Magnesium Reduces Insulin-Stimulated Glucose Uptake and Serum Lipid Concentrations in Type 1 Diabetes

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A magnesium (Mg) deficit has been described in patients with type 1 diabetes, and it has been related to the development of cardiovascular disease. We tested the hypothesis that type 1 diabetic patients have deficits in dietary Mg intake and that proper long-term (24 weeks) oral Mg supplementation would reduce cardiovascular risk factors. Therefore, the Mg status, dietary Mg intake, and the effect of Mg supplementation were evaluated in 10 type 1 diabetic patients and 5 control subjects. Muscle Mg content was decreased by 7% in the type 1 diabetic patients, and it increased by 5% after 24 weeks of oral MgO supplementation. Acute and chronic Mg supplementation decreased serum total cholesterol, serum low-density lipoprotein (LDL)-cholesterol, and apolipoprotein B. Insulin-stimulated glucose uptake decreased by 35% after 24 weeks of oral MgO supplementation. Eight of 10 patients with type 1 diabetes had a daily intake of Mg below 90% of the recommended daily allowance. In conclusion, a Mg deficit was found in type 1 diabetic patients. The deficit might be due partly to a relatively Mg-deficient diet. Mg repletion was associated with a decrease in atherogenic lipid fractions and a reduced insulin-stimulated glucose uptake.

A MAGNESIUM (Mg) DEFICIT has been described in patients with type 1 diabetes.[1-3] Such Mg deficits have been linked to the development of atherosclerosis,[4,5] and in patients with coronary atherosclerosis, a Mg deficit has been related to an atherogenic lipid profile.[6] Therefore, a Mg deficit in patients with type 1 diabetes could be associated with the development of late diabetic complications, especially macroangiopathy.[3] Both hyperinsulinemia and hyperglycemia increase renal Mg excretion,[7,8] and even moderate improvement in metabolic control decreases renal Mg excretion.[3] Little is known regarding the Mg intake of type 1 diabetic patients. In a study of 18 women with type 1 diabetes, Smith et al[9] found a mean daily intake of Mg averaging 85% of the recommended amount using 3-day diet records. Gebre-Medhin et al[10] found that children with type 1 diabetes had a sufficient intake of Mg. In type 2 diabetes, Mg supplementation improves insulin-stimulated glucose uptake.[11] This has led to a number of studies on the effect of Mg supplementation on glycemic control in patients with type 2 diabetes, so far without any appreciable effect on metabolic control.[12-16]

The aims of the present investigation were to test the hypotheses that: (1) type 1 diabetes mellitus is associated with Mg deficiency, which is reflected by lowered serum and tissue content; (2) type 1 diabetic subjects normally (at random sampling) have a deficit in dietary Mg intake; (3) long-term (24 weeks) oral administration of an adequate Mg supplementation will positively affect variables supposedly related to the development of atherosclerosis in patients with type 1 diabetes mellitus, ie, serum lipid concentrations and insulin sensitivity; and (4) acute Mg loading will positively affect serum lipid concentrations in patients with type 1 diabetes mellitus.

SUBJECTS AND METHODS

Subjects

Ten patients with type 1 diabetes were included in the study. For every 2 patients with type 1 diabetes, 1 healthy control subject was matched by sex and age (±10 years). None of the subjects had acute illness within the month prior to the start of the study. No other medication was allowed besides insulin. Two patients had simplex retinopathy. Further clinical characteristics are listed in Table 1. Four type 1 diabetic subjects were included in the first quarter of the year and 2 patients were included in each succeeding quarter.

A 41-year-old type 1 diabetic woman with a 5-year history of diabetes withdrew from the study due to psychological disturbances. Her body mass index (BMI) was 21.8 kg/m^2. The last muscle biopsy was not taken in a 51-year-old type 1 diabetic male with a 21-year duration of diabetes, due to a vasovagal attack in conjunction with the preceeding muscle biopsy. His BMI was 23.0 kg/m^2. In the same male patient, indirect calorimetry was not performed due to claustrophobia. Indirect calorimetry was not performed in 1 female control, 37 years old with a BMI of 29.7, and in 1 male patient with type 1 diabetes, 25 years old with a 24-year duration of diabetes and a BMI of 22.3, due to unexpected absence of the equipment in both cases.

A reference interval for serum Mg concentration has been estimated on the basis of 139 healthy persons, collected to determine normal values. The material consisted of 2 different groups; both groups have been described in detail elsewhere.[3,17] The total sample consisted of 40 women and 99 males, with a median age of 36 years (range, 20 to 54).

Informed consent was obtained, and the study was approved by the regional ethical committee.

Methods

The design of the study is shown in Table 2.

Euglycemic, hyperinsulinemic study day. After an overnight fast and before morning insulin, the subjects delivered a 24-hour urinary

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Table 1. Clinical Characteristics of Patients With Type 1 Diabetes and Control Subjects

	Type 1 Diabetic Subjects	Controls
Age, years		
Median	41	37
Range	25 to 53	27 to 57
Women/men	4/6	2/3
Duration of diabetes (yr)		
Median	16.5	—
Range	5 to 38	
HbA_{1c} (%)		
Mean	6.9	5.1*
95% CI	6.4 to 7.4	4.0 to 6.2
Body mass index (kg/m^2)		
Mean	21.3	23.7
95% CI	15.8 to 26.7	18.7 to 28.7
Urinary albumin excretion rate ($\mu g/min$)		
Mean	14.7	10.5
95% CI	8.2 to 21.1	2.5 to 18.4
Serum creatinine ($\mu mol/L$)		
Mean	84.5	74.0
95% CI	78.1 to 90.9	60.4 to 87.6

*$P < .01$ (t test).

sample. After blood sampling, a muscle biopsy specimen was obtained. In the patients with type 1 diabetes, plasma glucose concentration was normalized by a variable infusion of insulin (Insulin Actrapid, NOVO Nordisk, Bagsvaerd, Denmark) or glucose as needed, aiming at a plasma glucose concentration of 5.0 ± 0.5 mmol/L. This was obtained within 120 minutes, and was followed by a basal period, lasting 120 minutes, during which plasma glucose concentration was maintained at 5.0 ± 0.5 mmol/L. After this basal period, the intravenous infusion of insulin, 240 pmol · m^{-2} · min^{-1}, was initiated in both controls and type 1 diabetic patients. The infusion lasted 120 minutes. Blood glucose concentration was maintained at 5.0 ± 0.5 mmol/L by the infusion of glucose, 200 g/L. A blood sample and a muscle biopsy specimen were obtained at the end of the euglycemic, hyperinsulinemic period.

Intravenous Mg supplementation. Between 2 and 14 days after the euglycemic, hyperinsulinemic study day an additional 24-hour urinary sample was obtained. Then 30 mmol $MgSO_4$ in 500 mL 0.9% NaCl was infused intravenously over 12 hours. The subjects met fasting, and a blood sample was collected before the infusion on the first day, as well as every morning for the following 4 days. A muscle biopsy specimen was obtained 24 hours after the start of the intravenous infusion of $MgSO_4$.

