Magnesium Supplementation Effect On Muscle Magnesium Content And Magnesium Loss In Humans During Hypokinesia

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Abstract

Objective: To study the impact of magnesium (Mg^{2+}) supplementation on muscle Mg^{2+} content and muscle Mg^{2+} loss during hypokinesia (HK; diminished movement).

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

Results: The muscle Mg^{2+} content decreased ($p<.05$) and plasma Mg^{2+} concentration, and Mg^{2+} loss in urine and feces increased ($p<.05$) in the SES and UES groups compared to their pre-experimental levels and the values in their respective control groups (SCS and UCS).The muscle Mg^{2+} content decreased ($p<.05$) more, and plasma Mg^{2+} concentration, and Mg^{2+} loss in urine and feces increased more ($p<.05$) in the SES group than in the UES group.

Conclusion: It is concluded that muscle Mg^{2+} content is not decreased by the Mg^{2+} deficient diet and bodily Mg^{2+} loss is not increased by the higher muscle Mg^{2+} content but due to the inability of the body to use Mg^{2+}; this is because muscle Mg^{2+} level cannot reduce with Mg^{2+} supplements and Mg^{2+} loss cannot increase with muscle Mg^{2+} deficiency unless Mg^{2+} deposition decreases.

ABBREVIATIONS

Hypokinesia (diminished movement) Hypokinesia (HK) Unsupplemented control subjects (UCS) Unsupplemented experimental subjects (UES) Supplemented control subjects (SCS) Supplemented experimental subjects (SES) Magnesium (Mg^{2+}) Adenosine triphosphate (ATP) Adenosine diphosphate (ADP)

INTRODUCTION

Muscular activity is a vital 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 result is increase of catabolism ([1,2,3]) and decrease of muscle cell mass ([4, 5]) and increase of electrolyte loss ([4,5,6,7]).

During HK, tissue electrolyte content decreases, and plasma electrolyte concentration and electrolyte loss increases ([4,5,6,7]). This is most likely attributable to many factors and primarily to the decreased muscle cell mass ([4, 5]). The muscle cell reduction can result in the higher plasma electrolyte level and electrolyte loss and electrolyte deficiency ([4,5,6,7]). The escalated plasma electrolyte level and electrolyte loss with tissue electrolyte deficiency indicates decreased electrolyte deposition, because with tissue electrolyte deficiency plasma electrolyte and electrolyte loss cannot increase unless electrolyte deposition decreases ([4,5,6,7]). Escalated plasma electrolyte level and electrolyte loss with tissue electrolyte deficiency ([4,5,6,7]) during HK indicates different mechanisms from those involved in the decreased plasma electrolyte level and electrolyte loss with tissue electrolyte deficiency during normal activity.

Magnesium (Mg^{2+}) is the fourth most abundant cation in the body and within the body the second only to potassium. There are three major roles for the Mg^{2+} in biological systems. The Mg^{2+} can compete with calcium for binding sites on proteins and membranes, and it can form chelates with important intracellular anionic ligands, notable adenosine triphosphate (ATP) ([8]). Magnesium catalyzes or activates more than 300 enzymes in the body. Magnesium also acts as an essential cofactor for enzymes ([9]) concerned with the 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 the 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+} supplements on enzymes activity, Mg^{2+} flux across cell membrane, ATP synthesis, Na^{+}-K^{+}-ATPase activity, Mg^{2+} deposition, Mg^{2+} loss and muscle Mg^{2+} deficiency during HK. Thus, we do not know how muscle Mg^{2+} deficiency and Mg^{2+} loss occurs or if muscle Mg^{2+} deficiency comes from Mg^{2+} shortage in the food consumed and Mg^{2+} loss from the inability of the body to use Mg^{2+} ([10],[11],[12],14). Each electrolyte has a well-defined and separate homeostatic mechanism to control Mg^{2+} content in a tissue at both cell membrane level and higher organ level. Being Mg^{2+} is a major electrolyte which functions as a coenzyme to keep nerve and muscle function, control body temperature, energy metabolism, and bone formation, it is vital to show if Mg^{2+} supplementation affects Mg^{2+} deposition and thus Mg^{2+} loss and muscle Mg^{2+} content during HK. To establish a potential of muscle Mg^{2+} deficiency and Mg^{2+} loss and lower Mg^{2+} deposition, it is important to study the effect of Mg^{2+} supplementation on muscle Mg^{2+} loss during prolonged HK.

