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Does Magnesium Dysbalance Participate in the Development of Insulin Resistance in Early Stages of Renal Disease?

K. ŠEBEKOVÁ, K. ŠTEFÍKOVÁ, D. POLAKOVIČOVÁ[1], V. SPUSTOVÁ, R. DZÚRIK

Institute of Preventive and Clinical Medicine and [1]Derer's Hospital, Bratislava, Slovakia

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Summary

We investigated the potential role of magnesium (Mg) dysbalance in the pathogenesis of insulin resistance (IR) in patients with mildly-to-moderately decreased renal function (creatinine: 142.8±11.0 mmol/l). The data were compared to those of 8 age- and sex-matched healthy controls (CTRL). The standard oral glucose tolerance test (oGTT) was performed in 61 patients. Twenty-two patients were classified as IR according to their values on fasting and after-load immunoreactive insulin concentrations. Serum and total erythrocyte Mg (tErMg) (atomic absorption spectrophotometry) and free erythrocyte Mg (fErMg) concentrations (^{31}P NMR spectroscopy) were determined prior to and two hours after the glucose load. Ten out of 39 insulin-sensitive (IS) patients, but only one out of 22 insulin-resistant (IR) patients, had a low basal fErMg concentration (<162.2 μmol/l, χ^2, $p<0.01$). IR patients had higher serum Mg, total erythrocyte Mg and bound erythrocyte Mg (bErMg) concentrations (both before and after glucose load) when compared with the IS group. Both groups responded to the glucose load with a significant decrease in serum Mg concentration (within the normal range), while the IR group also exhibited a decline in tErMg and bErMg. The mean sum of insulin needed to metabolize the same glucose load correlated positively with tErMg ($r=0.545$, $p<0.01$) and bErMg ($r=0.560$, $p<0.01$) in the IR patients. It is concluded that, at an early stage of renal dysfunction, IR is not associated with the decline in free erythrocyte Mg concentration, but the magnesium handling in red blood cells is altered.

Key words

^{31}P NMR spectroscopy • Erythrocyte • Magnesium • Insulin resistance • Obesity • Kidney Disease

Introduction

The clinical coexistence of hypertension, obesity, non-insulin dependent diabetes, left ventricular hypertrophy and accelerated atherosclerosis has suggested that they might be different aspects of the same underlying pathological process (Reaven 1988). The ionic hypothesis explains the concurrent appearance of these cardiovascular and metabolic syndromes by a generalized defect in cell ion handling, resulting in intracellular free calcium excess and lower intracellular free magnesium (fMg) and pH (Resnick 1992). Low free intracellular Mg concentrations in erythrocytes is associated with high blood pressure (Resnick *et al.* 1984) and Mg deficiency produces insulin resistance (IR) even in normal subjects (Nadler *et al.* 1993). The degree of peripheral IR in essential hypertension (EHT) is inversely correlated to free erythrocyte Mg (fErMg) concentration (Resnick *et al.* 1990), and is associated with an intracellular shift of Mg from its free to the bound form (Šebeková *et al.* 1994).

Fax+4202 24920590
E-mail: physres@biomed.cas.cz http://www.biomed.cas.cz/physiolres

A high prevalence of IR was already revealed at early stages of chronic renal insufficiency (CRI) (Dzúrik *et al.* 1995), and it is almost a constant finding in patients with advanced renal failure. In CRI, the incidence of hypertension and accelerated atherosclerosis increases. In patients with mildly to moderately decreased kidney function uremic toxins do not accumulate to the extent that they could cause IR. Therefore, another mechanism should be considered. These lines of evidence prompted us to investigate the potential role of disturbance in cell Mg handling in the pathogenesis of IR in patients with kidney disease prior to the development of renal failure. We supposed that if cell Mg mishandling participates in pathophysiology of IR, the basal Mg levels or at least the Mg response to a standard glucose load should differ from those of insulin-sensitive subjects. Therefore the serum, total (tErMg) and Mg concentrations bound to erythrocytes (bErMg) were investigated before and after a standard oral glucose load.

Methods

Patients

Sixty-one patients with kidney disease of various origin and mildly to moderately decreased kidney function were included. The diagnosis was obtained by clinical, laboratory and in most cases by kidney biopsy evaluation. The underlying disease was glomerulo-nephritis (n=12), interstitial nephritis (n=22), nephrosclerosis (n=4) and other afflictions were diagnosed in 23 patients. Diabetes mellitus, proteinuria in the nephrotic range, severe hypertension, systemic disease, analgetic nephropathy and signs of catabolism were the criteria for exclusion. Data were compared with those obtained from 8 age- and sex-matched healthy controls (CTRL). The study was explained in detail and a written informed consent was obtained from all participants.