Twenty-four weeks of oral MgO supplementation. Having finished the intravenous Mg supplementation, the subjects with type 1 diabetes received oral MgO, 500 mg twice daily (Magnesia, DAK, Roskilde, Denmark) for 24 weeks. The number of tablets returned was counted. It was found that 90.2% (range, 70.8% to 100%) of the prescribed dose of MgO was ingested. The patients were seen after 8 and 16 weeks. At the end of the oral MgO supplementation period, the patients were restudied as described above, except that the intravenous Mg supplementation was given 7 to 21 days after the euglycemic, hyperinsulinemic study. Treatment with MgO was withdrawn 7 days prior to the intravenous Mg supplementation. There were no side effects from the MgO supplementation.

Dietary intake of Mg. All 10 patients completed a 7-day weighed food record before the intervention with Mg. After 24 weeks of oral MgO supplementation, 4 men and 3 women completed a new 7-day weighed food record. The 5 controls completed a single 7-day weighed food record.

Blood sampling. In patients with type 1 diabetes and their matched controls, an indwelling plastic catheter was placed in a hand vein 1 hour prior to blood sampling. All samples were obtained through this indwelling catheter, which was kept open by 10 mL 0.9% NaCl. The hand with the indwelling catheter was placed in a heated plastic box to ensure arterialized venous blood,[18] and the person was kept supine for at least 1 hour prior to blood sampling. Blood was drawn using a plastic syringe. The first 3 mL of blood was discarded. In the healthy control subjects, used for establishing a reference interval, blood samples were obtained using venipuncture without stasis.

Urine sampling. Urine was collected in plastic containers without additives. The samples were weighed (Mettler PL3000, Greifensee, Switzerland; balance precision, ±0.15 g) and their specific gravities determined by refractorimetry (Reichert TS meter, American Optical, USA). The last voiding was always after blood sampling and muscle biopsy. The renal data were related to body surface area calculated as

Table 2. Design of the Study

Time		Blood Sampling(s)	24-Hour Urine Sampling	Muscle Biopsy Specimen (n)
−30 to 0 days	Run in			
0 days	Euglycemic, hyperinsulinemic clamp	x	x	2
2 to 14 days				
Day 1	Intravenous infusion of 30 mmol $MgSO_4$	x	x	
Day 2		x	x	1
Day 3		x	x	
Day 4		x	x	
Day 5	1 g MgO orally per day to type 1 diabetics	x	x	
8 weeks	Control visit	x		
16 weeks	Control visit	x		
24 weeks	Euglycemic, hyperinsulinemic clamp, then withdrawal of MgO	x	x	2
25 weeks				
Day 1	Intravenous infusion of 30 mmol $MgSO_4$	x	x	
Day 2		x	x	1
Day 3		x	x	
Day 4		x	x	
Day 5		x	x	

described by Gehan and George.[19] However, to ensure that the registered dietary intake and urinary samplings were correct, the total amount of urinary-excreted potassium was compared to the estimated oral intake of potassium.

Individuals were weighed on a weighing balance to a precision of ± 200 g.

Calculation of the electrolyte content of the diet. All diets including fluids were weighed (Soehnle 8000, Soehnle-Waagen, Murrhardt, Germany; precision, ±1 g) and registered. The Mg content of the subject's tapwater was measured and a clinical dietician calculated the daily mineral intake using a program developed by Odense University Hospital. The program is continuously updated.

Measurements. Muscle Mg content was determined on biopsy specimens from vastus lateralis m. quadriceps femoris using freeze-drying and dissection as described in detail elsewhere.[20]

Serum insulin concentrations were measured using a double-antibody radioimmunological method (Pharmacia Diagnostics AB, Uppsala, Sweden). The free insulin concentration was determined in patients with type 1 diabetes mellitus. After 10 minutes centrifugation at 37°C, the immunoglobulin-bound insulin was precipitated with polyethyleneglycol at 37°C, after which the samples were centrifugated for 30 minutes at 37°C.

HbA_{1c} was determined by a high-performance liquid chromatography (HPLC) method, standardized to the level of the Diabetes Control and Complications Trial.

Serum fructosamine was measured on a Cobas Fara (Hoffman-La Roche, Basle, Switzerland) using the NBT/Formazane reaction and corrected with serum albumin concentration.[21] Serum albumin was determined by the Bromcresol green method on a Technicon RA-XT (Tarrytown, NY).

Serum lipid concentrations were determined by routine methods.

Mg concentrations were measured by atomic absorption using a Perkin-Elmer 403 (Überlingen, Germany). These analyses have been described in detail elsewhere.[17,20]

Potassium (K) concentrations were measured by flame emission spectrophotometry.

Insulin-stimulated glucose uptake was determined as the infusion rate of glucose during the last 30 minutes of the euglycemic, hyperinsulinemic clamp corrected for body surface area.

Metabolic rates were determined with indirect calorimetry using a flowthrough canopy gas analyzer system (Deltatrac; Datex, Helsinki, Finland). After an equilibration period of 10 minutes, the average gas exchange rates recorded over the final 25-minute periods of both the basal state and the insulin-stimulated period were used to calculate rates of glucose and lipid oxidation corrected for body surface area. Nonoxidative glucose disposal rate during insulin-stimulation was calculated as the glucose infusion rate minus the glucose oxidation rate.

Statistics

Statistical analysis was performed using the SPSS/PC+ package (SPSS, Chicago, IL). All variables were tested for normality using the Kolmogorow-Smirnov's test. Where a Gaussian distribution could not be rejected, variables are reported as means and their 95% confidence intervals (95% CI), whereas non-Gaussian distributed variables are reported as median and range. Fisher's exact test, the binomial test (bin), *t* test, analysis of variance (ANOVA), and repeated measures ANOVA have been applied as indicated. All variables were tested for a possible variation between sexes, and if such a difference was encountered, it was included in the statistical analysis. The associations between 2 variables were evaluated by Pearson's correlation coefficients. Correlation coefficients are reported together with their 95% CI calculated as described by Gardner and Altman.[22]

Mg, K, lipid, and insulin concentrations or contents before and after 24 weeks of oral MgO supplementation were determined as the mean of the value before the euglycemic, hyperinsulinemic clamp and the value before the infusion of $MgSO_4$.

The reference interval for serum Mg concentration was determined using the IFCC-recommended statistical treatment of reference values (REFVAL).[23]

RESULTS

Serum Mg Concentration

In the basal state, before Mg supplementation, serum Mg concentration was found to be lower in patients with type 1 diabetes (0.74 [95% CI, 0.69 to 0.80] mmol/L, n = 10) compared with the control group (0.82 [95% CI, 0.76 to 0.88] mmol/L, n = 5, $P < .05$). Oral MgO supplementation for 24 weeks given to the patients with type 1 diabetes induced an increase in serum Mg concentration from 0.75 (95% CI, 0.69 to 0.81) mmol/L to 0.80 (95% CI, 0.73 to 0.87) mmol/L, n = 9, $P < .05$.

