

## Update on the assessment of magnesium status

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Magnesium (Mg) is the fourth most abundant mineral in the body and the most abundant intracellular divalent cation, with essential roles in many physiological functions. Consequently, the assessment of Mg status is important for the study of diseases associated with chronic deficiency. In spite of intense research activities there is still no simple, rapid, and accurate laboratory test to determine total body Mg status in humans. However, serum Mg <0.75 mmol/l is a useful measurement for severe deficiency, and for values between 0.75 and 0.85 mmol/l a loading test can identify deficient subjects. The loading test seems to be the gold standard for Mg status, but is unsuitable in patients with disturbed kidney and intestinal functions when administered orally. There is also a need to reach a consensus on a standardized protocol in order to compare results obtained in different clinical units. Other cellular Mg measurements, such as total or ionized Mg, frequently disagree and more research and systematic evaluations are needed. Muscle Mg appears to be a good marker, but biopsies limit its usefulness, as is the case with bone Mg, the most important but heterogeneous Mg compartment. The development of new and non invasive techniques such as nuclear magnetic resonance (NMR) may provide valuable tools for routinely analysing ionized Mg in tissues. With the development of molecular genetics techniques, the recent discovery of Transient Receptor Potential Melastatin channels offers new possibilities for the sensitive and rapid evaluation of Mg status in humans.

**Loading test: Serum: Erythrocyte: Lymphocytes: Muscle: Bone: Ionized Mg**

Magnesium (Mg) is the fourth most abundant mineral in the body, and the most abundant intracellular divalent cation, and is essential for a diverse range of physiological functions. Mg deficiency, either from inadequate intake, excess excretion or altered homeostasis, is often suspected to be associated with the initiation of many symptoms and diseases<sup>(1)</sup>. In spite of its multiple and ubiquitous roles, Mg status can be assessed in severe deficiency and a large number of studies have investigated various markers during nutritionally induced or pathological mild and chronic deficiency. A review published in 1991 stated that the assessment of Mg status was difficult as there was no simple, rapid and accurate test to indicate total body Mg status<sup>(2)</sup>. More recently, another review indicated that whilst functional and/or biological markers are available for many nutrients, there is still a need for indicators to specifically diagnose Mg deficiency<sup>(3)</sup>. Another difficulty associated with Mg is related to its metabolism. It is known that the equilibrium and exchange of Mg between body compartments and tissue pools occurs slowly, so determining Mg concentration in one tissue may not provide information about Mg status in another. This review provides the most up-to-date information on the assessment of Mg status.

### Metabolism

The metabolism of Mg and its body distribution have been investigated in animals and humans using the radioactive

isotope  $^{28}\text{Mg}^{(4,5)}$  but its use is limited by the short half-life of 21 hours, and for human studies by exposure to radiation. Thus,  $^{25}\text{Mg}$  and  $^{26}\text{Mg}$  stable isotopes have been used in human to accurately assess Mg absorption, excretion, bioavailability, pool sizes, and turnover. However, the relatively high abundance of the isotopes used (10.13 % for  $^{25}\text{Mg}$  and 11.17 % for  $^{26}\text{Mg}$ ) imposes the administration of doses of Mg for analytical precision that may be absorbed and eliminated dose dependently. The mean biological half-life of Mg has been estimated to be between 41 and 181 days<sup>(6,7)</sup>. These values are consistent with the observation that it takes 3 months to normalize a 20 % depletion of body Mg stores<sup>(8)</sup>.

### Compartments

Observations from stable isotope studies in children and adolescents indicate that it is not possible to directly relate Mg deficiency to changes in the exchangeable pool size or pool turnover<sup>(9)</sup>. In adults, compartmental analysis has shown that only 25 % of total body Mg can be studied using stable isotopes, i.e. that which rapidly exchanges between the plasma compartment and two extra-plasma pools. Consequently, the majority of total body Mg escapes this analysis and is transferred to long-term storage pools, particularly bone<sup>(10)</sup> where approximately half the body's Mg content (1 mol) is found. Less than 1 % of total body Mg is present in blood, with approximately 0.3 % in serum<sup>(2)</sup>.

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## Biomarkers and tests for status assessment

### Serum

Blood samples must be prepared carefully to prevent Mg contamination with anticoagulant, and generally blood concentrations are determined in serum rather than plasma. Haemolysis can increase the Mg concentration because erythrocytes contain more Mg than serum. In addition to sample preservation, a survey of the quality of the measurements of routine laboratories has been performed and it was concluded that these sources of error are significant and may reduce the diagnostic potential of serum total Mg<sup>(11)</sup>.

Serum Mg concentrations are dependent on dietary intake and intestinal absorption as well as kidney function. Kidney filtration and reabsorption are essential to maintain stable serum levels between 0.75 and 0.96 mmol/l, a range observed for healthy adult subjects. Serum Mg concentration is the most frequently performed analysis, and whilst some studies have found correlations between serum and tissues values<sup>(12,13)</sup> others have not<sup>(14–16)</sup>. Bone is the primary storage site for both calcium and Mg, and provides a labile pool for the release of Mg to maintain serum concentrations. Decreases in serum Mg can be observed when drugs are taken, particularly diuretics. Otherwise, lower serum Mg values indicate deficiency and impaired metabolic control as observed in diabetes, renal tubular disorders, alcoholism and malabsorption. By comparison, higher serum values are observed when subjects take Mg medication such as Mg-rich antacids or cathartics<sup>(17)</sup> or in the case of renal failure. In conclusion, serum values lower or higher than the 0.75–0.96 mmol/l range require diagnosis and treatment; but values in the normal range also do not rule out the possibility of total body deficit compensated for by the release of Mg from the bone pool.

**Diabetes and obesity.** Hypomagnesaemia has been reported in type 2 diabetes but after 3-months of treatment with 30 mmol/day given orally, the values increased from  $0.73 \pm 0.8$  to  $0.81 \pm 0.1$  mmol/l, equivalent to control group concentrations<sup>(18)</sup>. A comparison of serum Mg concentrations in 109 type 2 diabetics with 156 age- and sex-matched healthy controls in Switzerland showed significantly lower values for the diabetic subjects,  $0.77 \pm 0.08$  and  $0.83 \pm 0.07$  mmol/l, respectively. Serum Mg concentrations were below the normal reference range in 37.6% of the diabetic patients and 10.9% of the control subjects ( $P < 0.001$ ). This study highlights the fact that serum Mg concentrations indicate that poor Mg status is common in type 2 diabetics in Switzerland<sup>(19)</sup>.

In obese patients, a significant negative correlation was found between waist-to-hip ratios and serum Mg concentrations, whilst no such correlation was observed for either erythrocyte or platelet Mg<sup>(20)</sup>.

