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Utilization of magnesium during hypokinesia and magnesium supplementation in healthy subjects

Yan G. Zorbas M.D. [a], Kostas K. Kakuris M.D. [a], Yuri F. Federenko Ph.D. [a], Viktor A. Deogenov Ph.D. [b,*]

[a] *Institute of Hypokinetic Biochemistry, Sophia, Bulgaria*
[b] *European Foundation of Environmental Sciences, Athens, Greece*

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ABSTRACT

Objective: The incompleteness of electrolyte utilization during hypokinesia and electrolyte supplementation is the defining factor of electrolyte metabolic changes, yet the effect of electrolyte supplementation and HK upon electrolyte utilization is poorly understood. To determine the influence of magnesium (Mg^{2+}) supplementation and hypokinesia (diminished movement) on magnesium utilization, we investigated the use of Mg^{2+} supplementation to establish its effect upon muscle Mg^{2+} content and $Mg2^{2+}$ losses.

Methods: This study was conducted in 40 physically healthy male volunteers during a pre-experimental period of 30 d and an experimental period of 364 d. Subjects were equally divided into four groups: unsupplemented control subjects (UCSs), unsupplemented experimental subjects (UESs), supplemented control subjects (SCSs), and supplemented experimental subjects (SESs). A daily supplementation of 3.0 mmol of magnesium-chloride per kilogram of body weight was given to subjects in the SCS and SES groups.

Results: Muscle Mg^{2+} content decreased ($P < 0.05$) and plasma Mg^{2+} concentration and Mg^{2+} loss in urine and feces increased ($P < 0.05$) in the SES and UES groups compared with their pre-experimental levels and values in their respective control groups (SCS and UCS). Muscle Mg^{2+} content decreased more ($P < 0.05$) and plasma Mg^{2+} concentration and Mg^{2+} loss in urine and feces increased more ($P < 0.05$) in the SES group than in the UES group.The muscle Mg^{2+} content and plasma Mg^{2+} level and Mg^{2+} losses did not change in the control groups.

Conclusion: Daily Mg^{2+} supplementation during prolonged hypokinesia decreases more muscle Mg^{2+} content and Mg^{2+}-deficient muscle increases more Mg^{2+} loss in healthy subjects indicating lower Mg^{2+} utilization with than without Mg^{2+} supplementation.

Introduction

Muscular activity is an important determinant of normal electrolyte homeostasis. The mechanisms by which muscular activity affects electrolyte homeostasis are not known, but in its absence, such as during hypokinesia (HK; diminished movement), the consequences are energy deficiency, body weight losses [1–3], fluid shifting to the lower part of the body, and reduction of total cell mass [4,5] and circulating blood volume.

During prolonged HK tissue electrolyte content (sodium, potassium, chloride, magnesium, calcium, and phosphate) decreases and plasma electrolyte level and electrolyte loss increase [4–7]. This is primarily attributable to the decreased electrolyte utilization; the decreased electrolyte utilization is attributable to several factors inherent to prolonged HK and primarily to energy deficiency, body weight losses [1–3], reduction of total cell mass [4,5], fluid shifting to the lower part of the body and hypovolemia. The decreased electrolyte utilization can result in a higher plasma electrolyte concentration, which potentially can lead to the higher electrolyte loss [4–7]. In electrolyte-deficient tissue the reduction of electrolyte utilization, which is accompanied by a higher plasma electrolyte level, can result in higher electrolyte loss [4–7]. During prolonged HK the increase of plasma electrolyte level and electrolyte loss in electrolyte-deficient tissue [4–7] indicates different mechanisms from those involved in the decreased plasma electrolyte concentration and electrolyte loss in electrolyte-deficient tissue during normal muscular activity.

Magnesium (Mg^{2+}) is the fourth most abundant cation in the body and second only to potassium within the body. There are three major roles for Mg^{2+} in biological systems. The Mg^{2+} can

* Corresponding author: Tel.: +30210-762-8676; fax: +30210-762-8675.
E-mail address: vdeogenov@yahoo.com (V. A. Deogenov).