The reference interval for serum Mg concentration was 0.75 to 0.97 mmol/L.

Muscle Mg Content

In the basal state, muscle Mg content was lower in the group of patients with type 1 diabetes compared to the control group (Table 3).

Oral MgO supplementation for 24 weeks increased muscle Mg content (Table 3). The patients with the lowest muscle Mg content showed the greatest increase in muscle Mg content. Of

Table 3. Muscle Mg Content Before and After an Euglycemic, Hyperinsulinemic Clamp, After the Infusion of 30 mmol $MgSO_4$, and After Oral MgO Supplementation for 24 Weeks

	Basal	After Euglucemic Hyperinsulinemia	After Infusion of $MgSO_4$	Men/Women
Patients with type 1 diabetes before oral MgO supplementation (mmol/kg dry weight)	31.4 (30.1 to 32.7)	30.7 (29.0 to 32.4)	32.6 (30.7 to 34.5)	6/4
Patients with type 1 diabetes after oral MgO supplementation for 24 weeks (mmol/kg dry weight)	32.9* (31.7 to 34.1)	31.7† (30.2 to 33.2)	33.4 (31.2 to 35.5)	6(5)/3
Controls (mmol/kg dry weight)	33.6‡ (30.1 to 37.1)	32.7 (31.6 to 33.9)	34.5 (31.1 to 38.0)	3/2

NOTE. Values are mean (95% CI).

*Repeated-measures ANOVA: Difference before/after oral MgO supplementation to patients with type 1 diabetes: $P < .05$.

†Repeated measures ANOVA: the effect of euglucemic hyperinsulinemia, $P < .02$. The effect of oral MgO supplementation upon muscle Mg content, $P < .02$.

‡ANOVA: difference between patients with type 1 diabetes before oral MgO supplementation and control, $P < .05$.

the 9 patients who completed the 24 weeks of oral MgO supplementation, muscle Mg contents were below the lowest values for the same sex in the control group in 6 of the type 1 diabetic patients. All of these 6 patients demonstrated an increased muscle Mg content after 24 weeks of oral MgO supplementation (bin[0;6;0.5] = 0.03). Of the remaining 3 patients, 2 showed decreased muscle Mg content, while muscle Mg content increased in the last patient.

Intravenous Mg infusion did not induce significant changes in muscle Mg content, while the infusion of insulin and glucose reduced muscle Mg content (Table 3).

Renal Mg Excretion

In the basal state, the patients with type 1 diabetes had the same renal Mg excretion (3.68 [95% CI, 3.01 to 4.36] μmol · min^{-1} · 1.73 m^{-2}, n = 10) as the controls (3.26 [95% CI, 2.59 to 3.94] μmol · min^{-1} · 1.73 m^{-2}, n = 5, $P < .40$).

Oral MgO supplementation for 24 weeks increased the renal Mg excretion from 3.78 (95% CI, 3.06 to 4.51) μmol · min^{-1} · 1.73 m^{-2} before Mg supplementation to 5.05 (95% CI, 3.59 to 6.50) μmol · min^{-1} · 1.73 m^{-2}, n = 9, $P < .02$ after oral MgO supplementation. The increase in the renal Mg excretion correlated positively with the increase in serum Mg concentration, $r = .77$ (95% CI, 0.21 to 0.95), n = 9, $P < .02$.

Serum Lipid Concentrations

There were higher basal serum high-density lipoprotein (HDL)-cholesterol concentrations and a tendency towards lower serum low-density lipoprotein (LDL)-cholesterol values in patients with type 1 diabetes compared to controls. Patients with type 1 diabetes had a mean serum HDL-cholesterol concentration of 1.69 (95% CI, 1.33 to 2.06) mmol/L compared to 1.25 (95% CI, 0.93 to 1.57) mmol/L in controls. The mean serum LDL-cholesterol concentration was 3.04 (95% CI, 2.50 to 3.58) mmol/L in type 1 diabetic patients and 3.46 (95% CI, 2.07 to 4.84) mmol/L in controls. This resulted in a higher HDL/LDL ratio in type 1 diabetic patients (0.61 [95% CI, 0.41 to 0.82], n = 10) compared to controls (0.38 [95% CI, 0.27 to 0.48], n = 5, $P < .05$). Likewise, the patients with type 1 diabetes have lower concentrations of serum triglycerides than controls at the start of the study (Fig 1). Basal serum total cholesterol and apolipoprotein A1 and B did not differ between groups (Figs 1 and 2).

Twenty-four weeks of oral MgO supplementation reduced serum total cholesterol (Fig 1) and apolipoprotein B (Fig 2). Likewise, serum LDL-cholesterol decreased from 2.96 (95% CI, 2.38 to 3.54) mmol/L to 2.67 (95% CI, 2.24 to 3.10) mmol/L, n = 9, $P < .05$, whereas serum HDL-cholesterol was unchanged, 1.74 (95% CI, 1.35 to 2.14) mmol/L before and 1.71 (95% CI, 1.29 to 2.13) mmol/L after oral MgO supplementation. The HDL/LDL cholesterol ratio was unchanged: 0.65 (95% CI, 0.42 to 0.87) before and 0.68 (95% CI, 0.46 to 0.90) after oral MgO supplementation, as were serum triglycerides (Fig 1). The changes in serum cholesterol and apolipoproteins were only present after 24 weeks of oral MgO supplementation, whereas no changes were found in these parameters after 8 or 16 weeks. Regarding serum total cholesterol, the mean value was 4.97 (95% CI, 4.47 to 5.47) mmol/L before oral MgO supplementation, 5.00 (95% CI, 4.60 to 5.41) mmol/L after 8 weeks of oral MgO supplementation, and 4.95 (95% CI, 4.53 to 5.37) mmol/L after 16 weeks of oral MgO supplementation.

