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Magnesium Deficiency in Type 2 Diabetes

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Abbreviations

AAS	atomic absorption spectrometry
ADA	American Diabetes Association
ARIC	Atherosclerosis Risk in Communities (Study)
ATP	adenosine triphosphate
cAMP	cyclic adenosine monophosphate
Casr	extracellular Ca^{2+}/Mg^{2+}-sensing receptor
cTAL	cortical thick ascending limb of the loop of Henle (kidney)
DCT	distal convoluted tubule (kidney)
EAR	Estimated Average Requirement (USA)
ECG	electro-cardiogram
GLUT	glucose transporter protein
HbA_{1c}	glycosylated hemoglobin A_{1c}
HDL	high density lipoprotein
ICP-MS	inductively coupled plasma mass spectrometry
ICP-OES	optical emission spectrometry with inductively coupled plasma
IGT	impaired glucose tolerance
IRS	insulin receptor substrates
LDL	low density lipoprotein
MBC	mononuclear blood cells
MDCT	mouse distal convoluted tubule (cell line for in vitro studies)
Na/K-ATPase	sodium/potassium-adenosine triphosphatase (enzyme)
NHANES	National Health and Nutrition Examination Survey
PTH	parathyroid hormone
RDA	Recommended Dietary Allowances (USA)
RDI	recommended dietary intake
TIMS	thermal ionization mass spectrometry
UKPDS	United Kingdom Prospective Diabetes Study
VLDL	very low density lipoprotein

Summary

Background: Magnesium deficiency is a common finding in patients with type 2 diabetes. In the USA, 25 to 39 % of outpatient diabetics have low concentrations of serum magnesium. Although low serum magnesium concentrations in diabetics have also been found in several European countries, including Austria, Germany, Italy, France and Sweden, there are no published data from Switzerland. Magnesium depletion has a negative impact on glucose homeostasis and insulin sensitivity in people with type 2 diabetes as well as on the evolution of complications such as retinopathy, thrombosis, and hypertension. The reasons why magnesium deficiency occurs in diabetes are not clear. They may include increased urinary loss, lower dietary intake, or impaired intestinal absorption of magnesium compared to healthy individuals.

Determination of magnesium status is problematic because magnesium is primarily an intracellular cation with less than 1 % of total body content present in the extracellular fluids. Serum magnesium concentration is a specific but insensitive measure of Mg status and can be normal despite depletion of intracellular magnesium. Other diagnostic tests, such as the assessment of intracellular magnesium in blood cells, muscle or bone, or physiologic assessment tests, either have shown equivocal results or are too invasive.

Aims: The objective of this thesis was to determine the prevalence of magnesium deficiency in Swiss type 2 diabetics, to investigate the etiology of magnesium deficiency in type 2 diabetes, and to perform a pilot study for a physiologic test for the assessment of magnesium status.

Methods: To determine magnesium status in Swiss diabetics, plasma magnesium concentrations were determined in 109 type 2 diabetics and 156 age- and sex-matched healthy controls living in the Zurich area.

To investigate the etiology of magnesium deficiency in type 2 diabetics, dietary intake, intestinal absorption and urinary excretion of magnesium were compared between type 2 diabetics and healthy controls. The magnesium intake of 97 patients with type 2 diabetes and 100 healthy, non-diabetic controls matched for age and sex

was estimated using a diet history method. During winter and summer periods, mean daily magnesium intakes were calculated from detailed information given by the test subjects about their eating habits over the previous two months. The calculations were performed using EBIS, a computer program based on a German nutrient data base (BLS 2.3), with food items specific to Switzerland added or directly analyzed when necessary. Magnesium absorption, and its excretion and retention were evaluated in 12 type 2 diabetics and 10 healthy controls using a stable isotope technique. The subjects received a test meal labeled with 10 mg of ^{26}Mg isotopic label and collected stools and urine for 10 and 6 days, respectively. Apparent absorption was calculated as the difference between the oral dose of ^{26}Mg isotopic label and the total amount of isotopic label excreted in feces. Magnesium retention was calculated from the apparent absorption and the urinary excretion of ^{26}Mg isotopic label in the 6 days after administration.

A modified version of the magnesium retention test using a small dose of ^{26}Mg was evaluated for assessment of magnesium status in 22 healthy subjects. Muscle magnesium concentration was used as reference for magnesium status. A muscle biopsy was taken from the lateral portion of the quadriceps muscle from each subject. Six to ten weeks later, 11 mg of ^{26}Mg (as $MgCl_2$ in 14 ml water) was injected i.v. over a period of 10 minutes and all urine was collected for the following 24 hours. Excretion of the isotopic label was expressed as percentage of the administered dose excreted in urine within 24 hours.

Results: Mean±SD plasma magnesium concentrations of the patients with type 2 diabetes (0.77 ±0.08 mmol/l) were significantly lower than the controls (0.83 ±0.07 mmol/l) (p < 0.001). Plasma magnesium concentrations were below the normal reference range in 37.6 % of the diabetic patients and 10.9 % of the control subjects (p<0.001). Plasma magnesium was not correlated with glycemic control as measured by HbA_{1c}.

The mean±SD daily magnesium intake of the male diabetic and male control subjects was 423.2 ±103.1 mg and 421.1 ±111.0 mg, respectively. The mean daily magnesium intake of the female diabetic and female control subjects was 419.1 ±109.7 mg and 383.5 ±109.7 mg, respectively. There were no significant differences in daily magnesium intake between the diabetic and the non-diabetic subjects and mean intakes in both groups exceeded Swiss recommended dietary intakes. Mean

fractional magnesium absorption±SD in the diabetic patients and controls was 59.3 ±7.0 % and 57.6 ±8.5 %, respectively. Mean urinary magnesium excretion and mean magnesium retention ±SD in diabetics and controls was 11.2 ±2.6 vs. 11.7 ±3.8, and 54.2 ±7.1 % vs. 51.4 ±6.1 %, respectively. There were no significant differences between the two groups neither in absorption, excretion nor retention.

For the evaluation of the status assessment method, mean±SD magnesium concentration in muscle was 3.85 ±0.17 mmol/100g fat-free dried solids. Mean±SD excretion of the injected dose within 24 hours was 7.9 ±2.1 %. No correlation was found between muscle magnesium concentration and excretion of the isotopic label (r^2=0.061, p=0.27).

Conclusions: Lower plasma magnesium concentrations are common in type 2 diabetics in Zurich, Switzerland, similar to reports from the USA and other European countries. The reasons for magnesium deficiency in type 2 diabetes do not appear to include lower intestinal absorption or insufficient dietary intake of magnesium. Moreover, in reasonably well-controlled diabetics urinary magnesium excretion is not different from controls. The main causes for impaired magnesium status in patients with type 2 diabetes may therefore include increased urinary magnesium excretion in patients with poor metabolic control and in those using diuretics, and decreased cellular uptake of magnesium due to insulin resistance.

The modified magnesium retention test did not correlate with muscle magnesium and did therefore not discriminate magnesium status. Further studies would be necessary to determine its potential role in the assessment of magnesium status.

Zusammenfassung

Hintergrund: Magnesiummangel ist ein häufiger Befund bei Typ 2 Diabetikern. In den USA weisen 25 bis 39 % der ambulanten Diabetiker erniedrigte Serummagnesiumspiegel auf. Obwohl auch in verschiedenen europäischen Ländern wie z. B. Österreich, Deutschland, Italien, Frankreich und Schweden erniedrigte Serummagnesiumspiegel bei Diabetikern gefunden wurden, gibt es keine publizierten Daten für die Schweiz.

Magnesiummangel hat einen negativen Einfluss auf den Glukosehaushalt und die Insulinsensibilität in Typ 2 Diabetikern, sowie auf die Entstehung von Komplikationen wie Retinopathie, Thrombose und Hypertonie. Die Gründe für die Entstehung von Magnesiummangel bei Diabetikern sind bisher unklar. Mögliche Ursachen sind erhöhte Ausscheidung mit dem Urin, geringere Zufuhr mit der Nahrung oder verminderte Absorption im Darm im Vergleich zu gesunden Individuen.

Die Bestimmung des Magnesiumstatus ist nicht einfach, da Magnesium hauptsächlich intrazellulär vorkommt und sich weniger als 1 % des totalen Körpergehalts in den extrazellulären Flüssigkeiten befindet. Die Messung des Serummagnesiumspiegels ist eine spezifische aber wenig empfindliche Methode um den Magnesiumstatus zu bestimmen. Dabei kann trotz normalem Magnesiumspiegel ein intrazelluläres Magnesiumdefizit vorliegen. Andere diagnostische Tests wie z. B. die Bestimmung von intrazellulärem Magnesium in Blutzellen, Muskeln oder Knochen oder physiologische Tests haben entweder nicht eindeutige Resultate gezeigt oder sind zu invasiv.

Zielsetzung: Das Ziel dieser Dissertation war die Abschätzung der Prävalenz von Magnesiummangel bei Typ 2 Diabetikern in der Schweiz und die Untersuchung der Ursachen von Magnesiummangel bei Diabetikern. Weiterhin wurde eine Pilotstudie für einen physiologischen Test zur Bestimmung des Magnesiumstatus durchgeführt.

Methoden: Um den Magnesiumstatus von Diabetikern in der Schweiz zu bestimmen, wurden die Serummagnesiumspiegel von 109 Typ 2 Diabetikern und 156 gesunden Kontrollpersonen vergleichbar in Alter und Geschlecht in der Region Zürich bestimmt.

Um die Ursachen für den Magnesiummangel in Typ 2 Diabetikern zu untersuchen, wurde die Zufuhr mit der Nahrung, die Absorption im Darm und die Ausscheidung mit dem Urin von Magnesium zwischen Typ 2 Diabetikern und gesunden Kontrollpersonen verglichen. Die Magnesiumzufuhr mit der Nahrung wurde mit Hilfe einer Ernährungserhebung (Diet History) bei 97 Typ 2 Diabetikern und 100 gesunden Nicht-Diabetikern vergleichbar in Alter und Geschlecht erfasst. Die durchschnittliche Magnesiumzufuhr wurde aufgrund detaillierter Informationen der Testpersonen über ihre Ernährungsgewohnheiten während der letzten zwei Monate berechnet. Die Berechnungen wurden mit EBIS, einem Computerprogramm basierend auf der deutschen Nährwertdatenbank BLS 2.3, durchgeführt. Lebensmittel, die spezifisch nur in der Schweiz vorkommen, wurden angefügt oder falls nötig direkt analysiert. Die Magnesiumabsorption, Ausscheidung und Retention wurden in 12 Typ 2 Diabetikern und 10 gesunden Kontrollpersonen mittels einer stabilen Isotopentechnik untersucht. Dabei erhielten die Probanden eine Testmahlzeit versetzt mit 10 mg ^{26}Mg und sammelten anschliessend ihren gesamten Stuhl während 10 Tagen und den gesamten Urin während 6 Tagen. Die scheinbare Absorption wurde als Differenz zwischen der oralen ^{26}Mg Dosis und der totalen ^{26}Mg Menge im Stuhl berechnet. Die Magnesiumretention wurde anhand der scheinbaren Absorption und der Urinausscheidung des ^{26}Mg-Markers in den 6 Tagen nach der Verabreichung berechnet. Für die Bestimmung des Magnesiumstatus wurde eine modifizierte Version des Magnesiumretentionstests mit einer kleinen Dosis ^{26}Mg in 22 gesunden Probanden evaluiert. Als Referenz für den Magnesiumstatus wurde die Muskelmagnesiumkonzentration verwendet. Von jeder Testperson wurde eine Muskelprobe vom seitlichen Teil des Quadrizeps-Muskels genommen. Sechs bis 10 Wochen später wurden 11 mg ^{26}Mg (als MgCl$_2$ in 14 ml Wasser) intravenös über einen Zeitraum von 10 Minuten injiziert und der gesamte Urin wurde während der folgenden 24 Stunden gesammelt. Die Ausscheidung des isotopischen Markers im Urin innerhalb von 24 Stunden wurde als Prozent der injizierten Dosis ausgedrückt.

Resultate: Der durchschnittliche ±SD Plasmamagnesiumspiegel der Typ 2 Diabetiker (0.77 ±0.08 mmol/l) war signifikant tiefer als derjenige der Kontrollpersonen (0.83 ±0.07 mmol/l) ($p < 0.001$). In 37.6 % der Diabetiker und 10.9 % der Kontrollpersonen waren die Plasmamagnesiumspiegel unterhalb des normalen

Bereichs (p<0.001). Der Plasmamagnesiumspiegel korrelierte nicht mit der Blutzuckerkontrolle (gemessen als HbA$_{1c}$).

Die durchschnittliche ±SD tägliche Magnesiumzufuhr mit der Nahrung der männlichen Diabetiker und Kontrollpersonen war 423.2 ±103.1 mg beziehungsweise 421.1 ±111.0 mg. Die durchschnittliche tägliche Magnesiumzufuhr der weiblichen Diabetiker und Kontrollpersonen war 419.1 ±109.7 mg beziehungsweise 383.5 ±109.7 mg. Es gab keine signifikanten Unterschiede zwischen Diabetikern und Nicht-Diabetikern und die durchschnittliche Magnesiumaufnahme beider Gruppen übertraf die Schweizerischen Referenzwerte für die Magnesiumzufuhr. Die durchschnittliche ±SD prozentuale Absorption war 59.3 ±7.0 % bei den Diabetikern und 57.6 ±8.5 % bei den Kontrollpersonen. Die durchschnittliche Magnesiumausscheidung mit dem Urin und die durchschnittliche Magnesiumretention bei Diabetikern und Kontrollpersonen war 11.2 ±2.6 vs. 11.7 ±3.8 beziehungsweise 54.2 ±7.1 % vs. 51.4 ±6.1 %. Weder in der Absorption, noch in der Ausscheidung mit dem Urin, noch in der Retention wurde ein signifikanter Unterschied zwischen den beiden Gruppen gefunden.

Bei der Evaluierung der Methode für die Bestimmung des Magnesiumstatus war die durchschnittliche ±SD Magnesiumkonzentration im Muskel 3.85 ±0.17 mmol/100g fettfreie Trockenmasse. Die durchschnittliche ±SD Ausscheidung der injizierten Dosis innerhalb von 24 Stunden war 7.9 ±2.1 %. Es wurde keine Korrelation zwischen der Muskelmagnesiumkonzentration und der Ausscheidung des isotopischen Markers gefunden (r^2=0.061, p=0.27).

Schlussfolgerungen: Erniedrigte Plasmamagnesiumspiegel sind häufig bei Typ 2 Diabetikern in Zürich, Schweiz, ähnlich wie in anderen europäischen Ländern und den USA. Die Gründe für Magnesiummangel bei Typ 2 Diabetikern scheinen weder eine verminderte Absorption im Darm noch eine ungenügende Magnesiumzufuhr mit der Nahrung zu beinhalten. Zudem ist die Ausscheidung von Magnesium im Urin bei gut kontrollierten Diabetikern nicht signifikant verschieden von gesunden Kontrollpersonen. Die Hauptgründe für den verminderten Magnesiumstatus bei Typ 2 Diabetikern bestehen wahrscheinlich aus einer erhöhten Magnesiumausscheidung im Urin bei Patienten mit schlechter Blutzuckerkontrolle und solchen unter Diuretika und einer verminderten Magnesiumaufnahme in die Zellen verursacht durch Insulinresistenz.

Der modifizierte Magnesiumretentionstest korrelierte nicht mit der Muskel-magnesiumkonzentration und ist deshalb nicht aussagekräftig in Bezug auf den Magnesiumstatus. Weitere Studien sind nötig, um seine potentielle Rolle in der Bestimmung des Magnesiumstatus zu beurteilen.

Introduction

Type 2 diabetes is an increasing cause of morbidity and mortality in industrialized countries. In Switzerland, approximately 250'000 people are diabetic, > 90 % of them with type 2 diabetes. Type 2 diabetes is a chronic disease characterized by a disorder of glucose metabolism associated with a reduced ability of tissues to respond to insulin (insulin resistance). Resulting chronic hyperglycemia damages blood vessels and nerves throughout the body producing microvascular diseases including retinopathy, neuropathy and nephropathy. Moreover, the risk for cardiovascular disease is considerably elevated in patients with type 2 diabetes compared to the general population. Therefore, type 2 diabetes represents a major public health problem causing high economic costs in industrialized countries.

Low magnesium status has repeatedly been demonstrated in patients with type 2 diabetes. Magnesium deficiency appears to have a negative impact on glucose homeostasis and insulin sensitivity in patients with type 2 diabetes (Durlach & Rayssiguier, 1983; Nadler *et al.*, 1993) as well as on the evolution of complications such as retinopathy, thrombosis, and hypertension (McNair *et al.*, 1978; Mather *et al.*, 1982; Nadler *et al.*, 1992). In the USA, 25 to 39 % of outpatient diabetics have low concentrations of serum magnesium (Nadler & Rude, 1995) and numerous studies have shown lower serum magnesium concentrations in type 2 diabetics compared to healthy controls (Nadler *et al.*, 1992; Ma *et al.*, 1995). Although low serum magnesium concentrations in diabetics have also been found in several European countries, there are no reported data for Switzerland.

The reasons why magnesium deficiency occurs in diabetes are not clear. They may include increased urinary loss, lower dietary intake, or impaired absorption of magnesium compared to healthy individuals. Several studies have reported increased urinary magnesium excretion in type 1 and 2 diabetes (Johannson *et al.*, 1981; Fujii *et al.*, 1982; McNair *et al.*, 1982; Sjögren *et al.*, 1988), some reporting a correlation between glycemic control and urinary magnesium loss (McNair *et al.*, 1982). Low dietary intake may also contribute to low magnesium status in diabetics. Patients with type 2 diabetes are often overweight, and may consume a diet higher in fat and lower in magnesium density than non-diabetics. However, the few studies

that have reported magnesium intake in type 2 diabetes are equivocal (Schmidt *et al.*, 1994; Ma *et al.*, 1995) and none of them has been performed in Europe. Impaired intestinal absorption might also contribute to low magnesium status in diabetics. In alloxan-induced diabetic rats, fractional magnesium absorption was decreased compared to controls (Schneider & Schedl, 1974). However, there are no published data on magnesium absorption in humans with diabetes.

Despite the growing realization of the importance of magnesium in human health and disease, measurement of magnesium status remains problematic. Serum magnesium concentrations can be normal despite depletion of intracellular magnesium (Resnick *et al.*, 1993). Measurement of intracellular magnesium in erythrocytes and leukocytes has been used, but the results are equivocal (Elin, 1991). Muscle and bone contain the majority of body magnesium and are therefore potentially important tissues for assessment of status; however, muscle and bone biopsies are invasive, time consuming and not suitable for ambulatory diagnosis. An alternative is the magnesium retention test, which is a physiologic assessment method and has been proposed, applied and validated by several authors as method for the determination of magnesium status (Bohmer & Mathiesen, 1982; Ryzen *et al.*, 1985; Gullestad *et al.*, 1992). However, the test is time-consuming and requires hospitalization of the subjects. Therefore, to study magnesium deficiency in relation to various diseases, there is still a lack of an easily performable and valuable test for assessing magnesium status.

This thesis begins with a literature review followed by four manuscripts. The literature review is subdivided into three chapters covering various aspects of type 2 diabetes, the function of magnesium in human health and disease, and the relationship between magnesium and type 2 diabetes. The first manuscript reports a study comparing plasma magnesium concentrations between Swiss type 2 diabetics and healthy controls. The second and the third manuscripts investigate the etiology of magnesium deficiency in diabetes, namely dietary intake and intestinal absorption of magnesium. The fourth manuscript evaluates a modified version of the magnesium retention test using a small dose of a stable magnesium isotope as a potential future status indicator.

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LITERATURE REVIEW

1   Type 2 Diabetes

With an estimated 151 million cases worldwide (Lieberman, 2003), diabetes mellitus is the most common metabolic disease in the world. The term diabetes mellitus includes a group of different metabolic disorders resulting in an elevated blood glucose level (hyperglycemia) secondary to either insulin deficiency or abnormal insulin action. The symptoms of untreated diabetes include excessive thirst, excessive urine production, increased hunger and poor wound healing. Type 2 diabetes is the most common form, accounting for approximately 90 % of all cases.

1.1   Etiology and Pathology

Type 2 diabetes, also called adult-onset diabetes or non-insulin-dependent diabetes, is caused by insulin resistance which is characterized by a decreased effectiveness of insulin. In contrast to type 1 diabetes in which the pancreatic islets are destroyed and no insulin can be synthesized anymore, in type 2 diabetes insulin secretion is normal, elevated or reduced. Unlike in patients with type 1 diabetes, symptoms do not appear abruptly, but set on gradually so that the disease often remains undiagnosed for a long time. The criteria for the diagnosis of type 2 diabetes established by the American Diabetes Association (ADA) are: symptoms of diabetes plus a casual plasma glucose concentration $\geq$ 11.1 mmol/l, or fasting plasma glucose $\geq$ 7.0 mmol/l, or a 2-hour oral glucose tolerance test with plasma glucose $\geq$ 11.1 mmol/l. Impaired glucose tolerance, a pre-diabetic stage, is defined as fasting plasma glucose < 7.0 mmol/l and a 2-hour plasma glucose $\geq$ 7.8 and < 11.1 mmol/l (ADA, 2003).

Type 2 diabetes probably affects 5-7 % of the adults in Western countries and 10 % of people over 70 years of age. In contrast, in most developing countries the prevalence of type 2 diabetes is less than 1 % (Cruickshank, 1997). However, there are large differences between ethnic groups. In certain groups who have undergone rapid westernization such as the Pima Indians or the inhabitants of the Micronesian island of Nauru, the prevalence is as high as 40-50 %. Urbanization has had similar effects in African populations, and in immigrant populations such as Chinese moving to Mauritius, Japanese emigrating to Hawaii, or Asian Indians living in the UK or South Africa (Cruickshank, 1997). The prevalence of type 2 diabetes increases with

age and obesity in all populations and represents a major public health problem causing high economic costs.

1.1.1 Insulin resistance

Insulin resistance is characterized by a decreased effectiveness of insulin. Himsworth & Kerr (1939) were the first to describe insulin resistance, based on studies during which the ability of insulin to blunt the rise of plasma glucose was assessed following glucose infusion. They observed that in obese, older patients insulin injections had a reduced impact. In general, defects of both insulin action and insulin secretion must be present to produce overt type 2 diabetes, although the relative importance of these may differ between population groups (Kruszynska & Olefsky, 1996). Tissue insulin sensitivity and insulin secretion are both influenced by genetic and environmental factors and there is a variable interplay between them. Several conditions are related to insulin resistance, ranging from normal (aging, starvation, puberty, pregnancy) to abnormal (obesity, diabetes, polycystic ovarian syndrome, Cushing syndrome, acromegaly) (Valdez, 2000).

In 1979, DeFronzo *et al*. (1979) developed the hyperinsulinemic euglycemic glucose clamp method which enables to quantitatively assess insulin resistance during in vivo human studies. Insulin resistance is expressed by the degree of impairment of the insulin-mediated glucose uptake. It must be noted that a high number of individuals with normal glucose tolerance could have an insulin-mediated glucose uptake that is as impaired as that of people with type 2 diabetes, but their β-cells compensate by secreting more insulin (Valdez, 2000). Glucose tolerance, which is a measure of the disappearance of glucose after a glucose load, is usually assessed by a glucose tolerance test, either oral (OGTT) or intravenous (IVGTT). Large epidemiologic studies usually use fasting plasma insulin concentration as indicator for insulin resistance. This concentration is considered an acceptable indicator of insulin resistance, particularly among subjects with impaired glucose tolerance (Laakso, 1993).

1.1.2 Mechanism

Insulin was discovered in 1921, but only recently the mechanism by which it promotes glucose uptake into the cells has been understood. Insulin resistance

primarily affects skeletal muscle, adipose tissue and the liver. There are different families of glucose transporters. The Na-linked glucose transporters are restricted to the intestine and the kidney where they actively transport glucose against a glucose-concentration gradient. The other group of transporters convey glucose by facilitated diffusion down glucose-concentration gradients (Shepherd & Kahn, 1999). This group consists of five homologous transmembrane proteins, GLUT-1, 2, 3, 4, and 5. The GLUT proteins have different substrate specificities, kinetic properties, and tissue distributions. GLUT-4 is the main insulin-responsive glucose transporter and is located primarily in muscle cells and adipocytes. The most likely explanation for the development of insulin resistance is a post-receptor defect in propagation of the message induced by the binding of insulin to its receptor (see Figure 1).

Figure 1: Insulin-signaling pathway (Shepherd & Kahn, 1999)
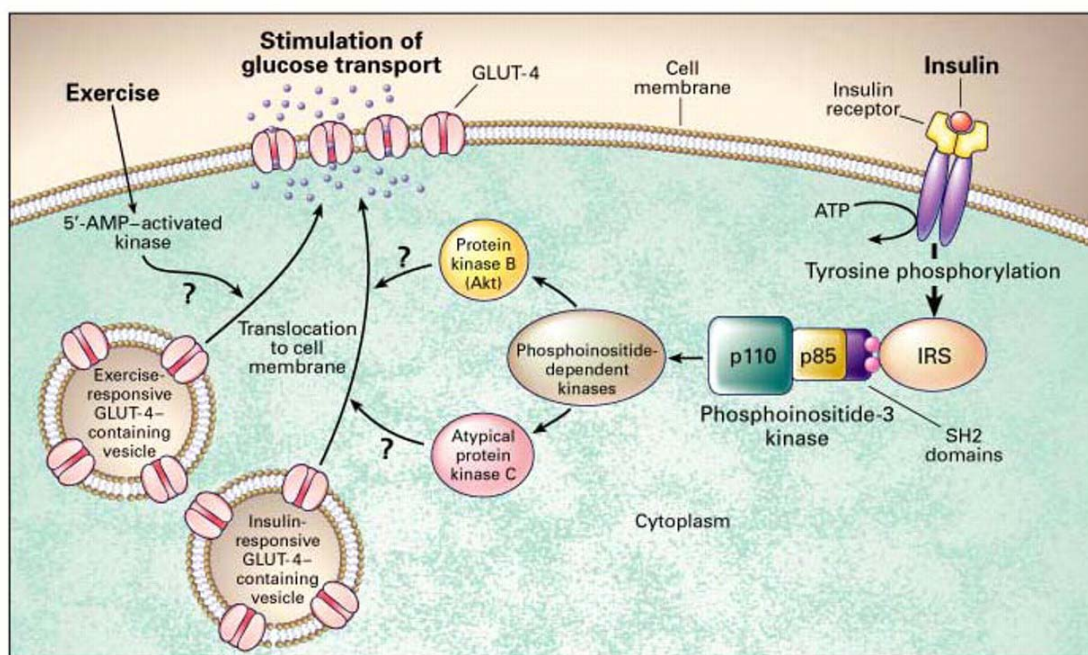
In the absence of insulin, about 90 % of GLUT-4 is sequestered intracellularly in vesicles. In response to insulin or exercise, the vesicles containing GLUT-4 move to the plasma membrane, fuse with it, and increase thus the rate of glucose transport into the cells. On removal of insulin stimulation, GLUT-4 is re-internalized into intracellular storage pools (Shepherd & Kahn, 1999). Insulin acts by binding to its receptor in the plasma membrane resulting in phosphorylation of the receptor and different insulin-receptor substrates (IRS). These substrates form complexes with

docking proteins leading to the activation of phosphoinositide-3 kinase. This enzyme activates phosphoinositide-dependent kinases participating in the activation of protein kinase B and atypical forms of protein kinase C. Both these kinases are involved in promoting the vesicles containing GLUT-4 to move to the plasma membrane. In contrast, exercise stimulates glucose transport by pathways that are independent of the insulin-signaling pathway and may involve 5'-AMP-activated kinase (Shepherd & Kahn, 1999).

It is thought that insulin resistance arises from a defect in the insulin-signaling pathway that regulates the translocation of GLUT-4 containing vesicles to the plasma membrane. Studies have shown that the concentrations of phosphorylated insulin receptor and IRS-1, and the activity of phosphoinositide-3 kinase are reduced in skeletal muscle of obese subjects with insulin resistance and of patients with type 2 diabetes (Goodyear *et al.*, 1995; Bjornholm *et al.*, 1997). In response to the increased blood glucose levels, the pancreatic islets produce greater and greater quantities of insulin. The resulting hyperinsulinemia may additionally worsen the situation by a 'down-regulation' of the number of insulin receptors (Chausmer, 1998). Although increased insulin secretion may at first compensate the reduced insulin action, the pancreatic β-cells eventually become overstrained, thus leading to decreasing insulin production and type 2 diabetes. Both, reduced insulin sensitivity and reduced insulin secretion must be present to produce overt type 2 diabetes. Stimuli such as muscle contraction and hypoxia activate GLUT-4 pools distinct from those activated by insulin, and that pathway of glucose uptake is normal in muscle of obese or diabetic subjects (Hayashi *et al.*, 1997). Moreover, the chronic elevation of serum free fatty acids due to high fat diets or obesity may contribute to insulin resistance (Roden *et al.*, 1996). In obese subjects, especially those with visceral obesity, circulating free fatty acid levels are elevated due to lipolysis of stored triglyceride (Rebuffé-Scrive *et al.*, 1990). In a study with healthy subjects, lipid infusion over 5 hours decreased insulin-mediated glucose uptake in association with a loss of the ability of insulin to stimulate phosphoinositide-3 kinase activity in muscle (Dresner *et al.*, 1999). Findings from a rat study confirm that high-fat feeding produces impaired activity of phosphoinositide-3 kinase associated with alterations in both protein kinase B and atypical protein kinase C activities in skeletal muscle (Tremblay *et al.*, 2001). The aggravation of insulin resistance is probably caused by

oxidation of the free fatty acids into acetyl CoA which stimulates glucose production in the liver and inhibits glucose oxidation in skeletal muscle, thus aggravating hyperglycemia (Jung, 1997).