Study protocol

Our study was approved by the Institutional Review Board and performed according to the Helsinki Declaration. Patients were free of drugs interfering with glucose metabolism and were not supplemented with magnesium or calcium for at least 2 months before the study. They were asked to consume an isocaloric, moderately high carbohydrate diet for at least one week preceding the standard oral glucose tolerance test (oGTT). They were instructed to avoid alcohol, caffeine and smoking.

OGTT was performed in the morning hours after 10-hour fasting using 75 g of glucose in 300 ml water. Blood samples were withdrawn before, 60 and 120 min after the glucose load from an antecubital vein. A fasting blood sample was also used for other analysis.

Methods

Glucose was determined by the glucose oxidase method (Beckman Glucose Analyzer 2); immunoreactive insulin (IRI) and C-peptide by the RIA method (RIA Ins, Laboratorium Saxoniae, Sebnitz). OGTT was classified according to WHO criteria. Patients were considered as insulin-resistant if their basal insulinemia was >20 μU/ml or >65 μU/ml at 120 min after the glucose load.

Parameters of Mg metabolism were determined before and 120 min after the glucose load. Serum Mg and tErMg (normal range in 52 healthy volunteers 1661- 2381 μmol/l) was determined by atomic absorption spectrophotometry, fErMg in red blood cells using ^{31}P NMR spectroscopy, as described in detail previously (Gupta *et al.* 1978, Šebeková *et al.* 1992). Briefly, 2 ml of heparinized venous blood were centrifuged immediately after the collection at 2500 rpm at 4 ^{o}C for 10 min. The supernatant and buffy coat were carefully discarded and erythrocytes were resuspended into an ice cold solution containing 10 mmol/l HEPES, 140 mmol/l NaCl, 10 mmol/l glucose, pH=7.2, to obtain a hematocrit value >60 % (60-84 %). ^{31}P NMR spectra were recorded in 5 mm cuvettes on a Varian RX-300 spectrometer operating at 7.05 T, at a frequency of 121.5 MHz and at 37 ^{o}C after 2048 accumulations. The data were Fourier transformed to produce red blood cell spectrum. Spectra were recorded in duplicate. The fErMg concentration was calculated from the chemical shift difference between the midpoint of the double peak of α-ATP (adenosintriphosphate) and the central peak of β-ATP according to the formula:

$$[Mg^{2+}]_f = K_D^{MgATP} (\varphi^{-1} - 1)$$

where K_D^{MgATP} is the dissociation constant of MgATP complex, at pH = 7.2, 38 μM, φ is the fraction of free vs Mg-bound ATP, calculated from analysis of the α- and β-phosphoryl resonance of ATP on ^{31}P NMR. The coefficient of variation estimated from measurements of 90 individual samples in duplicate was 0.7 %. The bErMg, and the ratios of free to total (F/T) and free to bound Mg erythrocyte (F/B) were calculated.

Serum lipids and creatinine were determined using standard methods. Blood pressure was measured on the right forearm in the sitting position. The body mass index (BMI) and clearance of endogenous creatinine (Cockcroft's formula) were also calculated.

Statistics

For evaluation, patients were divided into subgroups according to their insulin sensitivity, or according to BMI (BMI<25 kg/m^2 and >30 kg/m^2). As the distribution of some data did not fit to normal, both Student´s t-test and Wilcoxon sign-rank tests were used for comparing the means within paired and between unpaired groups. Glycemia, IRI and C-peptide concentrations during oGTT were evaluated by ANOVA and *post hoc* by the least square difference test. Single and stepwise multiple regression analysis was performed (Statgraphics V5 software, STSC). Results are given as mean ± standard error of the mean (S.E.M.), $p<0.05$ was considered significant.

