 4.14 (0.4) | 4.19 (0.3) | NS |
| K/erythrocytes | 97.0 (9.3) | 93.3 (16) | NS |
| K/urine | 46.8 (15) | 52.9 (18) | NS |

Values are given as means with SD in parentheses.

(Spectrosol, BDH, Poole, England) and $KNO_3$ (Spectrosol, BDH). All dilutions were prepared in glass-distilled water in acid-washed plastic (polypropylene) tubes.

**Statistics.** Values are presented as mean ± SD. Unpaired Student's *t*-test was used when mean values were compared, and linear regression analysis when correlations were studied. P-values of $< 0.05$ (*), $< 0.01$ (**), and $< 0.001$ (***) were considered as significant. N = number of subjects and NS = nonsignificant.

## RESULTS

Subjects with IDDM had significantly lower concentrations of magnesium in muscle ($P < 0.01$) and plasma ($P < 0.001$) as compared with the control group (Table 2, Figure 1). Magnesium levels in erythrocytes and mononuclear cells and in the urinary excretion of magnesium during 24 h were not significantly different from those of healthy control subjects (Table 2). The mean concentration of potassium in muscle was significantly lower ($P < 0.01$) in subjects with IDDM as compared with that of healthy controls (Table 3).

The $HbA_{1c}$ levels were significantly correlated with the concentrations of magnesium in muscle ($r = -0.62$, $P < 0.001$), plasma ($r = -0.62$, $P < 0.001$), and mononuclear cells ($r = -0.47$, $P < 0.05$) (Figures 2–4). No correlations were found between the $HbA_{1c}$ levels and the concentration of magnesium in erythrocytes ($r = -0.09$, NS) or the urinary excretion of magnesium ($r = 0.07$, NS). We also found a significant correlation ($r = 0.69$, $P < 0.001$) between the concentrations of magnesium in muscle and mononuclear cells (Figure 5). Only nonsignificant interrelations were found between different potassium parameters measured. The concentration of potassium in muscle was significantly correlated ($r = 0.52$, $P < 0.01$) with the corresponding magnesium level (Figure 6).

There were no significant differences between men and women in any of the parameters measured and the concentrations of magnesium and potassium in the different fluids and tissues analyzed were not correlated with the duration of the disease. Two of the magnesium parameters in diabetic subjects were correlated with age; the levels of magnesium in mononuclear cells and erythrocytes were significantly ($P < 0.01$ and $P < 0.05$) lower in diabetic subjects over the age of 40 yr (Table 4).

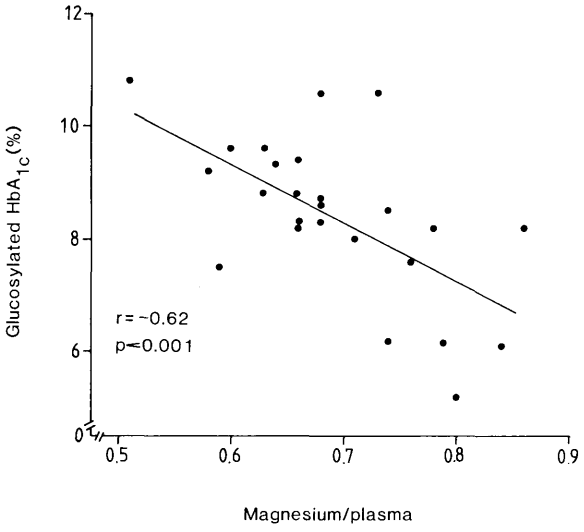


**FIGURE 3. The correlation between the concentration of magnesium in plasma (mmol/L) and the $HbA_{1c}$ level (%).**

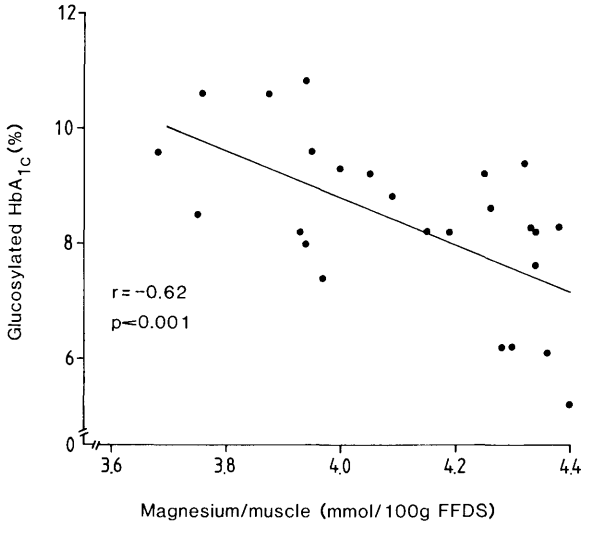


**FIGURE 2. The correlation between the content of magnesium in striated muscle (mmol/100 g FFDS) and the $HbA_{1c}$ level (%).**

