Magnesium Research 2008; **21** (3): 157-62

In vivo assessment of Mg^{2+} in human brain and skeletal muscle by ^{31}P-MRS

Stefano Iotti, Emil Malucelli

Dipartimento di Medicina Interna, dell'Invecchiamento e Malattie Nefrologiche, Università di Bologna, Italy

Correspondence: S. Iotti
<stefano.iotti@unibo.it>

Abstract. Phosphorus magnetic resonance spectroscopy offers a unique opportunity to measure *in vivo* the free cytosolic magnesium [Mg^{2+}] of different tissues. In particular, this technique has been employed in human brain and in skeletal muscle providing new hints on Mg^{2+} homeostasis and on its involvement in cellular bioenergetics. In skeletal muscle it has been shown that the changes of free Mg^{2+} concentration occurring during contraction and in post-exercise recovery are mainly due to the cytosolic pH influence. The possibility of assessing the free cytosolic [Mg^{2+}] in the human brain offered the chance of studying the involvement of Mg^{2+} in different neurological pathologies, and particularly in those where defective mitochondrial energy production represents the primary causative factor in the pathogenesis. The results obtained, studying patients affected by different types of mitochondrial cytopathies, helped to clarify the functional relationship between energy metabolism and free [Mg^{2+}], providing evidence that cytosolic [Mg^{2+}] is regulated in brain cells to equilibrate any changes in rapidly available free energy. Moreover, it has also been shown that the measurement of brain Mg^{2+} can help in the differential diagnosis of neurodegenerative diseases sharing common clinical features, such as Multiple System Atrophy and Parkinson's disease.

Key words: magnetic resonance spectroscopy, muscle exercise, mitochondrial cytopathies, migraine, Parkinson's disease

Many relevant pathological conditions, such as cardiovascular diseases [1], essential hypertension [2], diabetes mellitus [3] neuropsychiatric disorders [4, 5], metabolic syndrome [6, 7], different types of migraine [8, 9] and mitochondrial cytopathies [10] are associated with reduced Mg availability and/or increased excretion either at a systemic level or in specific tissues.

Moreover, the beneficial effect of Mg administration in brain pathological conditions has been also investigated. Several studies have examined the clinical efficacy of Mg^{2+} therapy in animal models of traumatic brain injury, showing that administration of Mg^{2+} pre- or post-injury effectively improved recovery of cognitive deficits following injury [11, 12] and several pieces of evidence establish a connection between magnesium deficiency and aging [13], providing a rationale for the administration of Mg as a co-adjuvant agent for the retardation of neurodegenerative processes typical of senescence.

Although the clinical effects of variations in serum [Mg^{2+}] have not been widely recognised [14], in routine clinical practice [Mg^{2+}] is assayed in serum and not directly in tissues.

Phosphorus magnetic resonance spectroscopy (^{31}P-MRS) offers a unique opportunity to measure *in vivo* free cytosolic magnesium concentration in several tissues [10, 15, 16]. The main phosphorylated molecules present in the cell cytosol detected by ^{31}P-MRS are inorganic phosphate (Pi), phosphocreatine (PCr) and ATP [17]. Magnesium is almost com-

doi: 10.1684/mrh.2008.0142

Presented in part at the European Meeting on Magnesium, EuroMag, Paris, May 15-17, 2008.

pletely bound to ATP in the cytosol matrix. The amount of magnesium bound to ATP shifts the resonance frequencies of signals coming from the three phosphoric groups of the molecule (chemical shift). Due to the chemical equilibrium between the Mg bound to ATP and free Mg^{2+}, the chemical shift of ATP signals is a function of free Mg^{2+} concentration. With the availability of calibration curves which take into account both: i) other ions present in the cell cytosol competing with Mg^{2+} in binding ATP such as: H^+, Na^+, K^+ and ii) other ligands as ADP, PCr and Pi competing with ATP in binding Mg^{2+}, it is possible to measure *in vivo*, not invasively and with high accuracy the cytosolic free Mg^{2+} concentration in brain and skeletal muscle in different metabolic conditions [18, 19].