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compete with calcium for binding sites on proteins and membranes, and it can form chelates with important intracellular anionic ligands, notably adenosine triphosphate (ATP) [8]. The Mg^{2+} catalyzes or activates more than 300 enzymes in the body. The Mg^{2+} also acts as an essential cofactor for enzymes [9] concerned with cell respiration, glycolysis, and transmembrane transport of other cations such as calcium and sodium. Notably, the activity of Na^{+}-K^{+}-ATPase depends on the Mg^{2+} stores. The Mg^{2+} can also affect enzyme activity by binding the active site of the enzyme (pyruvate kinase, enolase) by ligand binding (ATP-requiring enzymes), by causing conformational changes during the catalytic process of the Na^{+}-K^{+}-ATPase, and by promoting the aggregation of multiple enzyme complexes.

There has been limited information on the effect of Mg^{2+} supplementation during HK on the activity of enzymes, Mg^{2+} flux across the cell membrane, ATP synthesis, Na^{+}-K^{+}-ATPase activity, Mg^{2+} utilization, Mg^{2+} loss, and Mg^{2+} deficiency. We do not know how Mg^{2+} deficiency and Mg^{2+} loss occur and/or if Mg^{2+} deficiency comes from an Mg^{2+} shortage in the food consumed or if Mg^{2+} loss is caused by an inability of body to utilize Mg^{2+} [10–14]. Each electrolyte has a well-defined and separate homeostatic mechanism to regulate Mg^{2+} content in a tissue at the cell membrane level and higher organ level. Because Mg^{2+} is a major electrolyte that works as a coenzyme to keep nerve and muscle function and control body temperature, energy metabolism, and bone formation, it is important to determine the effect of Mg^{2+} supplementation during HK on Mg^{2+} utilization. To establish a potential of a disordered Mg^{2+} utilization it is vital to study the effect of Mg^{2+} supplementation and HK on muscle Mg^{2+} content and Mg^{2+} loss.

The aim of this study was to determine the effect of Mg^{2+} supplementation and HK on Mg^{2+} utilization, which aimed at determining the capability of the body to utilization Mg^{2+} under such conditions. Measurements of muscle Mg^{2+} content, plasma Mg^{2+} level, and Mg^{2+} loss in urine and feces with and without Mg^{2+} supplementation were carried out during prolonged HK.

Materials and methods

Forty physically healthy trained male volunteers 21.5 ± 3.0 y of age were chosen as subjects. They gave informed consent to take part in the study after a verbal and written explanation of risks and methods involved were given. During the experimental period there were no dropouts. There were no medical problems among the subjects and none of the volunteers was under any drug therapy that could have interfered with magnesium metabolism. Procedures were reviewed and approved by the committee for the protection of human subjects. Financial incentives were used to encourage compliance with the study protocol. Subjects were students and had been trained for the previous 2 to 3 y, at an average rate of 5.0 times per week at 45.1 ± 2.2 km/wk. Subjects ran average distances of 9.2 ± 1.2 km/d at a speed of 9.9 ± 1.2 km/h. They had a body weight of 70.1 ± 4.4 kg and a peak oxygen uptake of 58.4 ± 6.0 mL kg^{-1} min^{-1}. During the 30-d pre-experimental period subjects ran average distances of 9.0 ± 1.2 km/d at a speed of 9.7 km/h.

Assignment of subjects into four groups was conducted randomly and their conditions were as follows. In group 1, 10 healthy subjects ran an average distance of 9.0 ± 1.2 km/d for 364 d. They were assigned to the unsupplemented control subject (UCS) group. In group 2, 10 healthy subjects walked an average distance of 2.3 ± 0.1 km/d for 364 d. They were assigned to the unsupplemented experimental subject (UES) group. In group 3, 10 healthy subjects ran an average distance of 9.1 ± 1.2 km/d for 364 d and were supplemented with 3.0 mmol of Mg^{2+} chloride per kilogram of body weight per day. They were assigned to the supplemented control subject (SCS) group. In group 4, 10 healthy subjects walked an average distance of 2.3 ± 0.1 km/d for 364 d and were supplemented with 3.0 mmol of Mg^{2+} chloride per kilogram of body weight per day. They were assigned to the supplemented experimental subject (SES) group.

Protocol

The study consisted of a 30-d pre-experimental phase and a 364-d experimental phase. Diets were served as a 4-d menu rotation. Meals were prepared under standard conditions in a research kitchen. The mean daily energy values of the metabolic diet were 3430 ± 457, 2850 ± 310, 3487 ± 488, and 2855 ± 324 kcal and mean daily dietary Mg^{2+}intakes were 211 ± 10, 212 ± 11, 211 ± 13, and 213 ± 12 mmol for the UCS, UES, SCS, and SES groups, respectively.

