Tissue Magnesium Status in Diabetes Mellitus

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Summary. Leucocyte and erythrocyte magnesium was assayed in 17 healthy subjects and 17 insulin dependent diabetic patients. Plasma magnesium concentration (mean $\pm$ standard error of mean) was significantly lower in the diabetic patients (0.80 ± 0.02 mmol/l), compared with the healthy subjects (0.90 ± 0.02 mmol/l, $p < 0.001$), but the leucocyte and erythrocyte magnesium content was not significantly different in the diabetic patients (34.5 ± 0.8 and 6.2 ± 0.2 mmol/kg dry solids) compared with the healthy subjects (35.5 ± 0.8 and 6.5 ± 0.11 mmol/kg dry solids). In a separate study skeletal muscle obtained by needle biopsy was also assayed. Plasma magnesium in 10 diabetic patients (0.74 ± 0.01 mmol/l) was significantly lower than in 16 healthy subjects (0.85 ± 0.02 mmol/l, $p < 0.001$), but there was no significant difference in the mean muscle magnesium content (43.0 ± 0.7 compared with 40.7 ± 0.9 mmol/kg dry solids in the diabetic patients).

Key words: Magnesium, plasma, leucocytes, erythrocytes, skeletal muscle, diabetes mellitus

Hypomagnesaemia was until recently, a poorly recognised feature of diabetes mellitus [11, 20], but in a large survey was found to occur in about 25% of unselected diabetic outpatients [15]. Its origins are not known but it is associated with poor diabetic control [15]. It has been reported [16] that patients with severe diabetic retinopathy have a lower mean serum Mg concentration than those with minor changes or normal fundi, and suggested that hypomagnesaemia is a factor predisposing to the development and severity of diabetic retinopathy [16]. There is evidence also that Mg may be implicated in the pathogenesis and prognosis of ischaemic heart disease in non-diabetic subjects [19].

In order to investigate further the hypomagnesaemia in diabetes, tissue and plasma magnesium levels were measured as hypomagnesaemia is not necessarily accompanied by a decrease in cellular Mg stores [21]. The tissues analysed were leucocytes, erythrocytes, and skeletal muscle.

Patients

The first study involved 17 insulin-dependent diabetic patients. There were eight males and nine females, with ages ranging from 13 to 69 years. Reference values for plasma, leucocyte and erythrocyte electrolytes were obtained from 17 hospital personnel (12 males and five females) aged between 21 and 57 years. In the second study, ten insulin-dependent diabetic patients were selected who were found to have a plasma Mg below 0.74 mmol/l at a previous outpatient attendance. There were five males and five females aged between 22 and 54 years. Reference values for plasma and skeletal muscle electrolytes were obtained from 11 healthy non-diabetic patients undergoing minor surgery under general anaesthesia and from five healthy volunteers under local anaesthesia. There were ten males and six females, with ages ranging from 24 to 55 years. None of the diabetic patients or healthy subjects were receiving diuretic therapy and none had significant renal impairment as assessed by plasma creatinine or plasma urea concentration. No assessment of retinal disease was made in the diabetic patients. Two diabetic patients in the second study were receiving thyroxine but were euthyroid, and a third was taking an oral contraceptive agent. The mean insulin dose for the diabetic patients was 58 units/day (range: 24–110 units/day). Glycosylated haemoglobin was measured [22] in eight of the ten patients in the second study and gave a mean value of 12% (range: 10.1 to 14.7%, normal range: 6–9%).

All diabetic patients and healthy subjects gave their informed consent to the muscle biopsy and venepuncture. The study was approved by the St. George's Hospital Ethical Committee.

Methods

Venous blood samples were obtained without venestasis. In the second study, blood was collected immediately before the muscle biopsy. Plasma Mg concentration was estimated by atomic absorp-

Table 1a. Results of plasma and leucocyte analyses in diabetic and healthy subjects

		Plasma				Leucocytes				
		Mg	Na	K	Ca	Mg	K	Mg	K	Water content (1/kg dry solids)
		(mmol/l)				(mmol/l cell H ₂ O)		mmol/kg dry solids)		
Diabetic patients	<i>n</i>	17	17	17	17	17	14	17	14	16
	Mean	0.80	144	4.2	2.48	13.0	169	34.5	451	2.67
	SEM	0.02	1	0.1	0.04	0.3	2	0.8	5.0	0.03
Healthy subjects <i>n</i> = 17	Mean	0.90	142	4.2	2.47	13.5	165	35.5	435	2.65
	SEM	0.02	1	0.1	0.04	0.4	2	0.8	7.0	0.05
<i>p</i> ^a		<0.001	NS	NS	NS	NS	NS	NS	NS	NS

^a The significance of difference between the means was assessed by Student's *t* test

n = number of subjects or patients

Table 1b. Results of erythrocyte analyses in diabetic and healthy subjects

		Erythrocytes						
		Water content (1/kg dry solids)	Mg	Na	K	Mg	K	
			(mmol/l cell H ₂ O)			(mmol/kg dry solids)		
Diabetic patients <i>n</i> = 17	Mean	1.87	3.3	11.8	137	6.2	257	
	SEM	0.02	0.1	0.6	1	0.2	5	
Healthy subjects <i>n</i> = 17	Mean	1.79	3.6	13.3	145	6.5	258	
	SEM	0.02	0.1	0.8	1	0.1	3	
<i>p</i> ^a		<0.001	<0.001	NS	<0.001	NS	NS	

^a The significance of difference between the means was assessed by Student's *t* test

n = number of subjects or patients

tion spectrophotometry (Pye Unicam SP 2900). The coefficient of variation for this assay was 2.5%. Assays of plasma sodium, potassium, urea and albumin followed standard Technicon autoanalyser methods. Leucocytes were isolated by the method of Baron and Ahmed [5] with the modification that the leucocytes were resuspended in the subject's plasma for 30 min at 37°C [13]. Samples were analysed in duplicate, and the coefficient of variation of the leucocyte magnesium concentration was 6.1%. Erythrocyte analysis was performed on heparinised venous blood to which had been added a trace amount of ¹²⁵I-human serum albumin [6]. Samples were analysed in duplicate and the coefficient of variation for magnesium concentration was 4.0%.