In vivo assessment of Mg^{2+} in human skeletal muscle

Skeletal muscles contain approximately 35% of total human body magnesium. Magnesium ions influence the equilibria of many reactions involved in cellular bioenergetics by interacting with phosphorylated molecules [20] and interfere with the kinetics of ion transport across plasma membranes. In particular, Mg^{2+} is known to regulate Ca^{2+} traffic in smooth [21] and skeletal [22] muscle cells by acting as a blocker of Ca^{2+} channels. All this implies that an accurate knowledge of intracellular magnesium concentration ($[Mg^{2+}]$) is crucial for a deeper understanding of both cellular bioenergetics and reaction kinetics *in vivo.*, and that any change in cellular $[Mg^{2+}]$ may alter critical regulatory mechanisms, causing abnormal metabolism.

In skeletal muscle, variations of cytosolic pH, [PCr] and [Pi] occurring in the transitions from rest to exercise and vice-versa, influence the complex multi-equilibrium system of the molecular species which bind magnesium ions. As a consequence, free cytosolic $[Mg^{2+}]$ can change considerably in different metabolic conditions such as rest, exercise and recovery.

It has been shown by ^{31}P-MRS that the increase of cytosolic free $[Mg^{2+}]$ occurring in skeletal muscle of healthy subjects during exercise and initial recovery is matched by a decrease in cytosolic pH, *(figure 1)* and the changes in cytosolic free $[Mg^{2+}]$ were mainly the result of the predominant effect of $[H^+]$ [19]. This result was attributed to the mechanisms of the binding competition which exists between Mg^{2+} and H^+ towards the negatively-charged molecules present in the cell cytosol.

The values of cytosolic free $[Mg^{2+}]$ measured in the calf muscle of 42 healthy subjects during exercise and recovery, plotted as a function of pH, showed an exponential pattern with a sharp increase of $[Mg^{2+}]$ below pH 6.5 *(figure 2)*. However, this does not necessarily imply a causal relationship between pH and $[Mg^{2+}]$, as it could be argued that muscular exercise *per se* elicits an increase in cytosolic free $[Mg^{2+}]$. Therefore, to understand to what extent homeostasis of intracellular free Mg^{2+} is linked to pH, we studied patients affected by McArdle's and Tarui's disease, which are metabolic pathologies characterised by the absence of intracellular acidification [23]. The results of the study show that, in the calf muscle of these patients, the lack of intracellular acidification was accompanied by a lack of Mg^{2+} increase. This outcome provides experimental evidence that the increase in cytosolic $[Mg^{2+}]$ occurring in skeletal muscle during exercise is actually the consequence of an increase of H^+ concentration and not of other mechanisms related to muscle contraction.

In vivo assessment of Mg^{2+} in human brain

The free cytosolic $[Mg^{2+}]$ assessed in the human brain by ^{31}P-MRS was 0.182 mM [18]. This value measured in the occipital lobes of 36 healthy subjects is about half of that assessed in the human calf muscle [19]. This result is most likely related to the lower ATP concentration of brain tissue compared to that of skeletal muscle, ATP being the major binding site present in the cellular milieu. The possibility of assessing the free cytosolic $[Mg^{2+}]$ in the human brain offered the chance of studying the involvement of Mg in different neurological pathologies. Particularly interesting are mitochondrial cytopathies and migraines, in which the defective mitochondrial energy production is respectively the primary causative or putative pathogenetic factor.

