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Effect of Acute High-intensive Swimming Exercise on Blood Electrolytes and Metabolites

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Abstract : Magnesium (Mg^{2+}) is an essential co-factor for over 325 physiological and biochemical processes so that plays a central role of neuronal activity, cardiac excitability, neuromuscular transmission, muscular contraction, vasomotor tone, and blood pressure significantly related to physical performance. However, only limited information on blood ionized Mg^{2+} (iMg^{2+}) regarding to physical exercise is available and the data from blood total Mg^{2+} detection are inconsistent. This present study investigated the changes of blood iMg^{2+} correlated with metabolic demands during acute high-intensive exhaustive physical exercise in rats. After exhausted swimming (3-4 hours), blood pH, glucose, HCO_3^-, oxygen and ionized Ca^{2+} (iCa^{2+}) were significantly decreased, whereas lactate, carbon dioxide, iMg^{2+}, ionized Na^+ and ionized K^+ were significantly increased. During the exhausted swimming, the changes in iMg^{2+} showed a significant negative correlation with changes in pH, glucose, HCO_3^- and iCa^{2+}, however a significant negative correlation with changes in lactate and anionic gap. It is concluded that the acute high-intensive exhaustive physical exercise could produced hypermagnesemia, an increase in blood iMg^{2+} via stimulation of iMg^{2+} efflux following increase in intracellular iMg^{2+} from muscle induced by metabolic and respiratory acidosis.

***Key words* :** Ionized Mg^{2+}, swimming, acidosis, hypermagnesemia.

Introduction

Magnesium (Mg^{2+}) is the 4th most common mineral in body, the 4th most ion in blood and the 2nd most cation in cell (12). Mg^{2+} is an essential co-factor for over 325 physiological and biochemical processes, especially energy synthesis, storage and utilization, therefore it plays a central role of neuronal activity, cardiac excitability, neuromuscular transmission, muscular contraction, vasomotor tone, and blood pressure significantly related to physical performance (4). Likewise, there are evidences that Mg^{2+} deficiency may reduce physical performance and Mg^{2+} status may have an effect on exercise capacity in racing horse (20). Indeed exercise is a potent stressor that appears to lead to Mg^{2+} depletion through alterations on blood Mg^{2+} levels subsequent loss into sweat and urine excretion (4).

In blood, Mg^{2+} is divided into three fractions: 65% ionized (iMg^{2+} as active form), 27% protein-bound and 8% anion complex form (saris). Since only the free iMg^{2+} is biochemically active and could be moblilizble (12), the determination of iMg^{2+} concentration is supposed to give more reliable information than that of total Mg^{2+} (tMg^{2+}). However, only limited information on blood iMg^{2+} regarding to the exercise is available and the data for tMg^{2+} detection regarding to exercise are inconsistent (4). The blood iMg^{2+} may be affected by total serum protein concentration, pH, presence of carrier proteins and individual protein-binding affinity so that do not always correlate with the respective blood tMg^{2+} (17). Therefore, it would be advantageous to directly measure blood iMg^{2+} so that clinically relevant abnormalities are more reliably detected.

The goal of the study was to investigate the changes of whole blood iMg^{2+} correlated with metabolic demands after acute high-intensive swimming exercise in rats.

Materials and Methods

All experimental protocols employed herein were approved by the Committee on the Care of Laboratory Animal Resources, Chonbuk National University, and were conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication No. 85-23, revised 1996).

Animals

Thirteen male Sprague-Dawley rats (220~250 g, Samtako Biokorea, Daejeon, Korea) were used. They were housed in a temperature (23 ± 2°C) and humidity (50 ± 5%) with a 12 hours light/12 hours dark cycle. Food and water were available ad libitum.

Acute high-intensive forced swimming

We designed a forced swimming pool especially planned

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for swimming exercise of rat. The system consists of a glass chamber (height: 70 cm in height, 60 cm in length and 90 cm in width) filled with water of 55 cm in height, heating system and air pumping system. To prevent floating during swimming, water bubbling was produced by tubes connected to the air pump system. A heating system kept the temperature of water within glass chamber at 36 ± 1°C using a thermostatically controlled heater on the base of the chamber. Rats were individually forced to swim until exhaustion (about 3-4 hours) within the swimming pool.