Table 1. Characterization of the studied groups

	CTRL	All	IS	IR	BMI<25	BMI>30
Gender (F/M)	4/4	33/28	23/16	10/12	17/13	5/6
Age [years]	53.4±5.2	49.2±1.8	49.3±2.3	49.1±3.0	46.7±2.83	46.6±4.2
BMI [kg/m^2]	25.8±1.2	26.0±0.6	25.1±0.6	27.6±1.3 *	22.4±0.4	33.4±0.7$^{\#}$
Cr-S [mmol/l]	80.5±5.3	142.8±11.0^b	138.4±12.6^b	150.6±21.1^b	142.7±16.1^b	155.3±36.8^b
ClCr [ml/s]	1.6±0.1	1.1±0.1^b	1.1±0.1^b	1.1±0.1^b	1.02±0.1^b	1.4±0.2^b
SPB [mm Hg]	134.6±4.0	141.3±3.5	138.2±4.5	146.5±5.4^a	131.6±4.8	160.0±8.5$^{a,\#}$
DPB [mm Hg]	82.6±2.7	87.5±1.6	85.9±2.1	90.3±2.4^a	82.9±2.0	97.2±4.0$^{a,\#}$
Proteinuria [g/day]	ND	0.9±0.1	0.7±0.2	1.1±0.2	0.7±0.1	1.7±0.4$^{\#}$
Chol [mmol/l]	ND	5.4±0.3	5.4±0.3	5.4±0.3	5.3±0.3	5.3±0.3
TAG [mmol/l]	ND	2.1±0.2	1.9±0.2	2.5±0.4	1.8±0.2	2.6±0.5$^+$
HDL-Chol [mmol/l]	ND	1.1±0.1	1.2±0.1	0.9±0.1 **	1.2±0.1	0.8±0.1$^+$
LDL-Chol [mmol/l]	ND	3.2±0.2	3.2±0.2	3.2±0.2	3.2±0.3	3.2±0.2
VLDL-Ch [mmol/l]	ND	1.0±0.1	0.9±0.1	1.3±0.2 *	0.9±0.1	1.3±0.2$^+$
Hippurate [mmol/l]	ND	8.9±1.9	8.6±2.5	9.3±3.2	12.4±3.8	3.8±1.3
Pseudouridine [mmol/l]	ND	7.1±1.4	5.1±0.7	10.2±3.4	7.8±2.5	8.3±4.1

*Means ± SEM are given; CTRL: healthy controls; IS: insulin-sensitive patients; IR: insulin-resistant patients; BMI: body mass index; Cr-S: creatinine concentration in serum; ClCr: clearance of endogenous creatinine; SBP: systolic blood pressure; DBP: diastolic blood pressure; TAG: triglycerides; Chol: cholesterol; ND: not determined; a: p<0.05 vs CTRL; b: p<0.01 vs CTRL; *: p<0.05 IS vs. IR; **: p<0.01 IR vs IR, +: p<0.05 BMI <25 vs BMI >30; #: p>0.01 BMI <25 vs BMI >30;*

Results

Clinical characteristics of individual groups

Descriptive characteristics of the groups are given in Table 1. As expected, the control group had a lower blood pressure, plasma creatinine concentration and higher creatinine clearance values when compared to the patients. The IS and IR subgroups did not differ with regard to the gender, age, serum creatinine concentration, clearance of endogenous creatinine, blood pressure, proteinuria, total cholesterol concentration, or hippurate and pseudouridine accumulation. Serum urea and uric acid concentrations were similarly enhanced, and no significant differences in height, red blood cell count, hemoglobin, hematocrit and parameters of acid-base balance were found (data are not given because they were within the normal range). The prevalence of hypertension was similar in both groups (IS: 11/39; IR: 8/22). Higher BMI, marginally higher triglyceride levels (p=0.055) and a redistribution of cholesterol between lipoproteins of various density in comparison with IS was revealed in IR patients (Table 1).

To evaluate the potential contribution of obesity to Mg disturbance subgroups with BMI <25 kg/m^2 versus >30 kg/m^2 were compared. The proportion of IR patients was comparable in both groups: BMI<25: 14/31 patients; and BMI>30: 7/11, χ^2=NS. Patients with BMI>30 kg/m^2 had a higher blood pressure, proteinuria and triglyceride concentrations and showed a redistribution of cholesterol between lipoproteins of various density in comparison with BMI<25 kg/m^2 (Table 1).