Mg^{2+} in mitochondrial cytopathies

^{31}P-MRS was used to assess the free Mg^{2+} in the occipital lobes of patients affected by different types of mitochondrial cytopathies due to known enzyme and/or mitochondrial DNA defects, to clarify the functional relationship between energy metabolism and the concentration of cytosolic free magnesium [10]. Cytosolic free $[Mg^{2+}]$ was found to be abnormally low in all patients. Nine of the 19 patients investigated were treated with CoQ which improved the efficiency of the respiratory chain, as shown by an increased [PCr], decreased [Pi] and [ADP]. Administration of CoQ also increased cytosolic free

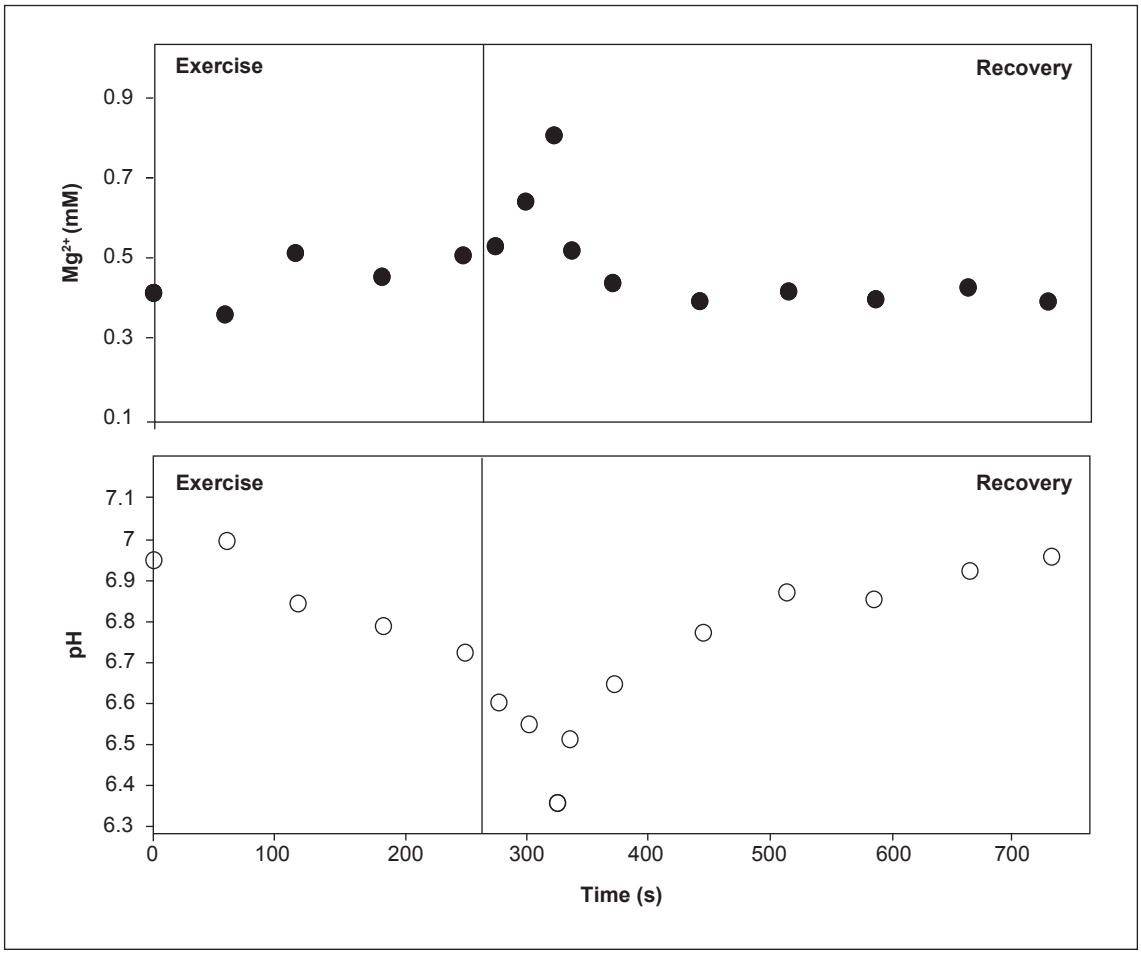

Figure 1. Typical pattern of cytosolic [Mg^{2+}] (upper panel) and pH (lower panel) assessed by ^{31}P-MRS in the calf muscle of a healthy subject during exercise and post-exercise recovery. The values at zero time are those assessed at rest.

