行政院國家科學委員會專題研究計畫 成果報告

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研究成果報告(精簡版)

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成果報告

鎂對於運動在能量代謝物質與細胞激素分析之影響及其應用

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整合型計畫

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Magnesium sulfate enhances exercise performance and manipulates dynamic changes in peripheral glucose utilization

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Abstract  The effect of magnesium supplementation on exercise performance remains controversial. In the present study, the effects of magnesium sulfate on exercise performance and blood glucose metabolism were examined. In order to provide a non-invasive measure of continuous exercise, we developed an auto-blood sampling system was coupled to a microdialysis analyzer to detect the dynamic changes in glucose metabolism in conscious and freely moving gerbils subjected to forced swimming. Gerbils were pretreated with saline or magnesium sulfate (90 mg kg$^{-1}$, ip) 30 min before exercise. The duration times were significantly increased by 71% in the magnesium sulfate-treated groups ($p < 0.01$) when compared with those in the control. Another group of gerbils were subjected to blood sampling assay. A catheter was implanted in the jugular vein of each gerbil for collecting blood samples by the computer-aided blood sampler. The basal levels of plasma glucose, lactate, and magnesium were $6,245 \pm 662$, $1,067 \pm 309$, and $590 \pm 50 \mu$M, respectively, with no significant difference between groups. Plasma glucose, lactate, and magnesium levels increased to 134 and 204%, 369 and 220%, and 155 and 422% of basal levels during swimming in both the control and magnesium sulfate-treated groups, respectively ($p < 0.05$). Pretreatment with magnesium sulfate elevated glucose and magnesium levels to 175 and 302% of the basal levels ($p < 0.05$), respectively, whereas pretreatment with magnesium sulfate reduced the lactate levels 150% of the basal level ($p < 0.05$) during swimming. Furthermore, the magnesium levels increased to about 152–422% of basal levels during forced swimming and the recovery period ($p < 0.05$). The present study demonstrates that magnesium sulfate improved the duration time of forced swimming exercise. In addition, magnesium raised glucose levels and attenuated lactate levels during forced swimming. These results indicate that positive effects of magnesium supplementation may contribute to the enhancement of exercise performance in athletes.

Keywords  Forced swimming · Glucose · Lactate · Magnesium

Introduction

Magnesium is a cofactor in more than 300 enzymatic reactions in which food is catabolized and new chemical products are formed (Lukaski 2000). Magnesium is also involved in cellular energy production, glycogen breakdown, and modulation of the activity of adenosine triphosphatase providing energy for cells; moreover, magnesium serves as a physiological regulator of neuromuscular functions (Ebel and Gunther 1980; Lin et al. 2002; Lukaski 2000; Saris et al. 2000). Thus, magnesium may be regarded
as one of the important elements that affect physical performance and exercise.

Accumulating evidence has shown a direct relationship between magnesium and exercise performance. Some studies have reported that serum or plasma magnesium concentration was decreased after exercise (Deuster and Singh 1993; Laires and Alves 1991; Laires et al. 1988, 1993; Lijnen et al. 1988; Mooren et al. 2005; Stendig-Lindberg et al. 1987). In general, long-term, high-intensity exercises, including hiking, swimming, and running, and taking the treadmill ergometer test resulted in a decrease in magnesium levels. In contrast, plasma or serum magnesium concentrations increased during short-term, intense exercise as a consequence of a reduction in plasma volume and a shift of cellular magnesium resulting from acidosis (Deuster et al. 1987; Joborn et al. 1985; Poleszak et al. 2005; Rama et al. 1993; Rayssiguier et al. 1990). These data suggest that the intensity and duration of exercise performance play critical roles in the regulation of magnesium homeostasis. On the other hand, magnesium regulates glycolysis (Saris et al. 2000), and hypermagnesemia reduces the rate of glucose metabolism in neural tissues by inhibiting glycolysis (Szabo and Crosby 1988). These studies indicate that prolonged exhausting exercise results in hypoglycemia and hypomagnesemia (Felig et al. 1982; Mooren et al. 2005), whereas short-term, high-intensity exercise induces hypermagnesemia and hyperglycemia (Gotoh et al. 1998; Poleszak et al. 2005; Rayssiguier et al. 1990). Accordingly, it is thought that magnesium may influence glucose homeostasis during exercise.