**Heart disease.** In patients with end-stage heart disease and Mg stores highly depleted, an intravenous administration of 64 mmol Mg significantly increased serum ( $0.68 \pm 0.06$  to  $0.82 \pm 0.3$  mmol/l), erythrocyte ( $1.44 \pm 0.3$  to  $1.75 \pm 0.4$  mmol/l) and lymphocyte Mg ( $1.23 \pm 0.7$  to  $1.52 \pm 0.8$   $\mu\text{g}/\text{mg}$  of protein) and also total urinary Mg excretion ( $3.4 \pm 1.27$  to  $17.5 \pm 8.1$  mmol). These changes observed after the 24-hour infusion of Mg ascorbate were all highly significant<sup>(21)</sup>.

**Crohn's disease.** After intravenous infusion of 60 mmol Mg to subjects with Crohn's disease, a significant increase in serum and mononuclear cell Mg concentrations was observed in addition to an increased muscle and body retention<sup>(22)</sup>.

**Asthma.** Mg status has been evaluated in healthy subjects and those with mild to moderate asthma. Measurements included total and ionized serum and erythrocyte Mg and the retention of an intravenous Mg load. The results showed that total serum Mg offers a useful clinical diagnostic tool and that ionized Mg is closely correlated but does not offer any diagnostic advantages<sup>(23)</sup>.

In conclusion, serum analysis is useful for the determination of Mg status in deficient subjects with pathologies leading to increased urinary excretion. In patients seen for routine medical care at an urban family centre, there was a 20% overall prevalence of hypomagnesaemia among this predominantly female African American population<sup>(24)</sup>. However, this observation did not rule out a higher prevalence of deficiency which could only be characterized by the measurement of low tissue Mg.

## Blood cells

### Erythrocytes

Normal erythrocytes contain a high concentration of Mg ions that are essential for ATP function and other metabolic processes<sup>(25)</sup>. The Mg content of erythrocytes has been shown to decrease in humans provided with low Mg diets. These changes were only observed after several weeks of low dietary intake, the delay being attributed to the erythrocyte pool which reflects long-term rather than current nutrient status<sup>(26)</sup>.

**Diabetes and obesity.** In non-insulin type 2 diabetics, both serum and intracellular ionized erythrocyte Mg concentration was significantly lower compared with non diabetic control subjects. Oral supplementation for 8 weeks with 400 mg/d Mg restored erythrocyte concentrations to normal values without changing serum concentrations<sup>(27)</sup>. Lower values of Mg in erythrocytes have also been reported in type 1 diabetics compared with control subjects ( $1.41 \pm 0.56$  v.  $2.94 \pm 1.12$  mmol/l, respectively), while serum concentrations were similar and urinary Mg excretion significantly elevated in the diabetic group<sup>(28)</sup>. A one year oral Mg supplementation study, with a daily dose of 13 mmol (300 mg) as Mg gluconate, in type 1 diabetic patients characterized by low erythrocyte Mg ( $< 2.3$  mmol/l) significantly increased these values from  $2.03 \pm 0.03$  to  $2.48 \pm 0.12$  mmol/l while serum Mg levels did not change:  $0.73$  and  $0.77$  mmol/l<sup>(29)</sup>. A recent study performed on diabetic children reports that erythrocyte Mg levels showed an inverse correlation with percentage of retained Mg load. Although erythrocyte and serum Mg were significantly lower in diabetic children compared with controls, serum Mg concentrations were in the normal range in both groups. The authors suggest that erythrocyte Mg measurement is preferred to serum Mg and that the load test is a reliable and sensitive method<sup>(30)</sup>.

In a group of normotensive obese patients, erythrocyte and platelet Mg concentrations, but not serum, were significantly lower than in healthy controls<sup>(20)</sup>.

**Migraine.** Total Mg levels in plasma, erythrocytes and lymphocytes were analyzed in a group of 29 migraine patients

and 18 control subjects. At baseline, results showed significantly lower concentrations of total erythrocyte Mg in migraine patients compared with controls ( $50.7 \pm 4.7$  v.  $52.9 \pm 4.7$  mg/l). Migraine patients then received a daily supplement of 11 mineral water containing 110 mg/l Mg for 2 weeks, which resulted in a significant increase in erythrocyte Mg concentrations from  $50.7 \pm 4.7$  to  $52.9 \pm 4.7$  mg/l with no observed effect on plasma Mg<sup>(31)</sup>.

**Chronic fatigue syndrome.** Shorter-term effects of intramuscular Mg administration given every week for 6 weeks to patients with chronic fatigue syndrome showed a significant increase in erythrocyte Mg concentrations ( $+0.57 \pm 0.192$  mmol/l). This study raises an important methodological problem. The erythrocyte Mg concentrations were different in two groups of patients with chronic fatigue syndrome, 1.29 and 1.60 mmol/l, whilst a normal range of 1.41–2.09 mmol/l have been suggested. This important difference was explained by the fact that the measurements were undertaken in different laboratories and under different conditions<sup>(32)</sup>. Although it is known that Mg slowly leaches out of red cells into the plasma, the length of sample storage time could not explain the differences observed.

**Critical illness.** From a study in critically ill postoperative patients and healthy controls, the best Mg parameter to measure hypo- or hypermagnesaemia was ionized Mg in erythrocytes when compared with total erythrocyte Mg and total ionized serum Mg. The prevalence of hypomagnesaemia was 15.9% from the measurement of total serum Mg, 22.2% from ionized serum Mg and 36.5% from ionized Mg in erythrocytes, a level almost twice as high as that observed in total or ionized serum Mg<sup>(33)</sup>.

**Asthma.** Since low Mg intake has been associated with airway hyper-responsiveness, a study was undertaken in 49 asthmatic patients in which Mg in erythrocytes, serum and urine was measured. The results showed lower erythrocyte and urine Mg as compared with healthy controls whereas serum concentrations did not differ<sup>(15)</sup>.

**Healthy subjects and genetic control.** It has been observed that intra-individual variations in plasma and red blood cell Mg concentrations over long time periods are small when compared with inter-individual variations. The analysis of family resemblance for Mg concentrations in serum and erythrocytes, based on data from nuclear families and twins showed that while adult plasma Mg varies linearly with age, erythrocyte Mg shows a non-linear trend: quadratic for males and fifth degree polynomial for females. Univariate and bivariate model analyses of these results strongly suggested that genetic factors were primarily responsible for the observed family resemblance and that one common genetic factor alone could not explain all the correlations<sup>(34)</sup>. Further analysis under a mixed model yielded significant support for a major gene effect on erythrocyte Mg, but not on plasma Mg. Parameter estimates indicated that the data are compatible with a common major gene for elevated erythrocyte Mg. About 5% of the population appeared homozygous for this gene and nonfamilial factors account for a small fraction of the total variance<sup>(35)</sup>. Genetic factors controlling intra- and extracellular Mg levels were shown amongst unrelated adult male blood donors to be composed of at least three components: the major histocompatibility complex (HLA and H-2)-associated genes, the non-major histocompatibility complex genes, and tissue

factors modulating the respective importance of the first two sets of factors<sup>(36)</sup>.