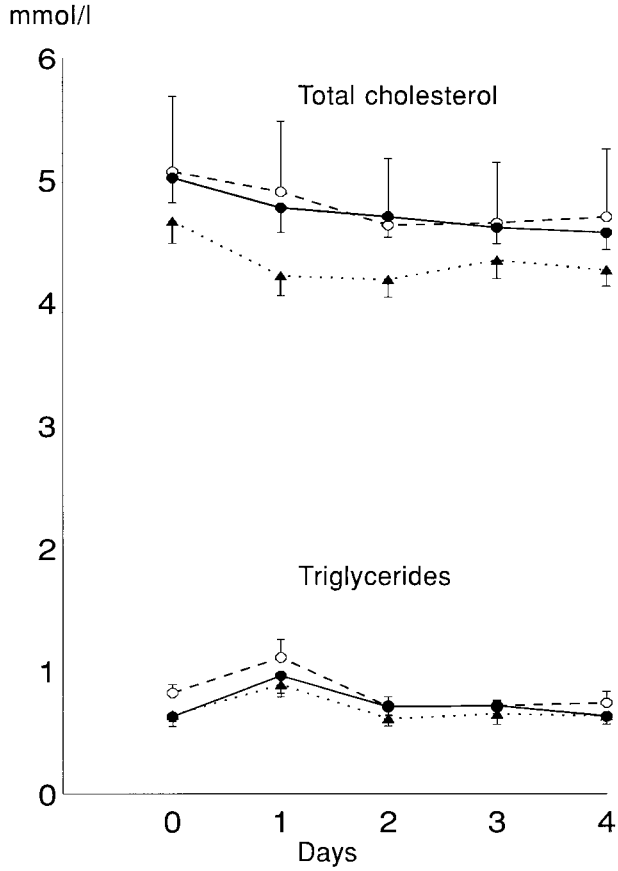

Fig 1. Serum total cholesterol (top) and serum triglycerides (bottom) before and after the intravenous infusion of 30 mmol $MgSO_4$. Mean and SEM. (●———●) Patients with type 1 diabetes before oral MgO supplementation, n = 10. (▲ . . . ▲) Patients with type 1 diabetes after 24 weeks oral MgO supplementation, n = 9. (○ - - - ○) Controls, n = 5. Repeated-measures ANOVA regarding serum total cholesterol: the effect of intravenous infusion of 30 mmol $MgSO_4$ on serum total cholesterol, $P < .001$. The 24 weeks of oral MgO supplementation reduced serum total cholesterol, $P < .02$, and serum total cholesterol decreased more due to the infusion of $MgSO_4$ after 24 weeks of oral MgO, $P < .05$. Considering only the effect of intravenous $MgSO_4$ on serum total cholesterol in controls, $P < .001$. No difference was found between patients with type 1 diabetes before oral MgO and controls, neither when all values were considered ($P < .9$), nor when the values before any Mg supplementation was considered separately, *t* test ($P < 1.0$). Repeated-measures ANOVA regarding serum triglycerides: the effect of intravenous infusion of 30 mmol $MgSO_4$ on serum triglycerides, $P < .005$. The 24 weeks of oral MgO supplementation did not change serum triglycerides, $P < .8$, and neither was there a difference between patients with type 1 diabetes and controls when all values were considered, $P < .5$. Difference between patients with type 1 diabetes and controls before any Mg supplementation, *t* test, $P < .05$.

The infusion of $MgSO_4$ induced a decrease in serum total cholesterol concentration both in patients with type 1 diabetes and in the control group (Fig 1). The mean decrease in serum total cholesterol during the 4 days after the infusion of $MgSO_4$ was 6.9% in the patients with type 1 diabetes before oral Mg

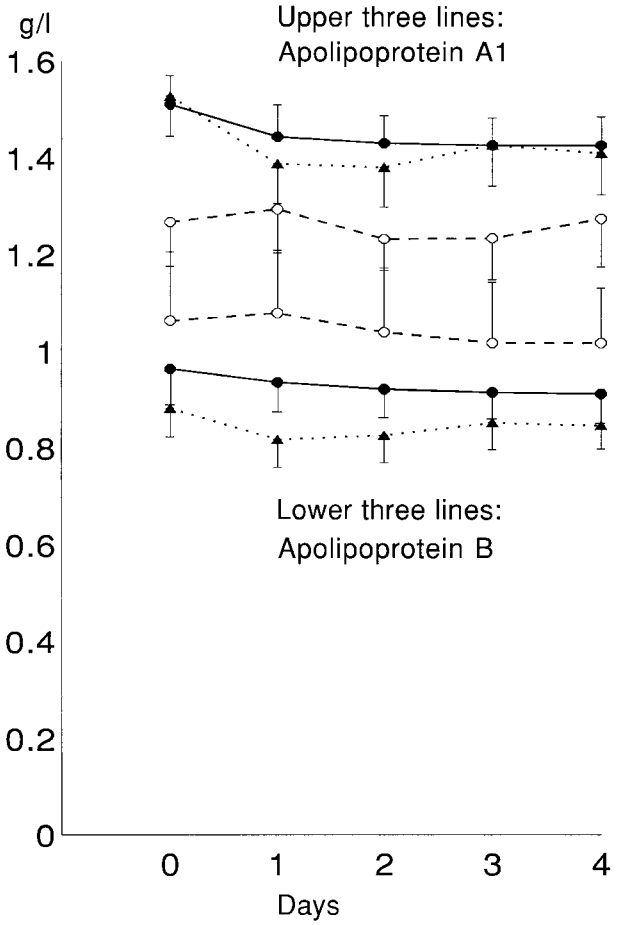

Fig 2. Serum apolipoprotein A1 (upper 3 lines) and serum apolipoprotein B (lower 3 lines) before and after the intravenous infusion of 30 mmol $MgSO_4$. Mean and SEM. (●— — —●) Patients with type 1 diabetes before oral MgO supplementation, n = 10. (▲ . . . ▲) Patients with type 1 diabetes after 24 weeks oral MgO supplementation, n = 9. (○ - - - ○) Controls, n = 5. Repeated-measures ANOVA before and after 24 weeks of oral MgO supplementation: the infusion of $MgSO_4$ decreased apolipoprotein A1 ($P < .0005$) and B ($P < .001$). The 24 weeks of oral MgO supplementation decreased serum apolipoprotein B, $P < .01$.

supplementation and 8.8% after oral Mg supplementation. The decrease was 6.9% in the control group (Fig 1). There were similar changes in serum LDL-cholesterol and serum HDL-cholesterol, except that the decreases in lipid concentrations induced by the infusion of $MgSO_4$ were identical after 24 weeks of oral Mg supplementation compared to before (data not shown).

Serum triglycerides increased and then decreased after the infusion of $MgSO_4$ in both groups, and the responses were the same after oral MgO supplementation (Fig 1). In the patients with type 1 diabetes, apolipoprotein A1 and B both decreased due to the infusion of $MgSO_4$ (Fig 2).

Insulin-Stimulated Glucose Uptake

The insulin-stimulated glucose uptake decreased 35% after 24 weeks of oral MgO supplementation, from 247.2 (95% CI, 172.7 to 321.7) mg · min^{-1} · m^{-2} before to 160.4 (95% CI, 107.8 to 213.1) mg · min^{-1} · m^{-2} after oral MgO supplementation, n = 9, $P < .01$. This was not due to a higher insulin-stimulated glucose uptake in the patients with type 1 diabetes compared to control subjects before Mg supplementation. On the contrary, the 10 patients with type 1 diabetes had an insulin-stimulated glucose uptake of 244.7 (95% CI, 161.5 to 305.846) mg · min^{-1} · m^{-2} before Mg supplementation, whereas the 5 control subjects had an insulin-stimulated glucose uptake of 287.6 (95% CI, 149.5 to 425.7) mg · min^{-1} · m^{-2}, $P < .4$. Body mass index was unaffected by the oral MgO supplementation: 21.2 (95% CI, 14.9 to 27.4) kg/m^2 before and 21.2 (95% CI, 15.0 to 27.4) kg/m^2 after 24 weeks of oral Mg supplementation. Plasma insulin concentrations during the euglycemic, hyperinsulinemic clamp were the same in the 2 groups, and they were unaffected by the 24 weeks of oral MgO supplementation (Fig 3). Plasma insulin concentrations during the last 30 minutes of the basal period before the infusion of insulin and glucose were likewise unaffected by the Mg supplementation (Fig 3). However, compared with the controls, a higher basal plasma insulin