1.1.3 Risk factors

The risk factors for the development of insulin resistance include obesity, physical inactivity, high-fat diets, low birth weight, aging, and unknown genetic factors (Valdez, 2000). The association between obesity and insulin resistance has now been well established (Abate *et al.*, 1995; Ludvik *et al.*, 1995). However, it is not currently known whether insulin resistance is the result of, or the cause of obesity. An alternative hypothesis is that obesity and insulin resistance are not directly related to each other but that they develop from a common underlying pathophysiologic process (Bessesen, 2000). The distribution of body fat appears to have an influence; obese individuals with truncal (or android) obesity have a higher risk of developing diabetes than individuals with gynoid obesity. Guidelines from the USA propose that a waist-hip ratio > 0.80 in women and > 0.95 in men is associated with an increased risk to health (Food and Nutrition Board, 1997). Interventions producing weight reduction such as diet and exercise have shown to be associated with an improvement in insulin sensitivity and a reduction in the risk of diabetes (see 1.6.1).

Regular exercise has a strong effect on the metabolic capacity of skeletal muscle. A relationship between physical activity and type 2 diabetes was suggested by studies in societies that had abandoned traditional lifestyles involving high physical activity and subsequently experienced major increases in rates of type 2 diabetes (Franz *et al.*, 2002). During exercise, glucose uptake is stimulated by increased translocation of GLUT-4 to the plasma membrane by an insulin-independent mechanism (Hayashi *et al.*, 1997). In addition, there is another, more persistent effect of exercise on insulin sensitivity which is mediated through changes in body composition and fat distribution (Kohrt, 2000).

Diets high in fat represent another risk factor. A high fat intake, especially saturated fat, appears to increase the risk for developing impaired glucose tolerance, hyperinsulinemia and type 2 diabetes (Feskens *et al.*, 1995; Marshall *et al.*, 1997). Furthermore, high levels of dietary fat have shown to increase the risk of progression from impaired glucose tolerance to type 2 diabetes (Marshall *et al.*, 1994).

The effect of age on insulin resistance is not clear. Earlier studies, such as the one of Rowe *et al.* (1983), have shown decreased insulin sensitivity in older individuals with normal glucose tolerance and physical activity in comparison to younger individuals. However, more recent studies have demonstrated that increased insulin resistance in older people was primarily due to higher abdominal obesity and that age had only little effect on insulin resistance (Kohrt *et al.*, 1993; Ferrannini *et al.*, 1996).

Low birth weight appears to be associated with the features of insulin resistance. Several studies, such as the one of Valdez *et al.* (1994), showed that normotensive, non-diabetic adults with a low birth weight had significantly higher levels of fasting serum insulin and a more truncal fat deposition pattern than individuals with a high birth weight. The human fetus adapts to undernutrition by metabolic changes, redistribution of blood flow and changes in the production of hormones (Barker, 1998), but the process that link thinness at birth with insulin resistance in adult life is not known. Thin babies have a low muscle mass as well as less subcutaneous fat. It has therefore been suggested that thinness at birth was associated with abnormalities in muscle structure and function which persist into adult life, interfering with the ability of insulin to promote glucose uptake (Barker, 1998).

Genetic influences play an important role in insulin resistance and type 2 diabetes, which has been demonstrated in numerous studies (Laws *et al.*, 1989; Cook *et al.*, 1994; McCarthy *et al.*, 1994). The most convincing evidence comes from twin studies. For example, in a study with 250 identical and 264 non-identical twin pairs, the concordance for diabetes was 58 % for identical twins compared to 17 % for non-identical twins (Newman *et al.*, 1987). Also, in first-degree relatives of patients with type 2 diabetes, insulin sensitivity has been shown to be reduced by 40 % compared with subjects matched for body fat, age, and waist-to-hip ratio (Jensen, 2000). The precise genetic factors involved remain however unknown and they may differ between population groups (Kruszynska & Olefsky, 1996). In populations adapting a Western lifestyle, an outburst of type 2 diabetes has often been observed. A 'thrifty genotype' has been suggested, which would favor fat storage maybe by inducing insulin resistance selectively in liver and skeletal muscle (Neel, 1962). Glucose would fail to enter the insulin-resistant skeletal muscle and would therefore be diverted into the relatively insulin-sensitive adipose tissue, where it would contribute to triglyceride synthesis. Moreover, amino acids derived from dietary protein would be converted by

unrestrained hepatic gluconeogenesis into glucose and thus to triglyceride. This would confer survival advantages under harsh conditions, but lead to obesity, insulin resistance and diabetes under conditions where food is readily available.

1.2   Diabetic complications

1.2.1   Microvascular complications

The microvascular complications in diabetes include retinopathy, nephropathy and neuropathy. The causes are not fully understood, but there are a few hypotheses. The 'glucose hypothesis' postulates that chronic hyperglycemia causes these complications, and that correction of hyperglycemia will prevent them (Herman & Crofford, 1997). Studies have documented associations between the duration and degree of hyperglycemia and the severity of microvascular and neuropathic complications. Pirart (1977) for example, followed 4'400 diabetic patients for up to 25 years and showed that poor glycemic control was clearly related to a higher prevalence of retinopathy, neuropathy and nephropathy. However, not all patients with hyperglycemia develop complications. Other factors, including age, sex, race, ethnicity, socioeconomic status, hyperlipidemia, and smoking have been implicated (Herman & Crofford, 1997). In type 1 diabetics, intensive therapy has shown to delay the onset and slow the progression of retinopathy, nephropathy and neuropathy by 35-70 %. For type 2 diabetes, up to now, there are no convincing data from prospective studies to support a beneficial effect of intensive therapy on microvascular complications (Herman & Crofford, 1997).

Hyperglycemia appears to damage tissues by causing both acute, reversible changes in cellular metabolism and cumulative, irreversible alterations in stable macromolecules such as extracellular matrix components and nucleic acids. The reversible abnormalities include abnormal polyol metabolism and the formation of early glycation products on matrix, cellular and plasma proteins (Giardino & Brownlee, 1997). The polyol pathway is based on a family of enzymes, which can utilize as substrates a wide variety of sugar-derived compounds and reduce these to their respective sugar-alcohols (polyols); for example glucose is converted to sorbitol. The first and rate-limiting step of the polyol pathway is governed by the enzyme aldose reductase, which is found in tissues such as nerve, retina, lens, glomerulus

and blood-vessel wall, in which glucose uptake does not require insulin. In these insulin-independent tissues, intracellular glucose concentrations rise in parallel with hyperglycemia leading to an increased activity of aldose reductase and therefore intracellular accumulation of sorbitol (Giardino & Brownlee, 1997). Sorbitol inhibits inositol transport leading to a fall in the intracellular inositol concentration which results in the inhibition of the membrane-bound Na/K-ATPase (see Figure 2). This has been supported by the observation that ouabain, an inhibitor of Na/K-ATPase activity, also impairs inositol transport (Grafton & Baxter, 1992).

Figure 2: Polyol pathway (Giardino & Brownlee, 1997)
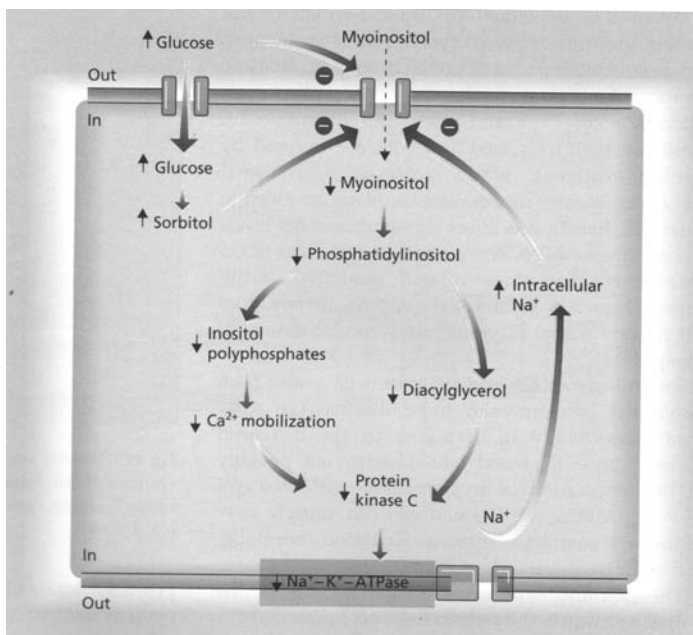
Myoinositol depletion has been associated with various functional abnormalities in the insulin-independent tissues susceptible to diabetic complications. In nerve, the inhibition of Na/K-ATPase activity may reduce Na extrusion and therefore lead to increased intracellular Na concentrations which on its part inhibits depolarization and slows nerve conduction rate. Abnormal polyol metabolism has also been implicated in the increased permeability of the blood-retinal barrier and in the development of hypertension in diabetes (Giardino & Brownlee, 1997).

Early glycation products form on proteins through the attachment of glucose to amino groups forming Schiff base adducts. These then undergo a so-called 'Amadori' rearrangement to form stable products analogous to glycated hemoglobin. In chronic exposure to hyperglycemia the early glycation products combine to form complex

cross-linked structures called advanced glycation end-products (AGE) which induce permanent changes (Giardino & Brownlee, 1997).

1.2.2 Macrovascular complications and metabolic syndrome

The macrovascular complications of diabetes mellitus include hypertension, lipid disorders and cardiovascular disease. Coronary heart disease is the most common cause of death in type 2 diabetes, followed by stroke (Gray & Yudkin, 1997). Macrovascular complications are more frequent in type 2 diabetes than in type 1 diabetes and are part of the metabolic syndrome. Insulin resistance is associated with a cluster of metabolic and vascular abnormalities known as 'syndrome X', or metabolic syndrome. The concept of syndrome X was introduced in 1988 by Reaven (1991) and is characterized by the concomitant occurrence of several conditions including insulin resistance, glucose intolerance, hyperinsulinemia, increased VLDL triglyceride, decreased HDL cholesterol, hypertension, and obesity.

Patients with type 2 diabetes are at a higher risk for hypertension and atherosclerosis than the general population. Reciprocally, it was repeatedly shown that patients with high blood pressure were more glucose intolerant, hyperinsulinemic or insulin resistant compared with individuals with normal blood pressure (Jarrett *et al.*, 1978; Persky *et al.*, 1979; Singer *et al.*, 1985; Shen *et al.*, 1988). Diabetes mellitus and hypertension are both recognized risk factors for developing cardiovascular disease. Numerous studies have found that already relatively minor degrees of glucose intolerance, comparable to those described in many individuals with high blood pressure, significantly increased the risk of developing cardiovascular disease (Pyörälä, 1979; Fuller *et al.*, 1980). Some evidence suggests that raised systemic insulin levels may be atherogenic. Hyperinsulinemia may also raise arterial blood pressure and cause changes in blood coagulability (Gray & Yudkin, 1997). The mechanisms linking the different conditions of the metabolic syndrome are however complex and not yet fully understood.

1.3 Pharmacologic treatment

Drugs used to treat type 2 diabetes should always be used in combination with attempts to modify the patient's lifestyle such as weight loss, restriction of dietary fat

and exercise. Patients with type 2 diabetes can be treated with oral hypoglycemics or with insulin, in mild cases diet and exercise may be sufficient.

Insulin was discovered in 1921 at the University of Toronto by FG Banting and CH Best (Banting & Best, 1922). Until the 1980s, insulin was extracted from porcine or bovine pancreas. Today, human insulin can be produced by genetic engineering (recombinant DNA technology). Insulin treatment in type 2 diabetics may be based on twice-daily injections of premixed insulin (e.g. 30:70 short-acting/isophane), or isophane at bedtime with short-acting insulin before meals. Combinations of once-daily isophane with an oral hypoglycemic during the day are also useful in some cases and may enhance endogenous insulin secretion (Groop, 1997).

Oral hypoglycemic agents include the sulphonylureas, the biguanides, the α-glucosidase inhibitors and the thiazolinediones. The sulphonylureas (e.g. chlorpropamide, glibenclamide, gliclazide, glimepiride) lower blood glucose primarily by stimulating the second phase of insulin secretion. The biguanide metformin lowers blood glucose levels through several mechanisms, including the inhibition of gluconeogenesis in the liver and the improvement of insulin sensitivity. Moreover it causes weight loss and is therefore useful for obese patients. The α-glucosidase inhibitor acarbose delays carbohydrate absorption in the gut by selectively inhibiting disaccharidases in the intestinal brush border (Groop, 1997). The thiazolinediones are an entirely new class of hypoglycemic agents. They act mainly through an improvement in insulin-mediated glucose disposal rates in peripheral tissues (Schoonjans & Auwerx, 2000).

1.4 Nutritional therapy

The nutritional guidelines for diabetics have very often been subject to changes during the past in parallel to new insights. In the late 19th century, the French diabetologist Bouchardat recommended "to eat as less as possible". He had actually observed during the famine caused by the Prussian siege in Paris in 1870, that the condition of the diabetic patients improved (Liebermeister, 2002). Indeed, the mortality due to diabetic coma could be reduced, but in return some patients died from starvation. In the beginning of the 20th century, diets poor in carbohydrates and proteins, but rich in fat were recommended. In 1921, insulin was discovered. At first,

insulin levels could not be very well adjusted, and to prevent hypoglycemia at least six meals had to be evenly spread over the day. Simple sugars were prohibited. But more and more, the proportion of carbohydrates in the diabetic diet increased. Moreover, dietary fat was found to increase the risk of cardiovascular diseases. In 1986, the American Diabetes Association (ADA) recommended that for patients with diabetes 10-20 % of total energy intake should come from proteins, less than 30 % from fat and the rest (50-60 %) from carbohydrates (ADA, 1987). However, more recent findings showed that replacing carbohydrates with monounsaturated fats reduced prostprandial glycemia and triglyceridemia. Moreover, data from the UKPDS[1] showed that normalization of hypertension, lipid levels and body weight was in some cases of type 2 diabetes more important than very tightly regulated blood glucose levels (Genuth *et al.*, 2002). Therefore, since 1994, the ADA recommends that monounsaturated fats and carbohydrates should together provide 60-70 % of energy intake (ADA, 1994).

The newest nutritional guidelines of the ADA were published in 2002. The goals of the 'medical nutrition therapy' are to attain and maintain optimal metabolic outcomes of blood glucose levels, lipid and lipoprotein profile and blood pressure, and the prevention and treatment of obesity, dyslipidemia, cardiovascular disease, hypertension, and nephropathy (ADA, 2002). The ADA recommends an intake of less than 10 % of total energy intake from saturated fats, up to 10 % from polyunsaturated fats, 10-20 % from proteins, leaving 60-70 % from monounsaturated fats and carbohydrates. A meta-analysis has confirmed that diets rich in monounsaturated fats improved lipoprotein profiles as well as glycemic control in patients with type 2 diabetes (Garg, 1998). However, there is concern that increased fat intake may promote weight gain and potentially contribute to insulin resistance (Marshall *et al.*, 1994; Feskens *et al.*, 1995). Thus the contributions of carbohydrate and monounsaturated fat to energy intake should be individualized based on nutrition assessment, metabolic profiles, and weight and treatment goals (Franz *et al.*, 2002). If obesity and weight loss are the primary issues, a reduction in dietary fat intake is an efficient way to reduce caloric intake and weight. If elevated triglycerides and VLDL cholesterol are the primary problems, a moderate increase of

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[1] UKPDS: United Kingdom Prospective Diabetes Study

monounsaturated fat up to 20 % of total energy and a more moderate intake of carbohydrates are recommended. In any case, the primary goal regarding dietary fat in diabetic patients is to decrease intake of saturated fat and cholesterol. For dietary cholesterol, a daily intake of less than 300 mg is recommended. The intake of trans-unsaturated fatty acids (formed through processing and hydrogenation of vegetable oils) should be as low as possible because their effect is similar to that of saturated fats in raising plasma LDL cholesterol. In patients with type 2 diabetes, postprandial glucose levels in response to complex carbohydrates and sugars are similar if the amount of carbohydrate is constant (Hollenbeck *et al.*, 1985; Peterson *et al.*, 1986; Abraira & Derler, 1988). Thus the intake of sucrose does not need to be restricted because of a concern about aggravating hyperglycemia. Non-nutritive sweeteners (e.g. saccharin, aspartame) can be recommended for patients who wish to loose weight. As for the general population, patients with diabetes should be encouraged to consume a variety of fiber-containing foods, such as whole grains, fruits and vegetables because they provide vitamins, minerals and dietary fiber. In individuals with type 2 diabetes, it appears that ingestion of very large amounts of fiber (> 50 g/d) has beneficial effects on glycemic control, hyperinsulinemia, and plasma lipids. It is however not clear, whether the palatability and the gastro-intestinal side effects of fiber in this amount would be acceptable to most people over long-term. Alcohol intake should be moderate and only in combination with food in patients treated with insulin or insulin secretagogues to avoid hypoglycemia (ADA, 2002).

1.5 Influence of micronutrients

The interrelationship between diabetes and various micronutrients is characterized by a certain reciprocity. Chronic hyperglycemia can cause significant alterations in the status of some micronutrients and on the other hand, some of these nutrients can directly modulate glucose homeostasis (Mooradian *et al.*, 1994). Especially deficiencies of certain minerals such as Mg, Zn and Cr have been shown to predispose a person to glucose intolerance and to promote the development of diabetic complications. The implication of some of the nutrients in diabetes is reviewed more in detail; for Mg see Chapter 3.

1.5.1   Chromium

Chromium is an essential element required for normal carbohydrate and lipid metabolism. The proposed mechanism includes the enhancement of insulin binding by increasing the number of insulin receptors and the activation of insulin receptor tyrosine kinase, thus leading to increased insulin sensitivity (Anderson, 2000b). Cr deficiency results in glucose intolerance and insulin resistance. In hyperinsulinemic obese rats, Cr supplementation has been found to increase glucose tolerance and enhance skeletal muscle GLUT-4 translocation (Cefalu *et al.*, 2002). Moreover, relatively large studies in Chinese diabetic subjects have shown that Cr supplementation may have beneficial effects on glycemia (Anderson *et al.*, 1997). However, it must be noted that these results may represent the effect of supplementation in a Cr-deficient population (Althuis *et al.*, 2002). There are controversial findings such as the results from two studies in Israel and Finland which have failed to demonstrate any significant effect of Cr supplementation in patients with diabetes or impaired glucose tolerance (Abraham *et al.*, 1992; Uusitupa *et al.*, 1992). Althuis *et al.* (2002) conducted a meta-analysis including 15 randomized placebo-controlled clinical trials of Cr supplementation and glucose-related factors in diabetic and non-diabetic subjects. Data showed no effect of Cr on glucose or insulin concentrations in non-diabetic subjects. Data for patients with diabetes were inconclusive because too few studies included diabetic subjects.

Insufficient dietary Cr intake has also been implicated as a possible risk factor for the development of diabetes. However, further studies are necessary to elucidate the mechanism of action of Cr and its role in the prevention and control of diabetes. Also, it is difficult to estimate Cr status and dietary Cr intake due to a lack of appropriate biomarker and reference databases, respectively.

1.5.2   Zinc

Zinc is involved in the synthesis, storage, secretion and conformational integrity of insulin (Chausmer, 1998). In the presence of Zn, insulin monomers assemble to a dimeric form for storage and secretion as crystalline insulin. In vitro, dimeric insulin assembles further into a hexamer in the presence of Zn. This form of insulin is relatively stable and it is this hexameric crystal which is the commonly used

pharmacological form. Conformational changes of this form may also affect the receptor binding of insulin (Chausmer, 1998).

Patients with type 2 diabetes were found to have decreased plasma Zn and intracellular Zn concentrations, and increased urinary Zn excretion compared to non-diabetic subjects (Kinlaw *et al.*, 1983; Chen *et al.*, 1995; Takakura, 1998). Decreased serum Zn concentrations in diabetes reflect impaired Zn status, which is caused by hyperzincuria and maybe impaired intestinal absorption. Hyperzincuria appears to be due to hyperglycemia rather than any specific effect of insulin on renal excretion (Chausmer, 1998). El-Yazigi *et al.* (1993) found a positive correlation between metabolic control (HbA$_{1c}$) and urinary Zn excretion in a study with 175 diabetic patients. Decreased intestinal Zn absorption has been proposed to contribute to impaired Zn status. In a study with type 1 diabetics using a stable isotope technique, absorption of ^{65}Zn tended to be lower in diabetics, but did not reach the level of statistical significance (Kiilerich *et al.*, 1990). In contrast, Rauscher *et al.* (1997) found no difference in Zn metabolism or absorption between type 2 diabetics and healthy controls using a stable isotope technique, although urinary Zn excretion was increased in diabetic males.

Supplementation with Zn may be associated with an improvement in glucose homeostasis and a decrease in lipid peroxidation (Anderson, 2000a). The antioxidant function of Zn is probably due to the protection of sulfhydryl groups in proteins against oxidation and to the inhibition of the production of reactive oxygen species by Fe and Cu (Bettger, 1993). Moreover, smaller studies in older subjects with diabetes have suggested some benefit from Zn supplementation in healing skin ulcerations (Mooradian *et al.*, 1994).

Low dietary Zn intake appears to be related to a higher risk for developing diabetes. In a cross-sectional survey in 3'575 Indian subjects, lower dietary Zn intake was associated with a higher prevalence of diabetes, glucose intolerance and coronary artery disease (Singh *et al.*, 1998).

1.5.3 Calcium

Numerous studies have shown diabetes to be associated with abnormal regulation of intracellular Ca. Elevated intracellular Ca has been noted in various tissues of

diabetic patients and animals (Levy *et al.*, 1994). In a large survey in Sweden with over 18'000 adults, total serum Ca concentrations were found to be correlated with serum glucose levels as well as with blood pressure and serum cholesterol levels (Lind *et al.*, 1988). These findings and others have led to the suggestion that altered intracellular Ca metabolism represented a common abnormality linking the metabolic, cardiovascular and microvascular manifestations of the diabetic disease (Levy *et al.*, 1994) (see also 3.4).

1.5.4 Vitamin C

Ascorbic acid is required for the synthesis of collagen and is also an important antioxidant. Moreover, it is involved in immunity and wound healing (Mooradian *et al.*, 1994). Diabetes has been associated with abnormalities in ascorbic acid metabolism. Several studies have reported lowered plasma concentrations of ascorbic acid in diabetics compared to healthy subjects (Som *et al.*, 1981; Stankova *et al.*, 1984). In some studies, plasma ascorbate concentrations were inversely correlated with HbA_{1c} levels (Yue *et al.*, 1990; Lysy & Zimmermann, 1992). However, in a study in which both diabetic and non-diabetic subjects had markedly higher than usual dietary vitamin C intakes, no differences between groups were detected (Lysy & Zimmermann, 1992). This supports the hypothesis that low vitamin C status in diabetes may be due to a higher turnover rate of ascorbic acid, with increased oxidation to the oxidized form dehydroascorbate (Som *et al.*, 1981). Another possible mechanism for the impaired ascorbate status is a competitive inhibition between glucose and ascorbic acid, which both share a close structural homology and possibly occupy common membrane transport sites (Pecoraro & Chen, 1987). Supplementation with ascorbic acid may have a beneficial effect. In a study with 27 type 2 diabetics, supplementation with ascorbic acid (2 g/d) improved glycemic control and fasting plasma glucose (Eriksson & Kohvakka, 1995). If the efficacy of vitamin C supplementation in diabetes is confirmed, a higher vitamin C intake than for the general population may be recommended for patients with diabetes.

1.5.5 Vitamin E

Vitamin E has been reported to play a role in the development of type 2 diabetes. In a follow-up study in 944 Finnish men, low plasma vitamin E concentration showed a strong association with the risk of diabetes at four years (Salonen *et al.*, 1995).

Felber & Golay (2002) suggested that the resistance of peripheral tissues to glucose uptake is a consequence of permanently high lipid oxidation. Vitamin E might therefore act as antioxidant and reduce lipid oxidation. This is supported by a study in which supplementation with vitamin E (900 mg/d) for 4 months was able to improve insulin action in type 2 diabetics and healthy subjects (Paolisso *et al.*, 1993). However, results from cross-sectional studies associating serum vitamin E or vitamin E intake with diabetes or insulin sensitivity are equivocal (Sanchez-Lugo *et al.*, 1997; Reunanen *et al.*, 1998). Furthermore, the Heart Outcomes Prevention Evaluation Trial did not confirm the efficacy of vitamin E in the prevention of macro- and microvascular diabetic complications. The study included 9'541 subjects, 38 % of whom had diabetes. Supplementation with vitamin E (400 IU/day) did not result in any significant benefit (Yusuf *et al.*, 2000).

The use of antioxidants such as vitamin C, vitamin E and β-carotene in the prevention of chronic diseases is controversial. Although small placebo-controlled or large observational studies have found beneficial effects of antioxidants on clinical outcomes, large placebo-controlled clinical trials have failed to show a benefit from antioxidants and in some cases have even suggested adverse effects (Franz *et al.*, 2002).

1.5.6 Others

The metabolisms of other micronutrients such as Cu, Fe, Mn and B vitamins have been reported to be altered in diabetes, but further studies are necessary to elucidate their implication in diabetes. Vanadium, an ultra-trace element, may have potential in improving glucose tolerance when used in pharmacological doses. Its role in diabetes has been explored in several small studies, but there is no clear evidence of efficacy and there is a potential for toxicity (Franz *et al.*, 2002).

1.6 Prevention of type 2 diabetes

The prevalence of type 2 diabetes is growing with increasing life expectancy, a more and more sedentary lifestyle and the migration of population groups (Lieberman, 2003). Type 2 diabetes represents a major public health concern in the western world because of its micro- and macrovascular complications. Treatment may prevent some of its devastating complications but does not usually restore normoglycemia or

eliminate all the adverse consequences. The disease is however preventable and efficient prevention strategies are therefore crucial. The three main strategies include the reduction of body weight, the increase of physical activity and the reduction of dietary fat.

1.6.1 Body weight

Obesity is one of the major risk factors for developing type 2 diabetes and more than 80 % of the patients are obese at the time of diagnosis (Lieberman, 2003). Wood & Bierman (1986) suggested that the incidence of type 2 diabetes could be cut in half by preventing obesity in middle-aged adults. Interventions producing weight reduction such as diet and exercise have clearly shown to be associated with an improvement in insulin sensitivity. Observational studies and smaller randomized placebo-controlled studies such as the 6-year Malmö Feasibility Study in Finland (Eriksson & Lindgarde, 1991) and the Da Qing IGT and Diabetes Study in China (Pan *et al.*, 1997) have demonstrated that diet and exercise can delay type 2 diabetes in at-risk people. A recent study, the Finnish Diabetes Prevention Study, included 522 overweight individuals with impaired glucose tolerance randomized to control or intensive lifestyle intervention including weight reduction, reduction of total and saturated fat, increased dietary fiber, and increased physical activity. The risk of diabetes was reduced by 58 % in the intervention group (Tuomilehto *et al.*, 2001). The largest study so far was the Diabetes Prevention Program, a major clinical trial comparing diet and exercise to treatment with metformin in 3'234 people with impaired glucose tolerance in the US (Knowler *et al.*, 2002). The lifestyle intervention (diet and exercise) reduced the incidence by 58 % and metformin by 31 % as compared with placebo; thus the lifestyle intervention was significantly more effective than metformin.

1.6.2 Physical activity

Physical activity increases the capacity for glucose uptake and disposal in the skeletal muscle (van Baak & Borghouts, 2000). During exercise, glucose uptake is stimulated by increased translocation of GLUT-4 to the plasma membrane by an insulin-independent mechanism (Hayashi *et al.*, 1997). Regular aerobic exercise has shown to be strongly associated with a reduced risk for developing type 2 diabetes, independent of body weight (Helmrich *et al.*, 1991). In addition, a large prospective

observational study demonstrated no elevated mortality risk in obese men if they were physically fit, and lean men had increased longevity only if they were physically fit (Lee *et al.*, 1999). The increase of insulin sensitivity after exercise appears to stay enhanced after training, but disappears within a few days of inactivity, thus indicating the need for regular exercise (van Baak & Borghouts, 2000). Protection from diabetes appears to occur from moderate intensity activities, such as brisk walking, as well as from participation in vigorous physical activity (Franz *et al.*, 2002).

1.6.3 Diet

The energy-dense diet consumed in industrialized countries, rich in fats and relatively low in carbohydrates and dietary fiber, largely contributes to the development of obesity and type 2 diabetes. The growing availability of fast food may add to the intake of energy-dense high-fat foods (Lieberman, 2003), but also traditional diets are often relatively rich in energy and fat. Overall decreasing physical activity, which is mainly due to changes in lifestyle such as decreasing physically strenuous work and increasing transport by automobile, implicates lower energy requirements.

There is some evidence that dietary composition, besides total energy intake, may be relevant in the development of type 2 diabetes (Jung, 1997). An analysis of over 11'626 adults in Scotland revealed a 19 % and 26 % prevalence of obesity in men and women with the highest fat intake, respectively, compared to 5 and 13 % in the lowest range of fat intake (Bolton-Smith & Woodward, 1994). In lean individuals, an increase in fat intake produces a corresponding increase in fat oxidation to maintain weight (Jung, 1997). In individuals with a predisposition to obesity, however, this does not seem to be the case. Astrup *et al.* (1994) have estimated that in people predisposed to obesity, an increase of 1.6 % in dietary fat intake may result in an increase of fat mass of 10 kg. In addition to its negative impact due to its energy density, dietary fat appears to be an important determinant of diabetes risk independently of total caloric intake. In a study with 338 Finnish and Dutch men, a high intake of fat, especially saturated fat, increased the risk for developing impaired glucose tolerance and type 2 diabetes after adjustment for age, body weight and energy intake (Feskens *et al.*, 1995). However, it is not clear whether all types of dietary fat (except n-3 fatty acids) have an adverse effect on insulin sensitivity.

Results are however most consistent for an adverse effect of saturated fat (Feskens *et al.*, 1995; Marshall *et al.*, 1997).

Dietary fiber, particularly soluble fiber, has been reported to lower postprandial glucose profiles and serum cholesterol significantly in patients with type 2 diabetes (Vinik & Jenkins, 1988). Furthermore, numerous recent prospective studies have provided evidence for reduced risk of diabetes with increased intake of whole grains and dietary fiber (Liu *et al.*, 2000; Meyer *et al.*, 2000; Fung *et al.*, 2002; Montonen *et al.*, 2003).