Measurement of blood ions and metabolites

Blood was collected from tail vein just before swimming and caudal vena cava after immediately following swimming. Blood collection, storage and measurement were following to the guideline (1,13,19). We used Nova Stat Profile® 8 CRT (NOVA Biomedical Corp, Waltham, MA, USA) for measuring pH, partial carbon dioxide tension (PCO_2), total carbon dioxide (TCO_2), partial oxygen tension (PO_2), oxygen content (o_2ct), oxygen saturation (o_2sat), alveolar oxygen (APO_2),arterial alveolar oxygen tension ratio (a/APO_2), arterial oxygen tension gradient ($AaDO_2$), partial oxygen tension/fraction of inspired oxygen (PO_2/FI), glucose, lactate, osmolality, hematocrit, hemoglobin, ionized Na^+ concentration (iNa^+), ionized K^+ concentration (iK^+), ionized Ca^{2+} concentration (iCa^{2+}), ionized Mg^{2+} concentration (iMg^{2+}), ration of iCa^{2+} per iMg^{2+} (iCa^{2+}/iMg^{2+}), normalized iCa^{2+} to ph 7.4 (nCa^{2+}), normalized iMg^{2+} to ph 7.4 (nmg^{2+}), ration of nCa^{2+} per nMg^{2+} (nCa^{2+}/nMg^{2+}), ionized Cl^- concentration (iCl^-), ionized HCO_3^- concentration ($iHCO_3^-$), standard bicarbonate concentration (SBC), base excess of extracellular fluid (BE-ECF), base excess of blood (BE-B) and anionic gap in whole blood.

Statistical analysis

The results were expressed as the means ± standard error of the mean (SEM). The data was analyzed via paired Student's *t*-test or correlation test via Spearman's rank correlation coefficient using Prism 5.03 (GraphPad Software Inc, San Diego, CA). A p value of < 0.05 was considered to significant.

Results

Effect of acute high-intensive forced swimming on blood pH, glucose, HCO_3^- and lactate

As shown as Fig 1, the acute high-intensive swimming produced a significant decrease in blood pH (7.21 ± 0.03 vs 7.44 ± 0.03 before swimming), glucose (87.6 ± 4.4 vs 148.1 ± 6.1 mM/L before swimming) and $iHCO_3^-$ (26.1 ± 0.8 vs 17.0 ± 0.8 mM/L before swimming), whereas a significant increase in lactate (11.5 ± 0.6 vs 6.0 ± 0.2 vs mM/L before swimming). The changes in osmolality (297 ± 2 vs 290 ± 1 mM/kg before swimming), hematocrit (43.4 ± 0.9 vs 42.7 ± 0.9% before swimming) and hemoglobin (14.5 ± 0.3 vs 14.3 ± 0.3 mM/L before swimming) were not significant.

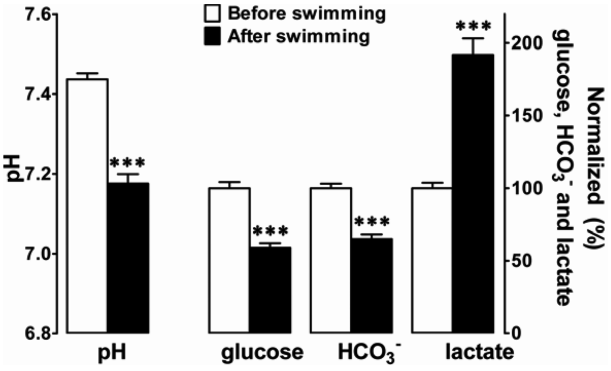

Fig 1. Effects of acute high-intensive swimming exercise on blood pH, glucose, HCO_3^- and lactate. The values of glucose, HCO_3^- and lactate. were normalized to those before swimming. Data are mean ± SEM of 13 different preparations. ***$p < 0.001$, paired Student's *t*-test versus before swimming.

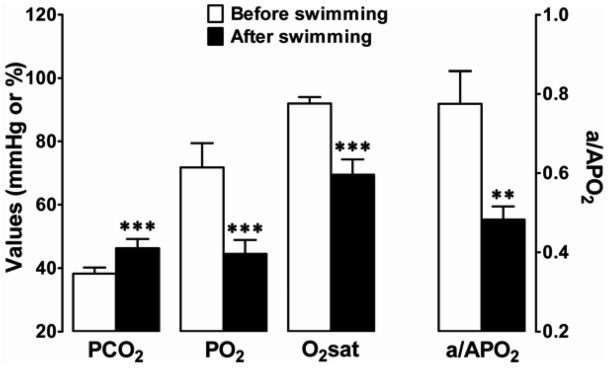

Fig 2. Effects of acute high-intensive swimming exercise on blood PCO_2, PO_2, O_2sat and a/APO_2. PCO_2, partial carbon dioxide tension; PO_2, partial oxygen tension; O_2sat, oxygen saturation alveolar oxygen; a/APO_2, arterial alveolar oxygen tension ratio. Data are mean ± SEM of 13 different preparations. **$p < 0.01$ and ***$p < 0.001$, paired Student's *t*-test versus before swimming.