Responses during oGTT: glucose, IRI ,C-peptide

The average venous plasma glucose, IRI and C-peptide (not determined in CTRL) values during oGTT are given in Table 2. Thirty-nine patients out of the 61 were insulin-sensitive with a normal fasting glycemia, immunoreactive insulin (IRI) and C-peptide levels as well as normal response to oGTT. No significant difference in mean fasting glycemia was found between the groups, while the corresponding mean concentrations of IRI and C-peptide were higher in IR patients. Moreover, the oral glucose load resulted in higher glycemia in IR patients, and caused elevation in IRI and C-peptide levels that persisted even after 120 min (vs. both CTRL and IS groups). Although IRI concentrations in IS patients 120 min after the glucose load remained elevated in comparison with CTRL, this was within the normal range.

Subdivision of the patients according to BMI resulted in a significant difference in after-load glucose concentrations, fasting and after-load IRI levels as well as fasting C-peptide concentrations. The latter were higher in patients with BMI >30 kg/m^2 if compared with both CTRL and BMI <25 kg/m^2 groups (Table 2). The postprandial (t=120 min) IRI levels were higher in patients with BMI <25 kg/m^2 than in the CTRL group. If the mean values of the investigated parameters at corresponding time intervals were compared between IS and non-obese patients, a significant difference was revealed only for IRI at 120 min ($p<0.01$), while no significant difference was observed between IR patients and those with BMI >30 kg/m^2.

Table 2. Glycemia, immunoreactive insulin (IRI) and C-peptide concentration during oral glucose tolerance test

	0 min	**60 min**	**120 min**
Glycemia			
CTRL	5.12±0.40	8.49±0.63**	5.74±0.15
IS	5.06±0.11	7.47±0.31**	5.82±0.18$^{**,++}$
IR	5.37±0.16	10.93±0.91$^{\#,**,b}$	8.63±0.60$^{\#,**,+,b}$
BMI <25 kg/m^2	4.99±0.13	7.69±0.58**	6.39±0.42$^{*,+}$
BMI >30 kg/m^2	5.56±0.21^{c}	10.61±0.93$^{\#,**,c}$	8.43±0.70$^{\#,**,++}$
IRI			
CTRL	6.02±0.64	50.76±9.12**	14.88±2.49**
IS	6.66±0.81	62.45±5.48**	35.09±2.26$^{**,\#\#,++}$
IR	14.61±1.74$^{\#\#,b}$	100.07±8.96$^{**,\#\#,b}$	96.29±8.50$^{**,\#\#,b}$
BMI <25 kg/m^2	7.26±0.96	61.27±5.25**	48.66±5.19$^{**,\#\#,+}$
BMI >30 kg/m^2	16.32±2.74$^{\#\#,d}$	111.55±17.0$^{**,\#\#,d}$	86.95±19.24$^{**,\#\#,+}$
C-peptide			
CTRL	ND	ND	ND
IS	0.64±0.04	2.76±0.24**	2.37±0.14**
IR	0.96±0.10^{b}	3.03±0.24**	3.70±0.33^{**,b}
BMI <25 kg/m^2	0.65±0.06	2.83±0.28**	2.71±0.26**
BMI >30 kg/m^2	1.04±0.16^{c}	2.92±0.37**	3.48±0.32**

*Means ± SEM are given; IS: insulin-sensitive patients; IR: insulin-resistant patients; BMI: body mass index; #: p<0.05 vs CTRL; ##: p<0.01 vs. CTRL; *: p<0.05 vs t0, **: p<0.01 vs t0, +: p<0.05 vs t60, ++: p<0.01 vs t60, a: p<0.05 vs IS, b: p<0.01 vs IS, c: p<0.05 vs BMI <25 kg/m^2, d: p<0.02 vs BMI <25 kg/m^2, ND: not determined*

Responses during oGTT: magnesium metabolism

Mean values of magnesium in the serum and erythrocytes before and 120 min after oGTT were within the normal range (Table 3). The mean values of all parameters of magnesium metabolism were normal in both groups, however, ten out of 39 IS patients had basal fErMg concentrations below normal (<162.2 μmol/l, while only one out of 22 IR patients had such low values (Fig. 1, $\chi^{2:}$ $p<0.01$). In IR patients the mean serum Mg levels, tErMg and bErMg concentrations (both before and after oGTT) were significantly higher than those of IS patients. Although intergroup comparison of the mean

fErMg concentrations were not significant, the increase in erythrocyte Mg concentration in IR patients was proportional, since there were no significant differences in the F/T or F/B erythrocyte Mg ratios.

Table 3. Parameters of magnesium metabolism during oral glucose tolerance test.