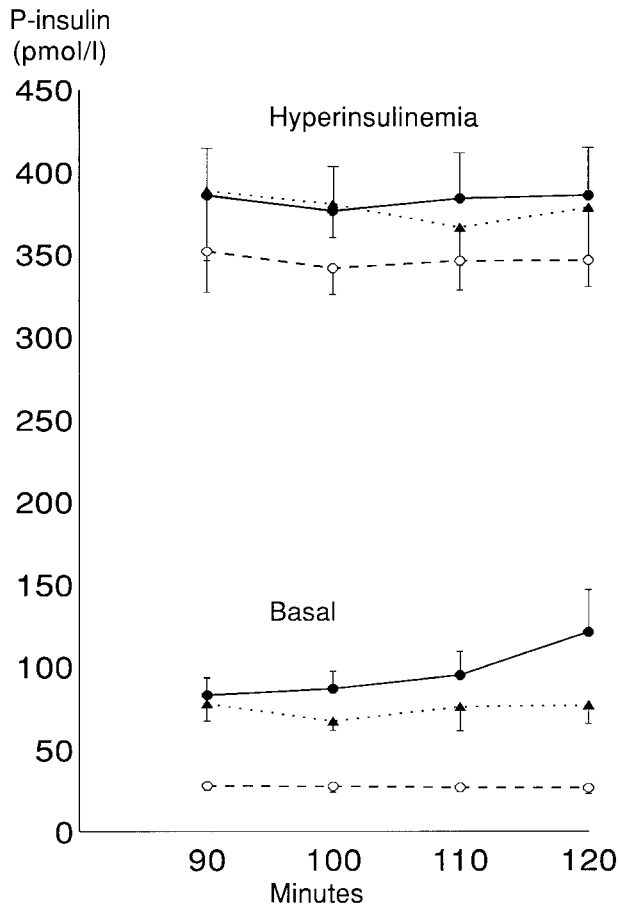

Fig 3. Plasma insulin concentrations in the last 30 minutes of the basal period and during hyperinsulinemia, respectively. Mean and SEM. (●— — —●) Patients with type 1 diabetes before oral MgO supplementation, n = 10. (▲ . . . ▲) Patients with type 1 diabetes after 24 weeks oral MgO supplementation, n = 9. (○ - - - ○) Controls, n = 5. Repeated-measures ANOVA using the values before oral MgO: control individuals had lower plasma insulin concentrations in the basal state, $P < .001$.

Table 4. Basal and Insulin-Stimulated Glucose and Lipid Turnover Rates Determined by Indirect Calorimetry

	Patients With Type 1 Diabetes Mellitus Before Oral MgO	Patients With Type 1 Diabetes Mellitus After Oral MgO	Controls
Glucose oxidation rate ($mg \cdot min^{-1} \cdot m^{-2}$)			
Basal	18.3	21.6	53.9
	(8.9 to 27.8)	(7.8 to 35.3)	(32.1 to 75.8)
Insulin-stimulated	70.5	64.2	112.2*
	(50.4 to 90.7)	(41.0 to 87.5)	(84.0 to 140.4)
Nonoxidative glucose uptake rate ($mg \cdot min^{-1} \cdot m^{-2}$)			
Basal	—	—	—
Insulin-stimulated	142.9	100.0†	213.3
	(70.8 to 214.9)	(42.0 to 158.1)	(106.8 to 319.7)
Lipid oxidation rate ($mg \cdot min^{-1} \cdot m^{-2}$)			
Basal	54.4	52.5	41.1
	(48.2 to 60.6)	(44.7 to 60.3)	(22.7 to 59.4)
Insulin-stimulated	30.7	32.4	17.1‡
	(19.8 to 41.5)	(19.9 to 44.9)	(14.8 to 19.5)
Men/women	4/4	4/3	3/1

NOTE. Values are mean (95% CI).

*Repeated-measures ANOVA: difference between patients with type 1 diabetes mellitus and controls, $P < .0005$, and the glucose oxidation rate increased during insulin-stimulation, $P < .0005$.

†Repeated-measures ANOVA: the effect of 24 weeks of oral MgO, $P < .02$.

‡Repeated-measures ANOVA: difference between patients with type 1 diabetes mellitus and controls, $P < .05$, and the lipid oxidation rate decreased during insulin-stimulation, $P < .0005$.

concentration was needed in order to obtain a plasma glucose concentration of 5.0 ± 0.5 mmol/L (Fig 3).

The intervention with Mg did not affect metabolic control or insulin dosage. The mean HbA_{1c} was 6.9% (95% CI, 6.4% to 7.5%) before and 7.2% (95% CI, 6.1% to 8.2%) after Mg supplementation, n = 9, $P < .6$; the mean albumin-corrected serum fructosamine was 1.2 (95% CI, 1.1 to 1.4) mmol/L before and 1.2 (95% CI, 1.1 to 1.3) mmol/L after Mg supplementation, n = 9, $P < .3$. Mean insulin dosage was 42.3 (95% CI, 34.4 to 50.3) IE before oral MgO supplementation and 43.3 (95% CI, 32.6 to 54.1) IE after 24 weeks of oral MgO supplementation, n = 9, $P < .6$.

Metabolic Rates

Compared to controls, the glucose oxidation rate was lower and lipid oxidation rate was higher in the patients with type 1 diabetes both in the basal state and during hyperinsulinemia, whereas the nonoxidative glucose uptake rate did not differ between groups (Table 4), but here the numbers of studied subjects are very small. A marked reduction was seen in nonoxidative glucose disposal after 24 weeks of oral MgO supplementation (Table 4).

Dietary Magnesium Intake

Eight of the 10 patients with type 1 diabetes had a daily intake of Mg below the recommended daily allowance (Fig 4). Three of the control subjects had a daily intake of Mg below recommended (Fig 4). Six of 10 patients with type 1 diabetes had a daily intake of Mg below 90% of the recommended daily intake, whereas all subjects from the control group had a daily intake of 90% or more of recommended (Fisher's exact test: $P < .05$) (Fig 4).

The daily intake of Mg was unchanged after oral MgO supplementation to the patients with type 1 diabetes, ie, 96.5% (range, 73% to 134%) of the recommended daily intake before oral MgO supplementation and 97.4% (range, 73% to 136%) after (n = 7). Five of 7 patients had a daily intake of Mg below recommended and 4 patients had a daily intake below 90% of recommended after oral MgO supplementation.

On average, tapwater contributed to 5.4% (95% CI, 3.6% to 7.3%) of the Mg intake, but the range was wide, from 1.9% to 13.5%. In accordance with the small geographical area from which the individuals were recruited, the variation in the content of Mg in the drinking water was limited, i.e., from 0.31 to 0.65 mmol/L with a mean of 0.49 mmol/L. There was, however, a relatively large variation in the intake of water from 0.49 to 3.25 L/d, with a mean of 1.38 L/d.