There is a long-standing debate regarding the effect of sugar intake on diabetes risk. Data on the subject are inconsistent, but reports from large epidemiological studies suggest that sucrose does not increase the risk of diabetes (Colditz *et al.*, 1992; Meyer *et al.*, 2000). Similarly, results from the Iowa Women's Health Study show no evidence for an effect of total carbohydrate intake on diabetes risk (Meyer *et al.*, 2000). However, the effect of glycemic load on diabetes risk is controversial. Although results from the Nurses' Health Study and the Health Professionals Follow-up Study show a positive association of glycemic load with diabetes risk (Salmeron *et al.*, 1997a; Salmeron *et al.*, 1997b), those from the Iowa Women's Health Study do not (Meyer *et al.*, 2000).

2 Magnesium

2.1 Magnesium metabolism

2.1.1 Functions of Mg

Magnesium is a ubiquitous element in nature and forms an estimated 2.1 % of the earth's crust. Mg plays an essential role in a wide range of fundamental biologic reactions. It is the second most abundant intracellular cation in the human body (second only to potassium) and the fourth most abundant total cation (Reinhart, 1988). Mg serves as cofactor in more than 300 enzymatic reactions involving energy metabolism and protein and nucleic acid synthesis (Elin, 1994). Free ionized Mg is the physiologically active form of the element. The intracellular level of free Mg^{2+} serves to regulate intermediary metabolism through activation of such rate-limiting enzymes as hexokinase, pyruvate dehydrogenase, enolase or creatine phosphokinase (Altura & Altura, 1996). In enzymatic reactions Mg interacts either by binding to the substrate or directly to the enzyme. Adenosine triphosphate (ATP) has an important function as 'energy supplier' for almost all cellular processes by providing high-energy phosphate. It exists in all cells primarily as MgATP (Shils, 1998). The second messenger cyclic adenosine monophosphate (cAMP) is involved in many reactions including the secretion of a number of hormones such as parathyroid hormone (PTH). Cyclic AMP is formed from MgATP and the enzyme adenylate cyclase and is activated by Mg through its two binding sites (Maguire, 1984). Further, Mg is required for DNA replication, transcription into mRNA and translation into proteins (Vernon, 1988). It is also involved in membrane stabilization, ion transport, and Ca channel activity (Weisinger & Bellorin-Font, 1998). As a consequence of its numerous biochemical cellular activities, Mg plays an important role in the control of neuronal activity, cardiac excitability, neuromuscular transmission, muscular contraction, vasomotor tone, blood pressure and peripheral blood flow (Altura & Altura, 1996).

2.1.2 Distribution in the body

The normal adult human body contains 20 to 28 g Mg. Mg is principally an intracellular cation, with less than 1 % of total body content present in the extracellular fluids. Approximately 53 % is in bone, 27 % in muscle and 19 % in other

soft tissues (Shils, 1998). Most intracellular Mg exists in bound form and only 0.25 - 1 mmol is present as free Mg^{2+} (Grubbs, 2002) out of a total Mg concentration ranging from 5 to 20 mmol (Rude, 1996). Hence, free Mg^{2+} constitutes only 1 to 5 % of total cellular Mg. In general, the higher the metabolic activity of the cell, the higher the Mg content (Rude, 1996). Within the cytoplasm, 80 % of Mg is complexed with ATP (Gupta & Moore, 1980). Other cell-type specific physiological chelators of Mg include creatine phosphate in muscle (Gupta & Moore, 1980) and 2,3 bisphosphoglycerate in red blood cells (Raftos *et al.*, 1999). Blood serum contains not more than 0.3 % of total body Mg. Of the total Mg in serum, around 67 % is present as free ionized Mg^{2+}, 14 % is complexed to anions (e.g. bicarbonate, citrate, sulfate) and 19 % is bound to proteins, mainly albumin (Altura & Altura, 1994).

2.1.3 Mg homeostasis

Mg is absorbed in the gastrointestinal tract and eliminated primarily through urinary excretion, to a smaller extent through gastrointestinal excretions and to a negligible part through sweat, menstrual losses and other corporal secretions.

Figure 3: Mg homeostasis in man (Shils, 1998)
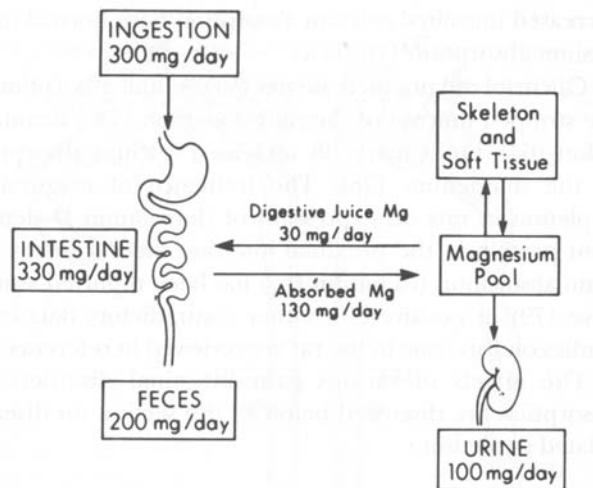
During periods of Mg deprivation, Mg homeostasis is maintained by increased fractional absorption of Mg in the kidney and the gastrointestinal tract, and by release from internal Mg stores such as bone and skeletal muscle (Rude, 1998). About one third of bone Mg resides on the surface of bone either within the hydration shell or on the crystal surface (Alfrey & Miller, 1973). This fraction is surface exchangeable and is thought to serve as a reservoir to maintain extracellular Mg concentration (Wallach,

1988). Bone provides the highest buffering potential, but skeletal muscle Mg contributes to the buffering and can lose an average of 15 % of its total Mg. Together, the bone and skeletal muscle pools can provide an average of 1.7 mmol/kg body weight equivalent to 15 % of total body Mg to maintain Mg homeostasis (Wallach, 1988).

2.1.4 Intestinal absorption

The mechanisms of intestinal Mg absorption are not entirely clear. The major sites of Mg absorption are probably the distal small intestine and the colon (Kayne & Lee, 1993). There is data to support the existence of both gradient-driven (passive) and saturable Mg absorption. It is not clear, however, which process predominates under normal conditions. A direct correlation between Mg absorption and the luminal or dietary Mg load has been shown in several studies (Kayne & Lee, 1993). Fine *et al*. (1991) describe the relationship between Mg absorption and Mg intake with a curvilinear function. This might reflect a Mg absorption process that simultaneously uses a mechanism that reaches an absorptive maximum (transcellular pathway), plus a mechanism that endlessly absorbs a defined fraction of ingested Mg (paracellular pathway). The paracellular pathway between the epithelial cells consists of tight junctions and the intercellular space. Flow and direction of Mg transport through this pathway depend on the electrochemical gradient or the flow of water. The second mechanism, the transcellular pathway, involves active absorption across the epithelial cells. Because hydrophilic compounds such as Mg^{2+} permeate only poorly across cell membranes, transport proteins in the luminal and basolateral membrane of the epithelial cells are necessary for transcellular transport (Schweigel & Martens, 2000). However, a few investigators (Hardwick *et al.*, 1990; Kayne & Lee, 1993) have postulated that the curvilinear transport pattern may represent transport across the paracellular route only. According to them, the observed changes in the transport rate with increasing luminal Mg concentration may simply reflect a progressive 'tightening' of the junction-complex with growing Mg concentration.

Mg is re-excreted in the intestine by pancreatic, bile and other intestinal excretions and by cell sloughing. There are only few data available on endogenous intestinal losses of Mg, with reports ranging from 2 to 38 mg/day (Graham *et al.*, 1960; Avioli & Berman, 1966; Freeman *et al.*, 1982).

Mg bioavailability or absorption can be determined by balance studies or by using radioactive or stable isotopes as tracers. The chemical balance technique is based on measuring the difference between intake and fecal content of Mg. However, the balance technique is time and labor consuming, and the precision is low due to problems in separating fecal content according to the corresponding dietary period. The use of isotopes as tracers helps to overcome this problem and allows moreover to determine true absorption or to study the influence of other dietary components on Mg absorption (Sandström et al., 1993). Four isotopes of Mg are available, the radioactive ^{28}Mg and the stable ^{24}Mg, ^{25}Mg and ^{26}Mg isotopes. In the late 1950s and 60s, the radioactive ^{28}Mg has been used to study Mg kinetics in man (Aikawa, 1958; Aikawa et al., 1960; Graham et al., 1960; Avioli & Berman, 1966). However, ^{28}Mg has a very short half-life of 21.3 h and is therefore not an ideal tool to study Mg absorption. Another disadvantage is the potential hazard of ionizing radiation. Of the three stable isotopes, two (^{25}Mg and ^{26}Mg) have low enough abundances (10.0 and 11.01 %, respectively) to be employed as enriched labels (Holden & Martin, 1983; Holden et al., 1983). When using stable isotopes as tracers, the isotopic pattern in the biological sample (blood, urine, feces) must be determined. Analytical techniques currently used to quantify stable isotopes include neutron activation analysis (NAA), thermal ionization mass spectrometry (TIMS), inductively coupled plasma mass spectrometry (ICP-MS), fast atom bombardment mass spectrometry (FABMS), gas chromatography mass spectrometry (GC-MS) and electron impact ionization mass spectrometry (EI-MS) (Hurrell et al., 1993). When designing a stable isotope study, the basic requirement is to achieve sufficient isotope enrichment in the body fluid of interest so that the added tracer can be quantified with the necessary degree of precision (Sandström et al., 1993). Apparent (or net) absorption can be determined by measuring the fecal excretion of an orally administered isotopic label (see Chapter 6). If true absorption is of interest, the extent of endogenous losses can be determined by simultaneous oral and intravenous administration of two different isotopes (van Dokkum et al., 1996; Bohn, 2003).

The fractional absorption of ingested Mg depends on the amount in the food, the presence of inhibiting or enhancing food components and Mg homeostasis. A balance study for whole diets has shown a Mg absorption of 21 to 27 % from self-selected diets with a mean daily Mg content of 323 and 234 mg in men and in

women, respectively (Lakshmanan *et al.*, 1984). Fractional Mg absorption from balance studies using differing diets or supplements have ranged from 10 to 65 % (Spencer *et al.*, 1980; Fine *et al.*, 1991). In stable isotope studies with adults, values for fractional net absorption varied from 20 to 60 % (Schwartz *et al.*, 1978; Schwartz *et al.*, 1984; Sabatier *et al.*, 2002; Bohn, 2003). Fractional Mg absorption is highly dependent on the amount of Mg in the meal; the larger the amount, the lower the fractional absorption (Fine *et al.*, 1991). Moreover, Mg absorption may be increased (protein, fructose) or inhibited (phytic acid, oxalic acid, zinc) by other food components (Brink & Beynen, 1992; Spencer *et al.*, 1994; Bohn, 2003). Finally, intestinal absorption is probably influenced by Mg homeostasis. A balance study showed significantly lower net absorption of Mg (17 %) in patients with chronic renal failure compared to patients with normal renal function (48.5 %). Because the patients with chronic renal failure presented decreased urinary Mg excretion, overall Mg balances did not differ significantly between the two groups (Spencer *et al.*, 1980). The study demonstrates that fractional intestinal absorption is modified in case of altered urinary Mg excretion in order to maintain Mg homeostasis.

2.1.5 Renal excretion

The kidney is the organ that most closely regulates Mg homeostasis. About 80% of the total plasma Mg is filtered through the glomerular membrane (ionized and complexed fractions). The major sites of Mg reaborption in the nephron are the proximal tubule (5-15 %), the thick ascending limb of the loop of Henle (70-80 %) and the distal convoluted tubule (5-10 %). Around 3 % of the filtered Mg normally appears in urine (Quamme, 1997). The mechanism by which Mg is reabsorbed is mainly paracellular in the cortical thick ascending limb of the loop of Henle (cTAL), and probably transcellular in the proximal and the distal tubules.

The cTAL plays a major role in the regulation of Mg reabsorption (Quamme, 1997). Factors controlling Mg absorption in this segment act through changes on the voltage and/or the permeability of the paracellular pathway (Wittner *et al.*, 1993). A large number of hormones are implicated in the control of renal Mg conservation. Parathyroid hormone (PTH), calcitonin, glucagon, vasopressin, insulin and steroid hormones (aldosterone, vitamin D) increase Mg reabsorption in the cTAL and the distal convoluted tubule (DCT), though by different cellular mechanisms (Quamme,

1997). However, none of these hormones seems to be involved in Mg homeostasis. Rather, it is believed that epithelial cells adapt their transport rate according to the availability of Mg (Quamme, 1997). In the presence of hypermagnesemia this occurs via the extracellular Ca^{2+}/Mg^{2+}-sensing receptor, Casr, present in the basolateral membrane of the TAL and the DCT, by inhibition of Mg and Ca absorption (Dai *et al.*, 2001). Animal studies with a mouse distal convoluted tubule (MDCT) cell line have demonstrated that elevated extracellular Mg or Ca inhibits fractional Mg transport through activation of the Casr (Bapty *et al.*, 1998).

Figure 4: Mg reabsorption in the nephron (Quamme, 1997)
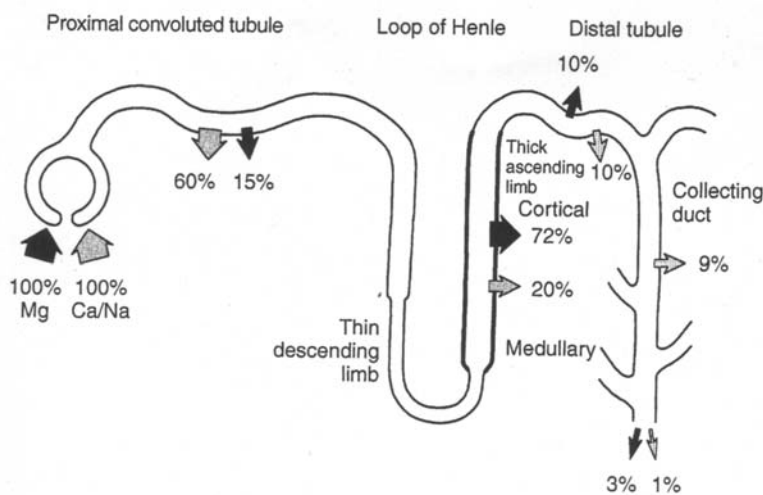
In the case of diminished extracellular Mg, another mechanism comes into play. Numerous human and animal studies show that Mg reabsorption in the kidney is increased in Mg deficiency. An animal study using microperfusion and micropuncture techniques has demonstrated a cellular adaptation of Mg transport in the loop of Henle following Mg restriction (Shafik & Quamme, 1989). This adaptation occurred very rapidly (within 5 hours), while plasma Mg did not fall until 20 hours after introduction of the Mg restricted diet. Moreover, the response was specific for Mg because there was no effect on Na and Ca transport. Based on studies with isolated distal cells, it was concluded that Mg transport may be controlled by genes that somehow respond to extracellular Mg by the formation of new transporters or channels (Dai *et al.*, 2001). However it is not clear, whether decreased plasma Mg concentrations or another mechanism is the trigger for this cellular adaptation. Several depletion studies in man indicate decreased Mg excretion from the start of

the depletion period while plasma levels begin decreasing only after several days (Dunn & Walser, 1966; Drenick *et al.*, 1969; Shils, 1969; Lukaski & Nielsen, 2002).

2.2 Mg deficiency in humans

The first observations of Mg deficiency in animal studies were made in 1932 by Kruse *et al.* (1932). In man, the first description of clinical depletion was published in 1934 (Hirschfelder & Haury, 1934). Although the diets consumed in Western countries usually do not lead to Mg deficiency, a number of clinical disorders have been associated with Mg depletion.

2.2.1 Etiology

The clinical disorders most often responsible for Mg deficiency are gastrointestinal disorders, renal disorders, alcoholism and diabetes mellitus. Gastrointestinal problems can lead to Mg deficiency through different mechanisms. Persistent nausea, cramping, or abdominal pain may impair food intake. Chronic diarrhea may cause Mg deficiency through loss of large volumes from the small intestine. Crohn's and Whipple's diseases, celiac disease, as well as other diseases associated with malabsorption also implicate impaired Mg absorption (Shils, 1998). Renal Mg wasting can be induced by metabolic disorders including hypercalcemia (caused by hyperparathyroidism or malignancy), alcoholism, diabetes mellitus or genetic disease, or by renal tubular dysfunction. Therapeutic agents such as diuretics, aminoglycoside antibiotics, cisplatin or ciclosporine also result in renal Mg wasting (Rude, 1996). Alcohol causes urinary Mg wasting, but other mechanisms related to alcoholism contribute to Mg deficiency, such as malnutrition and gastrointestinal problems (nausea, diarrhea) (Shane & Flink, 1991).

2.2.2 Symptoms

Mg deficiency is usually secondary to another disease or a therapeutic agent; hence features of the primary disease may mask or complicate Mg deficiency (Rude, 1996). In the 1950s and 60s, it was attempted to induce Mg deficiency in human subjects by dietary Mg restriction over several weeks or months (Fitzgerald & Fourman, 1956; Dunn & Walser, 1966; Shils, 1969). Only in one of these studies (Shils, 1969), marked symptoms of deficiency appeared, probably due to lower Mg content of the diet than the other studies (6-10 mg/day). Most common were neuromuscular signs

such as Trousseau's sign[1], Chvostek's sign[2], muscle fibrillations, muscle spasticity, tremor, and hyporeflexia. Further, general symptoms such as lethargy and generalized weakness, and gastrointestinal symptoms like anorexia, nausea and vomiting were noted. Urinary Mg excretion started declining rapidly from the first day of Mg restriction, plasma Mg decreased in most cases within a week, and erythrocyte Mg tended to fall more slowly. In addition, subjects developed hypocalcemia and hypokalemia despite adequate intake of Ca, vitamin D and K. These secondary Ca and K deficiencies probably contribute to the neuromuscular symptoms observed in Mg deficiency. In the same manner, observed electrocardiographic (ECG) changes may be related to hypokalemia and/or hypocalcemia.

Table 1: Symptoms and signs of Mg deficiency (adapted from Shils 1998 and Rude 1996)

Neuromuscular Positive Chvostek's and Trousseau's sign Spontaneous carpopedal spasm (tetany) Seizures Vertigo, tremor, ataxia, nystagmus Muscular weakness, tremor, fasciculation, wasting Psychiatric: depression, psychosis	**Cardiovascular** Arrhythmias ECG changes Myocardial ischemia/infarction Hypertension Atherosclerotic vascular disease
Gastrointestinal Anorexia Nausea and vomiting	**Bone metabolism** Hypocalcemia Impaired PTH secretion Renal and skeletal resistance to PTH Resistance to vitamin D
Potassium homeostasis Hypokalemia Renal K wasting, decreased intracellular K	

2.2.3 Hypocalcemia and osteoporosis

A major complication of Mg deficiency is hypocalcemia. Most hypocalcemic Mg-depleted patients have PTH concentrations which are either low or inappropriately normal for the degree of hypocalcemia (Rude *et al.*, 1976). Fatemi *et al.* (1991) demonstrated impaired PTH secretion in normal subjects after a 3-week low-Mg diet. There is also evidence for an end-organ resistance to PTH; Rude *et al.* (1976)

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[1] Trousseau's sign: In latent tetany, the occurrence of carpopedal spasm accompanied by paresthesia elicited when the upper arm is compressed, as by a tourniquet or a blood pressure cuff.

[2] Chvostek's sign: In tetany, tapping the muscles of the face causes them to go into spasm.

showed a reversible renal resistance to PTH in Mg-deficient patients. The reasons for the impaired PTH secretion and PTH end-organ resistance are unclear but may be secondary to disturbed adenylate cyclase function (Rude *et al.*, 1976). Since cyclic AMP is thought to mediate PTH secretion as well as PTH action on bone and kidney, altered adenylate cyclase function in Mg deficiency could account for both the impaired secretion and the resistance to PTH (Abbott & Rude, 1993). The effects of PTH are all with the objective of maintaining plasma Ca concentrations: activation of the osteoclasts in bone resulting in release of Ca and phosphate, enhancement of intestinal and renal Ca absorption, and inhibition of renal phosphate reabsorption. PTH deficiency or inefficacy leads to hypocalcemia. Because of this effect on Ca metabolism, Mg deficiency may also be a risk factor for osteoporosis (Abbott & Rude, 1993). Disorders in which Mg deficiency is prevalent such as chronic alcoholism, diabetes mellitus, and celiac disease have shown to be associated with a high prevalence of osteoporosis (Levin *et al.*, 1976; Crilly *et al.*, 1988; Rude & Olerich, 1996).

2.2.4   Hypokalemia and arrhythmias

Hypokalemia is another consequence of Mg deficiency. It appears to be due to renal K wasting, by a mechanism that has not been clarified (Shils, 1969; Whang, 1986). In addition, there is a loss of K from the cell with subsequent intracellular K depletion (Dorup, 1994). The ECG changes observed in Mg deficiency are similar to those observed in K depletion and may therefore be secondary to hypokalemia (Rude, 1996). The presence of arrhythmias has been described in hypomagesemic, nondigitalized patients, which disappeared by parenteral Mg infusions (Levine *et al.*, 1982; Hollifield, 1987). Arrhythmias associated with Mg depletion are often resistant to traditional antiarrhythmic therapy but reversible with i.v. Mg therapy (Iseri *et al.*, 1989). The arrhythmogenic effect of Mg deficiency may be related to the function of Mg in maintaining intracellular K. Mg acts as a coenzyme to stimulate the action of Na/K-ATPase. Thus, lack of Mg may impair active transport of K into the cell and of Na out of the cell (Dyckner & Wester, 1978; Wester & Dyckner, 1981). It is important to note that K supplementation alone may not correct a K deficit and concomitant Mg supplementation is needed (Whang, 1986).

2.2.5 Hypertension

The relationship between hypertension and Mg deficiency is not entirely clear. Mg modulates vascular tone and contractility by exerting regulatory effects on vascular smooth muscle and endothelial cells. Long-term Mg deficiency can induce hypertension by increasing peripheral resistance and altering mechanical properties of blood vessels (Laurant & Berthelot, 2001). Infusion of Mg in normal subjects resulted in a drop in blood pressure and a rise in renal blood flow (Rude *et al.*, 1989). There is some evidence for low Mg intake being a risk factor for developing hypertension in the Honolulu Heart Study (Joffres *et al.*, 1987) and a study conducted in Dutch people (van Leer *et al.*, 1995) in which dietary Mg intake was inversely associated with blood pressure. Mg seems to act as a naturally occurring Ca antagonist. A decrease in intracellular Mg (e.g. due to a disease or diuretics) results in an increase in cytosolic Ca within the small vessel smooth muscle fibers, thus leading to vasoconstriction (Altura *et al.*, 1987). Reports relating serum Mg concentrations to blood pressure are inconsistent, some finding a relationship (Albert *et al.*, 1958; Petersen *et al.*, 1977), and others not (Tillman & Semple, 1988). Supplementation studies also report conflicting results. In the study by Karppanen *et al.* (1984), patients were asked to substitute a K- and Mg-enriched salt mixture for their usual table salt. Consumption of the fortified salt was associated with a significant reduction of blood pressure. The same result was provided by a couple of controlled supplementation trials (Dyckner & Wester, 1983; Reyes *et al.*, 1984). In contrast, other randomized controlled trials of Mg supplementation in hypertensive patients did not find a decrease in blood pressure by supplementation (Cappuccio *et al.*, 1985; Zemel *et al.*, 1990). It must be noted that the patients enrolled in the studies of Dyckner & Wester and Reyes *et al.* were receiving diuretic therapy and were therefore more susceptible to develop Mg deficiency.

2.2.6 Cardiovascular disease

Depressed Mg status has also been described as a risk factor for myocardial infarction. Areas with increased water hardness (which is due to high Ca and Mg content) tend to have lower cardiovascular death rates (Luoma *et al.*, 1983; Rubenowitz *et al.*, 1996). However, dietary Mg intake was not a significant predictor of ischemic heart disease in a 16-year observation period of 2'512 Welsh males (Elwood *et al.*, 1996). In animal experiments, Mg deficiency has shown to be involved

in several steps of the atherosclerotic process, including the metabolisms of elastin and collagen, dyslipidemia, platelet aggregation and hypertension (Rayssiguier, 1984). In humans, the situation is more complex. A number of drugs commonly used to treat cardiovascular diseases can significantly affect Mg and K homeostasis. These include diuretics and digitalis drugs (Wester, 1992). Muscle Mg depletion was observed in 43 % of 297 patients with congestive heart failure and/or hypertension treated with diuretics, of whom 239 were also receiving digitalis therapy (Wester & Dyckner, 1986). Moreover, platelet hyperactivity is a recognized risk factor in the development of cardiovascular disease. Nadler *et al*. (1992) showed a decrease in platelet reactivity after supplementing Mg-deficient diabetic patients with Mg. Platelet hyperactivity in Mg deficiency may be related to increased [1]thromboxane A_2 synthesis (Nadler *et al.*, 1993).

2.3 Dietary Mg

2.3.1 Food Sources

Mg is widely distributed in plant and animal foods. Especially nuts, legumes, vegetables, cereals and chocolate contain high amounts of Mg. Hard drinking water may also be an important source of Mg. Nuts and seeds have the highest Mg-density (e.g. pumkin seeds: 402 mg, cashew nuts: 255 mg Mg/100g), but are consumed in rather low quantity. Leafy vegetables contain up to 85 mg Mg/100g (e.g. Swiss chard: 81 mg Mg/100g). The Mg content of black chocolate is as high as 228 mg/100g (data from the German Food and Nutrient Database BLS 2.3). The contribution of these sources to total Mg intake differs depending on dietary habits. In Switzerland, main sources for Mg are nuts and legumes (24 %), milk products (16 %), cereals (13 %) and vegetables (12 %) (Swiss Federal Office for Public Health, 1998).

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[1] Thromboxane A_2 = compound causing vasoconstriction and platelet aggregation

Figure 5: Contribution of food groups to Mg intake in Switzerland (data from the Swiss Federal Office for Public Health, 1998)
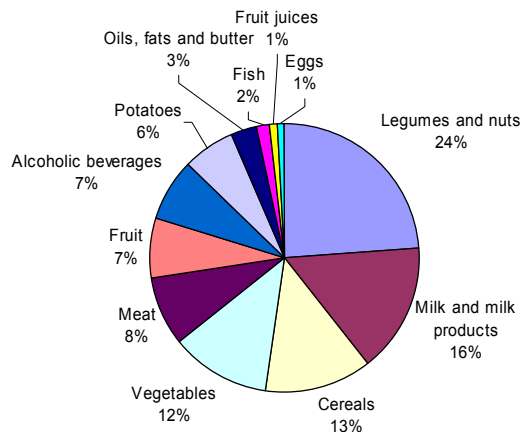
Bioavailability of dietary Mg is in average around 30 %, but depends strongly on the amount of Mg in the meal, the presence of inhibitory or enhancing food components, and Mg status (see 2.1.4). Estimates based on disappearance data from 1994/1995 suggest that the Mg intake is adequate in Switzerland: 406 mg/day in adults > 15 y (Swiss Federal Office for Public Health, 1998). However, refining, processing and preparing food may cause a substantial loss of Mg and thus, modern food technology may predispose people to an inadequate intake of Mg (Elin, 1988).

2.3.2 Mg requirements and dietary recommendations

The aim of dietary reference values is the maintenance of health, the prevention of disease and therefore quality of life. A nutrient intake of the order of the recommendations should prevent the occurrence of nutrient specific deficiency disorders (e.g. scurvy, rickets) and deficiency symptoms, but also prevent an over-supply of energy and nutrients such as fat or alcohol (D-A-CH, 2000). Most countries and the WHO publish their own recommended dietary intakes (RDI's) for macro- and micronutrients. Switzerland issues its RDI's in co-operation with Germany and Austria since 2000 (D-A-CH, 2000). Of worldwide importance are the US and Canadian Dietary Reference Intakes (Food and Nutrition Board, 1997) which are the successor of the former Recommended Dietary Allowances (RDA's). The RDA is the average daily dietary intake level that is sufficient to meet the nutrient requirement of nearly all (97 to 98 %) healthy persons in a particular life stage (life stage considers age and,

when applicable, pregnancy or lactation) and gender group. The RDA is set depending on the Estimated Average Requirement (EAR), which is the amount of nutrient that is estimated to meet the nutrient requirement of half the healthy individuals in a life-stage and gender group. If the EAR is normally distributed and the SD is available, the RDA is set at 2 SD's above the EAR (RDA = EAR + 2 SD_{EAR}).

For the US Dietary Reference Intakes, the EAR for Mg was determined based on balance studies. The aim of such balance studies is to determine the amount of Mg, which is just sufficient to maintain Mg balance. Using this method, the so determined average daily requirement for 31-50 y olds was 350 mg for men and 265 mg for women. By adding an assumed coefficient of variation of 10 %, the RDA was set at 420 mg for men and at 320 mg for women (Food and Nutrition Board, 1997).The RDI's for Switzerland, Germany and Austria (D-A-CH, 2000) were also based on balance studies and set corresponding to the recommendations of other countries with similar eating habits. For adults over 25 years, they are set at 350 mg for men and 300 mg for women. Mg RDI's for young adults (15 to 25 years) and pregnant and lactating women are slightly higher.

Requirements and recommendations of Mg are often expressed in mg/kg body weight per day which is independent from age and gender. Balance studies in normal healthy adults have shown that at Mg intakes of 4.5 mg/kg per day or above, Mg balance was uniformly positive (Food and Nutrition Board, 1997). The current average RDI in developed countries is therefore set at around 6 mg/kg body weight per day. However, requirements of Mg depend on lean body mass rather than on body weight because fat cells by weight need appreciably less Mg for their cellular functions (Shils & Rude, 1996). Thus, an older adult has a smaller Mg requirement than an adolescent of the same body weight. Abrams & Ellis (1998) showed a good correlation between fat-free mass and Mg kinetic values.