[Mg^{2+}] in all treated patients *(figure 3)*. These findings suggest that low brain free [Mg^{2+}] in mitochondrial cytopathies is secondary to failure of the respiratory chain, and they are consistent with the view that cytosolic [Mg^{2+}] is regulated in the intact brain cell to equilibrate, at least in part, any changes in rapidly available free energy.

Mg^{2+} in migraine

Migraine headache is a common feature in patients with mitochondrial encephalomyopathies where deficient brain mitochondrial oxidation is due to mutations of mitochondrial DNA. In addition, several studies have contributed to show an altered energy metabolism in the brain of patients with different types of migraine and cluster headache [24-26], although the molecular mechanisms leading to oxidative deficit in migraine and cluster headache are unknown. Total and ionised magnesium has been found to be reduced in serum and erythrocytes of patients with different forms of migraine and cluster headache [27, 28]. All these findings offered the rationale for an extended study to assess the brain Mg^{2+} by ^{31}P-MRS in different forms of migraines and in cluster headache [9]. This study was conducted in 78 patients with different forms of migraine in attack-free periods (7 with migraine stroke, 13 with migraine with prolonged aura, 37 with migraine with typical aura or basilar migraine, 21 with migraine without aura), and 13 patients with cluster headache.

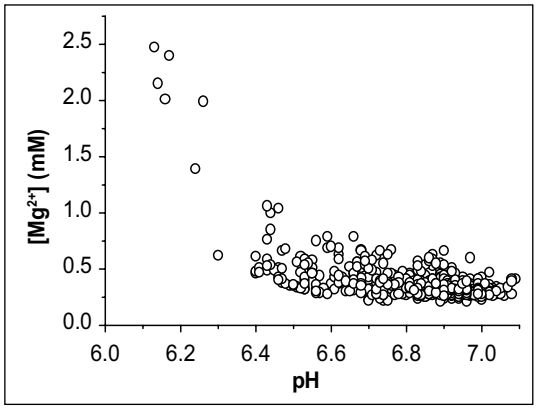

Figure 2. Patterns of free cytosolic [Mg^{2+}] in human calf muscle during exercise and recovery reported as a function of cytosolic pH. Both [Mg^{2+}] and pH were assessed *in vivo* by ^{31}P-MRS.

In the occipital lobes of all subgroups of migraine and in cluster headache patients, cytosolic free [Mg^{2+}] was significantly reduced. Among migraine patients the level of cytosolic free [Mg^{2+}] correlated with the severity of clinical phenotype and the bioenergetics deficit, showing the lowest values in patients with migraine stroke and the highest in patients with migraine without aura [9]. The results of this study confirm the hypothesis that the reduction in free [Mg^{2+}] in tissues with defective mitochondrial functionality is secondary to the bioenergetics deficit, excluding a primary role for low brain cytosolic free [Mg^{2+}] in the pathogenesis of headache. Nevertheless, a recent study [29] has shown a beneficial effect of magnesium supplementation in patients with migraine without aura, highlighting the possibility of using magnesium as a prophylactic agent in the treatment of migraine subtypes.

Mg^{2+} in multiple system atrophy and idiopathic Parkinson's disease

Multiple System Atrophy (MSA) is a group of multisystem degenerative diseases that have several clinical features of Parkinson's disease. Therefore, differentiating MSA from Parkinson's disease can be difficult and the diagnosis of Multiple System Atrophy (MSA) represents a clinical challenge. An *in vivo* study by ^{31}P-MRS showed that the combined measurement of [PCr], and free [Mg^{2+}], could help to differentiate patients with MSA from those with PD [30]. The study was carried out on the occipital lobes of 15 patients with multiple system atrophy (MSA), 13 patients with idiopathic Parkinson's disease (PD). The MSA group showed significantly reduced [PCr],