Simulation of hypokinesia

To simulate the effect of HK the number of kilometers walked per day was restricted to an average of 2.3 ± 0.1 km/d and was monitored daily by an accelerometer. Activities allowed were those that approximated the normal routines of hypokinetic individuals. Subjects were allowed to walk to dining rooms, lavatories, and various biochemical laboratories where the tests were administered. Climbing stairs and other activities that required greater efforts were not allowed. Subjects were mobile and were not allowed outside the hospital grounds.

Blood, urine, and fecal sample collection

To accommodate interindividual differences in bowel habits, urine and feces were collected daily and pooled to form 6-d composites, and plasma samples were collected every 6 d during the pre-experimental and experimental periods. Six-day (consecutive days) pooled samples were collected and mean ± standard deviations of the measurements were presented. Blood samples were taken from a superficial (antecubital) arm vein at rest and before the consumption of any meals. Subjects were fasted overnight for about 6–7 h. Blood samples were drawn under identical condition and between 08:00 and 09:00 h, without a venous stasis and after subjects had been sitting for 30 min. The blood volume sample was 7–9 mL. The blood samples collected with polypropylene tubes. To obtain plasma, blood samples were collected in heparinized ice-chilled tubes and were centrifuged immediately at 10 000 × *g* for 3 min at room temperature and separated using glass capillary pipettes that were washed in hydrochloric acid and deionized distilled water. The plasma samples were frozen on dry ice immediately after centrifugation and were stored at −20°C until analyses were conducted for plasma Mg^{2+}. The 24-h urine samples were stored at −4°C until needed for Mg^{2+} analyses. Creatinine excretion was measured by the colorimetric method using Jaffe's reaction to ensure 24-h urine sample collection. Feces were collected in plastic bags, dried, weighed, and stored at −20°C for Mg^{2+} analyses. Fecal samples wet ashed with acid, diluted in 5% nitric acid, and analyzed for Mg^{2+} content. To ensure complete recovery of feces, polyethylene glycol was used as marker.

Muscle, plasma, urine, and fecal magnesium measurements

Samples were analyzed in duplicate, and appropriate standards were used for measurements. The Mg^{2+} levels in muscle, plasma, urine, and feces were measured by an atomic absorption spectrophotometer (Perkin-Elmer 330, Perkin-Elmer Corp., Norwalk, CT, USA). Urine and fecal samples were diluted as necessary with deionized distilled water and aspirated directly into an atomic absorption spectrophotometer. The Mg^{2+} was determined by the atomic absorption spectrophotometer after diluting the specimen 1:50 with a solution of lanthanum-HCl to eliminate interference from anions including phosphate and protein and metal oxides.

Muscle preparations, magnesium extraction, and analysis

Muscle biopsies were performed by a percutaneous needle technique [15] under local anesthesia. Specimens were taken from the lateral portion of the quadriceps femoris muscle, 15–20 cm proximal to the knee. The muscle tissue (mean weight 14.5 mg) was placed on a piece of quartz glass and with non-metal tweezers carefully dissected free from all visible fat and connective tissue. All traces of blood were wiped off by rolling the specimens on the piece of quartz glass. The muscle tissue was then placed on a platinum hook and dried in an oven at 110°C to constant weight, extracted in 1 mL of petroleum ether for 2 h, and dried to constant weight again, and the weight of fat-free dry solids was calculated. The Mg^{2+} was extracted from the muscle tissue by treatment with 250 μL of 2.5 M HNO_3 for 24 h. From each sample, 100 μL of supernatant was diluted to 10 mL with 0.25% $SrCl_2$ and analysis for Mg^{2+} in muscle tissue was performed by atomic absorption spectrophotometry on a Perkin-Elmer 330 (Perkin-Elmer Corp., Norwalk, CT, USA). Results were calculated in millimoles per 100 g of fat-free dry solids.

Data analyses

The Mg^{2+} values in muscle, plasma, urine, and feces were subjected to a three-way analysis of variance to answer the question of whether muscle Mg^{2+} content would be affected by Mg^{2+} supplements and Mg^{2+} loss by muscle Mg^{2+} content; the three-way interaction (pre-experimental/experimental periods values, supplemented/ unsupplemented groups of subjects, control/experimental