To this end the aim of this study was to determine the effect of Mg^{2+} supplementation on muscle Mg^{2+} content and total bodily Mg^{2+} los which aimed at determining the ability of the body to use Mg^{2+} during prolonged HK. Measurements of muscle Mg^{2+} content, plasma Mg^{2+} level and Mg^{2+} loss with and without Mg^{2+} supplementation were carried out during HK.

MATERIALS AND METHODS

Forty physically healthy trained male volunteers 21.5 ± 3.0 years 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. There were no medical problems among the subjects and none of the volunteers was under any drug therapy which could have interfered with the magnesium metabolism. Procedures were reviewed and approved by the Committee for the Protection of Human Subjects. Financial incentives were used to encourage the compliance with the study protocol. Subjects were students and had been trained for the last 2 to 3 years, at an average rate of 5.0 times per week at 45.1 ± 2.2 km. $week^{-1}$. Subjects ran average distances of 9.0 ± 1.2 $km.day^{-1}$ at a speed of 9.7 ± 1.2 $km.h^{-1}$. 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-day pre-experimental period subjects ran average distances of 9.0 ± 1.2 $km.day^{-1}$ at a speed of 9.7 $km.h^{-1}$.

Assignment of subjects into four groups was conducted randomly and their conditions were as follows: Group 1: Ten healthy subjects ran an average distance of 9.0 ± 1.2 $km.day^{-1}$ for 364-days. They were assigned to the unsupplemented control subjects (UCS) group. Group 2: Ten healthy subjects walked an average distance of 1.3 ± 0.1 $km.day^{-1}$ for 364-days. They were assigned to the unsupplemented experimental subjects (UES) group. Group 3: Ten healthy subjects ran an average distance of 8.7 ± 1.2 $km.day^{-1}$ for 364-days and were supplemented with 3.0 mmol Mg^{2+} chloride/kg body weight per day. They were assigned to the supplemented control subjects (SCS) group. Group 4: Ten healthy subjects walked an average distance of 1.3 ± 0.1 $km.day^{-1}$ for 364 days and were supplemented with 3.0 mmol Mg^{2+} chloride/kg body weight per day. They were assigned to the supplemented experimental subjects (SES) group.

PROTOCOL

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

SIMULATION OF HYPOKINESIA

To simulate the effect of hypokinesia the number of km walking per day was restricted to an average of 1.3±0.1 km.day-^{1} and was monitored daily by an accelerometer. Activities allowed were those that approximated the normal routines of sedentary individuals. Subjects were allowed to walk to dining tables, 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 inter-individual differences in bowel habits, urine and feces collected daily and pooled to form 6-day composites, while plasma samples were collected every 6-days during pre-experimental and experimental period. A 6-day (consecutive day) pooled samples were collected and mean ± SD's 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 8.00-9.00 a.m., without a venous stasis and after subjects had been sitting for 30 min. The volume of blood sample was 7 to 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 x g for 3 min at room temperature and separated using glass capillary pipettes which 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 colorimetric method 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, were 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. 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 weight14.5 mg) was placed on a piece of quartz glass and with nonmetal 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 fat-free dry solids (FFDS) weight was calculated. The Mg^{2+} extracted from the muscle tissue by treatment with 250 µL 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 Model, Perkin-Elmer Corp., Norwalk, CT. Results calculated in mmol/100 g $^{-1}$ FFDS.

