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Intracellular and extracellular magnesium depletion in Type 2 (non-insulin-dependent) diabetes mellitus

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Summary. To investigate alterations of magnesium metabolism in Type 2 (non-insulin-dependent) diabetes mellitus, we utilized a new magnesium-specific selective ion electrode apparatus to measure serum ionized magnesium (Mg-io) in fasting subjects with and without Type 2 diabetes, and compared these values to levels of serum total magnesium, and of intracellular free magnesium (Mgi) analysed by ^{31}P-NMR spectroscopy. Both Mg-io (0.630 ± 0.008 vs 0.552 ± 0.008 mmol/l, $p < 0.001$) and Mgi (223.3 ± 8.3 vs 184 ± 13.7 mmol/l, $p < 0.001$), but not serum total magnesium, were significantly reduced in Type 2 diabetes compared with non-diabetic control subjects. Furthermore, a close relationship was observed between serum Mg-io and Mgi ($r = 0.728$, $p < 0.001$). We suggest that magnesium deficiency, both extracellular and intracellular, is a characteristic of chronic stable mild Type 2 diabetes, and as such, may predispose to the excess cardiovascular morbidity of the diabetic state. Furthermore, by more adequately reflecting cellular magnesium metabolism than total serum magnesium levels, Mg-io measurements may provide a more readily available tool than has heretofore been available to analyse magnesium metabolism in a variety of diseases.

Key words: Magnesium, nuclear magnetic resonance spectroscopy, Type 2 (non-insulin-dependent) diabetes mellitus, erythrocytes, ion selective electrode.

Although the cellular physiology of magnesium metabolism is still poorly understood, it has long been observed that insulin stimulates cellular magnesium uptake [1]. Accordingly, in acute diabetic ketoacidosis consequent to insulin deficiency, considerable total body deficits of magnesium routinely occur [2, 3]. However, it is unclear whether chronic stable diabetes, especially Type 2 (non-insulin-dependent) diabetes mellitus, is also a state of magnesium depletion [4–6]. This is a clinically relevant question, since magnesium deficiencies have been implicated in the pathogenesis of hypertension, atherosclerosis, and cardiac arrhythmias, all present to an increased extent in subjects with chronic Type 2 diabetes [7–10].

The assessment of magnesium status has been difficult, due largely to a lack of adequate technologies for measuring magnesium. Indeed, spontaneous hypomagnesaemia is an uncommon finding in subjects with diabetic or vascular syndromes. Our group has recently utilized non-invasive nuclear magnetic resonance (NMR) techniques to study intracellular free magnesium concentrations (Mgi), and have reported consistent significant reductions of free magnesium in erythrocytes from fasting Type 2 diabetic subjects [11], in non-diabetic essential hypertensive subjects [12], and in relation to the degree of insulin resistance present [13]. To complement these studies, and to design easier, more clinically applicable techniques, we have employed a new ion-specific magnesium electrode apparatus to measure extracellular, serum ionized magnesium levels [14].

The present study, based on the above newer techniques, and reported in abstract form previously [15], suggests the presence of both cellular and circulating magnesium depletion in mild, diet-controlled Type 2 diabetes. We believe these findings have both pathophysiologic as well as therapeutic implications in diabetes generally, and reinforce the link between altered glucose and divalent cation metabolism [16].

Subjects and methods

Subjects arrived at the Cardiovascular Center of the New York Hospital-Cornell Medical Center after an overnight fast, and had venous blood drawn while in the seated position. None of the subjects were receiving medication for any current medical condition, or had been on antidiabetic therapy. Type 2 diabetes ($n = 22$) was diagnosed on the basis of fasting whole blood glucose values greater than 7.8 mmol/l (140 mg%) and elevated HbA_{1c} levels, both on at least two consecutive occasions at least one month apart. Non-diabetic

Table 1. Extracellular and intracellular free magnesium levels in normal and Type 2 diabetic subjects

	Mg-t (mmol/l)	Mg-io (mmol/l)	Mgi (mmol/l)
Control subjects ($n = 30$)	0.86 ± 0.01	0.630 ± 0.008	223.3 ± 8.3
Type 2 diabetes ($n = 22$)	0.81 ± 0.05	0.552[a] ± 0.008	184.1[a] ± 13.7

[a] $p < 0.001$ vs control subjects
Mg-t, Serum total magnesium; Mg-io, serum ionized magnesium; Mgi, intracellular free magnesium