Studies performed on mice demonstrated that animals selected for low and high Mg levels exhibited significant different total ( $25.1$  v.  $18.0$  mg/l) and ionized ( $11.8$  v.  $9.1$  mg/l) plasma Mg, total erythrocyte Mg ( $54.5$  v.  $40.7$  mg/l) and decreased tibia ( $4.45$  v.  $3.56$  mg/g) and kidney ( $955$  v.  $869$  mg/kg) Mg concentrations. They also had a higher urinary excretion and changes in the size and exchange rates of other compartmental pools. The population examined were the second generation between 4 inbred strains, and pairs with the highest and the lowest erythrocyte Mg concentrations were selected for reproduction. After the same selection for 18 consecutive generations, erythrocyte Mg values diverged rapidly and regularly and remain constant. These two strains were homozygote for all the relevant alleles<sup>(37,38)</sup>. It was also shown that the genetic impact was not only observed with erythrocytes but also affects total body Mg metabolism<sup>(37)</sup>. Today, with the discovery and progress in transient receptor potential-melastatin (TRPM) research, we anticipate that a better understanding of the genetic expression at the cellular and tissue levels of these channels controlling epithelial Mg transport will explain the differences observed in Mg homeostasis.

In spite of this genetic regulation, the Mg content of erythrocytes has been studied as an index of Mg status in 20 healthy women with erythrocyte Mg concentrations below the 15th percentile ( $\leq 1.97$  mmol/l). Supplementation of 250 mg/day Mg for 3 weeks resulted in an increase in erythrocyte Mg concentration of only 1.6% while plasma Mg level was significantly increased by 5.3%. The authors concluded that erythrocyte Mg is not a useful measurement for monitoring the effect of Mg supplementation in individuals<sup>(39)</sup>. This opinion is shared by others<sup>(40)</sup> although it has been reported that a 10% lower erythrocyte Mg concentration in adults with marginal deficiency was restored to normal values after 2 weeks of supplementation with 15.6 mmol Mg (360 mg) as Mg pyrrolidone carboxylate<sup>(41)</sup>.

**Low Mg diet in healthy subjects.** The effect of a low Mg intake (112 mg/day) for 92 days preceded and followed by control periods of 35 and 49 days with a daily supplement of 200 mg showed a significant decrease of Mg in erythrocytes and muscle and a higher Mg retention<sup>(16)</sup>. These study protocols with induced deficiency and recovery in healthy subjects are effective to test both kinetic and quantitative changes in Mg cell and tissue pools as well as in urine. With further data, these intervention studies will allow the validation of status biomarkers by demonstrating their sensitivity and specificity for measuring Mg status.

#### *Leukocytes*

In both animal and human studies, the Mg content of white blood cells such as lymphocytes was shown to be a better index of intracellular Mg in skeletal and cardiac muscle<sup>(42,43)</sup>. It was even stated<sup>(44)</sup> that lymphocytes have advantages over other tissues, such as erythrocytes and muscle, for assessing intracellular Mg because during experiments on Mg deficient rats, the magnitude of the Mg loss from lymphocytes was similar to that of cardiac and skeletal muscle.

**Diabetes and obesity.** Mg was analyzed in plasma, mononuclear cells, erythrocytes, urine and in muscle biopsies from

25 subjects with type 1 diabetes and the results were compared with those of 28 healthy controls. Mg in mononuclear cells was suggested to be an index of intracellular Mg and a significant correlation between muscle and mononuclear cells was reported in patients<sup>(45)</sup>.

**Crohn's disease.** After intravenous infusion of 60 mmol Mg to 30 subjects with Crohn's disease, a significant increase in the Mg concentrations in mononuclear cells (65–94 % lymphocytes, 5–30 % monocytes, 0–3 % basophilic cells and 0–1 % granulocytes) was observed along with increases in plasma, muscle and an increased body retention of Mg. However, the authors concluded that the analysis of muscle Mg and an estimation of Mg retention during an intravenous infusion are superior markers for confirming suspected Mg deficiency<sup>(22)</sup>.

**Migraine.** In migraine patients, mononuclear blood cell Mg concentrations were significantly lower than in controls subjects ( $8.52 \pm 3.64$ ,  $7.93 \pm 2.84$  and  $10.6 \pm 3.38$  mg/d DNA, respectively)<sup>(46)</sup>. Total Mg levels in plasma, lymphocytes and erythrocytes and ionized Mg in lymphocytes were analyzed in a group of 29 migraine patients and 18 control subjects. Results showed significantly lower concentrations of ionized lymphocyte Mg ( $12.0 \pm 3.5$  v.  $14.2 \pm 3.8$  mg/l) and total Mg in erythrocytes ( $50.7 \pm 4.7$  v.  $53.5 \pm 2.9$  mg/l) in migraine patients compared with controls. After a 2 week daily supplementation with 1 l of mineral water containing 110 mg/l Mg, a significant increase in all intracellular Mg concentrations with no effect on plasma Mg was observed in migraine patients. Among the analyzed parameters, ionized lymphocyte Mg appeared to be the most sensitive index of Mg deficiency with a 15 % decrease in migraine patients when compared with controls and a 16 % increase after 2 weeks of a Mg-rich mineral water intake<sup>(31)</sup>.

**Hypertension and heart disease.** Mg content in lymphocytes and skeletal muscle biopsies from 28 subjects demonstrated no significant correlation between these values except in a group of three normal volunteers and nine patients with mild arterial hypertension<sup>(47)</sup>. Another study showed that total intracellular Mg content was significantly lower in lymphocytes from hypertensive patients compared with healthy subjects ( $0.07 \pm 0.03$  v.  $0.11 \pm 0.04$  mmol/g protein) while serum and erythrocyte Mg and ionized platelet Mg<sup>(48)</sup> were not significantly different.

Patients with congestive heart failure experienced cardiac arrhythmias due to digitalis toxicity. Although serum Mg concentrations were within normal ranges lymphocyte content was decreased, suggesting the existence of cellular Mg depletion. Intravenous bolus administration of Mg sulphate, followed by intramuscular Mg repletion, abolished the digitalis-toxic arrhythmia<sup>(49)</sup>.

**Critical illness.** Because the Mg content of mononuclear blood cells was suggested to be a better index of Mg status than serum concentrations, these measurements were performed in critically ill patients who were either moderately or severely hypomagnesaemia ( $\geq 0.4$  to  $\leq 0.6$  mmol/l and  $\leq 0.4$  mmol/l, respectively) and receiving a 24-hour intravenous Mg replacement therapy (0.5 and 0.75 mmol/kg of intravenous Mg sulfate, respectively). Serum concentrations increased significantly from baseline to 48 h ( $0.5 \pm 0.1$  to  $0.8 \pm 0.2$  mmol/l) while intracellular Mg content did not change significantly within the study period ( $2.6 \pm 1.0$  to

$3.0 \pm 1.3$  fmol/cell). In this group of trauma patients from an Intensive Care Unit, serum Mg was a better index of Mg status than mononuclear blood cell Mg<sup>(50)</sup>.

**Healthy subjects.** Although Mg deficiency has been diagnosed using low Mg levels in leucocytes, their concentration varies according to the pathology and the study population<sup>(40)</sup>. Some studies do not find a correlation between mononuclear blood cells, such as monocytes and lymphocytes, and serum or erythrocyte Mg. A 2-fold larger inter-individual coefficient of variation for the Mg content of mononuclear blood cells than for serum and erythrocytes introduced by the separation step and washing of the cells may explain the lack of correlation<sup>(51)</sup>.