DATA ANALYSES

The Mg^{2+} values in muscle, plasma, urine, and feces were subjected to a 3-way analysis of variance (ANOVA) to answer the question would HK induce decrease in muscle Mg^{2+} content and increase Mg^{2+} loss and would this be attenuated with Mg^{2+} supplement; the 3-way interaction(time of experimental period, pre-experimental/experimental values, supplemented /unsupplemented groups of subjects, control/experimental groups of subjects) was used. ANOVAS for each time point measurements were used. A Tukey-Kramer post-hoc test was used to establish which means were significantly different from each other. Predetermined level of significance was set at $p<0.05$. The results obtained are reported as mean ± SD.

RESULTS

During the pre-experimental period, muscle Mg^{2+} content, plasma Mg^{2+} level, and urine and fecal Mg^{2+} loss did not change in the control and experimental groups of subjects. The Mg^{2+} supplementation did not alter the muscle Mg^{2+} content, plasma Mg^{2+} concentration, and urine and fecal Mg^{2+} loss in the control and the experimental groups of subjects (Tables 1).

During the experimental period, muscle Mg^{2+} content and plasma Mg^{2+} concentration and urine and fecal Mg^{2+} excretion did not show any changes in the SCS and UCS groups compared to their baseline values (Table 1). The muscle Mg^{2+} content decreased ($p<0.05$), while plasma Mg^{2+} concentration, and urine and fecal Mg^{2+} loss increased ($p<0.05$) in the SES and UES groups compared to their pre-experimental levels and the values in their respective controls groups (SCS and UCS) (Table 1). However, the muscle Mg^{2+} content decreased more ($p<0.05$), while plasma Mg^{2+} concentration, urine and fecal Mg^{2+} loss increased more ($p<0.05$) in the SES group than in the UES group (Table 1).

Figure 1

Table 1: Magnesium Levels in Urine, Feces, Plasma, and Muscle Measured in Physically Healthy Subjects with and without Magnesium Supplementation at the Pre-experimental Period, and During the Ambulation Period, and the Experimental Period.