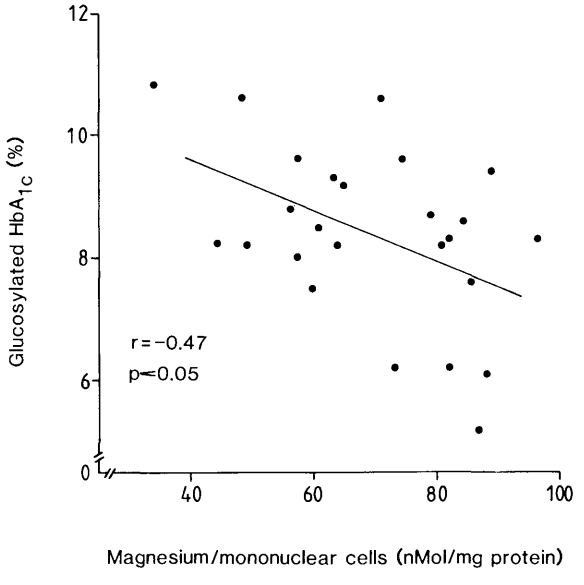


**FIGURE 4. The correlation between the content of magnesium in mononuclear cells (nmol/mg protein) and the $HbA_{1c}$ level (%).**

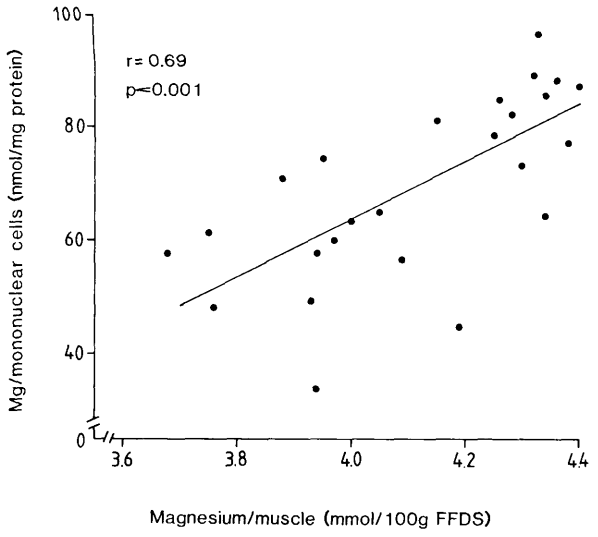


**FIGURE 5. The correlation between the content of magnesium in striated muscle (mmol/100 g FFDS) and mononuclear cells (nmol/mg protein).**

## DISCUSSION

It is generally agreed that disturbed body distribution of magnesium is often found in human subjects with diabetes mellitus. Whether magnesium deficiency is common or not in diabetic individuals is, however, a matter of controversy. Plasma or serum determinations of magnesium are often unreliable in confirming a magnesium deficiency,[19,27,29] although a low concentration may sometimes indicate magnesium deficiency.[28-30] Diabetic subjects were, in this study, found to have lower levels of magnesium in plasma as compared with healthy control subjects (Table 2). This finding confirms results presented by others.[8-15]

Analysis of magnesium in muscle biopsies gives information about the intracellular concentration of magnesium[19] and is considered to be reliable in confirming a suspected magnesium deficiency.[9,31] The contents of magnesium in striated muscle were, in this study, significantly lower in subjects with IDDM as compared with those of healthy control subjects (Table 2). The muscle biopsy technique is, however,

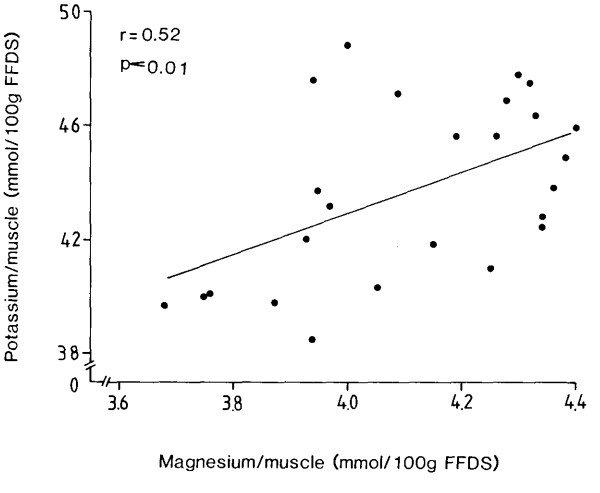


**FIGURE 6. The correlation between the content of magnesium (mmol/100 g FFDS) and potassium in striated muscle (mmol/100 g FFDS).**