The renal excretion of potassium was slightly less than the calculated intake. The patients with type 1 diabetes had a mean intake of potassium of 89.9 (95% CI, 73.7 to 106.1) mmol/d,

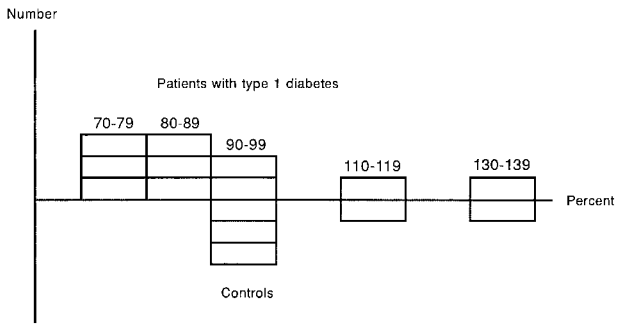

Fig 4. Dietary intake of Mg in patients with type 1 diabetes and in controls, plotted as a percentage of recommended daily intake of Mg, 300 mg/d (12.3 mmol/d) for women and 350 mg/d (14.4 mmol/d) for men.[48] Each person is symbolized as a box, patients with type 1 diabetes mellitus above the X-axis and controls below.

and a renal excretion of potassium of 80.2 (95% CI, 62.5 to 97.9) mmol/d before oral MgO supplementation and a dietary intake of potassium of 88.0 (95% CI, 76.3 to 99.7) mmol/d and a renal excretion of potassium of 86.5 (95% CI, 72.2 to 100.8) mmol/d after oral MgO supplementation. In the control group, the mean dietary intake of potassium was 85.8 (95% CI, 53.0 to 118.5) mmol/d and the renal excretion of potassium was 79.8 (95% CI, 52.1 to 107.5) mmol/d.

DISCUSSION

Our study demonstrates that Mg supplementation reduces insulin-stimulated glucose uptake. This has not been described in human studies before, and was surprising, as a previous human study has shown an increase in insulin-stimulated glucose uptake in patients with type 2 diabetes.[11] This latter finding of an increase in insulin-stimulated glucose uptake has prompted several studies on the effect of Mg supplementation on metabolic control in patients with type 2 diabetes.[12-16] However, only in one recent trial did the authors find a modest improvement in metabolic control in a subgroup.[15] In dogs, Mg deficiency is known to accelerate glucose disappearance after an intravenous glucose load.[24] In rats, a Mg-deficient diet has been found to reduce insulin concentration with an unchanged plasma glucose concentration[25,26] and to result in an increase in the effectiveness of insulin in stimulating glucose disposal.[27] In rat diaphragms, Mg deficiency increases the uptake of 2-deoxyglucose.[28] One human study found a reduction in insulin-stimulated glucose uptake during acute hypermagnesemia induced by Mg infusion.[29] These results are thus in keeping with the reduced insulin-stimulated glucose uptake found in the present study after oral MgO supplementation. Despite the decrease in insulin sensitivity, no significant change was observed in insulin dose or glycemic control. As the study was uncontrolled, this might be a "placebo" effect induced by the study. However, an increase in insulin sensitivity would have been more likely as a consequence of a placebo effect. Alternatively, an increased glucose-mediated glucose disposal, which might be related to the Mg supplementation, could have compensated for the reduced insulin sensitivity,[30] explaining the unchanged insulin dose and glycemic control. Furthermore, the insulin dosage was self-reported, and could be underestimated after Mg supplementation, and the absolute numerical value of both HbA_{1c} and insulin dosage increased after Mg supplementation, although not significantly so.

Basal plasma insulin concentration was increased in patients with type 1 diabetes both before and following intervention compared with controls, indicating insulin resistance in the type 1 diabetic patients. This is in agreement with previous studies,[31] and with the present study, where insulin-stimulated glucose uptake was decreased. As the effect of Mg supplementation was confined to the nonoxidative glucose disposal, Mg seems to inhibit one or more of the enzymes leading to the synthesis of glycogen. Interestingly, the activity of muscle glycogen synthase is reduced in patients with type 2 diabetes mellitus.[32] The results of the metabolic study are contrary to the findings of Paolisso et al, who found an increase in glucose oxidation rate after 4 weeks of Mg supplementation.[33] However, these data were obtained in type 2 diabetes, not type 1.

The moderate dose of MgO used in the present study induced insulin resistance at a physiologically obtainable plasma insulin concentration. Thus, equal doses of glucose will induce increased plasma glucose concentrations in the Mg-repleted state if plasma insulin concentrations are kept constant. Likewise, if plasma glucose concentrations are kept constant, plasma insulin concentration has to increase in the Mg-repleted state. We have shown previously that both hyperglycemia without glucosuria[8] and hyperinsulinemia with euglycemia[7] increase renal Mg excretion. Therefore, a model can be proposed that might serve as a basis for the physiological regulation of glucose homeostasis, insulin sensitivity, renal excretion of Mg, and whole body Mg content. When whole body Mg content increases, glucose uptake will decrease, leading to an increase in both plasma glucose and insulin concentrations, and thereby an increase in the renal excretion of Mg. This will tend to reduce whole body Mg, which, in turn, will reduce plasma glucose and insulin concentrations. This will tend to decrease the renal excretion of Mg. This model might explain the acute decrease in muscle Mg content induced by the infusion of insulin and glucose as found in the present study. The model would also explain why no improvement in metabolic control has been found in patients with type 2 diabetes when they are enriched with Mg using oral supplementation.[12-16] The model would further explain the Mg-deficit postulated to be found in patients with the so-called metabolic syndrome X,[34] where the Mg deficit could be viewed as a compensatory adaptation, aiming at improving the glucose uptake of skeletal muscles. Of course, the model will only account for some of the variations in plasma glucose concentration, insulin sensitivity, renal Mg excretion, and whole body Mg content, and it requires additional studies to prove this model, especially since this is an uncontrolled study. Hyperinsulinemia is a potential risk factor for the development of the diabetic late complications.[35] However, when insulin is used to improve the metabolic control in patients with type 1 diabetes mellitus, their insulin dose is increased.[3] Despite this, the incidence of the diabetic late complications is decreased when the metabolic control is improved.[36] Furthermore, it is possible that glucose enters the cell through routes unrelated to insulin as discussed above, thereby counteracting the effect of Mg on insulin sensitivity. Therefore, we do not think that normalization of whole body Mg status is harmfull in patients with type 1 diabetes mellitus. It is more questionable in patients with, or persons predisposed to, type 2 diabetes mellitus.

Before Mg supplementation, mean serum Mg concentration was below the lower reference limit determined in our laboratory. However, it should be kept in mind that the variation in serum Mg concentration within the individual is much less than the variation between individuals.[17] This implies that a value within the reference interval can be pathological for that particular individual.[17] In practice, a change in serum Mg concentration of 9.5% within an individual can be considered significant.[17] It is well known that patients with type 1 diabetes mellitus have lower serum Mg concentrations compared to controls,[1,37] and an increase in serum Mg concentration after oral Mg supplementation has previously been reported,[38] which is confirmed in the present study.