2.3.3 Assessment of Mg intake

The aim of a dietary assessment is the measurement of food and nutrient intake in individuals or population groups. There are four stages in any dietary assessment protocol: the measurement of food consumption, the calculation of the nutrient content of the consumed food, the estimation of absorbed intakes and the evaluation of the nutrient intakes in relation to recommendations (Gibson, 1993). Methods used

to measuring food consumption of individuals can be classified into two major groups: retrospective and prospective methods. Prospective methods measure food intake over a given period of time either by recording all food and drink consumed over this period by weighing (weighed food record) or estimating (estimated food record), or by making up a duplicate meal for analysis from each consumed meal (duplicate portion technique). Methods looking retrospectively at food intake are the 24-hour recall, the food frequency questionnaire and the diet history. The purpose of the 24-hour recall is to provide information on the subject's exact food intake during the previous 24 hours or the preceding day (Gibson, 1993). A food-frequency questionnaire evaluates dietary habits by assessing the frequency with which certain food items or food groups are consumed (e.g. daily, weekly or monthly) (Gibson, 1993). Usually, the questionnaire includes food items that contain the regarding nutrient to a certain extent and are consumed with a certain frequency in the studied population. The objective of the diet history is to obtain retrospective information on the usual food intake and meal pattern of individuals over a given period of time. The time periods covered often include the previous month, six months, or the previous year (Gibson, 1993). In an interview, breakfast, lunch, dinner and snacks are discussed in turn to determine which foods have been consumed, in which quantity and how often during the given time period. Calculation of the nutrient content of the consumed food and the evaluation of the nutrient intakes are mostly done using a computer software including an electronic nutrient database.

For Mg, not every method is appropriate as assessment tool. Weighed food records are very accurate and often used as 'golden standard'. However, they are time-consuming and intensive, and habitual eating patterns may be influenced by the recording process. Twenty-four-hour recalls are easy and rapid to carry out, but they evaluate food intake only on single days, and a minimum of 4 to 6 days are required to assess Mg intake in adults accurately (Nelson *et al.*, 1989). Advantages of the food-frequency questionnaire and the diet history are that they assess dietary habits over longer time periods. A food-frequency questionnaire is easy to perform, but because Mg is widely distributed in foods, a prohibitive number of food items need to be included in the questionnaire. The diet history also has a relatively low respondent burden compared to a weighed food record and is a good tool for assessing food

intake in subjects with relatively regular eating habits. As for the food frequency questionnaire, a good memory of the subject is required.

Dietary assessments are subject to many potential sources of error. The quality of dietary assessment methods is usually expressed by their validity and reproducibility (van Staveren *et al.*, 1985). The reproducibility of the diet history method based on repeated interviews has been found to be very good (Reshef & Epstein, 1972; Morgan *et al.*, 1978). Bloemberg *et al.* (1989) studied the reproducibility of a dietary history method by repeating the interviews after 3 months and 1 year. When considering the reproducibility of the different food groups, they found that bread, sugar products, milk products, and alcoholic beverages were very well reproduced, while meat and vegetables were poorly reproduced. They concluded that the reproducibility of the food groups that contain the most of a certain nutrient had a major effect on the reproducibility of the studied nutrient. Kune *et al.* (1987) found that reproducibility was good for most foods, but low for vegetables. Järvinen *et al.* (1993) compared the intakes of 32 food groups and 32 nutrients in interviews 4 to 8 months apart. They found a good correlation for most of the food groups (including vegetables) and for 90 % of the investigated nutrients (including Mg).

Validity describes the degree to which a method measures what it purports to measure (Klaver *et al.*, 1988). For the diet history method, the most objective validation method would require measuring the exact amount of food consumed over a prolonged period, and this is nearly impossible to obtain (Kune *et al.*, 1987). A diet history is often compared either to other dietary assessment methods (e.g. seven-day food record) or with clinical or biochemical parameters (e.g. urinary nitrogen excretion, doubly labeled water technique). When validating the diet history method with another dietary assessment method, it would be important to cover the same period of time. However, most of the validation studies have used shorter periods such as a 24-hour recall or a seven-day record (Jacob Sempach, 1995). In some studies, the diet history method has produced higher estimates of group mean intake than the food record (Gibson, 1990b). In contrast, other investigators have found similar or lower mean nutrient intakes when comparing the diet history with food records (van Staveren *et al.*, 1985).

2.3.4 Food composition tables

The accuracy of the used food composition table or database is essential for the evaluation of dietary assessments. However, nutrient contents of plant and animal foods may vary considerably depending on climate, soil conditions, fertilization, geographical and seasonal variations, feeding or breeding. It is difficult to choose a sample producing representative values when analyzed. Most countries have their own food composition table since nutrient contents and food habits vary between countries. Until very recently, Switzerland did not have an own food composition database, so that food composition tables from the surrounding countries were usually used. The German Food and Nutrient Database (BLS) for example, is an electronic database developed by the German Federal Health Department, for use in nutrition epidemiology and dietary assessment (see Chapter 5).

For Mg, the consistence between analyzed values and those from food composition tables is relatively good. A study comparing the nutrient content of diets as determined by laboratory analyses with the content determined by calculation from the German BLS 2.2 found a good correlation for Mg (r^2=0.786). Analyzed values for Mg were on average 8 % lower than calculated values (Ekmekcioglu *et al.*, 1999). A similar study performed in the US reported analyzed Mg values which were on average 7 % lower than those calculated from the USDA Nutrient Data Base for Standard Reference (Pennington & Wilson, 1990).

2.4 Determination of Mg status

Mg is principally an intracellular cation, with less than 0.3 % of total body content present in serum (Shils, 1998). Nevertheless, serum or plasma Mg measurement is the most available and most commonly employed test of status because it is easy and inexpensive to perform. Elin (1991) divides Mg assessment tests into three categories: tissue Mg, physiologic assessment of Mg, and free Mg. The equilibrium between some tissue pools occurs slowly and the biologic half-life for a large part of Mg in the body is more than 1000 hours (Avioli & Berman, 1966). Therefore, determining Mg in one tissue may not provide information about Mg status in other tissues (Elin, 1991).

Table 2: Laboratory tests for the assessment of Mg status (adapted from Elin 1991)

Tissue Mg	Physiologic assessment	Free magnesium
Serum/plasma Erythrocytes Mononuclear blood cells Muscle	Mg load/retention test Renal excretion of Mg Balance studies Isotope studies	Extracellular free Mg (serum) Intracellular free Mg

2.4.1 Serum or plasma Mg

Serum Mg is the most commonly used test, but is an insensitive indicator of Mg status for it does not correlate with any other tissue pool of Mg except for interstitial fluid (Elin, 1987). Most investigators did not find a relationship between serum Mg and the two largest Mg pools, bone and muscle. Dyckner & Wester (1978) and Ladefoged & Hagen (1988), who both analyzed serum and muscle Mg in a large number of subjects (107 and 93, respectively) found no association. Although Alfrey *et al*. (1974) showed a correlation between serum and bone Mg, this was not confirmed by repeat studies of other investigators. Nevertheless, serum Mg has some use as an indicator of Mg deficiency. In human studies, instituting a diet low in Mg produces a predictable decline in serum Mg (Shils, 1969; Rude *et al.*, 1991; Lukaski & Nielsen, 2002).

The reference interval for serum Mg varies depending on the literature. According to Dewitte *et al*. (2001), the most reliable source is the data from the first National Health and Nutrition Examination Survey (NHANES I) in the US (Lowenstein & Stanton, 1986). The data are based on a population sample of 15'820 healthy subjects aged 1-74 years and Mg was analyzed by atomic absorption spectrometry. They found that serum Mg concentrations were normally distributed with 95 % of adults, aged 18-74 years, falling between 0.75 and 0.95 mmol/L. Age and sex differences have shown to exist, but they are generally small. Changes in serum Mg concentration are usually caused by relatively acute changes in intake or excretion of Mg (Elin, 1991). Elevation of serum Mg occurs primarily in renal failure or after taking Mg-rich drugs such as antacids or laxatives. However, hypermagnesemia caused by orally ingested drugs is rare in healthy subjects. Mg-Al containing antacids may cause hypermagnesemia in subjects with renal insufficiency (Herzog & Holtermuller, 1982) and laxatives if used in high dose to treat toxic ingestion (Woodard *et al.*, 1990). Hypomagnesemia is caused by different chronic diseases or by the use of

drugs (see 2.2.1) However, there are a number of reports of low Mg values in various blood cells and tissues associated with normal serum Mg concentrations (Dyckner & Wester, 1982; Shils, 1998). In states of chronic Mg deficiency with a slow loss of Mg, serum Mg concentration may be maintained by Mg release from intracellular body stores such as the labile bone pool or skeletal muscle. In a study with 10 chronic alcoholics, 9 had diminished muscle Mg, but only 2 had concomitant low serum Mg concentration (Lim & Jacob, 1972).

Determination of Mg in serum or plasma can be done by a colorimetric method using calmagite, or by flame atomic absorption spectrometry (AAS). AAS is now generally used and considered to be the reference method. The method is very specific and not much susceptible to interferences. Plasma and serum can be directly measured after dilution in water (see Chapter 4). Several factors may confound the measurement of Mg in serum. These factors include hemolysis of erythrocytes (their Mg concentration is three times higher than that of serum), diurnal variations (values are lower in the morning than the evening), exercise (may reduce serum Mg), and serum albumin concentrations (Gibson, 1990a).

2.4.2 Mg in erythrocytes

Since 99 % of total body Mg is intracellular, determination of intracellular Mg might be a better indicator of Mg status. Because erythrocytes are easily accessible, measurement of erythrocyte Mg has been proposed for the estimation of intracellular Mg. Erythrocyte Mg can be measured by isolating red blood cells, lyzing them by acid digestion, and measuring Mg concentration by flame AAS. Erythrocyte Mg reflects chronic, rather than acute Mg status because of the long erythrocyte half-life (120 days). Concentrations of Mg in erythrocytes are dependent on the age of erythrocytes; the Mg content slowly declines as they age. In clinical conditions in which the survival time of erythrocytes is reduced (e.g. chronic renal failure, thalassemia, sickle cell anemia), erythrocyte Mg concentration may be higher because Mg-rich immature erythrocytes dominate (Gibson, 1990a). Most studies did not find a correlation between erythrocyte Mg and other Mg tissue pools. No correlation was found with serum Mg (Elin & Hosseini, 1985; Ladefoged & Hagen, 1988) or with Mg in mononuclear blood cells or lymphocytes (Elin & Hosseini, 1985; Girardin & Paunier, 1985; Ryschon et al., 1996). Ladefoged & Hagen (1988) did not

find an association between erythrocyte and muscle Mg in a group of 93 subjects. Moreover, Martin *et al*. (1997) measured raised erythrocyte Mg in patients with infections and pressure sores, but normal or low serum Mg. They concluded that high erythrocyte Mg in illnesses is likely to be due to alterations in characteristics of the erythrocytes rather than an indication of body Mg excess. Henrotte (1982) postulated that an apparent genetic regulation of erythrocyte Mg may be a factor in the lack of correlation with other tissue Mg pools. Erythrocyte Mg concentration has shown to be significantly lower in individuals with HLA[1]-Bw35. In conclusion, measurement of Mg concentration in erythrocytes does not seem to reflect intracellular Mg stores and is therefore of limited value as indicator of Mg status.

2.4.3 Mg in mononuclear blood cells

The determination of Mg in mononuclear blood cells (monocytes + lymphocytes) as status indicator has looked very promising during the last 2 decades. Mononuclear blood cells (MBC) can be separated from whole blood using a discontinuous Ficoll-Hypaque gradient with appropriate centrifugation, then be lyzed and analyzed by flame AAS. MBC Mg concentration is expressed per unit mass of protein, per cell, or per unit mass dry weight (Elin & Hosseini, 1985). Sjögren *et al*. (1986; 1987) found a significant correlation between MBC and muscle Mg in patients with type 1 diabetes (r=0.69) and in healthy subjects (r=0.63). Though, results are equivocal. At first view, Dyckner & Wester (1985) found a good correlation between MBC and muscle Mg among 3 healthy subjects and 6 patients with mild hypertension (r=0.74). However, when they added 16 patients with congestive heart failure, the correlation vanished (r=0.22). Arnold *et al*. (1995) performed a Mg load test in 16 critically ill patients and measured serum, MBC and erythrocyte Mg. Patients were divided into 2 groups according to their response to the loading test. Although one group was Mg-deficient, there was no significant difference in MBC and erythrocyte Mg between the two groups. Thus, it is difficult to judge the validity of MBC Mg as status indicator and further investigations should be done. Moreover, the determination of MBC Mg is not yet precise and reproducible enough to be a clinically useful tool. Problems include disease states that increase the relative proportion of monocytes, day-to-day individual variations and contamination by platelets (Tosiello, 1996).

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[1] HLA = human leukocyte antigen: a genetic fingerprint on white blood cells and platelets, composed of proteins that play a critical role in activating the body's immune system to respond to foreign organisms

2.4.4 Mg in skeletal muscle

Muscle and bone contain the majority of body Mg and are therefore potentially important tissues for the assessment of Mg status. Muscle Mg concentration is considered as a good indicator for Mg status (Lim *et al.*, 1969; Dyckner & Wester, 1985) with the disadvantage that the determination is invasive and time consuming, thus making it unsuitable for ambulatory diagnosis. The measurement involves needle biopsy of the muscle, preparation of the tissue and analysis of Mg by AAS, optical emission spectrometry with inductively coupled plasma (ICP-OES), or inductively coupled plasma mass spectrometry (ICP-MS). Various studies show that muscle Mg is lost during Mg deprivation (Drenick *et al.*, 1969; Wallach, 1988; Lukaski & Nielsen, 2002) and an average of 15 % of skeletal-muscle Mg can become available for extracellular buffering (Wallach, 1988). Muscle Mg may therefore provide a good measurement of body Mg stores, but may not react to acute changes (Dyckner & Wester, 1985). However, Dorup *et al.* (1988) found a significant rise in muscle Mg after supplementing four Mg-deficient patients (on diuretics) for two weeks with oral MgO.

Mg content does not seem to vary significantly between different muscles from the same subjects. Dorup *et al.* (1988) analyzed Mg content from two subjects who died acutely in four different muscles with different fibre composition and found only modest variations. The lowest value (soleus) was only 8.5 % lower than the highest (vastus lateralis). However, there is no reference range for muscle Mg concentration. Depending on the method used for analysis, values can vary considerably. When expressing the results as mmol/100 g fat-free dried solids (FFDS) there is somewhat more consistency. Holm *et al.* (1987) uses the value of 3.48 mmol/100 g FFDS as cut-off for Mg deficiency. According to Forsberg *et al.* (1991), muscle composition varies in relation to age and sex. In their study, muscle Mg was decreased in subjects aged 61-85 y compared to 19-40 y olds, and in women compared to men. The differences are probably due to the increased fat content of muscle in the older age group and the women.

2.4.5 Mg load test

The Mg load test is a physiological assessment method and has been proposed, applied and validated by several authors as method for the determination of Mg

status (Jones *et al.*, 1969; Lim & Jacob, 1972; Bohmer & Mathiesen, 1982; Ryzen *et al.*, 1985; Gullestad *et al.*, 1992; Lasserre *et al.*, 1996). It consists of measuring the retention of a parenterally administered dose of Mg in the body. A certain amount of Mg (5 to 30 mmol) is infused over 4 to 12 hours, and the Mg content in 24 hour-urine is determined to calculate the retention of the administered load. Mg retention is expressed as %loading dose retained. Ryzen *et al.* (1985) have established a reference interval: retention of > 50% is defined as definite Mg deficiency, retention between 20–50 % as probable deficiency and < 20% as non-deficiency. The test is based on the fact that the normal kidney regulates body stores of Mg, excreting an excess in the normal subject and retaining the mineral in deficiency (Caddell *et al.*, 1984). Moreover, rat studies have shown that tissues from Mg-deficient rats had a higher Mg uptake after intraperitoneal injection of ^{28}Mg than controls (Caddell *et al.*, 1981). Mg is administered with a slow infusion rate (< 30 mmol/12 h) to avoid the development of excessively high serum levels. This should be avoided for two reasons: to prevent toxic effects and to eliminate the possibility of exceeding the tubular maximum for Mg causing the loss of a large portion of Mg (Dyckner & Wester, 1982). A disadvantage of the Mg load test is that it is affected by dietary Mg intake, basal urinary Mg and fecal Mg excretion, all of which have an influence on the urinary Mg excretion after an i.v. loading dose. This is the reason for the necessity of administering a high dose of Mg.

The test has given satisfying results in Mg deficiency states related to malabsorption syndromes, alcoholism, cirrhosis and diabetes mellitus. Several studies have diagnosed Mg deficiency based on this test with no significant difference in serum Mg between experimental and control groups (Holm *et al.*, 1987; Rasmussen *et al.*, 1988; Goto *et al.*, 1990). Moreover, the Mg load test seems to be a valid test since retention correlates with Mg concentration in bone (Cohen & Laor, 1990) and skeletal muscle (Jones *et al.*, 1969; Lim & Jacob, 1972; Jeppesen, 1986; Holm *et al.*, 1987; Sjögren *et al.*, 1988c) of Mg-deficient patients. However, Danielson *et al.* (1979) have not found any association between muscle Mg and retention in healthy subjects. A major drawback of the load test is that it can not be used in patients with decreased renal function. Moreover, it might be of limited value in Mg deficiency states, which are characterized by reduced tissue Mg uptake such as diabetes mellitus. In summary, the Mg load test seems to provide good information on Mg status, but is

time-consuming and requires hospitalization of the subjects. Further, the amount of the Mg load, the length of time of infusion, and the length of time of urine collection has not been standardized (Elin, 1991) and it is difficult to relate the %retention to the total body deficit of Mg.

2.4.6 Free ionized Mg

Commonly, total Mg concentration is determined. However, the most important fraction is the free ionized Mg since it is the physiologically active proportion. Recent advances in technology permit to determine extracellular ionized Mg by ion-selective electrodes, and cytosolic free Mg^{2+} by fluorescent indicators (e.g. mag-fura-2) or ^{19}F- or ^{31}P-nuclear magnetic resonance spectroscopy (Elin, 1991). Altura & Altura (1996) used ion-selective electrodes to determine free Mg^{2+} in serum, plasma or whole blood and measured a mean concentration of ionized Mg in blood of around 0.6 mmol/L (0.54-0.65 mmol/L, 95% CI), thus 65-72% of total Mg being free Mg^{2+}. According to a review by Grubbs (2002), it appears that all mammalian cells maintain free cytosolic Mg^{2+} within the fairly narrow range of 0.25-1 mmol.

Altura & Altura (1996) showed reduced ionized Mg but normal total Mg in serum in a variety of diseases such as ischemic heart disease, hypertension, myocardial infarction and type 2 diabetes. Rude *et al*. (1991) determined free Mg^{2+} in erythrocytes of different Mg-deficient patient groups and of subjects on dietary Mg restriction. Due to promising results they suggested the assessment of intracellular free Mg^{2+} for determination of Mg status. It is however unclear how the decrease in free Mg^{2+} concentration in erythrocytes develops. It may reflect exchange with Mg^{2+} of the extracellular fluid or decreased availability of Mg during erythropoiesis. When looking at the results in the different patient groups, free erythrocyte Mg^{2+} correlates fairly well with serum Mg, which would support the first hypothesis. In patients with abnormal protein concentration in serum, the measurement of ionized Mg might be superior to total serum Mg. In such cases, low serum Mg concentrations may be measured despite normal Mg status due to lower protein-bound Mg concentration. In these patients, free ionized Mg would thus better reflect Mg status than total serum Mg (Huijgen *et al.*, 2000). In summary, measurement of free ionized Mg seems to have potential. However, technologies have yet to be simplified and further

evaluations have to be done to see if the test has some value as indicator for Mg status.

3 Magnesium and Type 2 Diabetes

3.1 Mg and diabetes risk

Low Mg intake may play a role in the development of diabetes and insulin resistance. Data from large epidemiological studies such as the Nurses' Health Study (84'360 US women), the Health Professionals Follow-up Study (51'529 US men) and the Iowa Women's Health Study (35'988 women) have shown an inverse association between Mg intake and the development of diabetes (Colditz *et al.*, 1992; Salmeron *et al.*, 1997a; Meyer *et al.*, 2000). It might be argued that the effect could be due to dietary fiber rather than Mg because both are found in large quantities in whole-grain cereals and vegetables. However, in the Iowa Women's Health Study, the association remained after adjustment for cereal fiber and grain intakes and the Nurses' Health Study did not even find an association between dietary fiber and diabetes risk. Humphries *et al.* (1999) compared dietary Mg intake with insulin resistance in non-diabetic adults and found a negative correlation. In addition, results from the Atherosclerosis Risk in Communities (ARIC) study indicated low serum Mg to be a strong, independent predictor of the development of type 2 diabetes (Kao *et al.*, 1999).

3.2 Evidence of Mg deficiency

Diabetes mellitus is one of the most common pathological states in which Mg deficiency occurs. As early as 1946, hypomagnesemia was noted in patients with diabetic ketoacidosis (Martin *et al.*, 1958). In 1968, a survey of 5'100 consecutive patients at a diagnostic clinic in the USA, revealed diabetes to be the most common condition associated with hypomagnesemia (Jackson & Meier, 1968). Twenty percent of the patients with hypomagnesemia (Mg < 0.74 mmol/l) were diabetics. Besides, the second most common condition was arteriosclerotic heart disease with 17.5 % of the patients. Moreover, hypomagnesemia was frequently observed in patients on diuretic (13.8 %) or digitalis (12.5 %) therapy. In 1995, the American Diabetes Association (ADA) stated in its clinical practice recommendations that Mg deficiency may play a role in insulin resistance, carbohydrate intolerance and hypertension, and that Mg deficiency should be corrected but only if hypomagnesemia could be demonstrated (ADA, 1995).

3.2.1 Hypomagnesemia in diabetes

In the USA, 25 to 39 % of outpatient diabetics have low levels of serum Mg (Nadler & Rude, 1995). Similarly, low serum Mg levels have been reported in type 1 and 2 diabetes in numerous European countries. The following table shows an overview of the studies which have compared mean serum or plasma Mg concentrations between type 2 diabetics and healthy control subjects. Also studies which did not differentiate between type 1 and 2 diabetes are listed.

Table 3: Low serum/plasma Mg concentrations in type 2 diabetes

Authors	Country	Diabetes Type	N Diabetics	N Controls	Mean plasma/serum Mg [mmol/l]	p	% below reference range
Sullivan et al. 1979	USA	1, 2	19	37	0.77 vs. 0.80	NS	25 vs. 0.7%[1]
Mather et al. 1979	UK	1, 2	582	140	0.74 vs. 0.81	< 0.001	
Johannson et al. 1981	Sweden	1, 2	45	106	0.74 vs. 0.84	< 0.001	
Ceriello et al. 1982	Italy	1, 2	56	30	0.77 vs. 0.92	< 0.001	
Fujii et al. 1982	Japan	1, 2	109	33	0.80 vs. 0.83	< 0.05	
Cohen et al. 1983	Israel	2	15	10	0.86 vs. 0.93	NS	
Yajnik et al. 1984	UK	2	55	30	0.95 vs. 0.89	< 0.01	
Fialip et al. 1985	France	1, 2	50	12	0.72 vs. 0.82	< 0.005	
Sjögren et al. 1988a	Sweden	2	18	35	0.70 vs. 0.77	< 0.01	
Schlienger et al. 1988	France	2	14	309	0.80 vs. 0.98	< 0.01	
Paolisso et al. 1988	Italy	2	12	12	0.84 vs. 0.88	< 0.05	
de Valk et al. 1992	Netherlands	2	53	72	0.85 vs. 0.92	< 0.01	3.8 vs. 1.4%[2]
Schnack et al. 1992	Austria	2	50	50	0.79 vs. 0.88	< 0.001	
Nadler et al. 1992	USA	2	20	16	0.80 vs. 0.95	< 0.05	
Resnick et al. 1993	USA	2	22	30	0.81 vs. 0.86	NS	
Ma et al. 1995	USA	1, 2	282	6707	0.79 vs. 0.83		
Corica et al. 1996	Italy	2	45	15	0.62 vs. 0.70	< 0.01	
de Lenardis 1999	Germany	2	114	116	0.84 vs. 0.88	< 0.001	31 vs. 12%[3]

cut-offs in mmol/l: [1]0.69, [2]0.70, [3]0.80

The reference range for serum/plasma Mg is 0.75 to 0.95 mmol/l (Lowenstein & Stanton, 1986). Of the studies listed above, all but one show a decreased serum Mg concentration in the diabetic group compared to the control group, and in most cases the difference is significant. Resnick et al. (1993) found no significant difference when analyzing total serum Mg, however when they analyzed serum ionized Mg they found

a significantly lower concentration in the diabetic group (0.552 vs. 0.630 mmol/l, p < 0.001). Moreover, they noted a correlation between serum ionized Mg and intracellular free Mg in erythrocytes (r=0.73).

In some of the studies, although mean serum Mg concentration is decreased in the diabetic group, it is still well within the reference range. However, the mean serum Mg concentrations of the control groups also vary considerably between the different studies, although mean serum Mg of a healthy population would be expected to be around 0.85 mmol/l. The differences between the studies may in part be due to inter-laboratory measurement variations. The concomitant measure of a reference serum sample may reduce such variations, such as the Seronorm Trace Elements Serum (Nycomed, Oslo, Norway).

Some authors have described a correlation between glycosylated hemoglobin (HbA$_{1c}$) and serum Mg in type 1 diabetics (Yajnik *et al.*, 1984; Sjögren *et al.*, 1986; Schlienger *et al.*, 1988). However, no such correlation was found in type 2 diabetes (Yajnik *et al.*, 1984; Vanroelen *et al.*, 1985; Schlienger *et al.*, 1988; de Valk, 1992). Moreover, although treatment with oral hypoglycemics decreased HbA$_{1c}$ levels of 50 type 2 diabetics, it did not improve hypomagnesemia (Schnack *et al.*, 1992). In contrast to type 1 diabetes, hypomagnesemia in type 2 diabetes may be a reflection of insulin resistance rather than being related to metabolic control.

3.2.2 Intracellular Mg deficiency

Mg deficiency in diabetes also affects intracellular Mg stores. Animal studies have shown reduced Mg concentrations in bone of streptozocin-induced diabetic rats (Rosholt & Hegarty, 1981) and in different soft tissues of alloxan-induced diabetic rabbits (Bhimji *et al.*, 1986). The following table summarizes human studies which have assessed intracellular Mg in type 2 diabetic patients and healthy controls in different tissues such as bone, skeletal muscle, erythrocytes, lymphocytes, platelets and mononuclear blood cells.

Table 4: Intracellular Mg deficiency in type 2 diabetes

Authors	Tissue	Diabetes Type	N Diabetics	N Controls	Mean tissue Mg	p
de Leuww *et al.* 1978	Trabecular bone	2	62	14	1.93 vs. 2.23 µg/mg dry bone	< 0.01
Johannson *et al.* 1981	Skeletal muscle	1, 2	45	106	3.4 vs. 3.7 mmol/100 g FFDS [1]	< 0.05
Fuji *et al.* 1982	Erythrocytes	1, 2	109	33	2.39 vs. 2.47 mmol/l	NS
Cohen *et al.* 1983	Trabecular bone	2	15	10	173 vs. 190 mmol/kg ash	< 0.05
	Lymphocytes				42.5 vs. 39.2 mmol/kg dry w.	< 0.05
Paolisso *et al.* 1988	Erythrocytes	2	12	12	1.87 vs. 2.37 mmol/l	0.001
Sjögren *et al.* 1988a	Erythrocytes	2	18	35	2.14 vs. 2.09 mmol/l	NS
	Skeletal muscle				3.79 vs. 4.29 mmol/100 g FFDS	< 0.001
	Mononucl. cells				61.6 vs. 72.3 nmol/mg protein	< 0.05
Schlienger *et al.* 1988	Erythrocytes	2	14	309	1.88 vs. 2.01 mmol/l	NS
Nadler *et al.* 1992	Erythrocyte free Mg	2	20	16	166 vs. 204 µmol/l	< 0.05
Resnick *et al.* 1991	Erythrocyte free Mg	2	12	25	206 vs. 232 µmol/l	0.05
Rude *et al.* 1991	Erythrocyte free Mg	2	37	29	172 vs. 207 µmol/l	< 0.001
Resnick *et al.* 1993	Erythrocyte free Mg	2	22	30	184.1 vs. 223.3 µmol/l	< 0.001
Corica *et al.* 1996	Platelets	2	45	15	60.7 vs. 67.9 µg/10^8 cells	NS
	Erythrocytes				2.02 vs. 2.18 mmol/l	< 0.01

[1]FFDS = fat-free dried solids

Most of the studies listed above show reduced intracellular Mg in type 2 diabetes, especially in bone and skeletal muscle. Cohen *et al.* (1983) found decreased bone Mg, though increased Mg in lymphocytes and a normal Mg retention in the Mg load test. De Leuww *et al.* (1978) found decreased bone Mg in insulin-treated patients but not in patients treated by oral hypoglycemics. Several authors (Fujii *et al.*, 1982; Schlienger *et al.*, 1988; Sjögren *et al.*, 1988a) found no significant difference in erythrocyte Mg concentration between the diabetic and the control groups despite lower serum Mg concentrations in the diabetic groups. However, it must be noted that the use of total erythrocyte Mg as indicator for Mg status is equivocal (see 2.4.2). It is possible however, that the measure of free intracellular erythrocyte Mg has more potential (see 2.4.6).

3.3 Etiology of Mg deficiency

The reasons for Mg deficiency in diabetes are not clear, but they may include lower dietary intake of Mg, lower intestinal Mg absorption, increased losses of urinary Mg or decreased Mg uptake into the cells compared to healthy individuals.