We have previously demonstrated that short-term forced swimming decreased brain extracellular glucose and pyruvate levels, whereas it increased extracellular lactate levels as measured by microdialysis in gerbils. Pretreatment with magnesium sulfate immediately increased glucose and pyruvate levels, whereas it attenuated lactate levels (Cheng et al. 2007). However, there has been considerable interest in whether magnesium supplementation and dosages can induce an improvement in exercise performance. The aim of this study was to determine the effects of magnesium sulfate supplementation on exercise performance and blood glucose metabolism during forced swimming exercise. In order to provide a non-invasive measure of continuous exercise, a second aim of the present study was to develop an auto-blood sampling system (DR-II) that could be coupled with a microdialysis analyzer (CMA/600) to measure blood metabolites during exercise.

Materials and methods

Animals

Adult and naïve male gerbils (n = 24), weighing between 70 and 80 g, were obtained from the Laboratory Animal Center of Taichung Veterans General Hospital. All animals were housed in a temperature (25°C) and light (12:12 h light–dark cycle)-controlled room with free access to rat chow and tap water. Animal care and experimental procedures (# La-95225, TCVGH, Taiwan) were in accordance with the Guide for the Care and Use of Laboratory Animals issued by the U.S. Department of Health and Human Services and with the policy statement regarding the care and use of experimental animals by the American College of Sports Medicine. The gerbils were separated into two groups. One group (first experimental group, n = 12) was subjected to forced swimming during the forced swimming. In the blood sampling group, a PUC-40 tubing was connected to the jugular vein of each gerbil and both was withdrawn according to the method described by Harms and Ojeda (1974). Through a subcutaneous vein in the neck from the back, the PUC-40 was connected to the DR-II, the auto-blood-sampling device.

Forced swimming

The gerbils were randomly divided into the control (saline) group or the magnesium sulfate supplement group (n = 6, each group). In the first experiment, each gerbil was placed on a polystyrene board floating in a Plexiglas cylinder that was 40 cm in diameter, 35 cm high, and filled with warm water (about 35°C, no heat loss or gain occurred) to a height of 18 cm (Cheng et al. 2007; Lin 1988). Saline or magnesium sulfate (90 mg kg\(^{-1}\), intraperitoneal injection) was injected 30 min prior to the forced swimming task. The total duration time of forced swimming was measured during the first experiment. Gerbils were judged to be immobile when they floated passively with the head above water (Komiya et al. 2006). After the forced swimming task, each gerbil was removed from the water, and then carefully and rapidly dried with a towel and a hair blower.

In the second experiment, the polystyrene board was removed gently, and each gerbil was forced to swim for 15 min (if a gerbil was judged to be immobile when it floated passively with the head above water, it was allowed to rest for 10 s, and then forced to perform the swimming exercise again). After the forced swimming exercise, each gerbil was removed from the water, and then carefully and rapidly dried with a towel and a hair blower. Each gerbil was then re-placed on the polystyrene board, and the blood samples were collected during the 3 h recovery period.

Sampling

Blood samples were automatically collected via an implanted catheter in the jugular vein by the computer
Aurora Bio-Medical Technology Inc. The sidetracked sample then was centrifuged at 1,000 g for 10 min at 4°C. Aliquots of the supernatant were directly injected into the microdialysis analyzer (CMA/600, Car-Nege Medicin), and a flame atomic absorption spectrometer for detecting glucose, lactate and magnesium concentrations, respectively.

Analysis of energy metabolites and magnesium

A Perkin-Elmer Model 5100 flame atomic absorption spectrometer (FAAS, Perkin-Elmer, Uberlingen, Germany) was used for analyzing magnesium levels. Twenty-five microliter of supernatant was taken and diluted with 975 µl of 0.2% HNO₃ for further analysis by the FAAS. All reagents used were of analytical grade and were purchased from E. Merck (Merck-Schuchardt, Darmstadt, Germany). All containers were soaked with 20% of nitric acid, rinsed with water and then dried in a clean room for later use.

The microdialysis analyzer was employed for determination of plasma glucose and lactate concentrations. Glucose and lactate are oxidized by glucose and lactate oxidase, respectively. Peroxidase catalyzes the reaction between the hydrogen peroxidase formed phenol, and 4-amino-antipyrine to form the red–violet colored quinone imine, which is detected at 546 nm.

Statistical analysis

All data are expressed as mean ± SEM. A repeated-measures two-way analysis of variance (ANOVA) and Fisher’s PLSD-test were used to analyze statistically significant differences between the saline control and the magnesium sulfate-treated groups. Differences were considered statistically significant at p < 0.05.

Results

The average duration times for the forced swimming were 219 ± 4 and 374 ± 12 s in the control and magnesium sulfate-treated groups, respectively. The duration times were significantly increased by 71% in the magnesium sulfate-treated group (p < 0.01) when compared with the control, as shown in Fig. 1.