	CTRL	All	IS	IR	BMI<25	BMI>30
Mg-S_0	883.9±43.5	832.4±13.6	809.4±17.6	873.2±18.7^{b}	814.0±21.2	869.6±31.1
Mg-S_{120}	854.6±46.8**	817.5±13.5**	793.4±17.5**	860.1±8.2^{**,b}	789.3±20.6**	865.1±30.0
tErMg$_0$	2081.3±11.1	2029.3±4.1	1967.7±37.4	2138.6±62.1^{b}	1978.0±53.7	2200.0±55.0^{a}
tErMg$_{120}$	2071.3±17.9	2016.2±36.5	1960.5±39.6	2115.0±69.0^{*,a}	1972.7±57.6	2184.6±61.6^{a}
fErMg$_0$	194.6±3.7	191.5±5.0	188.3±6.8	197.0±6.8	190.4±9.0	191.2±7.2
fErMg$_{120}$	200.7±13.6	195.8±7.1	192.4±10.4	201.6±7.3	190.3±8.8	188.5±13.3
bErMg$_0$	1886.7±102.7	1838.7±33.0	1779.1±35.7	1941.5±60.3^{b}	1787.6±49.6	2008.8±53.2^{a}
bErMg$_{120}$	1870.6±104.9	1819.2±36.1	1764.7±40.1	1913.4±66.4^{*,a}	1782.4±55.4	1996.0±58.7^{a}
F/T$_0$ [%]	9.4±0.5	9.5±0.2	9.6±0.3	9.3±0.4	9.6±0.4	8.7±0.4
F/T$_{120}$ [%]	9.7±0.2	9.8±0.4	9.9±0.5	9.7±0.4	9.8±0.5	8.7±0.6
F/B$_0$ [%]	10.4±0.6	10.5±0.3	10.7±0.4	10.3±0.4	10.7±0.5	9.6±0.4
F/B$_{120}$ [%]	10.7±0.2	11.0±0.5	11.1±0.7	10.7±0.5	10.9±0.6	9.5±0.8

*Mg concentrations are given in μmol/l; Mg-S: magnesium in serum; tErMg: total erythrocyte magnesium; fErMg: free erythrocyte magnesium; bErMg: bound erythrocyte magnesium; F/T: free to total erythrocyte magnesium ratio; F/B: free to bound erythrocyte magnesium ratio; $_0$: before glucose load; $_{120}$: 120 min after oral glucose load of 75g (oGTT); IS: insulin-sensitive patients with kidney disease; IR; insulin-resistant patients with kidney disease; BMI: body mass index, *:p<0.05; **:p<0.01 comparing intragroup means (0 to 120 min values); a: p<0.05; b: p<0.01 for intergroup comparison of corresponding intervals.*

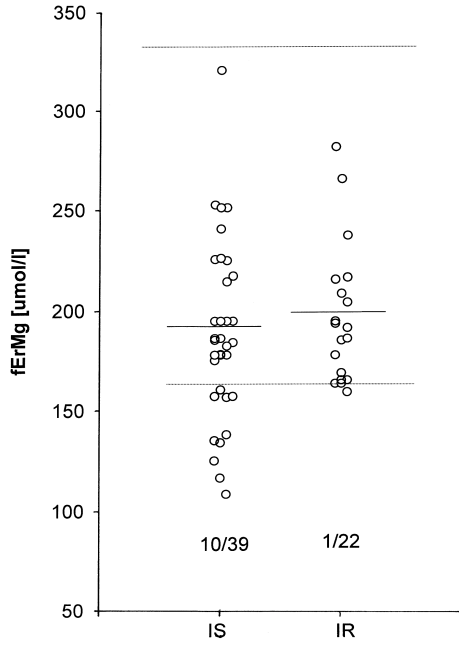

Fig. 1. *Individual data on basal free red blood cell magnesium (fErMg) concentration in insulin-sensitive and insulin-resistant patients with mild to moderate decline of kidney function. Mean value in each group is depicted in bold line, normal range of fErMg concentration is given by dotted lines.*

All groups responded to glucose load with a decline in serum magnesium concentration that was within the normal range. However, in IR patients a significant decline in tErMg and bErMg was observed after oGTT. In spite of this, F/T and F/B erythrocyte Mg ratios were not influenced significantly by the oral glucose load.