3.3.1 Impaired dietary Mg intake

It has been suggested that low dietary intake may contribute to low Mg status in diabetics (Durlach & Rayssiguier, 1983; Sheehan, 1991; White & Campbell, 1993). Patients with type 2 diabetes are often overweight, and may consume a diet higher in fat and lower in Mg density than non-diabetics. Foster (1987) reported that, in both the USA and Canada, mortality from diabetes tended to be increased where environmental Mg and Ca levels were low, i.e. in soft-water areas. Studies that have reported Mg intake in type 2 diabetes in the US are equivocal. Schmidt *et al.* (1994), using a 3-day food record in 50 type 2 diabetics, found an intake of 336.8 mg/d in men and 216.5 mg/d in women (mean age 57.2 ±10.2 y.). This is rather low (RDI for men: 350 mg/d, for women: 300 mg/d), however, the results have not been compared with the intake of a control group. The ARIC study (Ma *et al.*, 1995) investigated dietary Mg intakes in 15'248 participants using a food frequency questionnaire. In contrast, this study showed a higher Mg intake per kcal in diabetics than in disease-free subjects. In white male and female diabetics they found a Mg density of 175 mg/1000 kcal and 174 mg/1000 kcal, respectively, compared to 157 mg/1000 kcal and 170 mg/1000 kcal in white disease-free subjects.

3.3.2 Impaired intestinal Mg absorption

Several authors have suggested that impaired intestinal absorption might contribute to low Mg status in diabetics (Durlach & Rayssiguier, 1983; Nadler *et al.*, 1993; Tosiello, 1996). In diabetic individuals, enteric neuropathy and microvascular disease can alter intestinal absorption of carbohydrates, amino acids, and minerals (Casirola *et al.*, 1994; May & Goyal, 1994; Eliasson *et al.*, 1995). Intestinal absorption of Ca and Zn are decreased in diabetic rats and humans, compared to healthy controls (Schneider & Schedl, 1972; Kiilerich *et al.*, 1990; Verhaeghe *et al.*, 1990). Studies in diabetic rats suggested that Mg absorption may also be impaired. Schneider & Schedl (1974) reported lower fractional Mg absorption in alloxan-induced diabetic rats (18%) compared to control animals (27%). Net Mg absorption was not

significantly different between groups because the diabetic rats were hyperphagic and their Mg intake was 60% higher than the controls. Miller & Schedl (1976) measured Mg absorption by in situ perfusion of intestinal segments in streptozocin-induced diabetic rats. Mg absorption was significantly reduced in the diabetic rat intestine and the authors attributed these findings in part to abnormal vitamin D metabolism observed in the diabetic rats. However, there are no published data on Mg absorption in humans with diabetes.

3.3.3 Increased urinary Mg excretion

The reasons for increased Mg excretion in diabetes may include poor metabolic control, impaired renal reabsorption and the use of diuretics. Glucosuria leads to osmotic diuresis and hypermagnesuria by attenuating the transepithelial voltage difference in the loop of Henle which results in decreased tubular Mg reabsorption (Anwana & Garland, 1990). Several studies have reported increased urinary Mg excretion in both type 1 and 2 diabetics (Johannson *et al.*, 1981; Fujii *et al.*, 1982; McNair *et al.*, 1982; Sjögren *et al.*, 1988a; Walter *et al.*, 1991; Roffi *et al.*, 1994; Brown *et al.*, 1999), and increased urinary Mg excretion in diabetes has been associated with elevated fasting blood glucose or HbA$_{1c}$ (McNair *et al.*, 1982). Studies in type 1 diabetics have shown that urinary Mg excretion increases in response to a glucose infusion (Djurhuus *et al.*, 2000). Increased urinary Mg excretion was also found in diabetic patients with osmotic diuresis secondary to glycosuria (Johannson *et al.*, 1981; Fujii *et al.*, 1982; McNair *et al.*, 1982). However, there are a few studies which have not found significant differences in urinary Mg excretion between diabetics and healthy controls (Sjögren *et al.*, 1986; el-Yazigi *et al.*, 1993).

Insulin increases Mg absorption in the thick ascending limb of the loop of Henle (cTAL). Mandon *et al.* (1993) studied the effect of insulin in perfused mouse cTAL segments and showed that insulin raised transepithelial voltage and stimulated Mg absorption. A study with a mouse distal convoluted tubule (MDCT) cell line also demonstrated that insulin-mediated Mg uptake in the MDCT cells was associated with a concomitant increase in insulin-mediated cAMP generation (Dai *et al.*, 1999). The lack of insulin or insulin resistance would therefore impair Mg reabsorption in the kidney and lead to increased urinary Mg excretion. However, there are contradictory

reports to this hypothesis. Öscenyi *et al.* (1988) found an increase in urinary Mg excretion after an i.v. bolus injection of insulin in healthy children. This observation is supported by Djurhuus *et al.* (1995) who demonstrated increased urinary Mg excretion rates during an euglycemic, hyperinsulinemic clamp in healthy adults. According to their findings, hyperinsulinemia rather than insulin resistance would play a role in the development of Mg deficiency in type 2 diabetes.

Hypertension and cardiovascular disease are frequent complications of diabetes and often require treatment with diuretics. Diuretics, especially loop diuretics, are known to increase urinary Mg excretion. Loop diuretics block the reabsorption of Na and other minerals such as Mg and K in the loop of Henle. The effects of thiazide diuretics on Mg excretion are less well established; some authors have reported decreased serum Mg concentrations during treatment with thiazides (Hollifield, 1986), while others have found no effect on serum Mg levels and/or intracellular Mg stores (Cohen *et al.*, 1985; Siegel *et al.*, 1992). Following i.v. administration of hydrochlorothiazide, no significant alteration in Mg clearance was detected (Heidland *et al.*, 1973). Reyes & Leary (1984) explain this by a delayed action of thiazides on Mg excretion. Rather than by inhibiting Mg reabsorption, the effect of thiazides on Mg excretion may be secondary to alterations in Ca, parathyroid hormone or the renin-angiotensin-aldosterone system. Some diuretics such as K-sparing diuretics or sulfonamide derivatives do not increase Mg excretion (Ryan, 1986; Taylor *et al.*, 1988).

3.3.4   Decreased tissue Mg uptake

Insulin stimulates Mg uptake in insulin-sensitive tissues. Lohstroh & Krahl (1973) were the first to demonstrate in vitro that insulin promotes a net increase in the accumulation of Mg and K in uterine smooth muscle cells. The same was shown by Sanui & Rubin (1978) in chicken embryo cells. Therefore, if tissue Mg uptake is normally regulated by insulin, impairment of this process by insulin resistance could either cause or exacerbate intracellular Mg deficiency (Alzaid *et al.*, 1995). This is supported by Paolisso *et al.* (1988) who demonstrated that insulin increased intracellular Mg levels in vitro in erythrocytes of both type 2 diabetics and control subjects. However, the insulin-induced erythrocyte Mg accumulation was lower in the diabetic subjects, which they attributed to a post-receptor defect. They showed that

this defect was correlated to impaired insulin-mediated glucose disposal. Similarly, Hua *et al.* (1995) found decreased insulin-induced accumulation of free Mg^{2+} in lymphocytes of type 2 diabetics. The regulatory role of insulin on cellular Mg uptake has also been shown in platelets by Hwang *et al.* (1993). These investigators further demonstrated that the insulin effect on cellular Mg uptake was totally abolished by a monoclonal antibody directed towards the insulin receptor, thus confirming that insulin-mediated Mg transport depends on the interaction of insulin with its receptor. As for glucose transport, a post-receptor defect appears to be responsible for the reduced insulin-mediated Mg uptake. Alzaid *et al.* (1995) studied the decrease of plasma Mg and the glucose disappearance during insulin infusion in type 2 diabetics and control subjects. Both measured parameters were lower in the diabetics, and the authors concluded that insulin resistance in type 2 diabetic patients impaired the ability of insulin to stimulate Mg as well as glucose uptake. The mechanism by which insulin increases Mg uptake is not clear, but the interaction of insulin with its receptor appears to activate transport systems, including the Na/K-ATPase pump (Lostroh & Krahl, 1973).

3.4 Consequences of Mg deficiency

3.4.1 Glucose homeostasis and insulin sensitivity

Magnesium depletion has shown to have a negative impact on glucose homeostasis and insulin sensitivity in patients with type 2 diabetes. Nadler *et al.* (1993) demonstrated a decrease in insulin sensitivity (by modified intravenous glucose tolerance test) in normal subjects after induction of Mg deficiency by a low-Mg diet (< 0.5 mmol/d) for 4 weeks. Similarly, Yajnik *et al.* (1984) found an inverse association between plasma Mg concentration and glucose disposal in type 2 diabetics. Also several in vitro studies have pointed out the role of Mg in insulin action. Paolisso *et al.* (1987; 1988) showed reduced Mg uptake in response to insulin in erythrocytes of type 2 diabetics and hypertensives. They further demonstrated that this abnormality was associated with an increase in erythrocyte membrane microviscosity and therefore suggested that changes in the physical state of the plasma membrane as well as insulin resistance were both responsible for the lower erythrocyte Mg level found in these patients (Paolisso *et al.*, 1987). Also, these changes in plasma membrane liquid composition might impair the interaction of insulin with its receptor

and thus decrease glucose tolerance (Tongyai *et al.*, 1985). Suarez *et al.* (1995) have studied the effect of Mg deficiency on glucose disposal and insulin action in rats and have found an impairment of both insulin secretion and sensitivity. The impairment of insulin sensitivity seems to be related, at least in part, to a defective tyrosine kinase activity of the insulin receptor. Also in human non-diabetic subjects, low plasma Mg has been associated with relative insulin resistance, glucose intolerance, and hyperinsulinemia (Rosolova *et al.*, 1997). In fact, intracellular Mg deficiency appears to be associated with an impaired function of several enzymes involved in glucose metabolism, which need high energy phosphate bonds and thus require Mg as a cofactor (Paolisso *et al.*, 1990b). This may lead to impairment of insulin action and worsening of insulin resistance in diabetic and hypertensive patients (Paolisso & Barbagallo, 1997). The same seems to happen in obesity; two studies show insulin resistance along with reduced erythrocyte Mg accumulation after glucose loading in non-diabetic obese subjects (Paolisso *et al.*, 1990a; Corica *et al.*, 1999). In summary, the relationship between Mg deficiency and insulin resistance is a vicious circle. Low Mg status contributes to the development of insulin resistance, which in turn attenuates Mg uptake in insulin-sensitive tissues.

3.4.2 Metabolic syndrome

In 1993, Resnick (1993) came up with the "ionic hypothesis" in which the frequent clinical coexistence of hypertension and altered insulin metabolism is explained by abnormalities of cellular ion handling, resulting in excess levels of intracellular free Ca (Ca_i), depletion of intracellular free Mg (Mg_i) and lowered intracellular pH. These cellular ion alterations may have tissue-specific consequences such as vasoconstriction and elevated blood pressure in vascular tissue, insulin resistance in skeletal muscle and fat, and hyperinsulinemia in pancreatic β-cells. This could in fact explain the interrelationship between the different clinical components of the metabolic syndrome. Resnick's group performed oral glucose tolerance tests in diet-controlled type 2 diabetics and non-diabetics, with and without concurrent hypertension. Among non-diabetics they found that the insulinemic response to glucose loading was significantly higher in hypertensive patients than in normotensive subjects. They discovered that Mg_i in erythrocytes was inversely correlated to systolic and diastolic blood pressure as well as to the hyperinsulinemic response to glucose loading (Resnick *et al.*, 1990). They therefore concluded that

low intracellular Mg_i was a common feature and maybe cause of both elevation of blood pressure and the post-receptor defect in insulin resistance. For increased intracellular Ca_i and lowered intracellular pH they observed similar relationships. Draznin (1993) hypothesized that elevations in cytosolic free Ca concentrations may represent a common intracellular abnormality that is responsible for the frequent co-existence of insulin resistance and hypertension. His group had shown that elevations of cytosolic free Ca in insulin-sensitive tissues, such as observed in patients with type 2 diabetes and obesity and in some patients with hypertension, may lead to the development of insulin resistance. They further found that the presence of a Ca channel blocker (nitrendipine) in the incubation medium prevented increases in cytosolic free Ca and ameliorated insulin resistance. Peripheral insulin resistance results in an increase of the glycemic stimulus on insulin secretion and thus in hyperinsulinemia. In addition to that, increased cytosolic free Ca in the pancreatic β-cells may additionally enhance insulin secretion (Hellman *et al.*, 1976).

3.4.3 Hypertension

Mg deficiency has been described as a risk factor for the development of hypertension (see 2.2.5). In Wistar rats receiving a severely Mg-deficient diet, a gradual rise of blood pressure was observed (Laurant & Berthelot, 2001). Mg has been found to act as a naturally occurring Ca antagonist. Mg may bind competitively to the same sites as Ca; by competing with Ca for a binding site but not exert an effort, by altering the flux of Ca across the cell membrane, or by displacing Ca from intracellular binding sites with a rise in intracellular Ca (Levine & Coburn, 1984). An increase in cytosolic Ca within the small vessel smooth muscle fibers leads to vasoconstriction, whereas Mg has been shown to produce significant vasodilatation of arterioles and venules (Altura *et al.*, 1987). Moreover, Mg deficiency has been found to enhance the angiotensin-induced aldosterone synthesis which results in increased renal Na reabsorption and therefore increased vasomotor tone (Nadler *et al.*, 1993).

3.4.4 Atherosclerotic disease and myocardial infarction

In animal experiments, Mg deficiency has shown to be involved in several steps of the atherosclerotic process, including the metabolism of elastin and collagen, dyslipidemia, platelet aggregation and hypertension (Rayssiguier, 1984). Increased

platelet aggregation is a recognized risk factor in the development of atherosclerotic disease. Nadler *et al.* (1992) showed a decrease in platelet reactivity (in response to a thromboxane A_2 analog) after supplementing Mg-deficient diabetic patients with Mg. In another study, they observed reduced synthesis of thromboxane A_2 after a Mg infusion in Mg-deficient normal subjects (Nadler *et al.*, 1993). In rabbits fed saturated fat, dietary Ca and Mg had a protective effect against atherosclerosis by decreasing platelet aggregation and reducing plasma cholesterol and triglycerides (Renaud *et al.*, 1983).

A link between Mg depletion and dyslipidemia has also been demonstrated, although the mechanism by which Mg affects lipid metabolism is not clear. Rayssiguier's group observed that Mg deficiency in weanling rats produced hypertriglyceridemia and altered plasma lipid composition (Rayssiguier *et al.*, 1981; Gueux *et al.*, 1991). In a study with cholesterol-fed rabbits, Ouchi *et al.* (1990) found that additional dietary Mg prevented the development of atherosclerosis by inhibiting lipid accumulation in the aortic wall. In human type 2 diabetics, Sheehan (1991) found a significant inverse correlation between serum Mg and hypertriglyceridemia.

In addition, an association seems to exist also between myocardial infarction and Mg deficiency. Hypomagnesemia has been described in patients following acute myocardial infarction (Hughes & Tonks, 1965) and it has been shown that the Mg content of ventricular muscle was significantly lower in patients who died from myocardial infarction than in those patients who died from another cause (Seelig & Heggtveit, 1974). Because Mg depletion predisposes patients to both hypertension and atherosclerotic disease, Mg depletion could worsen angina pectoris and precipitate acute myocardial infarction.

3.4.5 Microvascular complications

It is possible that a common mechanism might be responsible for some of the diverse complications of diabetes (Winegrad, 1987). For example, a reduced activity of Na/K-ATPase may play a role in the development of diabetic complications in tissues such as nerve (Das *et al.*, 1976), retina (MacGregor & Matschinsky, 1986) and kidney (Cohen, 1986). Na/K-ATPase requires Mg as a cofactor and thus its activity may be reduced in Mg-deficient states. Grafton & Baxter (1992) proposed implication of the polyol pathway, the activation of which results in inhibition of Na/K-ATPase activity

(see 1.2.1). Na/K-ATPase is involved in the maintenance of gradients of Na and K and in glucose transport, and requires Mg as a cofactor. Hence, Mg deficiency might affect the activity of Na/K-ATPase. The authors further observed that the affinity of the inositol transporter was increased by Mg (Grafton *et al.*, 1992). Mg deficiency may therefore amplify intracellular inositol depletion resulting in impaired activity of certain regulatory proteins and thus predisposing to the development of diabetic complications.

In 1978, Mc Nair *et al.* (1978) were the first to describe lower plasma Mg concentrations in diabetic patients with severe background or proliferative retinopathy compared to patients without retinopathy. These findings were confirmed by other cross-sectional studies (Ceriello *et al.*, 1982; Fujii *et al.*, 1982; Hatwal *et al.*, 1989). In addition, in a prospective study with 61 patients, the progression of retinopathy was related to plasma Mg concentration (de Valk *et al.*, 1999). However, both retinopathy and hypomagnesemia could theoretically be sole manifestations of poor glycemic control; Sheehan (1991) could not find a correlation between serum Mg concentrations and retinopathy in 254 patients.

Mg deficiency may also influence the development of diabetic nephropathy. Corsonello *et al.* (2000) measured significantly reduced serum ionized Mg concentrations in diabetic patients with microalbuminuria compared to normoalbuminuric patients (0.36 vs. 0.45 mmol/l). Bauer & Rob (1998) described possible mechanisms in a review. In progressing nephropathy, an increase of vasoconstrictive prostaglandins and thromboxane A_2 leads to a fall of glomerular filtration rate and increasing filtration pressure damaging the basal membrane and resulting in proteinuria (DeRubertis & Craven, 1993; Bauer & Rob, 1998). Furthermore, lipids appear to play a direct role in the progression of renal disease (Wanner *et al.*, 1997). In Mg deficiency, increased thromboxane A_2 synthesis has been observed (Nadler *et al.*, 1993), and triglycerides were found to be increased (Gueux *et al.*, 1991; Sheehan, 1991).

The development of foot ulcers in diabetes is primarily due to diabetic neuropathy and peripheral vascular disease. In a recent study, a strong relationship between serum Mg and foot ulcers was found in type 2 diabetics (Rodriguez-Moran & Guerrero-Romero, 2001).

3.4.6   Osteoporosis

Mg deficiency is known to increase the risk of osteoporosis (see 2.2.3). Decreased bone Mg in type 2 diabetics was described by several investigators (de Leeuw *et al.*, 1978; Cohen *et al.*, 1983). McNair *et al.* (1979) determined bone mass in type 1 diabetics and found that bone mineral content declined by 10% during the first 5 years of diabetes. Levin *et al.* (1976) measured bone density in diabetics; 60% of the type 2 diabetics had a bone loss of more than 10%. A possible reason for the loss of bone mass in diabetes could be decreased PTH secretion; Fialip *et al.* (1985) observed lower levels of PTH in type 1 and 2 diabetics, and this being more pronounced in poorly controlled patients. Two possible causes were proposed: Mg deficiency (see 2.2.3), and the increase of ionized Ca may both be responsible for the inhibition of PTH secretion. Decreased PTH secretion or action would result in decreased intestinal Ca absorption and increased urinary Ca loss. Another mechanism was proposed by Verhaeghe *et al.* (1990) who studied bone turnover in poorly treated diabetic BB rats and found that insulin deficiency resulted in a suppression of osteoblast activity. This is supported by Catakay *et al.* (1998) who found reduced bone formation but unaltered bone resorption in 35 type 2 diabetics.

3.5   Mg supplementation

Because low Mg status has a negative impact on glucose homeostasis and insulin sensitivity, as well as on the evolution of diabetic complications, oral Mg supplementation is expected to have a positive effect in patients with diabetes. The results of most published intervention studies are summarized in Table 5.

Table 5: Mg supplementation studies in patients with diabetes

Authors	Diabetes Type	Nb subjects	Dose [mmol/d]	Duration [weeks]	Mg parameters after supplementation	Metabolic parameters after supplementation
Sjögren *et al.* (1988b)	1	16	8.6	21	Muscle: *increased* Plasma: no change Urinary: no change Erythrocyte: no change MBC: no change	HbA$_{1c}$: no change Glucose: no change Insulin req.: *reduced*
De Leuww *et al.* (1997)	1	11	18.5 (+ i.v. dose)	10	Plasma: no change Erythrocyte: *increased* Ionized: decreased MLT: *decreased*	HbA$_{1c}$: no change Lipids: no change
Hägg *et al.* (1999)	1	28	20-30	52	Serum: no change Urinary: increased	HbA$_{1c}$: no change Lipids: no change
Djurhuus *et al.* (2001)	1	10	24.8 (+ i.v. dose)	24	Muscle: *increased* Serum: *increased* Urinary: increased	HBA$_{1c}$: no change Fructosamine: no change Glu disapp: reduced Cholesterol: *reduced* LDL: *reduced*
Eriksson & Kohvakka (1995)	1/2	56 29 T1 27 T2	24.7	12	Serum: *increased* (T2) Urinary: increased	Glucose: no change HbA$_{1c}$: no change Lipids: no change BP: *decreased* (T1)
Paolisso *et al.* (1989b)	2	8 cross	7.2	4	Plasma: *increased* Erythrocyte: *increased*	Glucose: *decreased* Insulin levels: no change Insulin resp: *increased* Glu disapp.: *increased*
Paolisso *et al.* (1989a)	2	8 cross	10.8	4	Plasma: *increased* Erythrocyte: *increased*	Glucose: *decreased* Insulin levels: no change Insulin resp.: *increased*
Paolisso *et al.* (1989c)	2	8	10.8	10	Erythrocyte: *increased*	Glucose: no change HbA$_{1c}$: no change Platelet react.: *reduced*
Nadler *et al.* (1992)	2	20	16.5	8	Serum: no change Erythrocyte: *increased*	Platelet react.: *reduced*
Purvis *et al.* (1994)	2	28 cross	16	6	Serum: no change Erythrocyte: no change	Glucose: no change Lipids: no change BP: *fall in systolic BP*
Corica *et al.* (1994)	2	43 26 v 17 p	16.2	4	Serum: no change Erythrocyte: no change	Glucose: no change HbA$_{1c}$: no change Cholesterol: *reduced* Triglycerides: no change LDL: *reduced* HDL: *increased*
Paolisso *et al.* (1994)	2	9 cross	15.8	4	Plasma: *increased* Erythrocyte: *increased*	Glucose: no change Glu disapp: *increased*
Gullestad *et al.* (1994)	2	54 25 v 29 p	15	16	Serum: no change	Glucose: no change HbA$_{1}$c: no change Lipids: no change BP: no change
Eibl *et al.* (1995)	2	40 18 v 20 p	30	12	Plasma: *increased* Urinary: increased	HbA$_{1c}$: no change Lipids: no change BP: no change

Authors	Diabetes Type	Nb subjects	Dose [mmol/d]	Duration [weeks]	Mg parameters after supplementation	Metabolic parameters after supplementation
Gilleran *et al.* (1996)	2	40 20 v 20 p	fort. salt (Mg + K)	39	Serum: no change Urinary: no change	HbA$_{1c}$: no change Insulin levels: no change Lipids: no change BP: *fall in systolic BP*
de Valk *et al.* (1998)	2	50 18 v 16 p	15	12	Plasma: *increased* Erythrocyte: no change Urinary: increased	Glucose: no change HbA$_{1c}$: no change Lipids: no change BP: no change
Lima *et al.* (1998)	2	128 35 L 39 H 54 p	 20.7 41.4	4	Plasma: *increased* (H) MBC: *increased* (H) Urinary: increased (H)	Glucose: no change HbA$_{1c}$: no change Fructosamine: *decreased* (H)
Rodriguez-Moran & Guerrero-Romero (2003)	2	63 32 v 31 p	26.3	16	Serum : *increased*	Glucose: reduced in both gr. Insulin levels: increased in both groups HbA$_{1c}$: *reduced* HOMA-IR : *improved* Lipids: no change BP: no change

MBC = mononuclear blood cells
Ionized = free ionized Mg in plasma
MLT = retention in i.v. Mg load test
Glucose = fasting plasma glucose
Lipids = serum lipids (e.g. cholesterol, HDL, LDL, triglycerides)
Insulin req. = insulin requirement
BP = blood pressure
Platelet react. = platelet reactivity

v = verum
p = placebo
cross = cross-over study
T1 = type 1 diabetics
T2 = type 2 diabetics
L = low dosage
H = high dosage

Glu disapp.= glucose disappearance during euglycemic glucose clamp (indicator for insulin sensitivity)
Insulin resp. = glucose-induced insulin response (indicator for insulin secretion)
Fructosamine = fructosamine level in serum (indicator for metabolic control)
HOMA-IR = homeostasis model assessment for insulin resistance (fasting glucose [mmol/l] x fasting insulin [μUI/ml] /22.5)

Generally, an increase in serum Mg and erythrocyte Mg concentrations was observed, but there was no consistent improvement in glycemic control. The effect of Mg supplementation on Mg status has mostly been assessed by measuring serum and erythrocyte Mg concentrations. However, erythrocyte Mg had not shown to be a very reliable indicator of intracellular Mg status, and because tissue Mg uptake might be diminished in type 2 diabetes, other parameters such as muscle Mg concentration would probably provide better information. In type 1 diabetics, Mg supplementation has shown to increase muscle Mg concentration. However, there are no published data on the effect of supplementation on muscle Mg in type 2 diabetics. Due to the insulin resistance in type 2 diabetes, the effect might be different from type 1 diabetes.

Most studies report no change in glucose levels or HbA_{1c} after Mg supplementation. However, there are three studies from the same institute which have found increased insulin secretion, and/or increased insulin sensitivity (determined by euglycemic hyperinsulinemic glucose clamp) in type 2 diabetics after 4 weeks of Mg supplementation using a cross-over study design (Paolisso *et al.*, 1989a; Paolisso *et al.*, 1989b; Paolisso *et al.*, 1994). Further supporting results come from the latest study, a randomized placebo-controlled study performed in 63 type 2 diabetics in Mexico. They found not only increased serum Mg, but also improved metabolic control and insulin sensitivity after supplementation for 16 weeks (Rodriguez-Moran & Guerrero-Romero, 2003). However, there are a few contradictory reports. Surprisingly, a study in patients with type 1 diabetes has demonstrated reduced insulin-mediated glucose uptake after Mg supplementation (Djurhuus *et al.*, 2001). Similarly, in rats, a Mg-deficient diet has resulted in an increase in the effectiveness of insulin in stimulating glucose disposal (Legrand *et al.*, 1987). And moreover, a human study has found a reduction in insulin-mediated glucose uptake during acute hypermagnesemia induced by Mg infusion (Zofkova *et al.*, 1988).

A few studies have shown that Mg supplementation may reduce platelet aggregation (Paolisso *et al.*, 1989c; Nadler *et al.*, 1992), most probably by decreasing thromboxane A_2 levels (see 3.4.4). Most investigators have found no effect on lipid levels, except for two studies, which found reduced total and LDL cholesterol after supplementation. Also the effect of Mg supplementation on blood pressure is equivocal; a few studies resulted in a moderate decrease of systolic blood pressure (Purvis *et al.*, 1994; Gilleran *et al.*, 1996), but others did not find a significant change (Gullestad *et al.*, 1994; Eibl *et al.*, 1995; de Valk *et al.*, 1998).

Mg supplementation may be more effective in the prevention of type 2 diabetes since low Mg intakes seem to be associated with an increased risk of diabetes (see 3.1). A study performed in elderly subjects without diabetes has shown that oral Mg supplementation improved both insulin sensitivity and secretion (Paolisso *et al.*, 1992). Furthermore, Mg supplementation was able to reduce the development of diabetes in obese Zucker[1] diabetic fatty rats (Balon *et al.*, 1995).

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[1] Zucker rats = genetically characterized by hyperinsulinemia and peripheral insulin resistance; rat model for type 2 diabetes

A consensus statement of the American Diabetes Association (1995) suggested that serum Mg levels should be determined in patients at risk for Mg deficiency. This includes patients with congestive heart failure or acute myocardial infarction, ketoacidosis, ethanol abuse, long-term parenteral nutrition, K or Ca deficiency, chronic use of certain drugs (e.g. diuretics, aminoglycosides, or digoxin) or pregnancy. Those patients should receive replacement therapy if hypomagnesemia is demonstrated. Although Mg may have a positive effect on insulin sensitivity and secretion, most intervention studies in type 2 diabetics have failed to show an improvement of glycemic control or diabetes-related complications. It seems that the disorders resulting from a Mg deficit in diabetics are due to intracellular depletion of the ion, which is not controllable by oral supplementation alone. If tissue Mg uptake is decreased due to insulin resistance, the effect of supplementation may be limited due to low availability of the supplemental Mg to the cells. A greater benefit may lie in subjects with impaired glucose tolerance or beginning type 2 diabetes, in which improving insulin sensitivity by increasing Mg intake may retard or attenuate the disease.

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MANUSCRIPTS

4 Low plasma magnesium in type 2 diabetes

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Running title: Plasma magnesium concentration in diabetes

Summary

Questions under study/principles: Magnesium depletion has a negative impact on glucose homeostasis and insulin sensitivity in type 2 diabetic patients. Low plasma magnesium concentration is a highly specific indicator of poor magnesium status. In the USA and some European countries, plasma magnesium concentrations have been found to be decreased in diabetics. The aim of this study was to compare plasma magnesium concentrations of type 2 diabetics and healthy controls in Switzerland.

Methods: Plasma magnesium concentrations were determined in 109 type 2 diabetics and 156 age- and sex-matched healthy controls.

Results: Mean (±SD) plasma magnesium concentrations of the diabetics and controls were 0.77 ±0.08 and 0.83 ±0.07 mmol/l, respectively (p < 0.001). Plasma magnesium concentrations were below the normal reference range in 37.6 % of the diabetic patients and 10.9 % of the control subjects (p<0.001). Plasma magnesium was not correlated with glycemic control as measured by HbA_{1c}.

Conclusions: Lower plasma magnesium concentrations and poor magnesium status are common in type 2 diabetics in Zurich, Switzerland.