This study confirms previous data showing decreased muscle Mg content in patients with type 1 diabetes,[1,2] even though normal values also have been found in two studies.[39,40] The present study further confirmed the finding by Sjøgren et al[2]

that it is, indeed, possible to increase muscle Mg content in patients with type 1 diabetes with 24 weeks of oral Mg supplementation. The patients with the lowest muscle Mg content clearly showed the greatest increase in Mg.

This study showed a decrease in serum total cholesterol, serum LDL-cholesterol, and serum apolipoprotein B with oral Mg supplementation, and a more generalized lipid-lowering effect of intravenous Mg supplementation. A decrease in serum total cholesterol with long-term Mg supplementation has previously been described in various diseases.[6,41] However, it was surprising that serum triglycerides increased 1 day after the infusion of Mg, even though they did decrease later on. Previous reports have found a reduction in serum triglycerides with Mg supplementation in various disease states,[6,41] although this is not a constant finding.[38] In the present study no reduction could be found in serum triglycerides due to long-term oral MgO supplementation. Oral MgO supplementation to patients with ischemic heart disease has previously been shown to decrease apolipoprotein B with an unchanged serum apolipoprotein A1 concentration,[6] as found also in this study. The present study has the disadvantage that no placebo group was included. Thus, even though the patients were included evenly throughout the year, neither a placebo effect nor a period effect can be excluded. A double-blind, placebo-controlled study by Hägg et al[42] showed no effect of Mg supplementation on lipid concentrations. However, of 14 patients in their Mg-supplemented group, 2 showed a major decrease in their metabolic control and 1 had a very large weight gain.[42]

After 24 weeks of oral MgO supplementation in the present study, the decrease in serum total cholesterol induced by the infusion of $MgSO_4$ was larger than before the oral MgO supplementation in the patients with type 1 diabetes. Furthermore, we could find no difference between the patients with type 1 diabetes and the control group in response to infused $MgSO_4$, indicating that the effect of Mg is not confined to persons with a Mg deficit. The decrease in serum total cholesterol, serum LDL-cholesterol, and apolipoprotein B induced by Mg supplementation could be part of the protective role of Mg against the development of atherosclerosis,[4,5,43] even though Mg also seems to have other effects.[4,44] Mg binds bile acids weakly.[45] This could possibly explain the changes in lipid profiles. However, the infusion of Mg also induced changes in lipid concentrations, so it seems reasonable to suggest that it is a specific effect of Mg on the metabolism of lipids.

The 7-day weighed food records and the collection of 24-hour urinary samples reported herein turned out to be valid. The patients with type 1 diabetes reproduced their dietary intake of Mg, and by comparing the renal excretion of potassium with the calculated intake of potassium, it could be seen that both the registration of the diets and the collection of urine were valid. Even though the collection of urine and the registration of diets were performed on different days, the intake of potassium exceded the renal excretion with approximately 10 mmol/24 hours, as would be expected at equilibrium.[46,47]

The relatively modest intake of Mg found in the present study is in accordance with a study by Smith et al.[9] Using 3-day diet records in 18 women with type 1 diabetes, they found a mean daily Mg intake of 255 mg/24 hours. Using the 24-hour recall method, Gebre-Medhin et al[10] found a larger intake of Mg in diabetic children compared with controls. However, the diabetic patients might be biased due to their knowledge about recommended diets. In the present study, tapwater was a major, but variable, factor in the daily intake of Mg, as there was a relatively large variation in the intake of water, whereas no major differences existed in the Mg content of the tapwater.

In conclusion, muscle Mg content and serum Mg concentration were decreased even in patients with well-controlled type 1 diabetes, and they both increased with Mg supplementation, indicating a Mg deficit, which could partly be due to a moderately Mg-deficient diet. The repletion of Mg was associated with a decrease in serum total cholesterol, serum LDL-cholesterol, and serum apolipoprotein B, thereby probably reducing the risk of developing atherosclerosis.

Surprisingly, Mg supplementation induced a reduction in insulin-stimulated glucose uptake. By combining this response with the known increase in renal Mg excretion, seen with both hyperglycemia and with hyperinsulinemia, a physiological model is proposed, which might partly be responsible for the regulation of whole body Mg, renal Mg excretion, glucose homeostasis, and insulin sensitivity.

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REFERENCES

1. Johansson G, Danielson BG, Ljunghall S, et al: Evidence for a disturbed magnesium metabolism in diabetes mellitus. Magnes Bull 2:178-180, 1981

2. Sjøgren A, Florén CH, Nilsson Å: Oral administration of magnesium hydroxide to subjects with insulin-dependent diabetes mellitus: Effects on magnesium and potassium levels and on insulin requirements. Magnesium 7:117-122, 1988

3. Djurhuus MS, Henriksen JE, Klitgaard NAH, et al: Effect of moderate improvement in metabolic control on magnesium and lipid concentrations in patients with type 1 diabetes. Diabetes Care 22:546-554, 1999

4. Altura BT, Brust M, Bloom S, et al: Magnesium dietary intake modulates blood lipid levels and atherogenesis. Proc Natl Acad Sci USA 87:1840-1844, 1990

5. Singh RB: Effect of dietary magnesium supplementation in the prevention of coronary heart disease and sudden cardiac death. Magnes Trace Elem 9:143-151, 1990

6. Rasmussen HS, Aurup P, Goldstein K, et al: Influence of magnesium substitution therapy on blood lipid composition in patients with ischemic heart disease. A double-blind, placebo controlled study. Arch Intern Med 149:1050-1053, 1989

7. Djurhuus MS, Skøtt P, Hother-Nielsen O, et al: Insulin increases renal magnesium excretion: A possible cause of magnesium depletion in hyperinsulinaemic states. Diabet Med 12:664-669, 1995

8. Djurhuus MS, Skøtt P, Vaag A, et al: Hyperglycaemia enhances renal magnesium excretion in type 1 diabetic patients. Scand J Clin Lab Invest 60:403-410, 2000

9. Smith RG, Heise CC, King JC, et al: Serum and urinary magne-

sium, calcium and copper levels in insulin-dependent diabetic women. J Trace Elem Electrolytes Health Dis 2:239-243, 1988

10. Gebre-Medhin M, Kylberg E, Ewald U, et al: Dietary intake, trace elements and serum protein status in young diabetics. Acta Paediatr Scand 320:38-43, 1985 (suppl)

11. Paolisso G, Sgambato S, Pizza G, et al: Improved insulin response and action by chronic magnesium administration in aged NIDDM subjects. Diabetes Care 12:265-269, 1989

12. Purvis JR, Cummings DM, Landsman P, et al: Effect of oral magnesium supplementation on selected cardiovascular risk factors in non-insulin-dependent diabetics. Arch Fam Med 3:503-508, 1994