#### Platelets

**Diabetes, obesity and hypertension.** The concentration of Mg in platelets has been measured in two groups of normotensive and hypertensive type 2 diabetic patients and in healthy subjects. Plasma and erythrocyte Mg concentrations were significantly lower in the diabetic patients. The concentrations of Mg in platelets were lower in patients but the difference was significant only for the the hypertensive diabetics<sup>(52)</sup>. Later, this research group showed that both microalbuminuria and proteinuria in type 1 diabetic patients was associated with altered Mg homeostasis and a negative correlation was found between glycated haemoglobin and both plasma and platelet Mg<sup>(53)</sup>. These studies do not demonstrate that platelets are a more sensitive and specific marker of Mg status. However, in another study performed on obese normo- or hypertensive patients, platelet Mg was significantly reduced compared with controls. However, other parameters were also significantly decreased such as Mg in plasma and in erythrocytes. The conclusion of the authors<sup>(20)</sup> was that intra platelet Mg assay was more reliable than the dosage of ionize Mg by NMR. Although the study did not investigate platelets, ionized Mg in erythrocytes showed similar but less striking changes in normotensive and hypertensive obese subjects when compared with non-insulin-dependent diabetes<sup>(54)</sup>.

#### Buccal cells

In a study investigating the effects of various Mg levels in drinking water in healthy subjects, Mg was determined in muscle biopsies and in sublingual mucous membrane cells. A negative correlation was found between muscle and sublingual cell Mg suggesting that these cells cannot be used to evaluate intracellular Mg status<sup>(55)</sup>. More studies are needed to evaluate whether these cells, which require non-invasive collection procedures, can provide valuable information. Contamination from Mg in saliva or the salivary microbiota and previously ingested beverages or water can affect the results and must be controlled.

#### Tissues

##### Bone and teeth

There are more experimental studies in animals than in humans on the effects of Mg deficient diets on bone. Rats fed a diet providing a surfeit of Mg and 2 others diets resulted



in two degrees of Mg deficiency. While the recommended Mg dose is 40 mg/100 g dry diet, the control group of this study received 150 mg/100 g and the two groups with deficiency received either a diet without Mg for one week or 5 mg/100 g for 2 weeks. There was no consistent difference between the Mg concentrations found in liver, heart, or skeletal muscle of Mg-deficient and control rats, but bone accurately reflected the level of dietary Mg. However, there was a significant difference between the Mg concentration of the anterior and posterior halves of the ribs, indicating irregular distribution of Mg within the bone. There were also significant differences in the Mg concentration of different bones from the same animals. Therefore one entire bone, such as the sternum or the rib, should be analysed<sup>(56)</sup>. Weanling rats fed six levels of dietary Mg, ranging from 0 to 150 mg/100 g purified diet, showed a linear decrease in Mg retention with increased bone Mg. As a negative relationship was found between Mg retention following the load test and the level of dietary Mg, the load test appears to be an acceptable means of indirectly assessing Mg status provided there is normal renal and cardiovascular status and normal water balance<sup>(57)</sup>. In a more recent study, it was observed that mice selected for their low Mg status had reduced total and ionized plasma Mg, and lower erythrocyte, tibia and kidney Mg levels<sup>(37)</sup>.

A study on a 2-month-old boy with congenital hypomagnesaemia has been published in which Mg concentrations were measured at 8 and 12 years of age in milk teeth lost naturally and the values compared with those of healthy control children<sup>(58)</sup>. A significantly lower Mg content was found in teeth, 4.80 v.  $6.82 \pm 0.77$  mg/g dry weight and 4.12 v.  $6.18 \pm$  mg/g dry weight at 8 and 12 years old respectively compared with controls. However, a greater significant difference was observed at 12 years for serum ( $0.60 \pm 0.02$  v.  $0.86 \pm 0.02$  mmol/l), erythrocyte ( $1.55 \pm 0.04$  v.  $2.20 \pm 0.15$  mmol/l), and lymphocyte Mg ( $1.42 \pm 0.14$  v.  $3.72 \pm 0.41$  fmol/cell). Similar differences were found at 8 years of age demonstrating that dental Mg is potentially interesting for the evaluation of calcified tissues<sup>(58)</sup>. In adult subjects, Mg concentrations in serum and bone were significantly reduced in a patient with chronic hypomagnesaemia. However, as reported previously in animals, Mg in bone is not homogeneously distributed and the values measured are dependent on sampling. It was shown that 30 % of bone Mg is in a surface limited pool present either within the hydration shell or on the crystal surface. The larger fraction of bone Mg was shown not to be associated with bone matrix but rather to be an integral part of the bone crystal. The authors concluded that the major factor determining Mg concentration in bone would appear to be the serum Mg level<sup>(59)</sup>. In a study of the same group where muscle, erythrocyte and bone Mg were measured in patients with reduced, normal and increased Mg levels, a highly significant correlation between serum and bone Mg was reported. The authors' conclusion was that bone Mg in man increased during Mg excess and decreased during Mg depletion<sup>(12)</sup>. The invasive sampling and the heterogeneous distribution of Mg in bone are the reasons why there is limited knowledge on the metabolism of Mg in bone.

### Muscle

A quarter of total body Mg is located in muscle. This tissue is a significant compartment and seems appropriate to assess Mg

status, however, the limited number of studies is explained by the need to perform invasive biopsies.

**Heart disease.** The Mg retention test used together with muscle biopsies in 5 patients with acute myocardial infarction (MI) and 6 healthy controls demonstrated a significantly higher mean retention of 42 % in the MI group compared with 22 % in the control group. A lower muscle content of Mg in the MI group supported the results of the retention test and indicated Mg deficiency<sup>(60)</sup>.

**Crohn's disease.** Mg status was evaluated in 30 subjects with Crohn's disease and 30 healthy controls. Subjects with Crohn's disease had significantly lower concentrations of Mg in muscle, mononuclear cells, and 24 h urine collections compared with controls. A significant increase in muscle, mononuclear cells, and plasma Mg concentrations was observed following intravenous infusion of 60 mmol Mg into the Crohn's disease patients. The retention of the infused Mg was significantly higher in subjects with Crohn's disease than in 11 healthy controls and was inversely correlated with muscle Mg content. The most sensitive measurements of Mg status and deficiency were the analysis of Mg in muscle and the estimation of Mg retention<sup>(22)</sup>.

**Lung disease.** Muscle biopsies and serum samples have been taken in patients with chronic obstructive pulmonary disease and acute respiratory failure and Mg measurements showed that 10 % of the patients had hypomagnesaemia ( $<0.7$  mmol/l) with normal muscle values (44 mmol/kg of fat-free solids), whereas low muscle values were found in 47 % of patients with normal serum Mg levels. As no significant correlation was observed between serum and muscle Mg, the authors concluded that serum Mg levels are of little value in the diagnosis of intracellular Mg deficits<sup>(61)</sup>.