To determine which of the metabolic and anthropometric variables correlated best with the mean sum of the IRI values sampled during oGTT in each individual (an index used instead of area under insulin curve - AUC_{IRI}) (Okša *et al.* 1994) stepwise multiple regression analysis was conducted. The independent variables entered into analysis included fErMg, bErMg, Mg-S, age, BMI, pH, HCO_3, proteinuria, cholesterol, HDL-, VLDL- and LDL cholesterol, apoB, triglycerides concentration, systolic and diastolic blood pressure, hippuric acid and pseudouridine concentration. With virtual AUC_{IRI} taken as the dependent variable, the multivariate analysis selected serum creatinine levels, bErMg and HCO_3 concentrations contributing significantly (n=37, p=0.0003, $r^2 = 0.428$, significance level for inclusion was p<0.05). Amount of insulin needed to metabolize the glucose load correlated with basal bErMg concentration (Fig 2). The simple

correlation coefficient for the AUC_{IRI} and the basal tErMg was r=0.386, p<0.002 in all patients, and r=0.545, p<0.009, in IR group. Correlation coefficients for the mentioned Mg parameters after glucose load were slightly lower (data not given). In the patient's group as a whole the basal and as well as after load serum Mg concentrations correlated positively with AUC_{IRI} (t_0: r=0.328, p<0.01; t_{120}: r=0.336, p<0.008). None of these correlation was revealed in the CTRL and IS group.

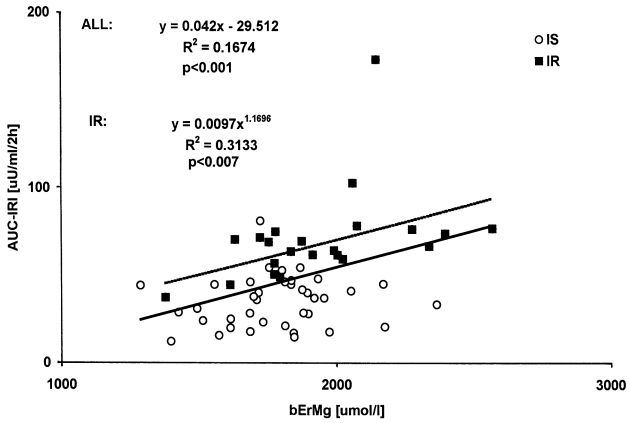

Fig. 2. *Correlation between mean insulin amount (AUC-IRI) needed to metabolize the 7 5g glucose load and basal level of bound erythrocyte magnesium (bErMg) in the group as a whole (solid line, n=61) and in insulin resistant patients (dotted line, n=22) with mildly to moderately decreased kidney function.*

Subdivision of the patients according to BMI revealed significantly higher basal and after-load levels of tErMg and bErMg in the subgroup of obese patients (Table 3). However, nor the pre-load, neither the after load F/T and F/B erythrocyte Mg ratios differed between the groups. Distribution of the patients with decreased basal fErMg was proportional in both groups (BMI <25: 6 out of 31, BMI >30: 2 out of 11, χ^2: NS). While patients with BMI <25 kg/m^2 responded to glucose load with significant decline in plasma Mg concentration (within the normal range), in obese patients only a trend towards the decrease was observed. The other studied parameters of Mg metabolism were not significantly influenced by the oral glucose load.

Neither single nor stepwise multiple regression analysis revealed any correlation between the mean sum of the IRI values and any other parameter either in the subgroup of the lean, or in the obese patients.

Discussion

Insulin resistance

Twenty two out of the 61 patients (36 %) suffered from insulin resistance. They had higher BMI and abnormal lipid pattern, typical for chronic renal failure patients. Incidence of IR was independent from age, sex, underlying disease, blood pressure and decline in renal function.

Magnesium metabolism

Basal values: Patients in end-stage of chronic renal failure on renal replacement therapy exhibit increased plasma and erythrocyte magnesium concentration, and the rate of Mg extrusion via Na^+/Mg^+ antiport is significantly higher in hemodialyzed patients (Vormann *et al.* 1994). These results indicate that erythrocyte Mg content is determined by the increased plasma Mg and increased Mg uptake. The patients studied suffered from mild to moderate decline in kidney function and their serum, as well as the mean tErMg and bErMg concentrations were normal. However, the incidence of low fErMg concentrations was significantly higher in IS group. Moreover, IR patients exhibited a significant increase in serum Mg concentrations and a proportional increase in erythrocyte Mg content compared to IS group. Stepwise multiple regression of the combined data (taking serum Mg, tErMg and bErMg, respectively, as dependent variables) selected AUC_{IRI} and hippurate or pseudouridine levels to contribute significantly (n=61, r^2=0.216-0.326, p<0.006-0.0003). This suggests a correlation between insulin resistance, uremic toxins and accumulation of magnesium. However, there is no data available on the effect of insulin, pseudouridine, or hippurate on Na^+/Mg^+ antiporter activity.