13. Eibl NL, Kopp HP, Nowak HR, et al: Hypomagnesemia in type II diabetes: Effect of a 3-month replacement therapy. Diabetes Care 18:188-192, 1995

14. de Valk HW, Verkaaik R, van Rijn HJ, et al: Oral magnesium supplementation in insulin-requiring type 2 diabetic patients. Diabet Med 15:503-507, 1998

15. Lima M de L, Cruz T, Pousada JC, et al: The effect of magnesium supplementation in increasing doses on the control of type 2 diabetes. Diabetes Care 21:682-686, 1998

16. Gullestad L, Jacobsen T, Dolva LO: Effect of magnesium treatment on glycemic control and metabolic parameters in NIDDM patients. Diabetes Care 17:460-461, 1994

17. Djurhuus MS, Gram J, Petersen PH, et al: Biological variation of serum and urinary magnesium in apparently healthy males. Scand J Clin Lab Invest 55:549-558, 1995

18. Liu D, Moberg E, Kollind M, et al: Arterial, arterialized venous, venous and capillary blood glucose measurements in normal man during hyperinsulinaemic euglycaemia and hypoglycaemia. Diabetologia 35:287-290, 1992

19. Gehan EA, George SL: Estimation of human body surface area from height and weight. Cancer Chemother Rep 54:225-235, 1970

20. Djurhuus MS, Klitgaard NAH, Tveskov C, et al: Methodological aspects of measuring human skeletal muscle electrolyte content and ouabain binding capacity. Anal Biochem 260:218-222, 1998

21. Johansen I, Milling-Eriksen R, Pedersen K, et al: Albumin-dependent correction of S-fructosamin (S-FAMN) in a reference population. Scand J Clin Lab Invest 46:141, 1986 (suppl 185, abstr)

22. Gardner MJ, Altman DG: Statistics With Confidence—Confidence Intervals and Statistical Guidelines. London, England, Br Med J, 1989

23. Solberg HE: Approved recommendation (1987) on the theory of reference values. Part 5. Statistical treatment of collected reference values. Determination of reference limits. J Clin Chem Clin Biochem 25:645-656, 1987

24. Kahil ME, Parrish JE, Simons EL, et al: Magnesium deficiency and carbohydrate metabolism. Diabetes 15:734-739, 1966

25. Lowney P, Gershwin ME, Hurley LS, et al: The effect of variable magnesium intake on potential factors influencing endurance capacity. Biol Trace Elem Res 16:1-18, 1988

26. Gueux E, Rayssiguier Y: The effect of magnesium deficiency on glucose stimulated insulin secretion in rats. Horm Metab Res 15:594-597, 1983

27. Legrand C, Okitolonda W, Pottier AM, et al: Glucose homeostasis in magnesium-deficient rats. Metabolism 36:160-164, 1987

28. Kahil ME, Simons EL, Brown H: Magnesium deficiency and sugar transport in muscle. Effect of acute insulin deficiency. Diabetes 17:673-678, 1968

29. Zofkova I, Zamrazil V, Simeckova A: Acute hypermagnesaemia retards glucose assimilation and inhibits insulin secretion during intravenous glucose tolerance test (IVGTT). Horm Metab Res 20:120-121, 1988

30. Henriksen JE, Alford F, Handberg Aa, et al: Increased glucose effectiveness in normoglycemic but insulin-resistant relatives of patients with non-insulin-dependent diabetes mellitus. A novel compensatory mechanism. J Clin Invest 94:1196-1204, 1994

31. Fasching P, Ratheiser K, Damjancic P, et al: Both acute and chronic near-normoglycaemia are required to improve insulin resistance in type 1 (insulin-dependent) diabetes mellitus. Diabetologia 36:346-351, 1993

32. Vaag A: On the pathophysiology of late onset non-insulin dependent diabetes mellitus. Current controversies and new insights. Dan Med Bull 46:197-234, 1999

33. Paolisso G, Scheen A, Cozzolino D, et al: Changes in glucose turnover parameters and improvement of glucose oxidation after 4-week magnesium administration in elderly noninsulin-dependent (type II) diabetic patients. J Clin Endocrinol Metab 78:1510-1514, 1994

34. Barbagallo M, Resnick LM, Dominguez LJ, et al: Diabetes mellitus, hypertension and ageing: the ionic hypothesis of ageing and cardiovascular-metabolic diseases. Diabetes Metab 23:281-294, 1997

35. Deedwania PC: Mechanism of the deadly quartet. Can J Cardiol 16:17E-20E, 2000 (suppl E)

36. The Diabetes Control and Complications Trial Research Group: The effects of intensive treatment of diabetes on the development and progression of long term complications in insulin-dependent diabetes mellitus. N Engl J Med 329:977-986, 1993

37. McNair P, Christensen MS, Christiansen C, et al: Renal hypomagnesaemia in human diabetes mellitus: Its relation to glucose homeostasis. Eur J Clin Invest 12:81-85, 1982

38. De Leeuw I, Engelen W, Vertommen J, et al: Effect of intensive IV + oral magnesium supplementation on circulating ion levels, lipid parameters and metabolic control in Mg-depleted insulin-dependent diabetic patients (IDDM). Magnes Res 10:135-141, 1997

39. Levin GE, Mather HM, Pilkington TRE: Tissue magnesium status in diabetes mellitus. Diabetologia 21:131-134, 1981

40. Naccarato R, Maschio G, Sirigu F, et al: The muscle in diabetes mellitus. A histologic (light and electron microscope) and biochemical study by means of needle biopsy. Virchows Arch B Cell Pathol 4:283-293, 1970

41. Kirsten R, Heintz B, Nelson K, et al: Magnesium pyridoxal 5-phosphate glutamate reduces hyperlipidaemia in patients with chronic renal insufficiency. Eur J Clin Pharmacol 34:133-137, 1988

42. Hägg E, Carlberg BC, Hillörn VS, et al: Magnesium therapy in type 1 diabetes. A double blind study concerning the effects on kidney function and serum lipid levels. Magnes Res 12:123-130, 1999

43. Freedman DS, Srinivasan SR, Shear CL, et al: The relation of apolipoproteins A-1 and B in children to parental myocardial infarction. N Engl J Med 315:721-726, 1986

44. Altura BM, Altura BT: Magnesium and cardiovascular biology: An important link between cardiovascular risk factors and atherogenesis. Cell Mol Biol Res 41:347-359, 1995

45. Stahlberg M, Jalovaara P, Laitinen S, et al: Adsorption of bile acids by sucralfate, antacids, and cholestyramine in vitro. Clin Ther 9:615-621, 1987

46. Zoccali C, Cumming AMM, Hutcheson MJ, et al: Effects of potassium on sodium balance, renin, noradrenaline and arterial pressure. J Hypertens 3:67-72, 1985

47. Brown RS: Potassium homeostasis and clinical implications. Am J Med 77:3-10, 1984

48. American Diabetes Association: Magnesium supplementation in the treatment of diabetes. Diabetes Care 15:1065-1067, 1992