**Asthma.** Skeletal muscle biopsies have been taken in asthmatics with and without oral  $\beta_2$ -agonists and Mg concentrations compared with healthy subjects. Muscle Mg was lower in the asthmatics both with and without oral  $\beta_2$ -agonists ( $3.62 \pm 0.69$  and  $3.43 \pm 0.60$  v.  $4.43 \pm 0.74$  mmol/100 g, respectively) while serum Mg was not different with the controls<sup>(62)</sup>.

**Low Mg diet in healthy subjects.** A more recent study compared the effect of a low Mg intake (112 mg/day) for 92 days preceded by a control period of 35 days where they received a daily supplement of 200 mg and followed by the same supplementation for 49 days. During the restriction periods skeletal muscle Mg decreased significantly ( $53.4 \pm 1.2$  and  $51.6 \pm 1.3$  v.  $48.1 \pm 1.3$  mmol/kg dry weight), as did erythrocyte Mg ( $6.74 \pm 0.08$  and  $6.68 \pm 0.08$  v.  $5.91 \pm 0.07$   $\mu$ mol/g haemoglobin) and Mg retention from Mg balance ( $+32$  and  $+38$  v.  $-42$  mg/day). There was a non-significant decrease of serum Mg during the restriction period confirming the lack of sensitivity of this measurement<sup>(16)</sup>.

**Healthy subjects.** Measurements of muscle Mg were undertaken in biopsies taken from 49 individuals living in two Swedish cities with 5.7 and 1.7 mg Mg/litre in the local drinking water. There were significantly higher skeletal muscle Mg concentrations ( $4.1 \pm 0.2$  v.  $3.9 \pm 0.3$  mmol/100 g fat-free dry weight) in subjects living in the area with the higher water Mg content. Dietary Mg intakes obtained from questionnaires were similar in the two groups, suggesting that muscle Mg content is a sensitive marker of Mg status<sup>(63)</sup>. However, another study from this research group did not

confirm these results<sup>(55)</sup>. No correlation between muscle and erythrocyte Mg was reported<sup>(64)</sup>. However, overall these results suggest that measurements of muscle Mg may be considered to be a reliable indicator of Mg status<sup>(65)</sup>.

In conclusion, more research is needed but muscle biopsies cannot provide a non-invasive method to assess Mg status.

#### Total, ionized Mg

Although Mg is either bound, in particular to protein, or free/ionized, only total Mg was able to be measured before ion-selective electrodes, fluorescent probes or nuclear magnetic resonance (NMR) were developed to identify and quantify this biological active form. Measurements of ionized Mg concentration were first undertaken in the 1970s on the axoplasm of squid axons<sup>(66)</sup> and then on muscle<sup>(67)</sup> and biological fluid<sup>(68)</sup>. Errors resulting from changes in electrolyte composition, electrolyte interactions and interferences in the electrode response were investigated at that time<sup>(69)</sup>. Many studies and publications only deal with the intra-method differences<sup>(70,71)</sup> in results, and errors in the analysis of ionized Mg<sup>(72–75)</sup>.

**Diabetes.** Low ionized Mg defined as serum concentrations lower than 0.46 mmol/l has been shown to be highly prevalent in diabetic subjects<sup>(76)</sup>.

**Kidney disease.** In haemodialysis patients, the values of ionized and total Mg in serum and mononuclear blood cells and total Mg in erythrocytes were shown to be significantly increased compared with a control population<sup>(77)</sup>. However, total serum Mg was not increased. In these patients, the two ionized Mg markers did not offer any advantages and total Mg concentration in serum remains the measurement of choice.

**Critical illness.** In critically ill children, ionized serum Mg was significantly lower than in a healthy group of children ( $0.37 \pm 0.10$  v.  $0.46 \pm 0.03$  mmol/l). Many critically ill children exhibit ionized hypomagnesaemia with normal total serum Mg concentrations and these children would not be recognized as Mg-deficient based on routine total serum Mg tests<sup>(14)</sup>.

**Healthy subjects.** In a study of healthy volunteers, the mean whole blood ionized Mg concentration was 0.52 mmol/l with a range of 0.44 to 0.59 mmol/l and an ionized Mg/total serum Mg ratio of 0.60 and a range of 0.50–0.69<sup>(78)</sup>. A positive correlation between ionized and serum total Mg was observed in 160 healthy children and the ratio was  $58.3 \pm 4.1\%$ <sup>(79)</sup>. There appears to be no demonstrable advantage to measuring ionized Mg as opposed to total Mg for evaluating Mg status.

#### Fluorescent probes

Fluorescent probes have made it possible to measure cytosolic free Mg using a two-excitation wavelength fluorometer. The probe penetrates the plasma membrane as an ester that is hydrolyzed in the cytosol, and a microscope connected to the fluorometer is used to measure the fluorescence ratio at two excitation wavelengths in individual cells. The probe Mag-Furan-2 has been mainly used for measurements in platelets, whilst; Mag-Indo-1 has been used to assess cytosolic free Mg in mononuclear blood cells and erythrocytes. When fluorescent probes are used in *ex vivo* samples, the

conservation of the sample is of major importance and validation of the method is necessary. Mag-Indo-1 has been used to determine the concentration of ionized Mg in lymphocytes of 29 migraine patients and 18 control subjects. The stability of lymphocytes in blood collected in either sodium citrate or heparin, during the isolation and staining process as well as the homogenous fluorescence distribution were tested to evaluate the reproducibility of the measurements. While there was no difference for total Mg in lymphocytes, significantly lower values were found for ionized Mg in migraine patients compared to controls, and after a 2 week Mg supplementation with 7.4–12 mmol Mg/d from mineral water (170–275 mg/d) the concentrations of ionized Mg in lymphocytes of migraine patients increased to those of the control subjects. In this study ionized intracellular Mg was a sensitive index to detect a deficit and the effect of a supplementation<sup>(31)</sup>. Using the same fluorescent probe, the effect of Mg supplementation on healthy volunteers showed a significant increase in ionized Mg in lymphocytes after 2 days with unchanged total Mg in lymphocytes. After 4 days supplementation, ionized Mg returned to the initial value while total Mg concentrations in lymphocytes increased significantly<sup>(80)</sup>. Intracellular ionized Mg concentrations have been measured in platelets using the fluorescent probe Fura-2<sup>(69)</sup>.