Skeletal muscle tissue was obtained from the lateral aspect of the quadriceps femoris muscle using a University College biopsy needle [10]. Superficial anaesthesia was obtained with 2% lignocaine, care being taken not to infiltrate below the fascia. Tissue samples usually weighed between 25 and 50 mg. Muscle samples were dissected free of adherent fibrous or adipose tissue, divided into two approximately equal parts and sealed into preweighed polythene envelopes. Light microscopy of the specimens showed a virtual absence of adipose tissue, hence lipid extraction was felt to be unnecessary. The subsequent analysis was similar to that of leucocytes and erythrocytes.

The proportion of extracellular fluid in the muscle biopsy specimen was not measured because of the relatively high concentration of intracellular compared with extracellular fluid Mg and the inadequacy of present techniques for measuring the extracellular fluid space [6].

Results

The findings for leucocytes and erythrocytes are summarised in Table 1. Values are given as mean $\pm$ standard error of mean (SEM). The significance of difference between means was assessed by Student's *t* test. Comparing diabetic patients with healthy subjects there was no significant difference in leucocyte or erythrocyte magnesium expressed as mmol/kg dry cell solids despite a highly significant difference in the mean plasma magnesium values. A small but significant increase in erythrocyte water associated with a reduction in erythrocyte magnesium, sodium and

Table 2. Results of plasma and skeletal muscle analyses in diabetic and healthy subjects

		Plasma				Skeletal muscle		
		Mg	Na	K	Albumin	Mg	Na	K
		(mmol/l)			(g/l)	(mmol/kg dry tissue)		
Diabetic subjects <i>n</i> = 10	Mean	0.74	139	4.4	39.6	40.7	88.0	423
	SEM	0.01	1	0.1	0.8	0.9	3.8	9
	<i>n</i>	16	12	12	11	16	16	16
Healthy subjects	Mean	0.85	141	4.2	39.8	43.0	84.2	433
	SEM	0.02	1	0.1	1.2	0.7	3.1	5
<i>p</i> ^a		<0.001	NS	NS	NS	NS	NS	NS

^a The significance of difference between the means was assessed by Student's *t* test.

n = number of subjects or patients

potassium was found when results were expressed as mmol/l erythrocyte water. These differences disappeared when results were expressed in terms of dry cell weight.

The findings of the second study are shown in Table 2. The biopsy results for the healthy subjects which were taken either under general anaesthesia or local anaesthesia have been combined, since no differences in the muscle electrolyte values were noted. There were no significant differences in muscle magnesium or potassium content between diabetic subjects and healthy subjects, despite a significant reduction in plasma magnesium concentration. There was no significant difference in mean plasma albumin between the two groups in the second study. Plasma albumin levels were measured in seven diabetic patients in the first study and did not differ significantly from normal. No significant correlation was found between plasma albumin and plasma magnesium.

Discussion

We found that the magnesium content of erythrocytes, leucocytes and skeletal muscle was normal despite hypomagnesaemia in insulin dependent diabetic patients.

Previous workers have also found that the correlation between plasma and muscle magnesium values in disease states is poor [2, 9]. Thus a normal plasma magnesium concentration has been associated with a reduction in muscle magnesium content in subjects with diarrhoea [14] and after prolonged fasting [8]. There are no previous reports of skeletal muscle magnesium content in human diabetes but un-

changed values were found in alloxan induced diabetes in rabbits [1].

The leucocyte results are in accord with the findings of others [18]. However, there are no studies comparing leucocyte magnesium content with another index of magnesium status, other than erythrocytes. Following jejuno-ileal bypass surgery for obesity, severe hypomagnesaemia is a common occurrence, and in this situation leucocyte and erythrocyte magnesium levels also remain normal [13].

The status of erythrocyte magnesium as an index of tissue stores is also uncertain. Results in diabetes have not revealed differences between patients and control subjects [12, 17, 18] in agreement with the findings of the present study. We found there was a small but significant decrease in erythrocyte magnesium concentration in the diabetic patients compared with the healthy subjects, presumably related to the small increase in erythrocyte water content, but this difference vanished when the magnesium was expressed in terms of dry cell solids.

By contrast with the soft tissue studies a significant decrease has been found in the trabecular bone Mg content of iliac crest biopsies from insulin dependent patients compared with non-diabetic subjects [7]. Bone magnesium has been shown to correlate well with serum magnesium in patients with the hypermagnesaemia of chronic renal failure [2, 3], but its relationship with hypomagnesaemia is less well defined. Nevertheless it has been suggested that bone and extracellular fluid magnesium are the major pools increased during magnesium excess and decreased during magnesium depletion [2, 3].

The significance of the hypomagnesaemia in diabetes, and its relationship to total body magnesium and soft tissue stores therefore remains

uncertain. It has been proposed that hypomagnesaemia might be a risk factor in the development of diabetic retinopathy [16], and a case for dietary magnesium supplements was tentatively put forward as a possible means of reducing vascular complications of diabetes [4]. While it still remains a possibility that long term hypomagnesaemia might exert a deleterious effect on vascular disease in diabetes, our findings suggest that hypomagnesaemia is not associated with depletion in the soft tissue content of this cation.

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