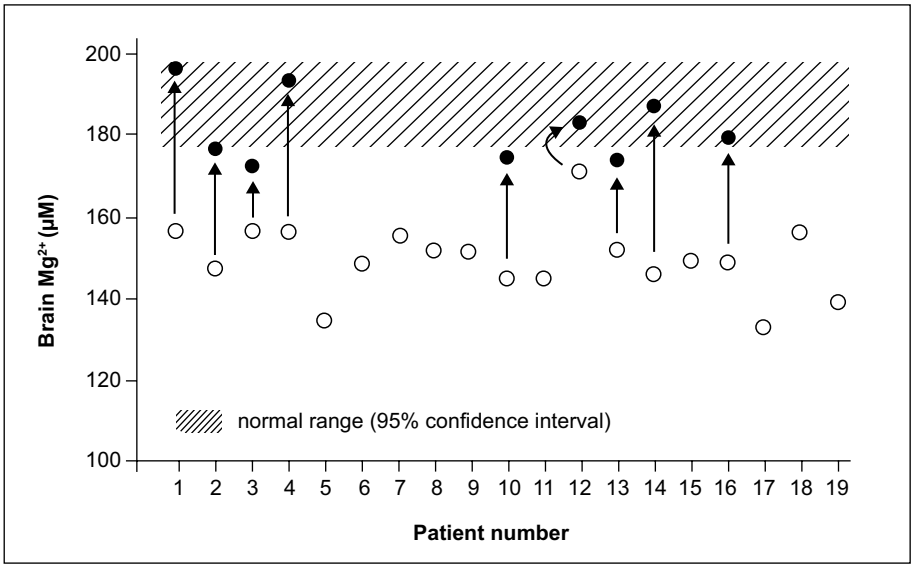

Figure 3. Brain cytosolic free [Mg^{2+}] from the occipital lobes of 19 patients with mitochondrial cytopathies (open symbols). The values from nine of these patients (cases 1-4, 10, 12-14 and 16) after treatment with CoQ are shown by black arrows and closed symbols. Dashed areas represent the 95% confidence intervals of 36 healthy control subjects (modified from [10]).

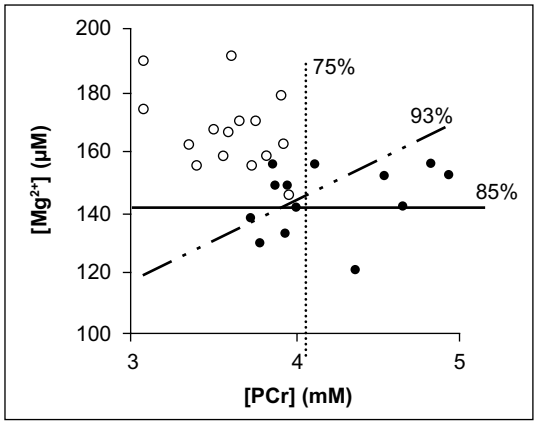

Figure 4. Distribution of 13 patients with Idiopathic Parkinson's Disease (closed circles) and 15 patients with Multiple System Atrophy (open circles) as a function of brain [PCr] and free [Mg2+] assessed by ^{31}P-MRS. Discriminant analysis using these two independent indicators correctly classified 93% of cases, while [PCr] and [Mg2+] alone were able to classify respectively 75% and 85% of the patients (modified from [30]).

increased [Pi] and unchanged cytosolic free [Mg^{2+}] and pH. On the other hand, PD patients showed a significantly increased [Pi], decreased cytosolic free [Mg^{2+}] and unchanged [PCr] and pH. Comparing the MSA vs. PD groups, [PCr] was significantly lower in MSA than in PD, while cytosolic free [Mg^{2+}] was significantly lower in PD. In spite of a certain degree of overlap of [PCr] and [Mg^{2+}] values between the two groups, by considering both variables at the same time it was possible to classify correctly 93% of cases by discriminant analysis *(figure 4)*. The results of the study revealed abnormal bioenergetics and Mg^{2+} contents in MSA and PD respectively, offering a new diagnostic clue that may help to differentiate MSA from PD.