Another promising technique is nuclear magnetic resonance (NMR). After blood sample measurements, the next consideration is non-invasive analysis of superficial body tissues, such as the skin and muscle. After the first determination of ionized Mg concentrations in human erythrocytes by <sup>31</sup>P NMR spectroscopy<sup>(81)</sup>, this technique was applied to measurements of intracellular erythrocyte Mg adenosine triphosphate and free Mg<sup>(25)</sup>. Ionized Mg has also been analyzed in human blood plasma using <sup>31</sup>P magnetic resonance spectroscopy with the addition of a ligand so that free and bound Mg have different resonances. The results showed that the magnetic resonance spectroscopy methods gave higher values for free ionized Mg than values obtained by ion-selective electrodes<sup>(82)</sup>. With *in vivo* <sup>31</sup>P NMR spectroscopy, intracellular free Mg in skeletal muscle and brain tissues was studied in 30 young volunteers after one month of daily supplementation with 12 mmol Mg ( $0.62 \pm 0.05$  v.  $0.71 \pm 0.03$  mmol/l, respectively). Only urinary excretion was increased and the distribution of Mg in brain tissue, and muscle and also in serum and erythrocytes was unchanged in these healthy young subjects apparently without Mg deficiency<sup>(83)</sup>. This non-invasive technique had previously demonstrated similar intracellular free Mg concentrations in skeletal muscle and brain tissues, approximately 0.3 mmol<sup>(84)</sup>. Skeletal muscle ionized Mg was measured with NMR in women over the course of one complete menstrual cycle. There was no evidence of a menstrual cycle effect on muscle ionized Mg or total Mg in serum, erythrocytes and mononuclear blood cells<sup>(85)</sup>. NMR brain and muscle *in vivo* measurement in children with migraine showed that brain intracellular ionized Mg concentrations were reduced by 25% in patients;  $0.139 \pm 10$  v.  $0.186 \pm 26$  mmol, respectively<sup>(86)</sup>. Recently, a noninvasive intracellular technique for ionized Mg measurements has been developed using energy dispersive X-ray microanalysis<sup>(87)</sup>.

In conclusion, these non-invasive methods are still in being developed and are not available to clinical laboratories, but in

the future they may become the most efficient and accurate way to routinely measure intracellular ionized Mg.

## Hair and nails

### Hair

The potential use of hair to assess Mg status is an attractive idea as it is the least invasive sampling procedure, and samples can be taken over a long period of time and can be stored until the analysis can be performed.

**Cattle and animal studies.** It has been reported that cattle suffering from grass tetany have blood serum Mg concentrations below 10 mg/litre compared with a mean normal value of 21 mg/l<sup>(88)</sup>; a higher Mg content was found in the hair of cattle when diet was supplemented with Mg. However, no difference was found in hair analyzed five times during the year (to determine seasonal effects) or in the Mg content of hair from cows with grass tetany<sup>(89)</sup>. In rats fed diets containing either 82 or 17 mg/100 g, significant changes in the Mg contents of various hair fractions were observed after 2 or 3 months<sup>(90)</sup>.

**Methods.** As hair can be contaminated by the environment, it is necessary to wash it before analysis. The effects of washing hair on its Mg content have been studied. The methods selected were a detergent wash, a hexane-ethanol wash and an acetone-ether detergent wash. There was a significant difference between various washing procedures, but it was shown that the Mg content was less than half that of the unwashed hair. These results demonstrate that Mg in hair is an unreliable sample to assess Mg status<sup>(91)</sup>.

**Human.** In end stage heart disease patients with significantly lower plasma and erythrocyte Mg levels, a lower Mg content was also found in hair ( $26.7 \pm 15.3 \mu\text{g/g}$ ) compared with healthy control subjects ( $54.5 \pm 19.8 \mu\text{g/g}$ ). The authors explained this exceptionally low content in hair by a chronic disturbance of Mg metabolism<sup>(21)</sup>. Hair Mg concentrations in patients with Fibromyalgia were significantly higher compared with those of healthy subjects ( $84.7 \pm 73.3$  v.  $46.8 \pm 28.9 \mu\text{g/g}$ ). As no other biomarker of Mg status was measured and no quality control procedures described, these results must be interpreted with caution<sup>(92)</sup>. In a 2-month-old boy with congenital hypomagnesaemia, hair Mg concentrations were measured at 8 and 12 years of age and compared with control healthy children with the same brown hair colour. Surprisingly, the Mg content of hair at 8 and 12 years was higher compared with controls:  $164 \pm 8.08$  v.  $52.7 \pm 21.7$  and  $244 \pm 11.5$  v.  $121.6 \pm 27.2 \mu\text{g/g}$  dry weight, respectively. Other trace element concentrations such as manganese, copper and zinc were also higher in hair. The authors concluded that hair acts as a sink for oligo elements, including Mg and did not reflect the severe deficiency as seen through the significantly low values for serum, erythrocyte, lymphocyte and teeth<sup>(58)</sup>. Analysis of hair and serum Mg in neonates and their mothers showed a negative correlation between maternal hair and ionized Mg in cord serum, and male neonates had higher levels of Mg in cord blood and hair than females<sup>(93)</sup>. These researchers also investigated the effect of two Mg multivitamin supplements given for 3 months (each tablet contained 24 mg Mg) and 4 months (tablets containing 100 mg Mg) at a dose of 7 mg Mg per kg body weight and

per day in 46 children aged 2–6 years. The results showed that hair Mg concentrations increased significantly from  $7.74 \pm 0.36$  to  $11.03 \pm 0.89 \mu\text{g/g}$  dry mass. This increase was observed in 40 children out of the 46 recruited, suggesting that 3 months Mg supplementation was effective for increasing Mg hair concentrations in children<sup>(94)</sup>.

### Nails

The mean loss of nail substance is approximately 3 g per year and the ratio between calcium and Mg is about 4.5/1 with some variability due to external adsorption<sup>(95)</sup>. The reproducibility over a 6-year period of the measurement in toenails of 16 trace elements, including Mg has been reported from 127 women in the United States. Toenail concentrations of some minerals can be used as biomarkers of exposure and a single sample may represent long-term exposure for toxic intakes of nutrients. For Mg, the mean value  $\pm$  SD was  $167 \pm 130 \mu\text{g/g}$  and was fourth most abundant after sulphur, calcium and chlorine<sup>(96)</sup>.

In conclusion, from these studies, there is a need to validate the measurement of both hair and nail Mg and to demonstrate that the sample represents a period of either deficient or excessive Mg intake. Up to now, it is unclear how to interpret Mg values found in hair or nail.

## Physiological tests

### Loading test

Balance studies are time consuming, labour intensive and need well trained staff. They are often performed in a metabolic unit and require complete urine and faecal collections; therefore it is not a method that can be applied as a routine test for the evaluation of Mg status. Loading tests are simplified balance studies where absorption is supposed not to be disturbed when Mg is given orally so that body retention is calculated from urine elimination. Mg administration during a loading test can be either oral or intravenous and it is important that the subjects have normal kidney function. Urine is collected for 24 hours following administration of the Mg load as Mg excretion by the kidney has been shown to have a circadian rhythm<sup>(97)</sup>. Under these conditions, the loading test is supposed to be a reliable indicator of Mg status<sup>(65)</sup>.

**Alcoholics.** Retention of a low dose of Mg, 0.2 mEq/kg lean body weight, given intravenously has been assessed in hypomagnesaemia patients and normomagnesaemic alcoholics. It was shown that they retained significantly higher amounts of the Mg load than normal subjects. After parenteral Mg repletion, the retention was normalized showing that this test is a more sensitive index of Mg deficiency than serum concentration<sup>(98)</sup>.