Days	Urinary Mg^{2+}, mmol/days	Fecal Mg^{2+}, mmol/days	Plasma Mg^{2+}, mmol/L	Muscle Mg^{2+} mmol/100/g[1]FFDS
	Unsupplemented Control Subjects (UCS), n=10			
Pre-experimental	4.05 ± 0.5	12.77 ± 1.7	0.78 ± 0.05	4.45 ± 0.52
60th	3.91 ± 0.6	11.76 ± 1.5	0.77 ± 0.04	4.46 ± 0.43
120th	4.00 ± 0.4	12.47 ± 1.5	0.76 ± 0.04	4.45 ± 0.62
180th	3.85 ± 0.6	11.84 ± 1.6	0.77 ± 0.05	4.46 ± 0.50
240th	4.01 ± 0.5	12.39 ± 1.4	0.76 ± 0.04	4.44 ± 0.42
300th	3.95 ± 0.7	11.77 ± 1.5	0.77 ± 0.05	4.45 ± 0.60
364th	4.01 ± 0.5	12.53 ± 1.7	0.76 ± 0.04	4.46 ± 0.50
	Unsupplemented Experimental Subjects (UES), n=10			
Pre-experimental	4.05 ± 0.5	12.80 ± 1.4	0.77 ± 0.04	4.46 ± 0.63
60th	5.85 ± 0.6*	18.87 ± 1.7*	0.85 ± 0.05[+]	3.67 ± 0.62[+]
120th	5.15 ± 0.7*	17.35 ± 1.5*	0.84 ± 0.05[+]	3.81 ± 0.50[+]
180th	6.70 ± 0.5*	21.61 ± 1.6*	0.87 ± 0.06[+]	3.54 ± 0.47[+]
240th	5.83 ± 0.7*	18.54 ± 1.5*	0.85 ± 0.05[+]	3.72 ± 0.55[+]
300th	7.25 ± 0.5*	23.75 ± 1.7*	0.88 ± 0.06[+]	3.46 ± 0.66[+]
364th	6.85 ± 0.6*	21.85 ± 1.4*	0.87 ± 0.04[+]	3.57 ± 0.73[+]
	Supplemented Control Subjects (SCS), n=10			
Pre-experimental	4.63 ± 0.5	14.83 ± 1.5	0.80 ± 0.04	4.57 ± 0.73
60th	4.60 ± 0.6	14.97 ± 1.6	0.82 ± 0.05	4.59 ± 0.72
120th	4.53 ± 0.5	14.86 ± 1.5	0.81 ± 0.07	4.60 ± 0.60
180th	4.65 ± 0.7	14.94 ± 1.4	0.83 ± 0.06	4.63 ± 0.54
240th	4.58 ± 0.4	14.87 ± 1.5	0.82 ± 0.04	4.62 ± 0.60
300th	4.52 ± 0.6	14.95 ± 1.6	0.83 ± 0.05	4.63 ± 0.53
364th	4.65 ± 0.5	14.80 ± 1.4	0.81 ± 0.06	4.62 ± 0.62
	Supplemented Experimental Subjects (SES), n=10			
Pre-experimental	4.61 ± 0.7	14.83 ± 1.7	0.80 ± 0.05	4.57 ± 0.62
60th	7.83 ± 0.6*[+]	26.77 ± 1.5*[+]	0.95 ± 0.07[+][+]	3.35 ± 0.54[+][+]
120th	6.91 ± 0.7*[+]	23.65 ± 1.8*[+]	0.92 ± 0.05[+][+]	3.41 ± 0.61[+][+]
180th	8.84 ± 0.5*[+]	32.02 ± 1.7*[+]	0.97 ± 0.06[+][+]	3.25 ± 0.57[+][+]
240th	7.90 ± 0.6*[+]	27.71 ± 1.6*[+]	0.95 ± 0.05[+][+]	3.31 ± 0.48[+][+]
300th	9.97 ± 0.7*[+]	36.06 ± 1.5*[+]	1.00 ± 0.08[+][+]	3.17 ± 0.65[+][+]
364th	9.05 ± 0.6*[+]	32.72 ± 1.6*[+]	0.97 ± 0.05[+][+]	3.23 ± 0.75[+][+]

FFDS, fat free dry solids. All values were expressed as mean ± SD.

*$p<0.05$ significant differences between the control and experimental groups of subjects. Each of the experimental groups was compared with their respective control groups (UCS vs UES and SCS vs SES).

[+] $p<0.05$ significant differences between the supplemented and unsupplemented experimental groups of subjects.

DISCUSSION

This study showed no changes of muscle Mg^{2+} level, plasma Mg^{2+} level, and urine and fecal Mg^{2+} loss in the supplemented or unsupplemented experimental and control subjects during the pre-experimental period. This is consistent with previous studies ([10,11,12,13,14]) where during the pre-experimental period, Mg^{2+} homeostasis, plasma Mg^{2+} level, and urine and fecal Mg^{2+} loss was relatively stable regardless of supplementation of Mg^{2+}. This shows that during the pre-experimental period, the consumed Mg^{2+} during the supplementation may have been taken up for deposition and was used by the body which in turn protected

the net muscle Mg^{2+} content, plasma Mg^{2+} level, and Mg^{2+} loss in urine and feces without showing any gross differences.