Effect of acute high-intensive forced swimming on blood gas composition

As shown as Fig 2, the acute high-intensive swimming produced a significant decrease in blood PO_2 (44.5 ± 3.7 vs 71.8 ± 7.0 mmHg before swimming), O_2sat (69.5 ± 4.7 vs 92.0 ± 1.9% before swimming) and a/APO_2 (0.48 ± 0.03 vs 0.78 ± 0.08 before swimming), whereas a significant increase in PCO_2 (46.3 ± 2.8 vs 38.2 ± 1.7 mmHg before swimming). Also, the acute swimming evoked a significant decrease in blood O_2ct (14.2 ± 1.0 vs 18.5 ± 0.6% before swimming, $p < 0.01$) and PO_2/FI (217 ± 18 vs 344 ± 217 before swimming, $p < 0.05$).

Effect of acute high-intensive forced swimming on blood electrolytes

As shown as Fig 3, the acute high-intensive swimming produced a significant decrease in blood nCa^{2+} (1.24 ± 0.02 vs 1.33 ± 0.02 mM/L before swimming) and nCa^{2+}/nMg^{2+} (2.05 ± 0.09 vs 2.60 ± 0.08 before swimming), whereas a significant

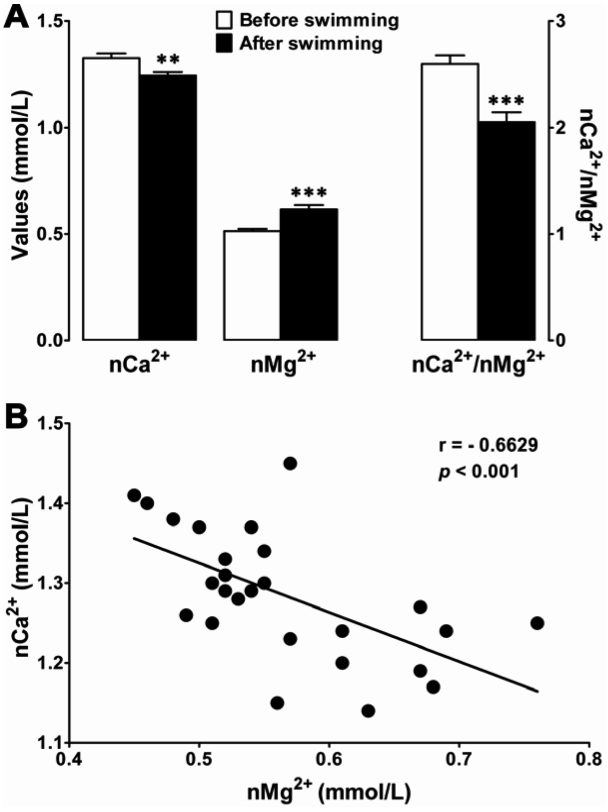

Fig 3. (A) Effects of acute high-intensive swimming exercise on blood nCa^{2+}, nMg^{2+} and nCa^{2+}/nMg^{2+}. nCa^{2+}, normalized ionized Ca^{2+} to pH; nMg^{2+}, normalized ionized Mg^{2+} to pH; nCa^{2+}/nMg^{2+}, ration of nCa^{2+} per nMg^{2+}. Data are mean ± SEM of 13 different preparations. **p < 0.01 and ***p < 0.001, paired Student's *t*-test versus before swimming. (B) The correlation between changes in nCa^{2+} and nMg^{2+} during the swimming exercise via Spearman's rank correlation coefficient. Data are mean ± SEM of values before and after swimming from 13 different preparations.

increase in nMg^{2+} (0.62 ± 0.02 vs 0.51 ± 0.01 mM/L before swimming). Because H^+ compete with iCa^{2+} and iMg^{2+} for protein binding sites in blood (19), the change of blood pH affects the binding of iCa^{2+} and iMg^{2+} to plasma proteins so that pH-normalizations of iCa^{2+} and iMg^{2+} are very important to accurate measurement of these ion concentration. Also, there was a significant correlation between nCa^{2+} and nMg^{2+} before and after swimming (r = -0.6629; $p < 0.001$). The changes in iNa^+ (148 ± 1 vs 144 ± 1 mM/L before swimming, $p < 0.05$) and iK^+ (5.8 ± 0.5 vs 4.5 ± 0.2 mM/L before swimming, $p < 0.05$) had significant, but the change in iCl^- (107.2 ± 1.4 vs 106.6 ± 0.6 before swimming) had not significant. Also, anionic gap was increased after swimming (22.9 ± 2.1 vs 11.7 ± 0.8 mM/L before swimming, $p < 0.05$).