**Crohn's and coeliac disease.** In Crohn's disease patients, the most sensitive measurement of Mg status and deficiency has been shown to be the analysis of Mg in muscle and also the estimation of Mg retention<sup>(22)</sup>. A 12-hour intravenous Mg loading test ( $30 \text{ mmol}/1.73 \text{ m}^2$ ) was used in children and adolescents with coeliac disease to evaluate the frequency of Mg deficiency. The cut-off level for tissue Mg deficiency was identified at the point when Mg retention was greater than 40 % of the load<sup>(99)</sup>.



**Critical illness.** A study was performed to assess the value of intracellular Mg in erythrocytes and mononuclear blood cells in critically ill patients sub-divided into Mg depleted and non-depleted groups according to their response to a loading test. There were no significant difference between the Mg depleted and non-depleted groups (plasma 0.81 and 0.90 mmol/l and red blood cell 2.34 and 2.18 mmol/l, mononuclear blood cell 25.16 and 18.1 mmol/kg dry weight, respectively). Thus, normal values of plasma, erythrocyte or mononuclear blood cell concentrations of Mg cannot exclude Mg depletion<sup>(13)</sup>. Mg deficiency was identified in critically ill patients using a loading test and was validated using measurements of serum ionized Mg. There was a significant increase in both serum ionized and total Mg concentrations by 43% and 59%, respectively on day 1 compared with the control group. Urinary Mg excretion also increased after a load of 30 mmol from  $4.8 \pm 2.3$  mmol/day during the 3-day study period to  $22.7 \pm 10.9$  mmol/day. The patients with an excretion lower than 70% of the total Mg were designated as functionally Mg-deficient retainers and patients who excreted more than 70% were non-retainers. Interestingly, the number of retainers on day 2 was ten patients and only six on day 3, indicating a replenishment of body Mg stores. In the retainer group, only two patients had a low serum ionized Mg concentration, while two other patients had low total serum Mg values. These results show that the Mg-loading test is effective and serum ionized Mg appears to be an insensitive biochemical marker of functional hypomagnesemia<sup>(100)</sup>.

**Chronic fatigue syndrome.** In a study population of 93 patients with unexplained chronic fatigue, only three subjects had plasma Mg concentrations lower than 0.6 mmol/l ( $0.82 \pm 0.10$  mmol/l) and normal erythrocyte Mg ( $1.97 \pm 0.22$  mmol/l). The Mg deficient group was identified from Mg retentions following an intravenous loading test with a four hour Mg infusion of 0.2 mEq/kg body weight. Patients with 20% or more Mg retention were diagnosed as moderately deficient and those with 50% or more as severely deficient. When 20% retention was taken as the cut-off value, 47% of the patients were classified as being Mg deficient<sup>(101)</sup>.

**Asthmatic.** In asthmatics, a significantly increased retention of Mg was observed in 58.9% of the patients after a loading test compared with 8.9% in normal subjects<sup>(15)</sup>.

**Renal transplant.** A one-hour intramuscular or intravenous infusion of 0.1 mmol Mg per kg body weight over 1 hour followed by a 24 h urine collection was designed for out-patients. Serum and urinary Mg were analyzed and the percentage retention before and after 4 months daily supplementation containing 5 mmol Mg showed a significant decrease from  $47 \pm 43$  in patients after renal transplantation to  $16 \pm 26$ . Thus, Mg supplementation successfully returned the percentage retention towards normal values after 4 months. In the placebo group of patients after renal transplantation, the percentage retention was  $58 \pm 27$ . Retention of 20% of the dose or more was considered evidence of deficiency. These high levels of retention are greater than the mean  $\pm 3SD$  of the control group and thus indicate Mg deficiency in spite of normal serum Mg level. The analysis of bone samples obtained from another group of patients undergoing hip replacement showed that short term Mg retention reflects femur-Mg content, the most relevant Mg store. In this study<sup>(102)</sup>, dietary intake, faecal excretion and basal urinary Mg output

were ignored and may explain the negative percentage retention values in healthy individuals. These data are however comparable with those obtained with 30 mmol (810 mg) Mg infused over 8 hours<sup>(103)</sup>.

**Low Mg diet in healthy subjects.** Recently, a double blind crossover study evaluated the effect of moderate Mg deprivation in postmenopausal women receiving either 4.4 mmol (107 mg) Mg from a basal diet or 13.45 mmol (327 mg) with a Mg supplement of 9.5 mmol (220 mg) added for 72 days. Mg deprivation significantly reduced the positive Mg balance when the supplement was given, decreased red blood cell membrane Mg, increased the calcium balance, decreased the faecal excretion of phosphorus and increased its urinary excretion, and decreased the urinary excretion of potassium. The authors suggest that a non-positive Mg balance and decreased red blood cell membrane concentration may be indicators of Mg deprivation<sup>(104)</sup>.

**Healthy subjects.** Mg status has been measured in healthy subjects by either oral or infused Mg loading tests and deficiencies have been diagnosed with this test, while serum Mg was not affected<sup>(105–107)</sup>. In one study urine was collected for 24 hours after an 8-hour infusion of 30 mmol Mg. The data demonstrated a Mg retention of 28 and 6% of the dose in elderly and younger subjects respectively, suggesting a higher prevalence of Mg deficiency in the elderly. It was concluded that a significant sub-clinical Mg deficit was present in these healthy elderly subjects that was not detected by serum Mg<sup>(108)</sup>. Their study also showed that a 3-week daily oral Mg supplementation with 9 mmol Mg improved Mg status as shown by increased urinary excretion and lower body retention. However, a parenteral loading test using Mg chloride (0.206 mmol/kg body weight) did not show any correlation between Mg retention and basal urinary excretion of Mg and plasma or erythrocyte Mg concentrations. This study raises the important point of the need to standardize the loading test procedure in order to produce the most sensitive and reproducible results<sup>(109)</sup>. The impact on Mg status of consuming drinking water of differing Mg content (1.6 mg and 25 mg/l) for 6 weeks has been evaluated using an oral Mg loading test (575 mg of Mg) administered in tablet form. The 24-hour urinary excretion of Mg was expressed as total Mg excretion and the Mg/creatinine ratio. There was no change when the urinary excretion was expressed as total Mg excretion but a significant change from 24.8 to 39.3 Mg/creatinine ratio was found suggesting that a small increase of Mg concentration in drinking water for 6 weeks can improve body Mg status<sup>(110)</sup>.

Although there is no standardized protocol and the relative Mg deficits identified through its use may not represent the total body Mg deficit, this test has been useful and could be improved in the near future.