Key words: magnesium, diabetes, hypomagnesemia, plasma magnesium

Introduction

Mg depletion has a negative impact on glucose homeostasis and insulin sensitivity in patients with type 2 diabetes (1, 2) as well as on the evolution of complications such as retinopathy, thrombosis and hypertension (3-5). Moreover, low serum Mg is a strong, independent predictor of the development of type 2 diabetes (6). In the USA, 25 to 39 % of outpatient diabetics have low concentrations of serum Mg (7) and several studies have shown lower serum Mg concentrations in type 2 diabetics compared to healthy controls (5, 8). Although low serum Mg concentrations in diabetics have also been found in several European countries, including Austria, Germany, Italy, France and Sweden (9-13), there are no reported data for Switzerland. Therefore, the aim of this study was to compare the plasma Mg concentrations of patients with type 2 diabetes and healthy controls in Switzerland.

Subjects, Methods and Materials

Subjects

One-hundred-and-nine type 2 diabetic patients and 156 non-diabetic controls matched for age and sex participated in the study (Table 1). The mean age (range) of the diabetics and controls was 61.3 (33-87) and 58.3 (46-74), respectively.

Table 1 Characteristics of the test subjects

Characteristics	Diabetics	Controls
No of subjects	109	156
Sex	76 men / 33 women	112 men / 44 women
Age (y) ±SD	61.3 ±10.3	58.3 ±7.2

The type 2 diabetics were recruited from the outpatient diabetes clinic at the University Hospital Zurich (44.0 %) and from a private endocrinologic practice in Winterthur (56.0 %). Median (range) duration of diabetes was 10.7 years (0 – 37 years). Of the diabetics, 61.5 % reported a history of hypertension and/or cardiovascular disease and 25.7 % a history of dyslipidemia. Fifty-eight were using insulin, 29 were taking oral hypoglycemics, 14 were using both, and 8 were not prescribed any antidiabetic drugs (Table 2). Because loop diuretics are associated with higher urinary Mg excretion, patients taking loop diuretics were excluded. None

were taking Mg supplements. Anonymous blood samples for control subjects were obtained from the local blood donation centre (Swiss Red Cross, Zurich) with specification of sex and year of birth for each subject. Exclusion criteria for blood donations included diabetes treated by insulin, infection, sexually transmittable diseases, cardiovascular diseases, cerebral incident, bleeding disorders, vascular diseases, chronic kidney diseases, autoimmunity diseases, epilepsy, cancer, hepatitis, and pregnancy. Written informed consent was obtained from each diabetic subject and the study protocol was approved by the Ethical Committee of the University Hospital Zurich.

Table 2 Characteristics of the diabetic patients

Characteristics		
Duration of diabetes [years] [1]		10.7 (0 – 37)
Medication	Insulin Oral hypoglycemics Insulin and oral hypoglycemics Diet only	53.2 % 26.6 % 12.8 % 7.3 %
Comorbidities	Hypertension Dyslipidemia	61.5 % 25.7 %

[1] median and range

Methods and Materials

Venous blood samples from the subjects were drawn in heparinized 10 ml tubes (Vacutainer, Becton Dickinson, Meylan, France). Whether the subjects were in the fed or fasting state was not specified. Plasma was separated from blood cells by centrifugation at 3000 rpm for 15 minutes (Omnifuge 2.0 RS, Heraeus GmbH, Hanau, Switzerland) and stored in plastic vials at -25 °C until analysis.

Quantitative analysis of Mg in plasma was done by flame atomic absorption spectrometry (SpectrAA 400, Varian, Mulgrave, Australia) at 285.2 nm, using parameters recommended by the manufacturer (14). Plasma samples were diluted 200-fold so that the Mg concentrations of the final diluted solutions were around 0.1 μg/ml. A commercial Mg standard (CertiPUR, Merck, Darmstadt, Germany) was used for internal calibration by standard addition to minimize matrix effects. Lanthanum nitrate (Fluka Chemie GmbH, Buchs, Switzerland) was added as a matrix modifier (5 mg La/ml in the final solutions), and 0.1 % Triton X-100 solution (Fluka Chemie

GmbH) to reduce the surface tension. Accuracy of the method was verified by analyzing a serum control sample for Mg (Seronorm Trace Elements Serum, Nycomed, Oslo, Norway). All samples were analyzed in duplicate and repeated if the difference between individual values relative to the mean was >10 %. All chemicals used were analytic grade. Water used for analytical procedures was purified by ion exchange and reverse osmosis (18 MΩ) (RD2000, Renggli AG, Rotkreuz, Switzerland; Nanopure Cartridge System, Skan AG, Basel, Switzerland).

For all diabetic subjects, the most recent HbA_{1c} concentration was collected. For the patients from the university clinic, the HbA_{1c} was determined from the same blood sample as the plasma Mg concentration. For the remaining subjects, the most recent HbA_{1c} was recorded from the medical record. For all subjects but one, HbA_{1c} was obtained within 2 months of the plasma Mg determination.

Data processing and statistical analysis were done using Excel 2002 (Microsoft, Seattle WA, USA) and SPSS for Windows 11.0 (SPSS Inc., Chicago IL, USA). Normal distribution of data was verified by calculating the quotient of the skewness divided by its standard error; normal distribution was assumed if the quotient was between -2.5 and +2.5. Normally distributed data were expressed as arithmetic means ±SD. Variables not normally distributed were expressed as medians and ranges. Differences between groups were evaluated using unpaired Student's t-test and considered statistically significant at $p < 0.05$. ANOVA was done to test for associations with plasma Mg concentration as the dependent variable.

Results

Mean (±SD) plasma Mg concentrations of the diabetics and the controls were 0.77 ±0.08 and 0.83 ±0.07 mmol/l, respectively ($p < 0.001$) (Figure 1). In 37.6 % of the diabetic patients and 10.9 % of the control subjects plasma Mg concentrations were below the normal reference range of 0.75 to 0.95 mmol/l (15).

Figure 1 Plasma Mg concentrations of type 2 diabetics and controls
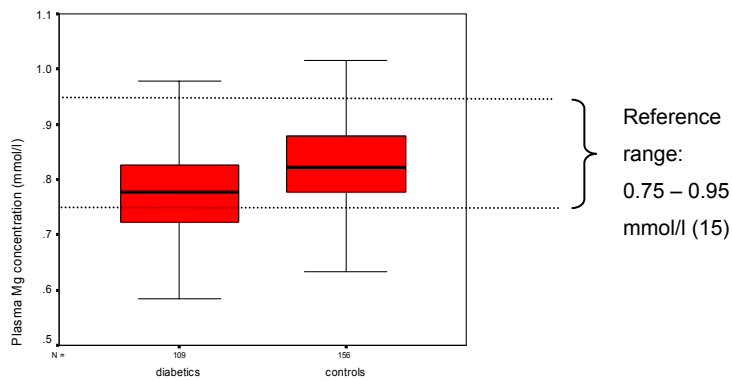
Box-whiskers plot showing medians, first and third quartiles, and minima and maxima

By ANOVA, sex and age were not significant predictors of plasma Mg in this sample. Median HbA_{1c} concentration (range) in the diabetic group was 7.1 % (5.1 – 11.5 %). By ANOVA, HbA_{1c} (Figure 2), duration of diabetes and diabetes treatment (medication) did not significantly predict plasma Mg concentration.

Figure 2 Relationship between plasma Mg concentration and glycemic control measured as HbA_{1c} in 109 diabetic patients
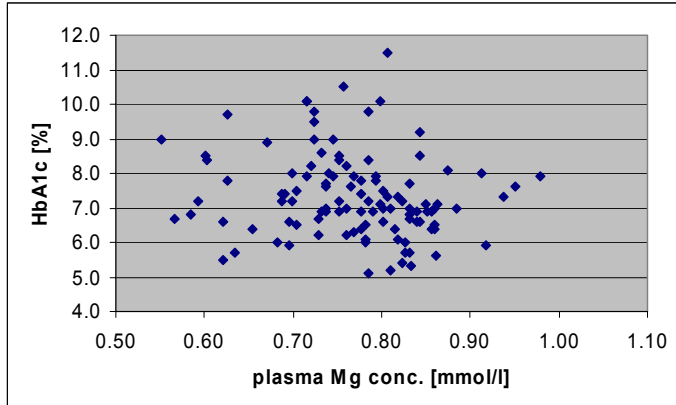
Discussion

Similar to findings from other countries in Europe and North America (5, 8), the mean plasma Mg concentration of the type 2 diabetics was significantly lower than in controls. The striking finding in this population was the high prevalence of low plasma Mg concentrations among the diabetic subjects. Plasma Mg concentrations of 37.6 % of the diabetics were below the reference range, a prevalence of low magnesium status that is similar to that reported in type 2 diabetics in outpatient clinics in the US (7). Mg depletion has a negative impact on glucose homeostasis and insulin

sensitivity in diabetic patients (1, 2) as well as on the evolution of complications such as retinopathy, thrombosis and hypertension (3-5). Preventing low Mg status in diabetics may therefore be beneficial in the management of the disease.

The reasons for the high prevalence of Mg deficiency in diabetes are not clear, but may include increased urinary loss, lower dietary intake, or impaired absorption of Mg compared to healthy individuals. Several studies have reported increased urinary Mg excretion in type 1 and 2 diabetes (13, 16-18). However, we have shown that low dietary intake does not appear to contribute to impaired Mg status in diabetics in Switzerland. A dietary assessment conducted in 97 type 2 diabetics and 100 healthy controls showed that only 5.4 % of the diabetic group and 9.1 % of the control group were predicted to have intakes of Mg below their individual requirements (19). In addition, we have recently shown that type 2 diabetics in reasonable metabolic control and without nephropathy absorb dietary Mg to a similar extent as healthy controls, and have similar rates of urinary excretion (20). Increased urinary Mg excretion due to hyperglycemia and osmotic diuresis may contribute to hypomagnesemia in diabetes (16-18). Several authors have described a correlation between HbA_{1c} and plasma Mg in type 1 diabetics (11, 21). However, no such correlation was found in type 2 diabetes (11, 22, 23), similar to our results.

Mg is mainly an intracellular cation, with less than 1 % of total body content present in the extracellular fluids. The Mg concentration in serum represents not more than 0.3 % of total body Mg (24). Nevertheless, serum or plasma Mg measurement is the most readily available and widely used test of Mg status. In human studies, instituting a diet low in Mg produces a predictable decline in serum Mg (25-27). However, there are a number of reports of low Mg values in various blood cells and tissues associated with normal serum/plasma Mg concentrations (24). It appears therefore that plasma Mg concentration is an insensitive, but highly specific indicator of low Mg status. Of the total Mg in serum, around 55 % is present as free ionized Mg^{2+}, 15 % is complexed to anions (e.g. bicarbonate, citrate, sulfate) and 30 % is bound to proteins, mainly albumin (28). It could therefore be argued that in diabetics with microalbuminuria, serum Mg might be reduced because of lower serum albumin concentration. However, Pickup et al. (29) found no difference in serum Mg concentration between type 1 diabetics with microalbuminuria or clinical proteinuria compared to diabetics with normal albumin excretion. In contrast, Corsonello et al.

(30) demonstrated significantly lower ionized serum Mg in type 2 diabetic patients with microalbuminuria or clinical proteinuria compared to a normoalbuminemic group. Free ionized serum Mg, however, is not associated with serum albumin levels. Moreover, microalbuminuria should not lower plasma albumin because plasma contains macro-amounts (35-52 g/l) of albumin. Therefore, we did not exclude subjects with microalbuminuria.

In summary, we have demonstrated that low Mg status is common in type 2 diabetics in the Zurich region. Because Mg depletion reduces insulin sensitivity and may increase risk of secondary complications, it may be prudent in clinical practice to periodically monitor plasma Mg concentrations in diabetic patients. If plasma Mg is low, an intervention to increase dietary intakes of Mg may be beneficial.

Acknowledgments

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5   Dietary magnesium intake in type 2 diabetes

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Abstract

Background: Magnesium deficiency is common in type 2 diabetes and may have a negative impact on glucose homeostasis and insulin resistance, as well as on the evolution of complications such as retinopathy, thrombosis, and hypertension.

Objective: To assess the dietary magnesium intake of patients with type 2 diabetes in Zurich, Switzerland and to compare the magnesium intake of diabetic and non-diabetic subjects.

Design: The magnesium intake of 97 randomly-selected patients with type 2 diabetes and 100 healthy, non-diabetic controls matched for age and sex was estimated using a diet history method. During winter and summer periods, mean daily magnesium intakes were calculated from detailed information given by the test subjects about their eating habits over the previous two months.

The calculations were performed using EBIS, a computer program based on a German nutrient data base (BLS 2.3), with food items specific to Switzerland added or directly analysed when necessary.

Results: The mean ±SD daily magnesium intake of the male diabetic and male control subjects was 423.2 ±103.1 mg and 421.1 ±111.0 mg, respectively. The mean daily magnesium intake of the female diabetic and female control subjects was 419.1 ±109.7 mg and 383.5 ±109.7 mg, respectively. There were no significant differences in daily magnesium intake between the diabetic and the non-diabetic subjects and mean intakes in both groups exceeded Swiss recommended dietary intakes.

Conclusions: Dietary intake of magnesium appears sufficient in Swiss adults with type 2 diabetes and is unlikely to contribute to the aetiology of magnesium deficiency.

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Descriptors: diabetes, magnesium, dietary intake, dietary assessment, magnesium deficiency

Introduction

Magnesium deficiency is a common characteristic of type 2 diabetes mellitus. In the USA, 25 to 39 % of outpatient diabetics have low levels of serum magnesium (Nadler, 1995). Low serum magnesium levels in patients with type 2 diabetes have also been reported in several European countries, e.g. Austria, Germany, Italy, France and Sweden (Schnack *et al,* 1992; De Lenardis *et al,* 1999; Paolisso *et al,* 1988; Schlienger *et al,* 1988; Sjögren *et al,* 1988). Magnesium depletion has a negative impact on glucose homeostasis and insulin resistance in people with type 2 diabetes (Durlach & Rayssiguier, 1983; Nadler *et al,* 1993), as well as on the evolution of complications such as retinopathy (McNair *et al,* 1978), thrombosis (Nadler *et al,* 1993), and hypertension. Low magnesium intake may play a role in the development of diabetes (Colditz *et al,* 1992) and in the development of insulin resistance in non-diabetic adults (Humphries *et al,* 1999).

The reasons magnesium deficiency is commonly found in diabetics are not clear, but may include increased losses of urinary magnesium, lower dietary intake of magnesium, or lower magnesium absorption compared to healthy individuals. Increased urinary losses due to glucosuria and osmotic diuresis are one of the causes of a low magnesium status in patients with diabetes (McNair *et al,* 1982; Fujii *et al,* 1982). Low dietary intake may also contribute to low magnesium status in diabetics (Durlach & Rayssiguier, 1983; Sheehan, 1991; White & Campbell, 1993). Patients with type 2 diabetes are often overweight, and may consume a diet higher in fat and energy and lower in magnesium than non-diabetics. However, if diabetics follow current dietary guidelines for diabetes which emphasise whole grains and vegetables, their intakes of magnesium may be higher than the general population. Studies that have reported magnesium intake in type 2 diabetes are equivocal (Schmidt *et al,* 1994; Ma *et al,* 1995). Schmidt *et al* (1994) found that diabetics had a low intake of magnesium (216.5 mg/day in women, 336.8 mg/day in men), however the results were not compared with the intake of a control group. In contrast, data from the Atherosclerosis Risk in Communities (ARIC) study (Ma *et al,* 1995), showed a higher magnesium intake per MJ in diabetics than in disease-free subjects (e.g. in white men: 42 mg/MJ (175 mg/1000 kcal) vs. 38 mg/MJ (157 mg/1000 kcal), respectively).

If low magnesium intake contributes to low magnesium status in type 2 diabetes, magnesium intakes could be increased by dietary emphasis on foods which are rich in magnesium or by magnesium supplements. Our objective was therefore to assess the dietary magnesium intake of patients with type 2 diabetes in Zurich, Switzerland and to compare the magnesium intake of diabetic and non-diabetic subjects.

Subjects and Methods

Subjects

Ninety-seven type 2 diabetics and 100 healthy non-diabetic subjects matched for age and sex participated in the study. The mean age (range) of the 54 diabetic and control men was 62.3 years (39-75) and 62.4 years (46-74), respectively. The mean age (range) of the 43 diabetic women and the 46 control women was 63.3 years (45-76) and 61.8 years (45-80), respectively (Table 1). All subjects lived in the Zurich metropolitan area, were of Central European origin, and lived in their own home (i.e. were not institutionalised). Sample size was set to allow detection of a difference of intake of 50 mg or more between the two groups with a significance level of 0.05 and a power of 90 %.

Table 1 Characteristics of the test subjects

Characteristics	Men		Women	
	Diabetics	Controls	Diabetics	Controls
No of subjects	54	54	43	46
Age (years)	62.3 ±8.8	62.4 ±7.9	63.3 ±9.7	61.8 ±8.4
Height (cm)	174.8 ±6.0	174.4 ±6.2	163.3 ±6.9	164.5 ±5.7
Weight (kg)	83.2 ±13.2	76.1 ±9.8	80.9 ±18.2	61.5 ±8.6
BMI (kg/m^2)	27.4 ±3.8	25.0 ±3.0	30.3 ±6.6	22.7 ±2.9

Data given as mean (SD)

The 97 diabetic patients were randomly selected from the register of the diabetic outpatient clinic at the University Hospital of Zurich. The response rate was 32 %. Thirty-seven were using insulin, 35 were taking oral hypoglycemics, 11 were using both, and 14 were not prescribed any antidiabetic drugs. The 100 non-diabetic subjects (54 men, 46 women) were recruited from two different sources. The city of Zurich provided 600 random addresses of Swiss nationals aged 45 to 75 years from

which we randomly selected 342 matched by age and sex to the diabetic group. From this group, 54 generally healthy non-diabetic subjects were recruited. The remaining 46 subjects were recruited from the program for older adults at the University of Zurich and from the staff of the Swiss Federal Institute of Technology Zurich.

Methods

The dietary habits of the subjects were assessed by recording the dietary history using a computer-aided interview. We used the EBIS computer software, a nutrient data base analysis software developed at the Robert-Bosch-Krankenhaus in co-operation with the University of Hohenheim, Stuttgart, Germany. The diet history portion of the program presents questions about the different meals and their components in form of a tree structure, starting with the single meals and ending with details of amounts of individual food items (Landig *et al,* 1998). The program is based on the German Food and Nutrient Data Base (Bundeslebensmittelschlüssel) BLS 2.3, an electronic data base developed by the Federal Health Department, for use in nutrition epidemiology and dietary assessment. The BLS includes approximately 11'000 food items and recipes with 166 nutrients. The nutrient values come from different European food tables and from analyses of the Federal Research Institutes. About 90 % of the analysed data derive from the German food table Souci-Fachmann-Kraut (1994). Missing values for which no analysed data are available are estimated from similar foods (Häussler *et al,* 1990).

The interviews were carried out during two different periods of the year, from November to December 1999 and from April to June 2000, to take seasonal variations into consideration. The subjects were asked to give detailed information about their eating habits during the previous two months. Breakfast, lunch, dinner and snacks were discussed in turn to determine which foods were consumed and how often. Detailed descriptions of all foods, beverages and supplements consumed, including cooking methods and brand names, were recorded. A photo catalogue was used to estimate portion sizes and a few pre-weighed samples of often consumed foods were showed to the subjects (e.g. slice of bread, piece of cheese). Cups and glasses with known volumes were also used to estimate quantities. The interviews were performed by five different interviewers. All of them were carefully trained by the

same person to ensure uniform data collection. If interviewers had difficulty coding a food item, it was recorded in written form and coded later by the person doing the evaluation of all the interviews. The time required for an interview was between 1 ½ and 2 hours. The EBIS diet history method for determining magnesium intake has been validated against weighed food records with good results (Landig *et al,* 1998).

Data analysis

Because certain Swiss foods differ from German foods, the BLS nutrient data base was adapted to Swiss customs and special food items were added to the data base. The adaptation of the data base to Swiss foods included the choice of food items from the BLS (only about 1100 food items from the total BLS were used for the interview) and the addition of missing foods and dishes. Missing foods were added to the data base when the exact nutrient values (e.g. for foods found in other food tables) was known, otherwise they were composed as recipes using unprocessed food items from the BLS. Added foods included different mineral waters on the Swiss market and missing food items for which data were found in other food composition tables. To estimate the magnesium value for ingested tap water, the mean magnesium content of Zurich's water supply was used. Recipes were composed for fortified foods on the Swiss market (breakfast cereals, fortified milk products, milky breakfast beverages, fortified flours, cereal bars, bakery products, fruit beverages) and for special Swiss dishes. Because Swiss recipes for breads differ from German ones, and because of their importance in the Swiss diet, breads were analysed for their magnesium content by atomic absorption spectroscopy (SpectrAA 400, Varian, Mulgrave, Australia). The fat content of many Swiss dairy products (yoghurts, curds, cheese), sausages and meat products differs from the fat content of German products. These products were carefully chosen from the total BLS or adapted by composing recipes.

The mean daily magnesium intake of the diabetic group was compared to both the mean intake of the control group and to the recommended dietary intakes (RDI) for Switzerland (D-A-CH Referenzwerte, 2000). The proportion of subjects not meeting the RDI was determined and compared between the two groups. The probability approach (Anderson *et al,* 1982; Gibson, 1990) was also used to compare magnesium intakes with the RDI. This method predicts the number of individuals

within a group with nutrient intakes below their own requirements and hence provides an estimate of the prevalence of inadequate intakes. The contribution of magnesium supplements to daily intake and the contribution of the different food groups to the magnesium intake were calculated and compared between the two groups.

The extent of underreporting in this study was estimated by comparing the reported individual energy intakes with estimated energy requirements (FAO/WHO/UNO, 1985; James & Schofield, 1990), with the physical activity level set at 1.55 for men and 1.56 for women based on a sedentary lifestyle or light activity.

Statistical analysis

Data processing and statistical analysis were done using Excel 97 (Microsoft, Seattle, WA, USA) and SPSS for Windows 10.0 (SPSS Inc., Chicago, USA). Normally distributed data were expressed as means and standard deviations (SD) and the unpaired Student's t-test was used for comparison. Variables not normally distributed were expressed as medians and ranges and compared with the Mann-Whitney test. Differences were considered statistically significant at $p < 0.05$.

Results

Mean magnesium intakes of the diabetic and the control group were not significantly different (Table 2). The mean ±SD daily magnesium intake in the male diabetic and male control subjects was 423.2 ±103.1 mg and 421.1 ±111.0 mg, respectively. The mean daily magnesium intake in the female diabetic and female control subjects was 419.1 ±109.7 mg and 383.5 ±109.7 mg, respectively. The recommended dietary intakes for Switzerland (350 mg for adult men, 300 mg for adult women) were not met by 24.8 % of the diabetics and 26.0 % of the control subjects. Using the probability approach (Gibson, 1990), 5.4 % of the diabetic group and 9.1 % of the control group were predicted to have intakes of magnesium below their individual requirements. In both, diabetics and controls, magnesium intake was also compared between quartiles of age, but no significant differences were found either in men or in women.

Table 2 Comparison of daily magnesium intake between diabetics and control subjects

	Men (RDI: 350 mg)			Women (RDI: 300 mg)		
	Diabetics	Controls	p	Diabetics	Controls	p
n	54	54		43	46	
Mean daily Mg intake (mg)	**423.2**	**421.1**	**0.92**	**419.1**	**383.5**	**0.13**
Standard deviation	±103.1	±111.0		±109.7	±109.7	
Mean daily energy intake (kJ)	8478	9693		7268	7798	
(kcal)	(2026)	(2317)		(1737)	(1864)	
Mg intake per 1 MJ (mg)	50	43	<0.001	55	47	0.001
per 1000 kcal (mg)	(209)	(181)		(229)	(198)	
% subjects below RDI for Switzerland	31.5	24.1		16.3	28.3	
% subjects with risk of inadequate Mg intake (Gibson 1990)	6.0	9.9		4.6	8.1	

RDI, recommended daily intake

Diabetics reported consuming less energy than the control subjects and foods with a significantly higher magnesium density (per 1 MJ) than the control subjects (Table 2). The mean magnesium density ±SD in the food consumed by the male diabetic and male control subjects was 50 ±10 mg/MJ (209 ±42 mg/1000 kcal) and 43 ±9 mg/MJ (181 ±38 mg/1000 kcal), respectively (p<0.001). The mean magnesium density ±SD of the female diabetic and female control subjects was 55 ±12 mg/MJ (229 ±49 mg/1000 kcal) and 47 ±10 mg/MJ (198 ±41 mg/1000 kcal), respectively (p=0.001). Women (diabetics and controls) consumed foods with a higher magnesium density than men (51 ±11 mg/MJ (213 ±47 mg/1000 kcal) vs. 47 ±10 mg/MJ (195 ±42 mg/1000 kcal), p=0.005).

Magnesium intake from different foods are shown in Table 3. The contribution of the different food groups to the magnesium intake was compared between diabetic patients and controls. Among the five major sources of magnesium in the diet (cereals and cereal products, milk products, vegetables, fruit and non-alcoholic beverages), there was no significant difference in the % magnesium intake from these sources in diabetics and non-diabetics. However, the magnesium intake of the diabetics from meat (6.6 % vs. 5.1 %), mineral water (6.1 % vs. 3.1 %) and soups and sauces (2.6 % vs. 2.1 %) was significantly higher than the control group. In contrast, the magnesium intake of the diabetics was significantly lower than the

control group from alcoholic beverages (0.5 % vs. 3.0 %), nuts and seeds (0.8 % vs. 2.0 %), and sugars, chocolate and sweets (0.8 % vs. 1.7 %). No significant differences were found when the other food groups were compared.

Table 3 Contribution of the different food groups (%) to the total magnesium intake with food in diabetics and control subjects

Food group	Diabetics		Controls		p
	Mean[1] / median[2]	SD[1] / range[2]	Mean[1] / median[2]	SD[1] / range[2]	
Cereals[1]	22.8	9.5	23.4	9.6	0.68
Milk products[1]	11.4	6.6	11.3	6.3	0.95
Vegetables[1]	11.6	6.2	10.3	4.3	0.08
Fruit[1]	8.8	5.5	10.0	5.9	0.11
Non-alcoholic beverages[1] (without mineral water & fruit juices)	9.4	5.7	8.6	4.0	0.25
Meat and meat products[1]	6.6	3.2	5.1	3.2	**0.001**
Potatoes[1]	3.2	1.9	3.2	1.8	0.77
Mineral water[2]	6.1	0-31.3	3.1	0-33.7	**0.005**
Alcoholic beverages[2]	0.5	0-20.9	3.0	0-40.1	**< 0.001**
Soups and sauces[2]	2.6	0-18.0	2.1	0-19.0	**0.045**
Nuts and seeds[2]	0.8	0-25.6	2.0	0-38.4	**< 0.001**
Fish and eggs[2]	2.2	0-9.7	2.3	0-11.2	0.995
Sugars, chocolate and sweets[2]	0.8	0-12.4	1.7	0-12.9	**0.001**
Legumes[2]	0.8	0-4.9	0.7	0-5.7	0.49

[1] normally distributed data: unpaired t-test was used
[2] not normally distributed data: Mann-Whitney test was used

Magnesium supplements accounted for 3.4 % of the total magnesium intake when diabetic and non-diabetic subjects were combined. Of the 40 subjects taking magnesium-containing supplements, 24 were taking supplements containing only magnesium and 14 were taking magnesium in combination with other minerals and vitamins (2 subjects were taking other medical preparations containing magnesium). Diabetics took more magnesium supplements than controls (23.7 % vs. 17.0 %) and women took more supplements than men (29.2 % vs. 13.0 %). Consumption of multivitamin and/or multimineral supplements did not differ between the diabetics and controls, but supplements containing magnesium alone were more frequently

consumed by diabetics (14.4 % in diabetics vs. 10.0 % in controls) and by women (20.2 % in women vs. 5.6 % in men).

Potential underreporting was compared between the diabetic and the control subjects. In the diabetic patients, mean ±SD reported energy intake (8478 kJ (2026 kcal) in men, 7268 kJ (1737 kcal) in women) was 21.6 ±18.7 % lower than the mean estimated energy requirement (10908 kJ (2607 kcal) in men, 9386 kJ (2243 kcal) in women). In the non-diabetic subjects, however, mean ±SD reported energy intake (9693 kJ (2317 kcal) in men, 7798 kJ (1864 kcal) in women) was only 6.9 ±19.7 % lower than the mean estimated energy requirement (10459 kJ (2500 kcal) in men, 8411 kJ (2010 kcal) in women). There were no significant differences between men and women in both groups.

Discussion

The magnesium intake of the patients with type 2 diabetes and healthy controls was considerably higher than the magnesium intakes reported in the ARIC study (Ma *et al,* 1995) and the study of Schmidt *et al* (1994), both from the USA. Schmidt *et al*, using a 3-day food record in 50 type 2 diabetics, found an intake of 336.8 mg/d for men and 216.5 mg/d for women (mean age 57.2 ±10.2 y.). The ARIC study investigated dietary magnesium intakes in 15'248 participants using a food frequency questionnaire. In white male and female diabetics they found a magnesium density of 42 mg/MJ (175 mg/1000 kcal) and 42 mg/MJ (174 mg/1000 kcal), respectively, compared to 38 mg/MJ (157 mg/1000 kcal) and 41 mg/MJ (170 mg/1000 kcal) in white disease-free subjects. Our findings agree with the ARIC study, in that diabetics consumed foods with a higher magnesium density than controls, but the overall density was considerably higher in our Swiss population.

The differences between our results and data from the US may reflect differences in overall dietary habits between the inhabitants of the two countries. The USDA's 1977-78 Nationwide Food Consumption Survey (Morgan *et al,* 1985) found that the magnesium intakes for all age/sex classes were below the 1989 US RDA (Food and Nutrition Board, 1989). Mean daily magnesium intake of adult men and women was 301 mg and 223 mg, respectively. Data from the Total Diet Study 1982-89 in the USA (Pennington & Young, 1991) produced similar results: means of 259 mg in 60 to 65

year old men and 195 mg in 60 to 65 year old women. In Switzerland, estimates based on disappearance data from 1994/95 suggest that the magnesium intake is adequate: 406 mg/d in adults > 15 years (4th Swiss Nutrition Report, 1998). Differences in dietary habits might be due to a higher consumption of magnesium from cereal products in Switzerland. In the USA, cereals account for 17 to 18 % of magnesium intake (U.S. Department of Health and Human Services, 1989; Pennington & Young, 1991) compared to 23 % in our study.