During the experimental period, the muscle Mg^{2+} content was decreased more in the SES group than in the UES group. Because the SES group show lower muscle Mg^{2+} content than the UES group, the SES group experiencing lower Mg^{2+} deposition than the UES group; because muscle electrolyte content cannot decreased more with than without electrolyte supplementation unless electrolyte deposition reduces more in the SES group than in the UES group. The lower muscle Mg^{2+} content in the SES group than in the UES group resulted from a more degraded Mg^{2+} deposition in the SES group than in the UES group; because with electrolyte supplementation, the lower electrolyte deposition and the greater tissue electrolyte deficiency ([4,5,6,7]). This kind of changes shows that the lower muscle Mg^{2+} content with than without Mg^{2+} supplementation during HK shows different mechanisms than those involved in the lowered muscle Mg^{2+} content without than with Mg^{2+} supplementation during normal muscular activity. Some research had shown that electrolyte content decreases more in tissue with less weight-bearing supporting function and morphology ([16,17,18]) and tissue electrolyte content decreases more with than without electrolyte supplementation ([4,5,6,7]). The lower muscle Mg^{2+} content in the SES group than in the UES group may have resulted from the lower Mg^{2+} deposition in the SES group than in the UES group, because the lower electrolyte deposition, the higher electrolyte loss and the greater tissue electrolyte deficiency with electrolyte supplementation ([4,5,6,7]). Thus, by contrast to the normal muscular activity during HK muscle electrolyte content decreases more with than without electrolyte supplementation.

The higher plasma Mg^{2+} level and Mg^{2+} loss could have been attributable to decreased Mg^{2+} deposition because plasma electrolyte level and electrolyte loss cannot increase with muscle electrolyte deficiency unless electrolyte deposition reduces ([10,11,12,13,14]). The decreased electrolyte deposition promotes electrolyte shifting in plasma leading to the higher plasma electrolyte level and electrolyte loss with electrolyte deficiency ([4,5,6,7]). The higher plasma Mg^{2+} level and Mg^{2+} loss with muscle Mg^{2+} deficiency during HK shows different mechanisms than those involved in the lowered plasma Mg^{2+} level and Mg^{2+} loss with Mg^{2+} deficiency during normal muscular activity. Some research had shown that plasma electrolyte level and electrolyte loss increase more with higher than lower tissue electrolyte deficiency and that tissue electrolyte deficiency increases more with than without electrolyte supplementation ([10,11,12,13,14]). Thus, the higher plasma Mg^{2+} level and Mg^{2+} loss in the SES group than in the UES group is related to the lower Mg^{2+} deposition, in the SES group than in the UES group, since plasma electrolyte level and electrolyte loss cannot increase more with greater tissue electrolyte deficiency unless electrolyte deposition decreases more ([4,5,6,7]). Because plasma Mg^{2+} level and Mg^{2+} loss increased more in the SES group than in the UES group, the SES group showed lower Mg^{2+} deposition than the UES group, because the lower electrolyte deposition, the higher plasma electrolyte level and electrolyte loss, and the greater tissue electrolyte deficiency ([4,5,6,7]).

Because the mechanisms of muscle Mg^{2+} deficiency with and without Mg^{2+} supplementation and higher plasma Mg^{2+} concentration and higher Mg^{2+} loss with muscle Mg^{2+} deficiency have not been identified it is difficult to discuss the mechanisms for this kind of reactions. Because muscle cell injury due to the energy deficiency and hypovolemia is considered as the main causes of muscle Mg^{2+} deficiency with and without Mg^{2+} supplementation and higher plasma Mg^{2+} level and higher Mg^{2+} loss with muscle Mg^{2+} deficiency some information which indicate muscle cell injury during HK is presented to support this contention.

Magnesium is extremely essential for proper adenosine triphosphate (ATP) formation and production, because ATP is stored in the body as a combination of Mg^{2+} and ATP, which is known as Mg^{2+} ATP. The Mg^{2+} is also an important cofactor of oxidative metabolism and its loss may lead to ATP deficiency. The activity of Na^{+}, K^{+}-ATPase depends on Mg^{2+} stores. Muscle Mg^{2+} deficiency can affect Na^{+}, K^{+}-ATPase activity. For the stability ATP requires Mg^{2+}. With muscle Mg^{2+} deficiency ATP would easily breakdown into other components of adenosine diphosphate (ADP) and inorganic phosphate. The exhaustion of the ATP supplies reduces cellular transport ([19]).Muscle Mg^{2+} deficiency increases cell membrane permeability and escalates intracellular calcium level which in turn leads to calcification and cell injury. The increase of intracellular calcium level can have a number of severe effects ([4, 5]); it can activate phospholipases and proteases causing further damage to the cell cytoskeleton and membranes, and lead to muscle cell injury ([20]). Muscle cell injury eventually impairs