The basal levels of plasma glucose were 6,245 ± 662 and 6,231 ± 745 µM in the control and magnesium sulfate-treated groups, respectively (Fig. 2). The glucose levels of the control group increased to about 134% of the basal level (p < 0.01) during forced swimming, then returned to the basal level after 30 min of recovery. In the magnesium sulfate-treated group, the glucose levels immediately increased to 150–175% of basal levels (p < 0.05), gradually increased to 204% of basal levels (p < 0.01), and returned to the basal level after 30 min of recovery. The plasma glucose levels of the magnesium sulfate-treated group during swimming and after 15 min of recovery were about 50–70% greater than those of the control group (p < 0.05).

The basal levels of plasma lactate were 1,067 ± 309 and 1,122 ± 234 µM in the control and magnesium sulfate-treated groups, respectively (Fig. 3). During swimming, the lactate levels increased to 220 and 369% of the basal level (p < 0.01) in the magnesium sulfate-treated and control groups, respectively, whereas lactate returned to the basal level after 30 min of recovery. The lactate levels during
swimming attenuated to about 150% of the basal level in the magnesium sulfate-treated group ($p < 0.05$) compared to those in the control group.

The basal levels of plasma magnesium were 590 ± 50 and 590 ± 140 μM in the control and magnesium sulfate-treated groups, respectively (Fig. 4). The magnesium levels of the control group increased to about 155% of the basal level ($p < 0.05$) during forced swimming and remained at that level throughout the recovery period. In the magnesium sulfate-treated group, the magnesium levels immediately increased to 302–347% of basal levels ($p < 0.05$). Moreover, the magnesium levels increased to 422% during swimming ($p < 0.01$) and then gradually diminished to about 150% of basal levels throughout the recovery period ($p < 0.05$). The magnesium levels of the magnesium sulfate-treated group during swimming and after 1 h of recovery were 120–267% greater, respectively, than those of the control group ($p < 0.01$).

The basal ratios of lactate/glucose level were 0.16 and 0.19 in the control and magnesium sulfate-treated groups, respectively (Fig. 5). In the control group, the ratio of lactate/glucose level increased to approximately 0.33 of the basal level ($p < 0.05$) during 15 min forced swimming. Then, it decreased to about 0.18 of the basal level at 30 min after forced swimming, and returned to about 0.11–0.16 of the basal level, where it remained throughout the rest of the recovery period. In the magnesium sulfate-treated group, the ratio of lactate/glucose level increased to approximately 0.25 of the basal level ($p < 0.05$) after 15 min forced swimming and gradually decreased to about 0.13 of the basal level at 30 min after forced swimming. These ratios returned to approximately 0.13–0.08 of the basal level after 30 min of recovery and remained at that level for an additional 2 h.

**Discussion**

In this study, a computer aided auto-blood sampling system (DR-II) coupled with a microdialysis analyzer (CMA/600) and a flame atomic absorption spectrometer was developed for the determination of the dynamic changes in plasma glucose, lactate, and magnesium levels. Our data indicated that magnesium sulfate enhanced exercise performance and increased plasma glucose and magnesium levels, and
decreased lactate levels during the short-term forced swimming exercise.

For obtaining repeated blood samples from small rodents, a number of techniques have been developed. Among these methods, three widely applied techniques are amputation of the tail-tip, tail incision, and retro-orbital puncture (Christensen et al. 2009; Durschlag et al. 1996; Fitzner Toft et al. 2006). However, the above assays are associated with stressful behavioral responses and require anesthesia. In addition, these techniques may also affect subsequent sampling of blood during a short period of time. A simple method for the implantation of silicone cannula into the rat jugular vein was developed by Harms and Ojeda (1974). In the present study, the blood sampling procedure was slightly modified according to this previously described method. Our procedure is much less stressful to the animal than the above-mentioned conventional methods. The implanted cannula can be used to either administer drugs or collect blood samples with less stress to the animals, which are obviously conscious during the exercise sessions. In addition, a computer program was used to ensure that a blood volume of 30 μl was collected automatically every 15 min during the baseline, exercise, and rest periods. During the sampling procedures, 30 μl of saline was also delivered to compensate for the blood volume withdrawn from the animal.