Large surveys in other European countries have demonstrated that magnesium intakes were slightly below the recommended dietary intakes. The German NVS study which assessed 23'000 subjects using a 7-day food record, showed that mean daily magnesium intake of the adult men was at or slightly below the RDI of 350 mg (325 to 354 mg, depending on the age group), and magnesium intake of the adult women was below the RDI in all age groups (270 to 282 mg) (Ernährungsbericht, 1996). Results of the SU.VI.MAX cohort in France which investigated magnesium intake in 5'448 subjects using six 24-hour recalls were similar. The mean magnesium intake in adult men and women was 369 mg and 280 mg, respectively (Galan *et al,* 1997). In Belgium, analysing duplicate portions of food collected over several 24-h periods, a mean magnesium intake of 271 mg/day was found (Hendrix *et al,* 1995). It is difficult to judge if magnesium intakes in Switzerland are truly higher than in other European countries as only disappearance data are available for Switzerland, which are usually higher than intake data from direct dietary assessments (Schneider, 1997).

Five food sources (cereals, milk products, vegetables, fruit, non-alcoholic beverages) accounted for 64 % of magnesium intake in both diabetics and non-diabetics. Magnesium intake from alcoholic beverages, and sugars, chocolate and sweets (including candies, ice-cream, honey, and syrup) of the diabetics was significantly lower than the control group, although intake from these sources was low (0.5 – 3 %). This is likely due to the fact that the diabetics in our study were advised by dieticians to avoid excess simple sugars and alcoholic beverages.

We chose the diet history method as the appropriate assessment tool for this investigation. Although 24-hour recalls are easier to carry out, they evaluate food intake only on single days, and a minimum of 4 to 6 days are required to assess

magnesium intake in adults accurately (Nelson *et al,* 1989). A food-frequency questionnaire also assesses dietary habits, but because magnesium is widely distributed in foods, a prohibitive number of food items need to be included in the questionnaire. Although weighed food records are accurate, they are time-consuming and intensive. The diet history provides information on habitual dietary intake and has a relatively low respondent burden compared to a weighed food record (Gibson, 1993). Our sample was moderately large and only group intakes were evaluated. In general, the diet history method yields good precision when used for a group, especially over a relatively short time frame (Van Staveren *et al,* 1985). The reproducibility of the diet history method used in this study is very good (Reshef & Epstein*,* 1972; Morgan *et al,* 1978). In some studies, the diet history method has produced higher estimates of group mean intake than the food record (Gibson, 1990). Other investigators have found similar or lower mean nutrient intakes when comparing the diet history with food records (Van Staveren *et al,* 1985). Landig *et al* (1998) compared nutrient intake obtained by the EBIS diet history method with a weighed food record over the same observation period and has received similar results for magnesium with both methods (with EBIS mean magnesium intake was 3.0 % lower than with the weighed food record). Major sources of potential errors in the diet history method are reliance upon memory, incorrect estimation of portion sizes, self-selection of participation subjects, coding errors and errors due to different interviewing techniques (Gibson, 1990). To minimise these potential sources of error, we used a photo catalogue to estimate portion sizes, a computer program to standardise the interviewing technique, and the nutrient data bank was checked and corrected for mistakes.

Underreporting occurs frequently in dietary assessment. In the present study, the energy intake reported by the diabetics was 21.6 % lower than their estimated energy requirement. This could have been due to underreporting, as patients with type 2 diabetes are often overweight and are aware they should eat less, or could reflect reduced energy intake in an attempt to lose weight. The tendency toward underreporting appeared to be higher among the diabetics than the control subjects. This suggests that if our data include biases due to underreporting, the measured magnesium intake of diabetics in this sample may actually be higher than reported here.

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6 Measurement of magnesium absorption and retention in type 2 diabetic patients with the use of stable isotopes

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Running title: Magnesium absorption and retention in diabetes

Abstract

Background: Magnesium deficiency has been associated with type 2 diabetes and may reduce insulin sensitivity and impair glucose tolerance. The etiology of magnesium depletion in diabetes is unclear. Animal studies have suggested diabetes may impair magnesium absorption, but there are no published studies in humans.

Objective: Magnesium absorption from a test meal, and its excretion and retention were compared between type 2 diabetics and healthy controls.

Design: A meal labeled with 10 mg of ^{26}Mg isotopic label was administered and stools and urine collected for 10 and 6 days, respectively. Apparent absorption was calculated as the difference between the oral dose of ^{26}Mg isotopic label and the total amount of isotopic label excreted in the feces. Magnesium retention was calculated from the apparent absorption and the urinary excretion of ^{26}Mg isotopic label in the 6 days after administration.

Results: Mean fractional magnesium absorption ±SD in the diabetic patients and controls was 59.3 ±7.0 % and 57.6 ±8.5 %, respectively (N.S.). Mean urinary magnesium excretion and mean magnesium retention ±SD in diabetics and controls was 11.2 ±2.6 vs. 11.7 ±3.8, and 54.2 ±7.1 % vs. 51.4 ±6.1 %, respectively (N.S.).

Conclusions: Dietary magnesium absorption and retention are not impaired in patients with reasonably well-controlled type 2 diabetes.

Key words: magnesium, absorption, excretion, retention, diabetes, stable isotopes, fecal monitoring

Introduction

Magnesium deficiency has been reported in type 2 diabetes mellitus. In the USA, 25 to 39 % of outpatient diabetics have low concentrations of serum Mg (1). Low serum Mg concentrations in type 2 diabetes have also been reported in several European countries, e.g. Austria, Germany, Italy, France and Sweden (2-6). Similarly, we have found significantly lower serum Mg concentrations in type 2 diabetics compared to healthy controls in a Swiss population (unpublished data, M Wälti 2002). Mg depletion has a negative impact on glucose homeostasis and insulin sensitivity in people with type 2 diabetes (7, 8) as well as on the evolution of complications such as retinopathy (9), thrombosis (8), and hypertension (8).

The reasons why Mg deficiency occurs in diabetes are not clear. They may include increased urinary loss, lower dietary intake, or impaired absorption of Mg compared to healthy individuals. We recently reported that low dietary intake does not appear to contribute to impaired Mg status in diabetics in Switzerland (10). Several studies have reported increased urinary Mg excretion in type 1 and 2 diabetes (6, 11-15), while other studies have found no significant differences in Mg excretion between diabetics and healthy controls (16, 17). Several authors have suggested that impaired intestinal absorption might contribute to low Mg status in diabetics (7, 18, 19). Fractional Mg absorption is decreased in alloxan-induced diabetic rats compared to controls (20). Using in situ perfusion of intestinal segments, Miller and Schedl (21) reported significantly lower Mg absorption in streptozocin-induced diabetic rats than in controls. However, there are no published data on Mg absorption in humans with diabetes.

Mg has 3 stable isotopes, two (^{25}Mg and ^{26}Mg) have low enough abundances (10.0 and 11.01 %, respectively) to be employed as enriched labels (22, 23). The objective of the present study was to compare Mg absorption and retention in patients with type 2 diabetes and healthy control subjects using stable isotopes.

Subjects, Methods and Materials

Subjects

Diabetic patients were recruited from the outpatient diabetes clinic at the University Hospital of Zurich and through advertisements in a local newspaper and a diabetes

newsletter. All had a $\geq$ 3 year history of type 2 diabetes, were in reasonable metabolic control (mean HbA$_{1c}$ 7.3 %) and had no history of nephropathy. Among the 12 diabetic patients enrolled, three were on insulin, 7 were taking oral antidiabetic agents and 2 were not taking any antidiabetic medication. Non-diabetic subjects were recruited from the local community and from the staff of the Swiss Federal Institute of Technology. All subjects in both the diabetic and control groups were of Caucasian origin. At screening, none had detectable urinary glucose, protein or ketone bodies in their urine (Keto-Diastix®/Albustix®, Bayer Corporation, Tarrytown NY, USA). None were taking regular diuretics, laxatives, or other medications known to directly affect urinary and/or bowel function. Inclusion criteria included a BMI $\geq$ 20 and $\leq$ 29.9.

In order to enroll subjects with normal Mg status, a venous blood sample was drawn two to three weeks prior to the study, and only individuals with normal plasma Mg concentrations (reference range 0.65 to 1.05 mmol/L (24)) were included in the study. Initially, 13 diabetics and 12 controls were enrolled. Two subjects (one in each test group) dropped out of the study after the test meal because of gastrointestinal discomfort. One subject in the control group did not completely collect stool. Thus, 12 type 2 diabetics and 10 healthy non-diabetic subjects completed the study. Sample size allowed detection of an absolute difference of 12 % in Mg absorption between groups with a significance level of 0.05 and a power of 90 %. Written informed consent was obtained from each subject and the study protocol was approved by the Ethical Committee of the Swiss Federal Institute of Technology Zurich.

Study protocol

The study protocol was based on the methodology described by Turnlund and Keyes (25) and by Bohn (26). The subjects were instructed to take no vitamin-mineral supplements in the two weeks before and during the entire study. The day before isotope administration, a fecal sample and a urine spot sample were collected in polyethylene containers/bottles for the determination of baseline Mg isotope ratios. Weight and height of each subject were measured. The most recent HbA$_{1c}$ level was recorded from the medical record of the diabetic subjects. For all subjects but one this value was obtained within 2 months of the study period. A standardized vegetarian diet was provided for the whole day (**Table 1**). At 8 p.m. on the evening

before receiving the test meal, 100 mg of Brilliant Blue, a fecal marker, was given orally in a gelatin capsule to indicate the start of the fecal collection period.

Table 1 Overview of the standardized diets provided on days 0 and 1 of the study

	Day 0	Day 1
Breakfast	Free choice	1 wheat bread roll (60g) 300 g isotopically labeled ultrapure water
Lunch	Vegetable lasagna (480g)	Vegetables and potato meal (600g)
Snack	1 apple and 1 cereal bar (17g)	1 apple and 1 cereal bar (17g)
Dinner	4 pancakes filled with mushrooms (240g) Carrot salad (160g)	Rice (140g) Ratatouille (160g)
Drinks	only tap water	only ultrapure water

After an overnight fast, a standardized test meal consisting of a wheat bread roll and 300 g isotopically labeled ultra-pure water was served for breakfast. The water was labeled with 10 mg of the ^{26}Mg isotopic label and 5 mg of ytterbium immediately prior to administration. Yb was used as a non-absorbable marker to control for complete stool collection (27). The exact amount of the ingested isotopic label and fecal marker were determined by weighing the water beaker before and after test meal administration. The bread roll and the drink were ingested simultaneously. No food or drink were allowed for 3 hours following intake of the test meal. A standardized diet and 2 liters of ultra-pure water as beverage were provided for the remainder of day 1 (**Table 1**). From day 2 onwards, the diet was unrestricted.

After isotope administration, subjects collected all urine and stool in pre-weighed polyethylene bottles/containers. Bottles for urine collection contained 5 ml concentrated HCl / liter urine to reduce precipitation. Six days after intake of the isotopic label, 100 mg Brilliant Blue was again given orally, and stool was collected in individual portions until the excretion of the second fecal marker was complete. Urine was collected until the evening of day 6. Stool and urine samples were weighed and then stored at –25 °C until analysis.

Materials

Reagents

All chemicals used were analytic grade. Nitric acid and hydrochloric acid were further purified by sub-boiling distillation. All water used for analytical procedures and as drinking water on day 1 was purified by ion exchange and reverse osmosis (18 MΩ) (RD2000, Renggli AG, Rotkreuz, Switzerland; Nanopure Cartridge System, Skan AG, Basel, Switzerland). Acid-washed labware was used for stool and urine sample analyses.

Bread rolls

To reduce the phytic acid content of the wheat bread rolls, commercial low-extraction wheat flour was used and the fresh dough was allowed to ferment for 5 hours. The dough was separated into identical portions of 74.0 g before baking. The baked bread rolls were kept frozen until use.

Isotopic label

For preparation of the isotopic label, highly enriched ^{26}MgO (96.8 %) was purchased from AMT Ltd. (St. Kiryat Ono, Israel) and converted into ^{26}MgCl$_2$ by dissolution in 4 mol/L hydrochloric acid. The ^{24}Mg/^{25}Mg isotope ratio was adjusted close to the natural ^{24}Mg/^{25}Mg isotope ratio of 7.899 (22, 23) using a ^{25}MgCl$_2$ solution prepared from ^{25}MgO (AMT Ltd., enrichment 97.86 %) by the same procedure as described for ^{26}MgCl$_2$. An adjustment of the ^{24}Mg/^{25}Mg isotope ratio in the isotopic label was necessary to allow for internal normalization of the data. Normalization of the isotope ratios increases reproducibility provided that the natural ^{24}Mg/^{25}Mg isotopic ratio is not altered significantly by addition of the label. After gravimetric preparation of the mixture, the isotopic composition was verified by thermal ionization mass spectrometry (TIMS, see below) against a commercial standard of natural isotopic composition (Titrisol, Merck, Darmstadt, Germany). The normalized ^{24}Mg/^{25}Mg isotope ratio (3 independent runs) of the isotopic label was 8.029 ±0.014.

Fecal markers

Ytterbium chloride hexahydrate (99.998 % purity, Aldrich Chemical Company, Milwaukee WI, USA) was used as a quantitative fecal marker and added to the drink

as aqueous solution. Gelatine capsules containing 100 mg Brilliant Blue per capsule were prepared using Brilliant Blue powder (food quality, Travosa AG, Goldach, Switzerland) and mannitol as a dilutant (pharmaceutical quality, Hänseler AG, Herisau, Switzerland).

Sample preparation and analysis

Venous blood samples were drawn in heparinized tubes (10 ml) to determine plasma Mg concentrations at recruitment. Plasma was separated from blood cells by centrifugation at 3000 rpm for 15 minutes (Omnifuge 2.0 RS, Heraeus GmbH, Hanau, Switzerland) and stored in plastic vials at -25 °C until analysis. Frozen stool samples were freeze-dried (Lyolab B, LSL Secfroid SA, Aclens-Lausanne, Switzerland) in the containers used for collection. Freeze-dried feces were ground with a pestle and dried in an incubator at 65°C for 20 hours (WTBbinder, Tuttlingen, Germany). The dried samples (including the first fecal sample dyed by Brilliant Blue and including all samples before the second appearance of Brilliant Blue) were milled using a centrifugal mill (1.0 mm mesh, Retsch Type ZM1, Retsch GmbH, Haan, Germany) in the order of collection. The samples from each subject were combined and mixed mechanically by overhead rotation at approximately 60 rpm for two hours. All urine samples were mechanically shaken for at least one hour before pooling. Six-day urine-pools were prepared by combining 1% of each sample (by weight) and were stored at -25 °C. Aliquots of stool pools (1.6 g) and urine pools (3 ml) were mineralized in duplicate using a mixture of concentrated nitric acid and H_2O_2 (30 %, suprapur quality, Merck) in a microwave digestion system (MLS 1200, MLS GmbH, Leutkirch, Germany).

Total Mg content of the mineralized samples was determined by flame atomic absorption spectrometry (SpectrAA 400, Varian, Mulgrave, Australia) at 285.2 nm, using parameters recommended by the manufacturer (28). A commercial Mg standard (CertiPUR, Merck) was used for internal calibration by standard addition to minimize matrix effects. Mg concentrations of the final diluted solutions were in the range of 0.1 to 0.4 μg/ml. Lanthanum nitrate (Fluka Chemie GmbH, Buchs, Switzerland) was added as a matrix modifier (5 mg La/ml in the final solutions). All samples were analyzed in duplicate and repeated if the difference between individual values relative to the mean was >5 %.

Quantitative analysis of Mg in plasma was done by flame AAS using standard addition after 200-fold dilution of the plasma samples and addition of lanthanum nitrate (5 mg La/ml). Accuracy of the method was verified by analyzing a serum control sample for Mg (Seronorm Trace Elements Serum, Nycomed, Oslo, Norway). Samples were analyzed in triplicate and repeated if the standard deviation relative to the mean was >10 %. Bread rolls were freeze-dried and mineralized and analyzed for their Mg content using the same AAS procedure as described for the stool and urine samples. Yb in fecal pools was quantified by electrothermal atomic absorption spectrometry (SpectrAA 400 and GTA-96 graphite furnace atomiser, Varian) at 398.8 nm, using parameters recommended by the manufacturer (29). A 5 point external calibration curve was established using a commercial Yb standard (Merck). Samples were analyzed in duplicate and repeated if the difference between individual values relative to the mean was >5 %.

For Mg isotopic analysis, Mg was separated from the mineralized samples by cation-exchange chromatography using a strongly acidic ion-exchange resin as described previously (26). Aliquots of the mineralized samples were evaporated to dryness in Teflon vials, the residue dissolved in 1.0 ml 0.7 mol/L hydrochloric acid and loaded on commercial Pyrex columns (Econo column, 1.0 cm x 10 cm, Bio-Rad, Hercules CA, USA) filled with ion-exchange resin (AG 50 W X-8, 200-400 mesh, Bio-Rad). After eluting Na and K with 0.7 and 0.9 mol/L hydrochloric acid, Mg was eluted with 1.4 mol/L hydrochloric acid. The Mg fraction was evaporated to dryness in Teflon vials, the residue dissolved in 0.5 ml concentrated hydrochloric acid, transferred to Eppendorff tubes, evaporated to dryness in a furnace at 70°C (WTBbinder) and re-dissolved in water.

Isotope ratios were determined by thermal ionization mass spectrometry (TIMS) with a single-focusing magnetic sector field instrument (MAT 262, Finnigan MAT, Bremen, Germany) equipped with a Faraday cup multiple collector device for simultaneous ion beam detection. About 20 nmol separated Mg from fecal/urine samples was loaded onto the metal surface of the evaporation filament of a double-rhenium filament ion source. Mg was coated with 5-10 μg silicagel, 0.8 μmol boric acid and 30 nmol aluminum as $AlCl_3$ (all chemicals from Merck). Each measurement consisted of 50 consecutive isotope ratio measurements. Systematic isotope fractionation effects in

the ion source were corrected by internal normalization techniques using an exponential law (30). Reproducibility for the ^{24}Mg/^{26}Mg isotope ratio (6 independent runs) was ±0.01 % (relative SD).

Data and statistical analysis

Apparent Mg absorption and retention from the isotopically labeled test meals were calculated based on the amount ratio of isotopic label to natural Mg present in 6-day fecal pools and 6-day urinary pools and isotope dilution principles (26). The amount of ^{26}Mg isotopic label in each fecal and urine sample was calculated from the amount ratio of ^{26}Mg isotopic label to natural Mg as determined by TIMS analysis and the total Mg amount in feces/urine as determined by AAS. Apparent absorption was calculated as the difference between the orally administered dose and the total amount of isotope excreted in feces and expressed as the percent of the administered dose. Retention of ^{26}Mg was calculated by subtracting the total amounts of ^{26}Mg isotopic label recovered in feces and urine from the administered dose. Complete collection of stool material was verified via Yb recovery. If Yb recovery was lower than 75%, subjects were excluded. Absorption was corrected when Yb recovery was between 75 and 90% using the following formula (31):

$$\text{Corrected absorption (\%)} = [1 - \frac{\%\ isotope\ dose\ excreted}{\%\ rare\ earth\ dose\ excreted}] \times 100$$

Yb recovery of 90 to 110 % was considered of being in the range of analytic error of the method.

Data processing and statistical analysis were done using Excel 97 (Microsoft, Seattle WA, USA) and SPSS for Windows 11.0 (SPSS Inc., Chicago IL, USA). Normal distribution of data was verified by calculating the quotient of the skewness divided by its standard error. Normal distribution can be assumed when this quotient lies between –2.5 and +2.5. Data were expressed as arithmetic means ± SD. Differences between groups were evaluated using unpaired Student's t-test and considered statistically significant at $p < 0.05$. ANOVA was done to test for associations with Mg absorption, excretion and retention as dependent variables.

Results

Baseline characteristics of the two groups are shown in **Table 2**. There were no significant differences in sex ratio, height, weight, BMI and plasma Mg concentrations between the groups. The mean Mg ±SD content of the wheat bread rolls was 13.5 ±0.7 mg. The mean total Mg content ±SD of the test meals (native Mg plus isotopic label) was 23.6 ±1.0 mg.

Table 2 Baseline characteristics of the subjects in the type 2 diabetic group and the healthy control group

Characteristics	Diabetics	Controls	p
No of subjects	12	10	
Sex	8 men / 4 women	7 men / 3 women	
Age (years)[1]	65.0 ±7.0	71.6±5.5	0.025
Height (cm)[1]	170.5 ±7.2	170.5±7.8	N.S.
Weight (kg)[1]	76.0 ±6.0	73.1±4.2	N.S.
BMI (kg/m^2)[1]	26.2 ±2.1	25.2±1.7	N.S.
Plasma Mg (mmol/L)[1]	0.83 ±0.08	0.87±0.07	N.S.
Duration of diabetes (years)[2]	7.5 (3-51)	-	
HbA$_{1c}$ (%)[1]	7.30 ±0.89	NA[3]	

Data given as mean ±SD[1] or median (range)[2]
[3]HbA$_{1c}$ not measured, but all tested negative for urinary glucose

Apparent absorption, urinary excretion and retention of Mg in the diabetic and control groups are shown in **Table 3**. There were no significant differences in mean fractional Mg absorption, mean urinary Mg excretion or mean Mg retention between groups. Data from one subject in the control group were excluded from analysis because of low Yb recovery (< 20 % of dose) and in three subjects (2 diabetics, 1 control) fecal Mg excretion was corrected based on Yb recovery (as described in 'Methods' section). In one diabetic subject only Mg absorption, but not retention, could be calculated due to incomplete urine collection. By ANOVA, sex, age, BMI, baseline plasma Mg, HbA$_{1c}$ levels, duration of diabetes and medication used to treat diabetes did not significantly predict the dependent variables Mg absorption, excretion and retention.

Table 3 Comparison of mean (±SD) Mg absorption, retention and excretion between the type 2 diabetics and the healthy controls

	Diabetics n = 11	Controls n = 10	p [2]
Apparent Mg absorption [% of aministered dose]	59.3 ±7.0 [1]	57.6 ±8.5	0.60
Urinary Mg excretion [% of absorbed dose]	11.2 ±2.6	11.7 ±3.8	0.75
Mg retention [% of aministered dose]	54.2 ±7.1	51.4 ±6.1	0.33
Total urinary Mg in 6-day pool [mg]	497.6 ±120.6	482.8 ±184.3	0.83
Mean daily Mg excretion [mg]	82.9 ±20.1	80.5 ±30.7	0.83

[1] n=12
[2] unpaired Student's t-test

Discussion

In diabetic individuals, enteric neuropathy and microvascular disease can alter intestinal absorption of carbohydrates, amino acids, and minerals (32-34). Intestinal absorption of calcium and zinc are decreased in diabetic rats and humans, compared to healthy controls (35-37). Studies in diabetic rats suggested that Mg absorption may also be impaired. Schneider and Schedl (20) reported lower fractional Mg absorption in alloxan-induced diabetic rats (18%) compared to control animals (27%). Net Mg absorption was not significantly different between groups because the diabetic rats were hyperphagic and their Mg intake was 60% higher than the controls. Miller and Schedl (21) measured Mg absorption by in situ perfusion of intestinal segments in streptozocin-induced diabetic rats. Mg absorption was significantly lower in the intestine of diabetic rats than of controls and the authors attributed these findings in part to abnormal vitamin D metabolism observed in the diabetic rats. These studies were performed in rat models for type 1 diabetes. In contrast to the findings of these animal studies, our data suggest that dietary Mg absorption and retention are not lower in humans with reasonably well-controlled type 2 diabetes compared to healthy controls. However, if we had enrolled subjects with advanced diabetes and established neuropathy and/or microangiopathy, we might have detected a difference in absorption. In addition, varying results could be due differences in methodology, or inherent differences between animal models of diabetes and the human disease.

In the present study, the mean apparent Mg absorption from our test meal was between 57.6 and 59.3 %. This is higher than that previously showed in a balance

study for whole diets, where Mg absorption was 21 to 27 % from self-selected diets with a mean daily Mg content of 323 and 234 mg in men and in women, respectively (38). Fractional Mg absorption from balance studies using differing diets or supplements have ranged from 10 to 65 % (39, 40). In stable isotope studies with adults, values for apparent absorption varied from 20 to 60 % (26, 41-43). Fractional Mg absorption is highly dependent on the amount of Mg in the meal; the larger the amount, the lower the fractional absorption (39). Moreover, Mg absorption may be increased (protein, fructose) or inhibited (cellulose, phytic acid, oxalic acid) by other food components (44). The high fractional Mg absorption from our test meal is presumably due to the very low Mg content (23.6 mg) of the meal, which contained no known inhibitors of Mg absorption.

Use of only an oral isotopic label does not allow determination of true Mg absorption but only apparent absorption. Mg is re-excreted in the intestine by pancreatic, bile and other intestinal excretions and by cell sloughing. There are only few data available on endogenous intestinal losses of Mg, with reports ranging from 2 to 38 mg/day (45-47). Measured apparent absorption is therefore lower than true absorption. As has been demonstrated previously in stable isotope studies, the absolute difference between apparent and true absorption is of the order of 2-10 % in normal healthy subjects (26, 42, 48) which is not of major practical significance for comparison of Mg absorption.

Several studies have shown increased urinary Mg excretion in both type 1 and 2 diabetics (6, 11-15), and increased urinary Mg excretion in diabetes has been associated with elevated fasting blood glucose or HbA$_{1c}$ (11). Studies in type 1 diabetics have shown that urinary Mg excretion increases in response to a glucose infusion (49). Urinary Mg excretion is also increased in diabetic patients with osmotic diuresis secondary to glycosuria (11-13). However, other studies have found no significant differences in urinary Mg excretion between diabetics and healthy controls (16, 17). In the present study, mean urinary Mg excretion in diabetics was not increased compared to controls. This may be due to the fact that the diabetic subjects were in reasonable metabolic control and none had detectable nephropathy.

Our sample size allowed detection of an absolute difference in Mg absorption of $\geq$ 12 % between groups. A beta error is possible considering the small sample size.

However, we judged that a difference in absorption of $\geq$ 12 % between groups would be of clinical relevance and could potentially contribute to low Mg status in diabetes. A potential source of error in our method is incomplete fecal or urine collection. In order to correct for incomplete collection of stools, Yb was administered together with the labeled test meal. Yb is a rare earth element whose absorption is less than 0.05 % (50) when administered orally. Rare elements have been evaluated in studies of iron and magnesium (26, 27, 51) and demonstrated to be a useful tool to correct for incomplete stool collection. Bohn (26) showed that the excretion of Yb and the Mg isotopic label was significantly correlated. Unfortunately, there is no suitable marker available to check for completeness of urine collection. Para-aminobenzoic acid (PABA) has been used as quantitative urine control marker (52-54), but its safety has been questioned (55). The use of creatinine as a quantitative control marker has low sensitivity, particularly in older age groups (53, 56). Therefore, we judged completeness of urine collection based on total volume and confirmation of compliance from our subjects.

In summary, the results from this study indicate that type 2 diabetic patients in reasonable metabolic control and without nephropathy absorb and retain dietary Mg to a similar extent as healthy controls. Because nephropathy is common in long-standing diabetes and good glycemic control is difficult to achieve, these findings may not be generalizable to the entire diabetic population. It is possible that poor Mg status is a problem only in poorly-controlled diabetics who have increased urinary Mg loss due to glycosuria. Additional studies in less well-controlled diabetics and/or patients with type 1 diabetes would be useful to confirm our findings that impaired Mg absorption does not contribute to poor Mg status in diabetic patients.

Acknowledgments

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7 Urinary excretion of an intravenous ^{26}Mg dose as an indicator of magnesium status: a preliminary study in healthy adults

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Running title: Muscle Mg concentration and excretion of an i.v. Mg dose

Abstract

Measurement of magnesium status is problematic because magnesium deficiency can be present without low serum Mg concentrations. A modified version of the magnesium retention test using a small dose of ^{26}Mg was evaluated for assessment of magnesium status in 22 healthy subjects. Muscle magnesium concentration was used as reference for magnesium status. A muscle biopsy was taken from the lateral portion of the quadriceps muscle from each subject. Two to four weeks later, 11 mg of ^{26}Mg (as $MgCl_2$ in 14 ml water) were injected i.v. over a period of 10 minutes and all urine was collected for the following 24 hours. Excretion of the isotopic label was expressed as percentage of the administered dose excreted in urine within 24 hours. Mean ±SD magnesium concentration in muscle was 3.85 ±0.17 mmol/100g fat-free dried solids. Mean ±SD excretion of the injected dose within 24 hours was 7.9 ±2.1 %. No correlation was found between muscle magnesium concentration and excretion of the isotopic label (r^2=0.061, p=0.27). In this preliminary study, the test did not discriminate magnesium status and further studies would be necessary to determine its potential role in the assessment of magnesium status.

Key words: magnesium, muscle, magnesium load test, excretion, stable isotopes

Introduction

Despite the growing realization of the importance of Mg in human health and disease, measurement of Mg status remains problematic. Mg deficiency is a common characteristic of various diseases such as diabetes, hypertension, cardiovascular disease or chronic alcoholism (1,2). Sometimes serum Mg values can be normal despite depletion of intracellular Mg (3). Therefore, to study Mg deficiency in relation to various diseases, there is still a lack of an easily performable and valuable test for assessing Mg status.