cellular transport and enhances intracellular electrolyte level ([4, 5]). The cell injury which changes the integrity of cell can lead to the release of intercellular contents into the plasma that can have severe effects on the electrolyte deposition because muscle cell injury diminishes the holding capacity of cell for Mg^{2+} that in turn results in Mg^{2+} loss with muscle Mg^{2+} deficiency ([4,5,6,7]).

Studies on the effect of HK on energy metabolism revealed significant changes in particular a reduction of ATP ([21]) and ADP formation ([22]). The total amount of creatine phosphate which is the first source of energy use for the reconstitution of ATP is decreased significantly ([1,2,3]), and because there is limited creatine phosphate there is little release of energy to cause bonding of a new phosphate ion to ADP to reconstitute ATP. The ADP is a stimulator of oxygen uptake and formation of new ATP molecules during oxidative phosphorylation a process which reduces markedly ([23]). The mitochondrial number reduction and/or function indicated as the most likely culprit to explain decreased oxidative phosphorylation. Because of inhibition of ADP formation and ATP production there is also a limitation of reserves of ATP. Moreover, the ATP becomes energetically less efficient ([1,2,3]) and for production of ATP more substrates are used, which causes an even greater shortage of the already sparse reserves of ATP. Any condition which decreases the production or use of ATP by muscle, or leads to ATP deficiency can result in muscle cell injury, thereby interfering with electrolyte deposition leading to the higher electrolyte loss with muscle electrolyte deficiency.

The decrease formation of ATP most probably reflects the functional condition of the entire body, because HK is associated with a reduction of energy production ([1,2,3]). The reduction of ATP level and increase of energy-producing processes may be indicative of increase in the rate of ATP-consuming processes, in particular increase of the ATPase transport due to the possible changes on membrane structure ([24]). There also occurs a reduction of the nucleotide pool due to the general catabolic orientation processes of the body ([1,2,3]). Changes in energy metabolism, i.e., decrease of ATP production and increase of glycolytic activity ([25]), are related to the triggering of compensatory mechanisms in response to the hypokinetic effect, and they are directed toward preserving the structural integrity of cell which is eventually affected due to the ATP deficiency and activation of glycolysis ([4, 5]). A uniform muscle necrosis, leukocyte and macrophage invasion of degenerated muscle fibers has been shown with muscle cell injury, and ultrastructural changes include the separation of myofibrils and other cellular elements by clear spaces ([26]). Decrease of Mg^{2+} deposition owing to cell injury because of energy deficiency may exacerbate Mg^{2+} loss with muscle Mg^{2+} deficiency ([4, 5]).