Glucose load: Patients responded to the glucose load uniformly by a slight but significant decline in serum Mg concentrations. This seems to be a physiologic reaction, since even the control subjects responded to oGTT with a drop in serum Mg concentration. This type of the reaction was observed in healthy volunteers even after glucose infusion (40 g within 60 min), and lasted throughout 3 hours after glucose load (Šebeková, unpublished data). However, no changes in tErMg or fErMg concentration in response to glucose load were observed in these subjects. On the other hand, Corica *et al.* (1999) found a decline in plasma Mg levels with an increase of red blood cell Mg content in healthy controls after oral glucose load. Thus, the reaction of IR patients

to glucose load is characterized by a significant decline in both total and bound erythrocyte Mg concentrations, while in healthy subjects and insulin-sensitive kidney-diseased patients the changes did not reach significance. Response revealed in IR patients with kidney disease corresponds to that of the obese subjects (Corrica *et al.* 1999), suggesting the same underlying mechanism. However, in our patients with kidney disease obesity itself had no significant impact on Mg status. The observed tendency towards an increase in fErMg concentrations after glucose load might probably reflect the direct effect of insulin. As shown by Barbagallo *et al.* (1993), in normal human erythrocytes insulin induced elevation of fErMg concentrations in a dose- and time-dependent manner. However, these *in vitro* effects peaked at much higher insulin concentrations (>200 μU/ml) than those reached *in vivo* in our patients. Slight but significant decline in both plasma and red blood cell Mg after glucose load may be explained by redistribution of Mg into other tissue stores, or, by urinary loss, but these parameters were not followed in our study.

An unexpected result of our study was the finding, that in contrary to essential hypertension, IR in patients with mildly to moderately decreased kidney function was not associated with low fErMg. The tErMg and bErMg concentrations of IR patients even correlated positively with the amount of insulin needed to metabolize the given glucose load. These data suggest a different pathophysiological mechanism for an early development of IR in kidney-diseased patients.

Effect of BMI

Obese patients with kidney disease had normal fasting plasma Mg concentration, and, in comparison to lean ones, slightly elevated tErMG and bErMg levels. Although no significant changes in fErMg levels were revealed, this rise seems to be proportional, as the F/T and F/B Mg ratio was not changed. Our finding corresponds to that reported by De Leeuw *et al.* (1992), who showed that non-diabetic obese humans (regardless of impaired glucose tolerance) are able to maintain normal plasma and erythrocyte Mg levels. On the other hand, in obese hypertensive subjects fErMg levels are suppressed (Resnick *et al.* 1991), while we found no significant decline of fErMg in obese patients with kidney diseases. Plasma glucose, IRI and C-peptide response to glucose load in obese patients were comparable to those of the IR patients. However, in contrast to the IR group, obese patients maintained their tErMg and bErMg levels after glucose load. Thus, in patients with kidney disease insulin resistance, not obesity, seems to be decisive for Mg dysbalance.

In summary: 1) insulin sensitive patients with mild to moderate decrease in renal function show a high prevalence of low fErMg; however, their intracellular Mg handling in response to glucose load seems not to be altered; 2) in IR patients there is a trend towards the accumulation of magnesium in serum and red blood cells, and they respond to a glucose load with a significant decrease of erythrocyte magnesium, particularly on the account of its bound fraction. Pathophysiological mechanism leading to these changes are still not clear, but the interaction of hyperinsulinemia and the action of inhibitors of glucose utilization (pseudouridine, hippuric acid) might be anticipated . In conclusion, the unexpected observation of the infrequent occurrence of low fErMg concentrations in IR patients with kidney disease does not support the assumption of the direct participation of Mg dysbalance in the development of IR.

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Reprint requests

K. Šebeková, MD. PhD.; Institute of Preventive and Clinical Medicine, Limbová 14, 833 01 Bratislava, Slovak Republic. Fax: +421-2-59369170. E-mail: sebekova@.upkm.sk