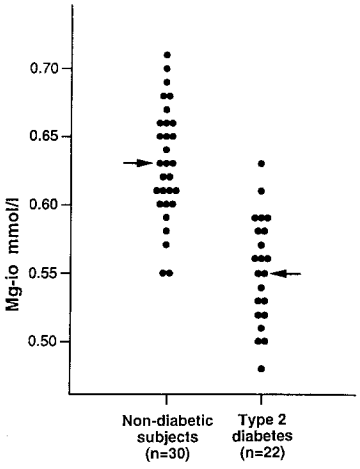

Fig. 1. Distribution of serum ionized magnesium (Mg-io) values in normal and diabetic subjects. Arrows indicate average values in non-diabetic (0.630 ± 0.008 mmol/l) compared to Type 2 diabetic subjects (0.552 ± 0.008 mmol/l), which were significantly different, ($p < 0.001$)

control subjects ($n = 30$) were obtained from among a population of healthy individuals followed-up prospectively to study the incidence of cardiovascular disease in a general population (NIH-SCOR). Non-diabetic individuals were matched for age, sex, and race with the diabetic subjects.

Blood obtained from these subjects was analysed for serum total magnesium, serum ionized magnesium (Mg-io), and for erythrocyte intracellular free magnesium (Mgi). All blood samples were obtained anaerobically through vacuum needle extraction. Serum total magnesium was analysed using standard Autoanalyzer techniques. Serum ionized magnesium (Mg-io) was analysed using blood drawn into air-evacuated glass tubes containing an inert cell-separating gel matrix. After clotting and centrifugation, the tube was inverted, and the serum drawn off under vacuum into a syringe, which was then capped and sent for analysis. Serum Mg-io was determined using a magnesium ion-selective electrode (ISE) containing a neutral carrier-based membrane, which provides measurement values within 2 min. This new ISE for ionized magnesium has been recently characterized, and Mg-io values obtained by this technique from whole blood, serum, and plasma are virtually identical [14]. Using this technique, venous pH values in these samples from both patient groups were within the published range of normal, and did not significantly differ from each other.

Erythrocyte intracellular free magnesium (Mgi) was measured by ^{31}P-NMR spectroscopic techniques, the details of which have been previously described [12]. Briefly, heparinized blood is centifuged, and the packed cells decanted into 10 mm, thin-walled NMR tubes, which are then placed in the NMR spectrometer for analysis. All spectra were obtained on a Varian XL 200 spectrometer, operating at 37 °C. Mgi levels were determined according to the formula [17],

$$Mgi = K_d(MgATP)\{\Phi^{-1} - 1\}$$

where Φ is the free, unbound fraction of ATP, calculated from the chemical shift differences of the alpha and beta phosphoryl resonances of ATP on the ^{31}P-NMR spectrum, and K_d(MgATP) is 38 mmol/l at 37 °C.

Statistical analyses

The various magnesium values were compared betweed Type 2 diabetic and control subjects using standard unpaired Student's *t*-tests. Relations between Mg-io and Mgi values were analysed using linear regression analysis and Pearson correlation coefficients. All values are reported as mean ± SEM.

Results

There were no significant demographic distinctions between the Type 2 diabetic ($n = 22$) and control, non-diabetic ($n = 30$) subjects. Specifically, age (60 ± 2.1 vs 56 ± 3.3 years, p = NS), and sex distribution (male/female = 8/14 vs 13/17, p = NS), did not differ among the groups. All study subjects were white. Fasting blood glucose (8.8 ± 0.4 vs 4.7 ± 0.3 mmol/l, $p < 0.001$) and weight (body mass index = 27.4 ± 0.6 vs 25.0 ± 1.0 kg/m^2, $p < 0.05$), were higher among the Type 2 diabetic subjects compared with the non-diabetic control subjects. The values for serum total magnesium, serum Mg-io, and erythrocyte Mgi among the two patient groups are shown in Table 1. Intracellularly, Type 2 diabetes was uniformly associated with a significant suppression of free magnesium levels. This was true of all the subjects, with almost no overlap of data points between Type 2 diabetes and non-diabetic control subjects. In the extracellular circulation, serum Mg-io levels, but not serum total magnesium levels, were also significantly lower in Type 2 diabetic subjects, compared with non-diabetic control subjects. The individual serum Mg-io values for control and diabetic subjects are displayed in Figure 1. For all subjects, a positive, significant relationship was observed between Mgi and extracellular serum Mg-io levels ($r = 0.728$, $p < 0.001$), as shown in Figure 2. Serum total magnesium was also related to Mgi levels, albeit more weakly ($r = 0.587$, $p < 0.05$).