The potential mechanisms of Mg^{2+} loss with muscle Mg^{2+} deficiency may also be attributable to the cell injury owing to inadequate blood supplies and oxygen delivery to the tissues. The absence of the muscular activity and the deconditioning of vessels of the lower part of the body results in the shifting of fluid to the lower extremities and the retention in them of a large fluid volume than what is the norm for the lower part of the body. Efflux of fluid to the lower half of the body occurs primarily from the thoracic region of the body and the fluid volume decreases ([27]) which results in the higher plasma electrolyte level and electrolyte loss. Because plasma electrolyte level and electrolyte loss increases with hypovolemia, it is in the nature of a reverse fluid volume regulating reflex ([28]). The higher plasma Mg^{2+} level and Mg^{2+} loss may be attributable to muscle cell injury due to hypovolemia; this is because the hypovolemia can indirectly result in cell injury ([20]) which alters the integrity of muscle cell and leads to the release of intracellular contents into the plasma which potentially can result to the higher electrolyte loss ([4, 5]). Depending on the magnitude of the hypovolemia and the compensatory ability of cells, the response at the cellular level may be one of compensation, dysfunction, or injury. Aerobic respiration apparatus of cell, i.e., oxidative phosphorylation by mitochondria, is the most susceptible to the inadequate blood supplies and thus oxygen delivery to the tissues. As blood flow and oxygen tension within the cell decreases, there is a significant reduction in oxidative phosphorylation ([23]) and the ATP production slows ([21]). The loss of ATP has widespread effects on the cellular function and morphology and the structure of cell. As the oxidative phosphorylation slows, cell shifts to anaerobic glycolysis which allows for the production of ATP from breakdown of cellular glycogen. During HK glycogen stores are depleted ([25]), while the anaerobic glycolysis is much less efficient than oxygen-dependent mitochondrial pathways and cell is eventually injured thereby interfering with the electrolyte deposition resulting in the higher electrolytes loss with tissue electrolyte deficiency ([4,5,6]).

The muscle Mg^{2+} deficiency may be a consequence of decreased Mg^{2+} deposition due to the cell injury owing to the energy deficiency and hypovolemia during HK. Because

of energy deficiency and hypovolemia occurs a change in the cell structure and Mg^{2+} deposition that in turn results in a higher Mg^{2+} loss because it cannot be retained in the body even in the face of muscle Mg^{2+} deficiency. Because Mg^{2+} is deposited in cell of different tissues a cell injury would alter Mg^{2+} deposition and lead to the extracellular buildup of Mg^{2+} resulting in the higher Mg^{2+} loss with muscle Mg^{2+} deficiency. In this instance, it becomes apparent that during HK restoration of muscle Mg^{2+} deficiency by means of Mg^{2+} supplements with food is impossible even with a normal regulation of Mg^{2+} metabolism at the cellular level, it is very difficult or impossible for the body to use Mg^{2+} until factors leading to the decreased Mg^{2+} deposition are restored to conform to the demands of body ($_4$, $_5$). Consequently the Mg^{2+} supplementation would fail to prevent muscle Mg^{2+} deficiency and muscle Mg^{2+} deficiency would fail to prevent Mg^{2+} loss in humans forced to restrict their muscular activity. The additional intake of Mg^{2+} therefore, can only be considered as a symptomatic therapy for Mg^{2+} deficient muscle in healthy subjects during prolonged HK.

CONCLUSION

The lower muscle Mg^{2+} content in SES group than in the UES group shows that muscle Mg^{2+} content decreases more with than without Mg^{2+} supplementation. The higher bodily Mg^{2+} loss with greater than smaller muscle Mg^{2+} deficiency in turn shows that the total bodily Mg^{2+} loss increases more with greater than smaller muscle Mg^{2+} deficiency. The dissociation between muscle Mg^{2+} content and Mg^{2+} supplementation and bodily Mg^{2+} loss and muscle Mg^{2+} deficiency demonstrates that the decreased Mg^{2+} deposition is the main cause of lower muscle Mg^{2+} content with Mg^{2+} supplementation and Mg^{2+} loss with muscle Mg^{2+} deficiency; because muscle Mg^{2+} content cannot decrease with Mg^{2+} supplementation and Mg^{2+} loss cannot increase with lower muscle Mg^{2+} level unless Mg^{2+} deposition reduces. It is concluded that muscle Mg^{2+} deficiency is more evident with than without Mg^{2+} supplementation and that the bodily Mg^{2+} loss is exacerbated more with greater than smaller muscle Mg^{2+} deficiency. In all, the reduction in the muscle Mg^{2+} content is not resulted from the consumed Mg^{2+} deficient diet and the total bodily Mg^{2+} loss is not caused by the excessive Mg^{2+} content in the skeletal muscle. Rather, it is caused due to the impossibility of muscle to use Mg^{2+} when physically healthy subjects are submitted to prolonged restriction of muscular activity.

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