Correlation between pH, lactate, glucose, HCO_3^-, anionic gap vs nMg^{2+} in acute high-intensive forced swimming

Before and after the acute swimming, pH (r = -0.6237; $p < 0.001$), glucose (r = -0.5191; $p < 0.01$) and HCO_3^- (r = -0.6835; $p < 0.001$) showed a significant negative correlation with nMg^{2+},

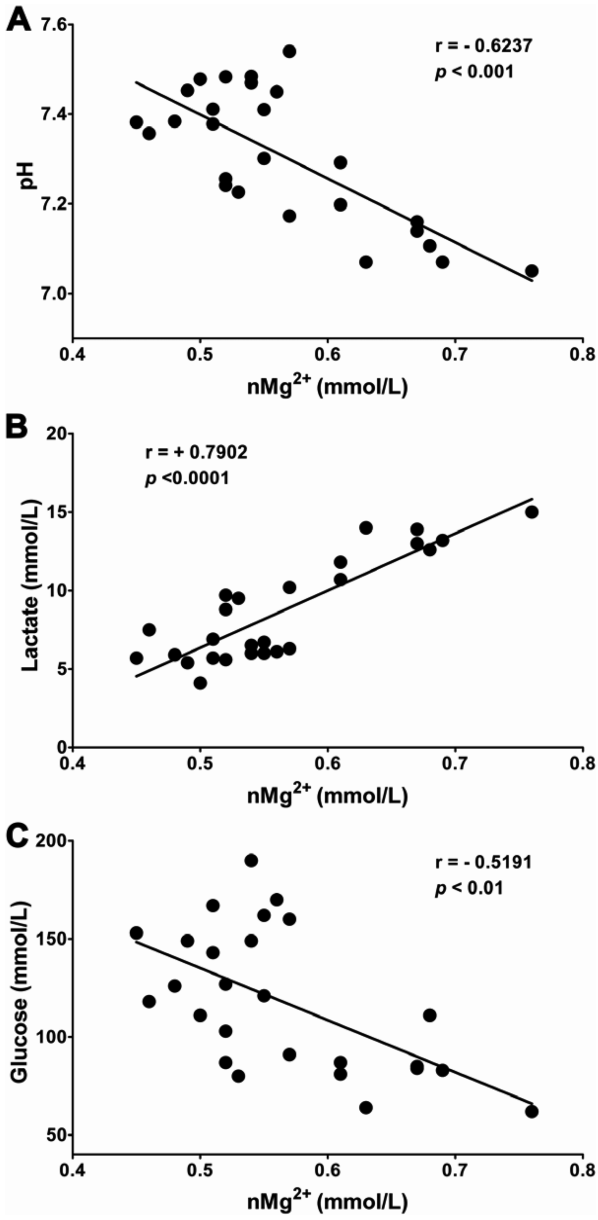

Fig 4. The correlation between changes in blood nMg^{2+} with pH (A), lactate (B) and glucose (C) during the swimming exercise via Spearman's rank correlation coefficient. Data are mean ± SEM of values before and after swimming from 13 different preparations.

however lactate (r = +0.7902; $p < 0.0001$) and anionic gap (r = +0.4741; $p < 0.05$) showed a significant negative correlation with nMg^{2+}.

Discussion

Recently, the determination of iMg^{2+} has been made possible in organ, tissue or cell by methodological advances like ion-sensitive fluorescent dyes, micro-electrodes and phosphorus magnetic resonance spectroscopy (17). There are only 2 types of ion-selective macroelectodes available for the mea-

surement of blood iMg^{2+} (18). This method has been used in human medicine and to recent extent in veterinary medicine (2,5,6,7). In this study, we found that the acute high-intensive exhaustive physical performing could induce a significant increase in blood iMg^{2+}, hypermagnesemia.