Discussion

The almost universal involvement of magnesium biochemically in a wide variety of cellular processes critical to both cardiovascular function and glucose and insulin metabolism is well appreciated. Nevertheless, the existence

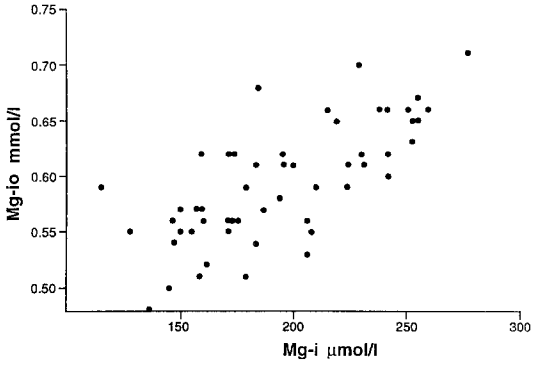

Fig. 2. Relation of serum ionized magnesium values (Mg-io) to intracellular levels of free magnesium. Mgi, intracellular free magnesium levels. Mg-io and Mgi were significantly related, $r = 0.728$, ($p < 0.001$)

and role of magnesium deficiency in various human disease states remains unclear. There are at least two reasons for this. First, intracellularly, it was believed that magnesium could not be a regulatory factor at physiologically occurring concentrations, since magnesium levels measured by standard methods are in the millimolar range, far in excess of the regulatory, K_m-micromolar concentration range of most Mg-dependent enzyme and ion pump mechanisms [18, 19]. Second, extracellularly, except for conditions inducing magnesium wasting, often iatrogenic, or in extreme presentations of more common diseases, such as diabetic ketoacidosis, serum Mg levels in e. g. hypertension, Type 2 diabetes, and other cardiovascular and metabolic diseases, have not been consistently measured outside the range considered normal.

The advent of new techniques to directly assess the ionic, freely available magnesium both intracellularly and in the circulation, have allowed us to investigate these issues more precisely. Our group has previously used ^{31}P-NMR spectroscopy to study Mgi levels in normal human subjects, as well as in subjects with essential hypertension, obesity, and Type 2 diabetes [11, 12]. Contrary to what had been suspected, Mgi exists in the regulatory concentration range (250–500 mmol/l) for most magnesium-dependent enzyme, channel, and pump mechanisms. This is true for cardiac muscle [20] and vascular smooth muscle [21], as well as for peripheral erythrocytes [12]. Furthermore, both essential hypertension and Type 2 diabetes are associated with significantly lower Mgi levels [11, 12]. That these findings are clinically significant is suggested by the close, inverse relationships observed in essential hypertension, between Mgi, blood pressure [12] and the degree of insulin resistance [13]; and in Type 2 diabetes, between Mgi and the level of fasting blood glucose [11]. Similarly, and even more recently, an Mg-selective ion electrode apparatus has been developed, which allows for a direct assessment of the free Mg-io in the extracellular space [14]. Normal circulating levels of Mg-io appear to exist within a narrow range, as is true for the control non-diabetic subjects studied here.

This report not only confirms our previous findings of intracellular magnesium depletion in Type 2 diabetes, but demonstrates at the same time that serum Mg-io levels are also significantly lower in diabetic, compared with non-diabetic control subjects, suggesting that in chronic stable Type 2 diabetes, depletion of extracellular magnesium occurs as well. This is further supported by the positive linkage we observed between Mgi levels and serum Mg-io measurements made in the same patient at the same time. Since obesity per se is not associated with significantly lower Mgi levels [11], it is doubtful that the differences reported here are due to the slightly increased body mass index of the Type 2 diabetic patients. Furthermore, our observed differences may actually underestimate those found in the general diabetic population, since our study subjects had only mild disease, with only minimal laboratory signs, and no symptoms of diabetes. Indeed, more than half of the Type 2 diabetic subjects studied here had not previously known of any abnormality of carbohydrate metabolism, and none had ever had any specific antidiabetic therapy.

These data suggest the overall notion that magnesium deficiency, defined on the basis of either Mgi levels or serum Mg-io concentrations, is a common, if not universal feature of the diabetic state. This concept, if supported by further studies of larger populations, has clinically significant implications. Pathophysiologically, magnesium depletion can directly cause vasoconstriction and frank hypertension [22], can predispose to cardiac arrhythmias and sudden death [10, 23], can increase platelet aggregation and thus the potential for in situ thrombosis [24–26], and can produce the pathologic lesions of atherosclerosis [27], all the above occurring clinically to an increased extent in diabetes. Hence, the present data suggest that the cardiovascular consequences of diabetes may be at least partly due to deficient magnesium.

Regardless of the possible implications of these data, the simple technical requirements and improved precision of the serum Mg-io measurement are certain to expand our clinical diagnostic capabilities. Its relevance to the diagnosis and clinical course of a variety of diseases is eagerly anticipated, once this selective ion electrode apparatus becomes more generally available.

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