### Isotope balance studies

The first analytical challenge when using stable isotopes to study the metabolic fate of Mg was the introduction of samples into a mass spectrometer. <sup>26</sup>Mg was chelated with tetramethylheptanedione, extracted and recovered by sublimation and introduced by solid probe into Mass Spectrometer for analysis at enrichment levels expected to be found in plasma, urine and faecal samples from subjects who had received this



isotope as a tracer<sup>(111)</sup>. The accuracy of these analyses has continuously improved and allows a precise evaluation of intestinal Mg absorption, faecal excretion, and body retention. In addition, using kinetic data obtained from the analysis of blood samples it is now possible to undertake compartmental modelling to determine pool sizes and turnover rates. Stable isotopes are routinely used to study gastrointestinal functions but for Mg, the availability and the cost of mass spectrometry measurements, the cost of the isotope and the rather complex protocol restrict the use of this method to research and not clinical studies. Among the studies published, stable isotopes have been used to evaluate the exchangeable Mg pool size in humans and to correlate these changes with Mg status. However, an 8-week Mg supplementation study of 366 mg Mg per day in 24 year old healthy women did not show any modification in the size of the exchangeable Mg pools, but plasma ionized Mg and urinary excretion were significantly increased, while total plasma and erythrocyte levels were unchanged. In these healthy subjects, and under these experimental conditions, the study of exchangeable Mg pool size is not a sensitive biomarker of the variations of Mg status<sup>(112)</sup>. To detect marginal Mg deficiency, an 11 ng dose of <sup>26</sup>Mg was injected into 22 healthy subjects with a wide range of plasma Mg concentrations from 0.68 to 0.95 mmol/l (mean value  $0.82 \pm 0.09$  mmol/l) and with adequate Mg intakes ( $438 \pm$  and  $464 \pm 138$  mg for men and women, respectively). This modified version of the loading test and Mg retention showed no correlation between the excretion of the isotopic label and muscle Mg concentration. Within 24 hours only 7.9% of the injected dose was excreted in the urine and the fraction excreted correlated with total urinary Mg excretion. In contrast to other loading tests, this dose was apparently insufficient to modify the size of the pools in subjects with marginal deficiency as defined by a range of Mg muscle concentrations from 3.50 to 4.19 mmol/100 g fat free dried solids<sup>(113)</sup>. Using double labelled Mg in healthy adult men, <sup>26</sup>Mg given orally and <sup>25</sup>Mg injected intravenously, blood, urine and faeces were collected for 12 days to build a compartmental model of Mg kinetics. However, this analysis only enables the exploration of 25% of the total body pool i.e. that which exchanges rapidly from the plasma compartment with two extra-plasma pools<sup>(10)</sup>.

### Physiological activities

Biomarkers such as ferritin for iron have not been found for Mg. It has been suggested that potential Mg markers could include Na/K ATPase, thromboxane B<sub>2</sub>, C-reactive protein and endothelin-1, but other biomarkers are needed<sup>(3)</sup>. Because of the ubiquitous role of Mg and the interactions of other minerals such as calcium, the identification of a specific marker of Mg deficiency is challenging. Mg has been shown to be associated with various physiological responses such as blood pressure, but in this case known parameters, such as calcium, and additional unknown factors will also affect blood pressure. Therefore, the relationship between intracellular free Mg and diastolic blood pressure cannot be a marker of Mg status<sup>(114)</sup>. The activities of alkaline phosphatase and creatine kinase, two Mg-requiring enzymes, have been evaluated in relation to plasma and erythrocyte Mg concentration in rats to determine their usefulness as indices of Mg status<sup>(115)</sup>.

The results showed that plasma Mg concentration is the most useful indicator of Mg status. A very strong correlation was observed between plasma and bone concentrations. Other sensitive markers of deficiency may be developed through the study of gene expression, which was shown in animal experiments to change with Mg deficiency<sup>(116)</sup>.

### Ex vivo or in vitro cellular methods

A new *in vitro* blood load test has been proposed to assess Mg status using stable isotopes. Blood cells were isolated and incubated with <sup>25</sup>Mg and it was proposed that high uptake would be triggered by Mg deficiency. The uptake in human erythrocytes was low compared to rat erythrocytes and higher enrichments were obtained for human lymphocytes and platelets. Thus, these latter cells seem more appropriate to test human Mg status using this *in vitro* system<sup>(117)</sup>. *In vitro* erythrocyte Mg fluxes were studied using stable isotopes in mice receiving Mg deficient diet and subsequently selected for their high or low erythrocyte Mg concentrations<sup>(38)</sup>. Although it is not possible to extrapolate the results obtained with severe Mg restriction in animals to the human situation, these *in vitro* studies can help to understand the role of TRPM channels in the specific regulation of intracellular Mg concentrations in blood cells and tissues. However, the authors correctly indicate that artificial erythrocyte Mg load tests performed *in vitro* may induce non-physiological cellular responses so any conclusions must be drawn with caution.

### TRPM (Transient Receptor Potential Melastatin) Channel

Hypomagnesaemia, first described in 1968<sup>(118)</sup> is an autosomal-recessive disorder of early infancy resulting in convulsions, muscle spasms or tetany. Extremely low serum Mg and low calcium levels are present, but the administration of high doses of Mg prevents permanent neurological damage and death. It was originally shown that the primary defect involved intestinal Mg absorption. TRPM6 protein has been identified and exhibits homology to TRPM7, which has been characterized as a calcium- and Mg-permeable ion channel regulated by Mg-ATP. The distribution of TRPM6 along the entire small intestine and colon as well as in distal tubule cells in the kidney shows how both the absorption and the excretion of Mg are controlled by this new family of cation channels. TRPM7 exhibits significant permeation to ionized Mg and is inhibited by cytosolic Mg ions and Mg-ATP. This recent progress in epithelial Mg transport will certainly help in understanding the fine tuning of Mg homeostasis and its impact on disturbed Mg status<sup>(119–121)</sup>. The recent identification of TRPM channels and their role in hypomagnesaemia may eventually also result in chronic and mild Mg deficiency being explained by these specific transporters. It may also be possible in the future to accurately evaluate the transport capacity of Mg through these channels as a diagnostic of the risk for Mg deficiency.

### Conclusions

A similar review of tests used to assess Mg status written 17 years ago concluded that there was no test that could readily be used in clinical medicine to assess the total body Mg

status of a patient. Although intense research activities have been dedicated to Mg, the difficulties of accessing total body Mg, and its main two compartments, namely bone and muscle, mean that today there is still no simple, rapid, and accurate laboratory test to indicate total body Mg status in human. However, taking into account all the more recent investigations, although serum Mg <0.75 mmol/l still remains a useful measurement for severe deficiency, for values between 0.75 and 0.85 mmol/l, a loading test must be performed to identify the deficient subjects. Loading tests appear to be the gold standard for Mg status but patients with disturbed kidney and intestinal functions should be excluded when the dose is given orally. There is also a need to reach a consensus on a standardized protocol to be used in order to compare results from different clinical units. Urinary Mg cannot replace the loading test as it does not reflect Mg status. Other cellular Mg measurements, such as total or ionized Mg are often equivocal and more research and systematic evaluations are needed. Muscle Mg appears to be a good marker but biopsies limit the use of this measurement to research. Bone, the most important Mg compartment cannot be used because of the invasiveness of the sampling and variations in measured Mg concentrations. The development of new and non invasive techniques such as NMR or *ex vivo* studies could in the future provide valuable tools for performing routine analyses of ionized Mg in tissues. With the development of molecular biology approaches and the recent discovery of TRPM channels, new, sensitive and fast evaluation of Mg status in humans may be developed in the near future.

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