Magnesium may be regarded as one of important elements for exercise as well as physical performance. Accumulating evidence has shown a direct relationship between magnesium and exercise performance. Some studies have reported that serum or plasma magnesium concentration was decreased after exercise (Deuster and Singh 1993; Laires et al. 1988; Laires et al. 1993; Mooren et al. 2005; Stendig-Lindberg et al. 1987). Exercise may increase the demand for magnesium and increase magnesium loss, potentially leading to magnesium deficit, which can result in muscle weakness, and neuromuscular dysfunction (Lijnen et al. 1988). Magnesium has many functions, so it is easy to understand why exercise performance is highly dependent on the regulation of magnesium homeostasis, and such performance seems to be impaired under conditions of magnesium deficiency (Clarkson 1995). Moreover, some studies have demonstrated that magnesium supplementation has positive effects on exercise capacity and performance (Bohl and Volpe 2002; Cinar et al. 2007; Coggan 1991; Nielsen and Lukaski 2006). Magnesium depletion may lead to changes in neuromuscular function and reduce physical performance and the efficiency of energy metabolism (Bohl and Volpe 2002), and exercise may deplete magnesium levels and impair energy metabolism efficiency. In this study, the duration time of forced swimming in the magnesium sulfate-treated group was significantly increased compared with that of the control group, as shown in Fig. 1. Magnesium supplementation is thought to provide a boost to exercise performance. According to the present data we propose that pre-treatment of magnesium sulfate has a beneficial effect during swimming exercise due to its ability to increase plasma glucose availability and reduce systemic lactate concentrations. In addition, numerous experimental studies have demonstrated the antidepressant- and anxiolytic-activity of various NMDA receptor antagonists (Poleszak et al. 2005, 2008). It has been shown that magnesium blocks the activation of the NMDA receptor ion channel in a voltage-dependent manner. In clinical trials, a low level of magnesium has been related to affective disorders and depressive symptoms. Moreover, magnesium administration reduces immobility time in the forced swimming test (Poleszak et al. 2005). We observed that magnesium enhanced the duration times of swimming (Poleszak et al. 2005; Rayssiguier et al. 1990). Our previous study indicated that short-term swimming resulted in extracellular glucose, and magnesium levels decreased in the brain (Cheng et al. 2007). However, the present study has indicated that the short-term swimming induced increases in plasma glucose, lactate, and magnesium levels, and magnesium remained at that increased level throughout the recovery period, as shown in Figs. 2, 3, and 4. With respect to blood extracellular magnesium, various studies have indicated that short-term, high-intensity anaerobic exercise leads to hypermagnesaemia as a consequence of the reduction in plasma volume and a shift in cellular magnesium resulting from acidosis (Cordova 1992; Rayssiguier et al. 1990). These data suggest that even the same exercise model can have a different effect on energy metabolites of the central and peripheral nervous systems. Evidence has indicated that acute swimming exercise induced activity in the hypothalamic–pituitary–adrenal axis in response to stress: i.e., elevations in corticosterone and adrenocorticotrophic hormone concentrations (Contartezte et al. 2008), which may result in hyperglycaemia. In the control group, glucose levels were stimulated and elevated at the beginning of swimming. The initial increased plasma glucose levels may be spiked by the forced swimming exercise, as shown in Fig. 2.

Moreover, we used the auto-blood sampling system to determine energy metabolites during short-term forced swimming. Pretreatment with magnesium sulfate increased
the glucose and magnesium levels, whereas it reduced the lactate level during swimming. The effects of supplemental magnesium on glucose levels may be via the regulation of both the oxidative phosphorylation and glycolysis pathways (Saris et al. 2000). In addition, magnesium is a cofactor in many rate-limiting enzymes such as hexokinase, pyruvate dehydrogenase, and creatine kinase (Altura 1991). Magnesium sulfate attenuated lactate levels and increased glucose during exercise. These data indicated that magnesium supplementation might increase energy for exercise and enhance the exercise performance.

Traditionally, lactate production has been considered to be associated with muscle fatigue during exercise, however current research has also suggested a positive effect for lactate during muscle contraction (Philp et al. 2005). In the present study, decreased lactate levels \( p < 0.01 \) were observed after magnesium supplementation. During the swimming period, the lactate/glucose ratio \( (L/G) \) increased to 0.25 of the basal level in the magnesium-sulfate-treated group \( (p < 0.05) \), whereas the \( L/G \) ratio increased to 0.33 of the basal level in the control group \( (p < 0.05) \), but the difference was not significant, as shown in Fig. 5. Magnesium supplementation may cause attenuation of lactate levels under the influence of high-intensity swimming exercise and might provide an additional energy source for enhancing exercise performance.

Accumulating evidence has shown that magnesium deficiency can result in a significant decrease in exercise performance. On the other hand, supplemental magnesium has been shown to improve exercise performance (Bohl and Volpe 2002; Clarkson 1991; de Haan et al. 1985; Lukaski and Nielsen 2002; Weight et al. 1988a, b). Moreover, magnesium sulfate increased glucose and pyruvate levels, whereas it decreased the rate of lactate formation in the brain and periphery during exercise (Cheng et al. 2007). Based on the above evidence, we believe that magnesium supplementation may be beneficial to exercise performance.

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