Concluding remarks

^{31}P-MRS has shown the unique capability of assessing *in vivo* cytosolic [Mg^{2+}] in human brain and skeletal muscle. This technique allowed us to prove that, in skeletal muscle, dramatic changes of free Mg^{2+} can occur in different metabolic conditions, in contrast to what is found in other cell types and tissues. It was demonstrated that these variations are modulated by pH, disclosing a new mechanism of cytosolic Mg^{2+} regulation. Moreover, the study of the involvement of Mg^{2+} in different neurological disorders provided new insights into the role of Mg2+ in cellular bioenergetics, also opening new diagnostic possibilities for some neurodegenerative diseases.

References

1. Touyz RM. Magnesium in clinical medicine. *Front Biosci* 2004; 9: 1278-93.
2. Altura BM, Altura BT, Gebrewold A, Ising H, Günther T. Magnesium deficiency and hypertension: correlation between magnesium-deficient diets and microcirculatory changes in situ. *Science* 1984; 223: 1315-7.
3. Resnick LM, Altura BT, Gupta RK, Laragh JH, Alderman MH, Altura BM. Intracellular and extracellular magnesium depletion in type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* 1993; 36: 767-70.
4. Helpern JA, Vande Linde AQ, Halvorson HR, Gdowski JW, Brown G, Levine SR, Brown E, Welch KMA. Brain pH and free Mg^{2+} in patients with dementia. *Neurology* 1990; 40(Suppl. 1): 372.
5. Barra A, Camardese G, Tonioni F, Sgambato A, Picello A, Autullo G, Silvia D, Bria P, Cittadini A. Plasma magnesium level and psychomotor retardation in major depressed patients. *Magnes Res* 2007; 20: 245-9.
6. Barbagallo M, Dominguez LJ. Magnesium metabolism in type 2 diabetes mellitus, metabolic syndrome and insulin resistance. *Arch Biochem Biophys* 2007; 458: 40-7.
7. Belin RJ, He K. Magnesium physiology and pathogenic mechanisms that contribute to the development of the metabolic syndrome. *Magnes Res* 2007; 20: 107-29.
8. Ramadan NM, Halvorson H, Vande-Linde A, Levine SR, Helpern JA, Welch KM. Low brain magnesium in migraine. *Headache* 1989; 29: 590-3.
9. Lodi R, Iotti S, Cortelli P, Pierangeli G, Cevoli S, Clementi V, Soriani S, Montagna P, Barbiroli B. Deficient energy metabolism is associated with low free magnesium in the brains of patients with migraine and cluster headache. *Brain Res Bull* 2001; 54: 437-41.
10. Barbiroli B, Iotti S, Cortelli P, Martinelli P, Lodi R, Carelli V, Montagna P. Low brain intracellular free magnesium in mitochondrial cytopathies. *J Cereb Blood Flow Metab* 1999; 19: 528-32.
11. Hoane MR. Assessment of cognitive function following magnesium therapy in the traumatically injured brain. *Magnes Res* 2007; 20: 229-36.
12. Hoane MR, Gilbert DR, Barbre AB, Harrison SA. Magnesium dietary manipulation and recovery of function following controlled cortical damage in the rat. *Magnes Res* 2008; 21: 29-37.
13. Killilea DW, Maier JAM. A connection between magnesium deficiency and aging: new insights from cellular studies. *Magnes Res* 2008; 21: 77-82.
14. Whang R, Ryder KW. Frequency of hypomagnesemia and hypermagnesemia. Requested vs routine. *JAMA* 1990; 263: 3063-4.

15. Gupta RK, Benovic JL, Rose ZB. The determination of the free magnesium level in the human red blood cell by 31P NMR. *J Biol Chem* 1978; 253: 6172-6.