TABLE 4
Mean level of the magnesium parameters in diabetic subjects in relation to age

| | Age <40 yr | Age ≥40 yr | |
|---|---|---|---|
| Mg/muscle | 4.14 (0.25) | 4.08 (0.19) | NS |
| Mg/plasma | 0.70 (0.13) | 0.64 (0.07) | NS |
| Mg/erythrocytes | 2.39 (0.29) | 2.15 (0.25) | $P < 0.05$ |
| Mg/mononuclear cells | 77.8 (12.3) | 58.8 (14.4) | $P < 0.01$ |
| Mg/urine | 3.41 (1.58) | 3.57 (1.07) | NS |
| Duration of disease | 21 (14) | 16 (9) | NS |

The data are divided into subjects older (N = 11) and younger (N = 14) than 40 yr. The same concentration ratios as in Table 2 are used. The duration (yr) of the disease in the two groups is given and the values are expressed as means with SD in parentheses.

both complicated to perform and time consuming. It is more convenient to obtain information concerning the intracellular level of magnesium from analysis of cells separated from a sample of peripheral venous blood. In this study, we performed analyses of magnesium in mononuclear cells and erythrocytes. The content of magnesium in mononuclear cells reflected the magnesium concentration in striated muscle (Figure 4). Furthermore, the content of magnesium in mononuclear cells was dependent on the metabolic control of the diabetic disease as measured by the $HbA_{1c}$ level (Figure 4). However, when the variabilty for each magnesium parameter (expressed as coefficient of variation) was studied, we found magnesium in mononuclear cells to be 23.1%, muscle 5.4%, erythrocytes 12.7%, plasma 11.6%, and urine 40.8%. The comparatively high degree of variation of the level of magnesium in mononuclear cells has previously been descibed[32] and limits the clinical use of this parameter. Furthermore, patients with IDDM did not have significantly lower magnesium content in mononuclear cells as compared with healthy control subjects (Table 2).

Potassium and magnesium are significantly interrelated[19-23] and magnesium deficiency is also often followed by potassium deficiency. This was confirmed in this study, as we found a significant correlation between the contents of potassium and magnesium in muscle (Figure 6), and the levels of both potassium and magnesium in muscle biopsies were significantly lower in the diabetic subjects as compared with those in healthy control subjects (Tables 2 and 3).

It is difficult to judge from earlier reports the extent to which different magnesium parameters are dependent on the degree of metabolic control of the diabetic disease. Before 1968, when the first report was published about abnormal hemoglobin ($HbA_1$) in blood from diabetic individuals,[33] most authors used the blood level and urinary excretion of glucose as the only measurement for the degree of metabolic control. The level of glycosylated hemoglobin ($HbA_1$) is, however, a better instrument and further investigations have indicated that determination of the concentration of $HbA_{1c}$ is even more reliable.[34-36] We found a significant correlation between the $HbA_{1c}$ level and the concentration of magnesium in muscle, plasma, and mononuclear cells (Figures 2–4). Plasma or serum levels of magnesium have been found by others to correlate inversely with blood glucose concentration[11,13,16,37] and the concentration of $HbA_{1c}$.[16] The latter finding was extended in this study by measuring intracellular magnesium (Figures 2 and 4).

The results presented here show that magnesium deficiency, as indicated by low magnesium levels in muscle, develops in the course of impairment of the metabolic control of the diabetic disease. The magnesium levels in plasma showed a profile of changes in diabetic subjects that resembled that of skeletal muscle; the mean concentration of magnesium in plasma was lower in diabetic subjects (Table 2), and the plasma values were significantly correlated with the levels of glycosylated $HbA_{1c}$ (Figure 3). However, the concentrations of magnesium in plasma and muscle biopsies were not significantly correlated with each other ($r = 0.38$, NS). Hence, even though muscular magnesium deficiency and/or a defective control of plasma magnesium is often present in diabetic subjects, the mechanisms beyond these phenomena may be completely different.

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