Mg is principally an intracellular cation, with less than 1 % of total body content present in the extracellular fluids. The Mg concentration in serum represents not more than 0.3 % of total body Mg (2). Nevertheless, serum or plasma Mg measurement is the most available and most commonly employed test of status because it is easy and inexpensive to perform. However, it is an insensitive indicator of Mg status for it does not correlate with any other tissue pool of Mg except for interstitial fluid (4). Magnesium deficiency can be present without low serum Mg concentrations (5). Since 99 % of total body Mg is intracellular, determination of intracellular Mg might be a better indicator of Mg status. According to Elin (6), measurement of Mg in erythrocytes and leukocytes has been used, but the results are equivocal. Muscle and bone contain the majority of body Mg and are therefore potentially important tissues for assessment of status; however, muscle and bone biopsies are invasive, time-consuming and not suitable for ambulatory diagnosis.

An alternative is the Mg load test which is a physiologic assessment method and has been proposed, applied and validated by several authors as method for the determination of Mg status (7-12). It consists of measuring the retention of a parenterally administered dose of Mg in the body. Five to 30 mmol of Mg is infused over several hours, and the Mg content in 24 hour-urine is determined to calculate the retention of the administered load. The Mg load test has been shown to correlate with Mg concentration in skeletal muscle in Mg-deficient patients (9,11,13-15). A disadvantage of the load test is that it is affected by dietary Mg intake, basal urinary Mg and fecal Mg excretion all of which have an influence on the urinary Mg excretion after an i.v. loading dose. In summary, the Mg load test seems to provide good

information on Mg status, but is time-consuming and requires hospitalization of the subjects.

The aim of the present study was to perform a pilot investigation of a modified method of the Mg retention test, which does not require hospitalization of the subjects. When using a stable isotope, a much smaller dose can be administered because the effects of dietary Mg intake and basal urinary Mg on the measurand are minimized. Moreover, a low dose has the advantage to allow an injection over a few minutes instead of an infusion over several hours, and therefore does not require hospitalization of the subjects. Because muscle Mg concentration has shown to be a good indicator for Mg status (16,17), it was used as reference.

Subjects, Methods and Materials

Subjects

Fifty apparently healthy subjects were recruited from the student population of the ETH and University of Zurich. A venous blood sample was taken for determination of plasma Mg concentrations. In order to assure a broad range of plasma Mg among the subjects, 22 subjects (11 women, 11 men) were selected. Plasma Mg concentrations ranged from 0.68 to 0.95 mmol/L. Exclusion criteria included pregnancy, lactation, cardiovascular, hepatic, bleeding, metabolic and/or renal disorders, and blood donations less than 3 weeks before the study. All subjects were of Caucasian origin. Baseline characteristics of the 22 selected subjects are shown in **Table 1**.

Table 1 Baseline characteristics of the subjects

Characteristics	Total	Women	Men	p
No of subjects	22	11	11	
Age (years)	25.2 ±3.1	25.3 ±3.1	25.1 ±3.3	N.S.
Height (cm)	174.4 ±6.0	170.4 ±4.5	178.4 ±4.6	0.001
Weight (kg)	65.1 ±6.5	61.4 ±5.5	68.7 ±5.3	0.005
BMI (kg/m^2)	21.4 ±1.8	21.2 ±1.8	21.6 ±1.8	N.S.
Plasma Mg (mmol/L) at recruitment	0.82 ±0.09	0.82 ±0.09	0.82 ±0.09	N.S.

Data given as mean ±SD

No medication (except oral contraceptives) or vitamin/mineral supplements were consumed during the entire study. Written informed consent was obtained from each subject and the study protocol was approved by the Ethical Committee of the Swiss Federal Institute of Technology Zurich.

Study protocol

Two to four weeks before the Mg test, a muscle biopsy was taken from the lateral portion of the quadriceps muscle similarly to the method described by Bergström (18) using a 14 G biopsy needle (ACN1410MF, Homedica, Hünenberg, Switzerland) and a special biopsy device (Pro-Mag 2.2, Homedica). Skin and subcutaneous tissue were locally anaesthetized with 10 ml mepivacain HCl 1 % (produced by the Cantonal Pharmacy, University Hospital Zurich). The method allowed drawing a muscle sample weighing between 13.2 and 34.5 mg of fresh muscle tissue. After biopsy, visible connective tissue or fat was cut, the muscle samples were transferred in plastic vials and frozen at – 25°C.

The day before the Mg test a urine spot sample was collected for determination of the baseline Mg isotope ratios. A standardized vegetarian diet and mineral water (20 mg Mg/L) were provided for the whole day (**Table 2**). Weight and height of each subject were measured. After an overnight fast, 11 mg of ^{26}Mg (as $MgCl_2$ in 14 ml water) were injected i.v. over a period of 10 minutes. A sterile injection system consisting of a 2-way catheter and a septum injection port was used. The isotope solution was injected quantitatively by flushing the system with physiological saline (10 ml). The exact amount of injected isotopic label was determined by weighing the syringe before and after injection. No food or drink was allowed for 1 hour following the isotope administration. A standardized diet and mineral water were provided for the remainder of the day (**Table 2**). Following the injection, the subjects collected all urine in pre-weighed polyethylene bottles for 24 hours. Bottles for urine collection (2 liter volume) contained 10 ml concentrated HCl to reduce precipitation. Exactly 24 hours after the injection, the subjects terminated urine collection. Between 22 and 25 hours after the injection a venous blood sample was taken from every subject (fasting).

Table 2 Overview of the standardized diets provided on days 0 and 1 of the study

	Day 0	Day 1
Breakfast	Free choice	2 bread rolls (100g) with butter and jam
Lunch	Rice (140g) Ratatouille (160g) 1 fruit yoghurt (180g)	Vegetables and potato meal (600g) 1 fruit yoghurt (180g)
Snack	1 nectarine	1 nectarine
Dinner	4 pancakes filled with cheese and leek (240g) Carrot salad (160g)	Vegetable lasagna (480g)
Drinks	Mineral water Vittel® (2 L)	Mineral water Vittel® (3 L)

Day 1 = day of the Mg injection

Habitual mean daily Mg intake of each subject was assessed by recording the diet history using a computer-aided interview. We used the EBIS computer software (Robert-Bosch-Hospital and University of Hohenheim, Stuttgart, Germany) which is based on the German Food and Nutrient Database BLS 2.3. The subjects were asked to give detailed information about their eating habits during the previous 2 months. The method has been described in detail previously (19).

Materials

Reagents

All chemicals used were of analytic grade. Nitric acid and hydrochloric acid were further purified by sub-boiling distillation. All water used for analytical procedures was purified by ion exchange and reverse osmosis (18 MΩ) (RD2000, Renggli AG, Rotkreuz, Switzerland; Nanopure Cartridge System, Skan AG, Basel, Switzerland). Acid-washed labware was used for urine, blood and muscle sample analyses.

Isotopic label

For preparation of the isotopic label, highly enriched ^{26}MgO (96.8 %) was purchased from AMT Ltd. (St. Kiryat Ono, Israel) and converted into ^{26}MgCl$_2$ by dissolution in 4 mol/L hydrochloric acid. The ^{24}Mg/^{25}Mg isotope ratio was adjusted close to the natural ^{24}Mg/^{25}Mg isotope ratio of 7.899 (20) using a ^{25}MgCl$_2$ solution prepared from ^{25}MgO (AMT Ltd., enrichment 97.86 %) by the same procedure as described for ^{26}MgCl$_2$. An adjustment of the ^{24}Mg/^{25}Mg isotope ratio in the isotopic label was necessary to allow for internal normalization of the data. Normalization of the isotope

ratios increases reproducibility provided that the natural ^{24}Mg/^{25}Mg isotope ratio is not altered significantly by addition of the label. Following, the ^{26}MgCl$_2$ was dried down and heated in a muffle furnace (thermicon P, Heraeus GmbH, Hanau, Switzerland) at 250°C for 30 minutes to destroy pyrogenes. Doses for i.v. administration were prepared in a sterile environment at the pharmacy of the University Hospital, Zurich. The isotopic label was re-dissolved in sterile water, acidified with HCl 32 % until complete dissolution and neutralized by adding NaHCO$_3$. The final injection solution contained 0.78 mg Mg/ml and had a pH of 6.3. The solution was sterilized by filtration, divided into individual doses of 15 ml, transferred into glass vials, capped and sealed. After preparation of the solution, the isotopic composition was verified by thermal ionization mass spectrometry (TIMS, see below).

Sample preparation and analysis

Sampling of urine and blood samples

All urine samples were weighed and mechanically shaken for at least one hour before pooling. Twenty-four-hour urine pools were prepared by combining 5 % of each sample (by weight) and were stored at -25 °C. Venous blood samples at baseline and 24 hours after isotope injection were drawn in heparinized tubes (10 ml). Plasma was separated from blood cells by centrifugation at 3000 rpm for 15 minutes (Omnifuge 2.0 RS, Heraeus GmbH, Hanau, Switzerland) and stored in plastic vials at -25 °C until analysis.

Quantitative analysis of baseline Mg in plasma was done by flame atomic absorption spectrometry (AAS) (SpectrAA 400, Varian, Mulgrave, Australia) at 285.2 nm, using parameters recommended by the manufacturer (21). A commercial Mg standard (CertiPUR, Merck, Darmstadt, Germany) was used for internal calibration by standard addition to minimize matrix effects. Plasma samples were diluted 200-fold so that the Mg concentrations of the final diluted solutions were in the range of 0.1 to 0.4 µg/ml. Lanthanum nitrate (Fluka Chemie GmbH, Buchs, Switzerland) was added as a matrix modifier (5 mg La/ml in the final solutions), and 0.1 % Triton X-100 solution (Fluka Chemie GmbH) to reduce the surface tension. Samples were analyzed in duplicate and repeated if the difference between individual values relative to the mean was >7.5 %.

Mineralization

Aliquots of urine pools (3 ml) and of blood plasma drawn after isotope injection (1 ml) were mineralized in duplicate using a mixture of concentrated nitric acid and H_2O_2 (30 %, suprapur quality, Merck) in a microwave digestion system (MLS-ETHOS plus, MLS GmbH, Leutkirch, Germany). Total Mg content of the mineralized samples was determined by flame AAS using standard addition technique after 417-fold (urine) or 200-fold (blood plasma) dilution of the samples and addition of lanthanum nitrate (5 mg La/ml). All samples were analyzed in duplicate and repeated if the difference between individual values relative to the mean was >5 %. Accuracy of the method was verified by analyzing a serum control sample for Mg (Seronorm Trace Elements Serum, Nycomed, Oslo, Norway).

Preparation for isotopic analysis

For Mg isotopic analysis, Mg was separated from the mineralized samples by cation-exchange chromatography using a strongly acidic ion-exchange resin as described previously (22). Aliquots of the mineralized samples were evaporated to dryness in Teflon vials, the residue dissolved in 1.0 ml 0.7 mol/L HCl and loaded on commercial Pyrex columns (Econo column, 1.0 cm x 10 cm, Bio-Rad, Hercules CA, USA) filled with ion-exchange resin (AG 50 W X-8, 200-400 mesh, Bio-Rad). After eluting Na and K with 0.7 and 0.9 mol/L HCl, Mg was eluted with 1.4 mol/L HCl. The Mg fraction was evaporated to dryness in Teflon vials, the residue dissolved in 0.5 ml concentrated HCl, transferred to Eppendorff tubes, evaporated to dryness in a furnace at 70°C (WTBbinder, Tuttlingen, Germany) and re-dissolved in water.

Isotopic analysis by TIMS

Isotope ratios were determined by thermal ionization mass spectrometry (TIMS) with a single-focusing magnetic sector field instrument (MAT 262, Finnigan MAT, Bremen, Germany) equipped with a Faraday cup multiple collector device for simultaneous ion beam detection. About 20 nmol separated Mg from urine/plasma samples was loaded onto the metal surface of the evaporation filament of a double-rhenium filament ion source. Mg was coated with 5-10 μg silicagel, 0.8 μmol boric acid and 30 nmol aluminum as $AlCl_3$ (all chemicals from Merck). Each measurement consisted of 50 consecutive isotope ratio measurements. Systematic isotope fractionation effects in the ion source were corrected by internal normalization technique using an

exponential law (23). The measured value for the ^{24}Mg/^{25}Mg isotope ratio of the isotopic label (5 independent runs) was 8.004 ±0.076 which was normalized to 7.899 (natural ratio). Reproducibility for the ^{24}Mg/^{26}Mg isotope ratio (6 independent runs) was ±0.01 % (relative SD).

Creatinine analysis

Creatinine concentrations of the urine samples were determined using the method described by Jaffé (24) which is based on the formation of a colored complex between picric acid (Riedel-de Haën, Buchs, Switzerland) and creatinine. A four point external calibration curve was established using a commercial creatinine powder (Fluka Chemie GmbH). Analysis was done with a spectrophotometer (Uvikon 940, Kontron Instruments, Rotkreuz, Switzerland) measuring the colored complex at 520.0 nm.

Analysis of muscle samples

Frozen muscle samples were freeze-dried (Lyolab B, LSL Secfroid SA, Aclens-Lausanne, Switzerland). Weighing of the dried samples was performed on an analytical precision balance (Mettler AT201, Mettler-Toledo AG, Greifensee, Switzerland) in an air temperature and air humidity controlled balance room. To correct for air buoyancy, the atmospheric parameters air temperature, air pressure and relative air humidity were determined using a precision climatic instrument (Klimet A30, meteolabor AG, Wetzikon, Switzerland) (25). The samples were mineralized in 1 ml HNO$_3$ 65% for at least 40 hours, then filled up to 7 ml with HNO$_3$ 2%. One ml of this solution was used for analysis. Quantitative analysis of Mg was done by optical emission spectrometry with inductively coupled plasma (ICP-OES) (Optima 3000, Perkin Elmer, Norwalk CT, USA) equipped with an ultrasonic nebulizer (U-5000 AT+, Cetac Technologies Inc., Omaha NE, USA). Measurements were done at three different wavelengths, 279.553, 280.271 and 285.213 nm. For the dilutions, only acid-washed PFA tubes were used. Internal calibration by standard addition with 5 points was used for 3 samples to minimize matrix effects, and the concentrations of the remaining samples were calculated based on the mean of the 3 slopes. Scandium was used as internal standard (0.5 ppm). Dilutions and measurements were done twice on different days.

Data and statistical analysis

Excretion of the isotopically labeled Mg was calculated based on the amount ratio of isotopic label to natural Mg present in 24-hour urinary pools and isotope dilution principles (22). The amount of ^{26}Mg isotopic label in each urine sample was calculated from the amount ratio of ^{26}Mg isotopic label to natural Mg determined by TIMS and the total Mg amount in urine determined by AAS. Mg excretion was expressed as the percentage of administered dose excreted in urine within 24 hours. Distribution of the isotopic label in the rapidly exchangeable pools excluding plasma within 24 hours was calculated by subtracting the amount of isotopic label in the urine pool and the amount in plasma from the injected dose. Plasma volume was calculated using empiric formulas based on sex, weight and height (26,27). ^{26}Mg excretion was compared to muscle Mg to test for correlation.

Data processing and statistical analysis were done using Excel 2002 (Microsoft Corp., Seattle WA, USA) and SPSS for Windows 11.0 (SPSS Inc., Chicago IL, USA). Normal distribution of data was verified by calculating the quotient of the skewness divided by its standard error. Normal distribution can be assumed when this quotient lies between –2.5 and +2.5.

Results

The mean ±SD habitual daily Mg intake in the men and women was 438 ±61 mg and 464 ±138 mg, respectively. The recommended dietary intake for Switzerland (350 mg for adult men, 300 mg for adult women, (28)) was met by all but one subject. The extent of under/over-reporting was estimated by comparing the reported individual energy intakes with mean estimated energy requirements (29) using physical activity levels calculated according to reported activities. In the men, mean ±SD reported energy intake (11'290 ±840 kJ) was 0.1 ±7.7 % higher than estimated energy requirement. In the women, mean ±SD reported energy intake (8'870 ±1160 kJ) was 4.8 ±10.9 % lower than estimated energy requirement.

Mean ±SD Mg concentration in muscle was 3.85 ±0.17 mmol/100g fat-free dried solids (FFDS). There was no significant difference between men and women. Mean ±SD excretion of the injected dose within 24 hours was 7.9 ±2.1 %. Mean ±SD distribution in the rapidly exchanging pools excluding plasma after 24 hours was 90.7

±2.1 %. For other measured parameters such as total urinary Mg excretion, plasma Mg concentrations, see **Table 3**. There was no correlation between muscle Mg concentration and excretion of the isotopic label within 24 hours (r^2=0.061, p=0.27) (**Figure 1**). The same result was obtained when men and women were analyzed separately, when Mg excretion was corrected by creatinine output or body weight. Either, no correlation could be found between muscle Mg concentration and distribution of the isotopic label in the rapidly exchangeable pools excluding plasma (r^2=0.061, p=0.27) or between muscle Mg and plasma Mg concentration (r^2=0.015, p=0.58).

Table 3 Results

Parameter	Mean	SD
Muscle Mg concentration [mmol/100 g FFDS]	3.85	0.17
%excretion of the labeled dose within 24 h	7.9	2.1
%distribution in rapidly exchanging pools after 24 h	90.7	2.1
Total urinary Mg excretion in 24 h [mg]	124.3	29.4
Plasma Mg conc. at baseline [mmol/L]	0.82	0.09
Plasma Mg conc. after Mg injection [mmol/L]	0.87	0.07

FFDS = fat-free dried solids

Figure 1 Urinary excretion of an i.v. dose of ^{26}Mg within 24 hours in relation to Mg muscle concentration in 22 healthy adults
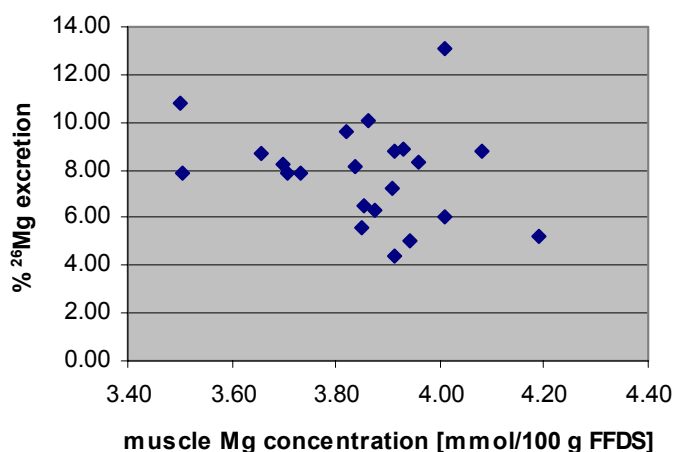
Plasma Mg concentrations measured at recruitment correlated well with those measured after the Mg test 6 to 10 weeks later (r^2=0.37, p=0.003), but were in average around 6 % higher (**Figure 2**). Fractional excretion of the isotopic label within 24 hours correlated with total urinary Mg excretion (r^2=0.51, p<0.001) (**Figure**

3). By ANOVA, sex, age, weight, plasma Mg concentration and dietary Mg intake did not significantly predict the dependent variables muscle Mg concentration and excretion of the isotopic label.

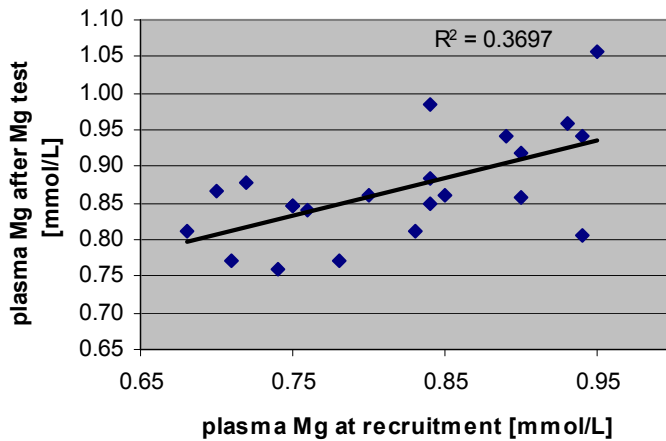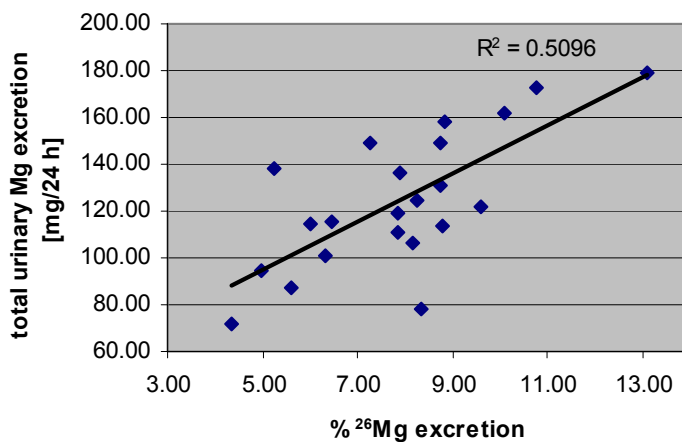**Figure 2** Correlation between plasma Mg concentration at recruitment and after the [26]Mg test (6 to 10 weeks later)

Figure 3 Correlation between the excretion of the i.v. [26]Mg dose and total urinary Mg excretion within 24 hours

Discussion

The classical Mg load test has shown to correlate with muscle Mg concentration in Mg-deficient patients (9,11,13-15). The test is based on the fact that the normal kidney regulates the body stores of Mg, excreting an excess in the normal subject and retaining the mineral in deficiency (30). Moreover, rat studies have demonstrated that tissues from Mg-deficient rats had a higher Mg uptake after intraperitoneal injection of [28]Mg than controls (31). The mechanisms by which more Mg is retained in Mg-deficient subjects are unknown, but they may include enhanced renal Mg

reabsorption (32) and repletion of intracellular Mg stores by increased cellular Mg uptake. During periods of Mg deprivation, Mg homeostasis is maintained by increased reabsorption of Mg in the kidney, increased absorption in the gastrointestinal tract and release from internal Mg stores such as bone and skeletal muscle (33). The kidney is the organ that most closely regulates Mg homeostasis by adapting Mg reabsorption in the loop of Henle (34). Numerous human and animal studies have shown that Mg reabsorption in the kidney is increased in Mg deficiency. An animal study using microperfusion and micropuncture techniques has demonstrated a cellular adaptation of Mg transport in the loop of Henle following Mg restriction (35). This adaptation occurred very rapidly (within 5 hours), while plasma Mg did not fall until 20 hours after introduction of the Mg restricted diet. Moreover, the response was specific for Mg because there was no effect on Na and Ca transport. Based on studies with isolated distal cells, it was concluded that Mg transport may be controlled by genes that somehow respond to extracellular Mg by the formation of new transporters or channels (36). However it is not clear, whether decreased plasma Mg concentrations or another mechanism is the trigger for this cellular adaptation. Several depletion studies in man indicate decreased Mg excretion from the start of the depletion period while plasma levels begin decreasing only after several days (37-40).

The Mg load test seems to provide good information on Mg status, but is time-consuming and requires hospitalization of the subjects. Further, the amount of the Mg load, the length of time of infusion, and the length of time of urine collection has not been standardized (6) and it is difficult to relate the %retention to the total body deficit of Mg. On theoretical ground, the fractional retention from a given i.v. Mg load should not be dose- nor duration-dependent provided the load does not exceed the renal threshold for Mg (10). In the classical load test, a high dose is administered to attenuate the effects of dietary Mg intake and basal urinary Mg as much as possible. When using stable isotopes, the influence of the fluctuations of dietary Mg intake and basal urinary Mg is minimized and therefore, the test would be expected to work in a similar way with a small dose. However, no correlation was found between the fractional excretion of the isotopic label and muscle Mg concentration in healthy subjects.

Kinetic studies with radioactive ^{28}Mg have shown that parenterally administered Mg is excreted very slowly by the kidneys, but that the overall balance is maintained by a compensatory excretion of endogenous Mg (41). This exchange of the labeled dose with body Mg implicates that only a small part of the administered dose is excreted within 24 hours. This amount might be too small to allow the detection of a difference between subjects with different muscle Mg concentrations. Moreover, the amount of excreted isotopic label is a function of total Mg excretion, which depends not only on Mg status, but also on the total Mg supplied during the test period (thus including Mg from food) and hormonal variations. Despite standardized meals the amount of total available Mg might have varied between individuals (e.g. because of differences in intestinal absorption).

Muscle Mg concentration is considered as a good indicator for Mg status (16,17) with the disadvantage that the determination is invasive and time-consuming, thus making it unsuitable for ambulatory diagnosis. Various studies show that muscle Mg is lost during Mg deprivation (37,39,42) and an average of 15 % of skeletal-muscle Mg can become available for extracellular buffering (42). However, there is no reference range for muscle Mg concentration. Depending on the method used for analysis, values can vary considerably. When expressing the results as mmol/100 g FFDS there is somewhat more consistency. It is therefore delicate to judge whether all of our subjects had a sufficient Mg status. The range of muscle Mg in our subjects was 3.50 to 4.19 mmol/100 g FFDS. Holm (13) uses the value of 3.48 mmol/100 g FFDS as cut-off for Mg deficiency. According to this, none of our subjects was Mg-deficient. In subjects with normal Mg status, excretion of Mg is not reduced and might thus not correlate with muscle Mg concentration. Similar to our findings, Danielson (43) found no correlation between the %retention of an infused Mg load and muscle Mg in healthy subjects.

In summary, the results of this study indicate that there is no association between muscle Mg concentration and the excretion of a parenterally administered dose of Mg in subjects with apparently normal Mg status. The most likely explanations for the failure of the test are that the range of Mg status in the selected subjects was too narrow, that the injected dose was too small compared to inter-individual variations of excretion, or that the analytical errors in the determination of excretion and muscle Mg were too high to permit clear distinction between individuals. Further studies in

subjects with a wider range of Mg status including clearly Mg-deficient subjects are necessary to obtain more results. Moreover, using a high dose of Mg labeled with an isotope in healthy, non-deficient subjects might provide further information.

Acknowledgments

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CONCLUSIONS AND PERSPECTIVES

Low plasma Mg concentrations were common in type 2 diabetics in Zurich, Switzerland, similar to findings from other countries in Europe and North America. Plasma Mg concentrations of 37.6 % of diabetics were below the reference range, a prevalence of low Mg status that is similar to that reported in type 2 diabetics in outpatient clinics in the US (Nadler & Rude, 1995). However, no correlation between glycemic control and plasma Mg concentration was observed, similar to other reports (Vanroelen *et al.*, 1985; Schlienger *et al.*, 1988; de Valk, 1992). Therefore, hypomagnesemia in type 2 diabetes may be a reflection of insulin resistance rather than being related to metabolic control.

The reasons for Mg deficiency in type 2 diabetes do not appear to include lower intestinal absorption or insufficient dietary intake of Mg. Moreover, in reasonably well-controlled diabetics urinary Mg excretion is not different from controls. However, urinary Mg excretion may be elevated in patients with poor metabolic control, in those using diuretics, and if renal reabsorption is impaired. In poorly controlled patients, glucosuria leads to osmotic diuresis and hypermagnesuria. The use of diuretics, especially loop diuretics, is known to increase the urinary excretion of Mg and other minerals. Mg reabsorption in the loop of Henle was shown to be increased by insulin (Mandon *et al.*, 1993; Dai *et al.*, 1999). The lack of insulin or insulin resistance would therefore lead to impaired reabsorption and increased urinary loss of Mg. Decreased cellular uptake of Mg may also play a role. Insulin stimulates Mg uptake in insulin-sensitive tissues, such as muscle (Lostroh & Krahl, 1973). It appears that the post-receptor defect found in insulin resistance may be responsible for both, reduced glucose and Mg uptake (Hwang *et al.*, 1993). Therefore, if tissue Mg uptake is normally regulated by insulin, impairment of this process by insulin resistance could either cause or exacerbate intracellular Mg deficiency (Alzaid *et al.*, 1995). Future research should be focused on investigating the impairment of cellular Mg uptake in patients with type 2 diabetes, and on approaches to improve cellular Mg uptake.

Because Mg depletion reduces insulin sensitivity and may increase the risk of secondary complications, it may be prudent in clinical practice to periodically monitor

plasma Mg concentrations in diabetic patients. If plasma Mg is low, an intervention to increase dietary intakes of Mg may be beneficial. However, the results of studies in which supplemental Mg was given to type 2 diabetics, have shown equivocal results. Generally, an increase in serum Mg and erythrocyte Mg concentrations was observed, but there was no consistent improvement of glycemic control or diabetes-related complications. It seems that the disorders resulting from a Mg deficit in diabetics are due to intracellular depletion of the ion, which is not controllable by oral supplementation alone. If tissue Mg uptake is decreased due to insulin resistance, the effect of supplementation may be limited due to low availability of the absorbed supplemental Mg to the cells. Therefore, in future supplementation studies, it would be essential to monitor intracellular Mg, preferably in an insulin-sensitive and Mg-rich tissue such as skeletal muscle.

Mg deficiency may be both a cause and a consequence of increasing insulin resistance. A greater benefit of Mg supplementation may therefore lie in subjects with impaired glucose tolerance or beginning type 2 diabetes, in which improving insulin sensitivity by increasing Mg intake may retard or attenuate the disease. Moreover, low Mg intake may play a role in the development of diabetes and insulin resistance (Colditz *et al.*, 1992; Humphries *et al.*, 1999). Therefore, high dietary Mg intake may contribute to the prevention of type 2 diabetes. Foods such as whole-grain cereals, leafy vegetables and legumes are especially rich in Mg, and have additional benefits on health issues due to their high density in dietary fiber, vitamins and minerals.

Determination of Mg status remains problematic. Preliminary experiments with the modified Mg retention test did not produce the expected results. The most likely explanations for the failure of the test are that either the range of Mg status in the subjects was too narrow, that the injected dose was too small, or that the analytical errors in the determination of excretion and muscle Mg were too high to permit clear distinction between individuals. Further studies with a wider range of Mg status including clearly Mg-deficient subjects would be necessary to obtain more results. Moreover, using a high dose of Mg labeled with an isotope in healthy, non-deficient subjects might provide further information. Because Mg is principally an intracellular cation and therefore difficult to assess, the use of physiologic assessment tests seems promising. A new in vitro blood load test has recently been developed at the

University of Auvergne based on the same hypothesis that cellular Mg uptake is increased in Mg deficiency (Feillet-Coudray *et al.*, 2003).

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