16. Halvorson HR, Vande Linde AM, Helpern JA, Welch KM. Assessment of magnesium concentrations by ^{31}P NMR *in vivo*. *NMR Biomed* 1992; 5: 53-8.

17. Iotti S, Lodi R, Frassineti C, Zaniol P, Barbiroli B. *In vivo* assessment of mitochondrial functionality in human gastrocnemius muscle by 31P-MRS. The role of pH in the evaluation of phosphocreatine and inorganic phosphate recoveries from exercise. *NMR Biomed* 1993; 6: 248-53.

18. Iotti S, Frassineti C, Alderighi L, Sabatini A, Vacca A, Barbiroli B. *In vivo* assessment of free magnesium concentration in human brain by 31P-MRS. A new calibration curve based on a mathematical algorithm. *NMR Biomed* 1996; 9: 24-32.

19. Iotti S, Frassineti C, Alderighi L, Sabatini A, Vacca A, Barbiroli B. *In vivo* ^{31}P-MRS assessment of cytosolic [Mg^{2+}] in the human skeletal muscle in different metabolic conditions. *Magn Reson Imag* 2000; 18: 607-14.

20. Lawson JWR, Veech RL. Effects of pH and free Mg^{2+} on the Keq of the creatine kinase reaction and other phosphate hydrolyses and phosphate transfer reactions. *J Biol Chem* 1979; 254: 6528-37.

21. Altura BM, Altura BT. Magnesium ions contraction of vascular smooth muscles: relationship to some vascular disease. *Fed Proc* 1981; 40: 2672-9.

22. Stephenson EW, Podolsky RJ. Regulations by magnesium of intracellular calcium movement in skinned muscle fibers. *J Gen Physiol* 1977; 69: 1-16.

23. Malucelli E, Lodi R, Martinuzzi A, Tonon C, Barbiroli B, Iotti S. Free Mg^{2+} concentration in the calf muscle of glycogen phosphorylase and phosphofructokinase deficiency patients assessed in different metabolic conditions by ^{31}P-MRS. *Dyn Med* 2005; 4: 7.

24. Barbiroli B, Montagna P, Funicello R, Iotti S, Monari L, Pierangeli G, Zaniol P, Lugaresi E. Abnormal brain and muscle energy metabolism shown by ^{31}P magnetic resonance spectroscopy in patients affected by migraine with aura. *Neurology* 1992; 42: 1209-14.

25. Montagna P, Cortelli P, Monari L, Pierangeli G, Parchi P, Lodi R, Iotti S, Frassineti C, Zaniol P, Lugaresi E. ^{31}P-magnetic resonance spectroscopy in migraine without aura. *Neurology* 1994; 44: 666-9.

26. Montagna P, Lodi R, Cortelli P, Pierangeli G, Iotti S, Cevoli S, Zaniol P, Barbiroli B. Phosphorus magnetic resonance spectroscopy in cluster headache. *Neurology* 1997; 48: 113-8.

27. Sarchielli P, Costa G, Firerize C, Morucci P, Abritti G, Galla V. Serum and salivary magnesium levels in migraine and tension-type headaches. Results in a group of adult patients. *Cephalalgia* 1992; 12: 21-7.

28. Soriani S, Arnaldi C, DeCarlo L, Arcudi D, Mazzotta D, Battistella PA, Sartori S, Abbasciano V. Serum and red blood cell magnesium levels in juvenile migraine patients. *Headache* 1995; 35: 14-6.

29. Köseoglu E, Talashoglu A, Gönül AS, Kula M. The effects of magnesium prophylaxis in migraine without aura. *Magnes Res* 2008; 21: 101-8.

30. Barbiroli B, Martinelli P, Patuelli A, Lodi R, Iotti S, Cortelli P, Montagna P. Phosphorus magnetic resonance spectroscopy in multiple system atrophy and Parkinson's disease. *Mov Disord* 1999; 14: 430-5.