In the present study, the acute high-intensive swimming exercise evoked a decrease in blood pH, glucose and $iHOC_3^-$ as well as increase in lactate, resulting in metabolic acidosis. Also, the acute high-intensive exercise produced a decrease in blood PO_2, O2sat and a/APO_2 as well as increase in PCO_2, resulting in respiratory acidosis. During the several minutes after starting the exhaustive high-intensity exercise met the high metabolic demands, oxidative and non-oxidative energy catabolism provide ATP and then later the high power output demand primarily meets anaerobic glycolysis (15). A relatively rapid supply of energy, ATP can be provided to working muscle in the absence of oxygen by the following equation termed anaerobic glycolysis: Glucose ↔ 2ATP + 2lactate + $2H^+$. The increased anaerobic glycolysis results in the increase in formation of lactic acid, which dissociates at normal physiological pH to lactate and free H^+. The generation of H^+ from lactic acid is primarily responsible for decreasing intramuscular pH during high-intensity exercise, resulting in cessation of exercise (3). In contrast to anaerobic glycolysis, oxidative catabolism of glucose or fatty acids can provide a constant wealth of ATP; Glucose + O_2 ↔ 36ATP + CO_2 + H_2O, Palmiate + O2 ↔ 130ATP + CO_2 + H_2O. During glycolysis, the formation of pyruvate from glucose, Mg^{2+}-dependent 7 enzymes are involved, for example, hexokinase is necessary for phosphorylating glucose and to activate it for further enzymatic reactions in energy metabolism (12). Mg^{2+} is also required in the citric acid cycle, the major energy producing mechanism in all aerobic organisms, e.g. for pyruvate and isocitrate dehydrogenase. In lipid metabolism, iMg^{2+} is necessary for the formation of phosphoglycerides in lipid synthesis, and for thiokinasis, catalyzing the first step in fatty acid degradation (11). iMg^{2+} therefore plays a predominant role in energy metabolism and is essential for muscle contraction because actions of actin and myosin filaments are ATP dependent. Accordingly, we demonstrated the changes in iMg^{2+} during acute high-intensive exhaustive physical exercise showed a positive correlation with metabolic and respiratory acidosis in this study.

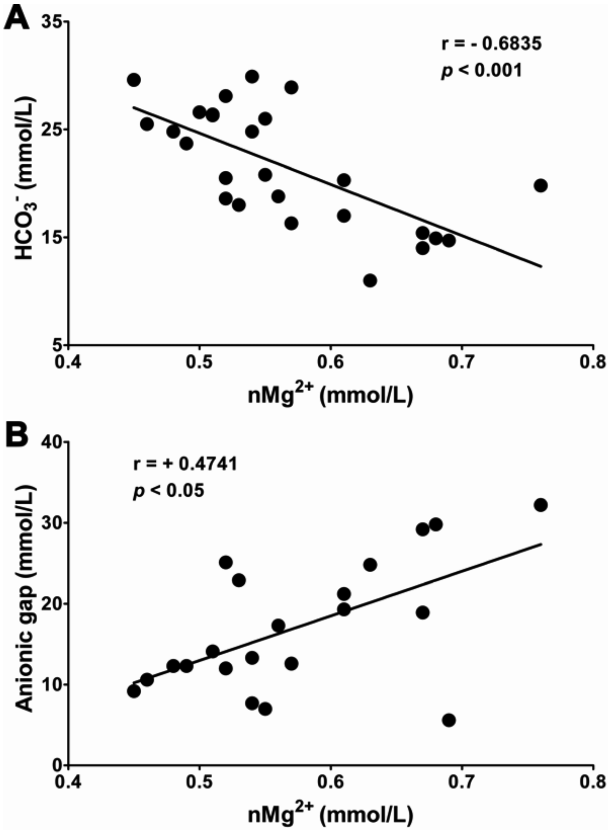

Fig 5. The correlation between changes in blood nMg^{2+} with HCO_3^- (A), and anionic gap (B) during the swimming exercise via Spearman's rank correlation coefficient. Data are mean ± SEM of values before and after swimming from 13 different preparations.

We founded that Hb and hematocrit did not change after exercise, suggesting no efflux from erythrocyte in which 2,3-bisphosphogluconate and hemoglobin are significant Mg^{2+} buffers. Muscular Mg^{2+} exists mainly as ATP^2-Mg^{2+}, bounded form (90%), whereas a small portion of intracellular Mg^{2+} is found as ionized free form (5-14 %) (16) Thus, the increase in iMg^{2+} could be a result from ATP^2-Mg^{2+} degradation due to the exercise associated ATP depletion (12). Whereas prolonged submaximal exercise is accompanied by hypomagnesaemia, short-term high intensity exercise leads to hypermagnesemia (4) in the studies which measured blood tMg^{2+}. Furthermore, we previously reported that extra- and intracellular acidification in ventricular muscle produced a significant increase in intracellular iMg^{2+} (9). The increased iMg^{2+} induced by the swimming could evoke an increase in iMg^{2+} efflux. This hypothesis is supported by secondary Mg^{2+} efflux following α_1-agonists-induced increase in $[Mg^{2+}]_i$ in rat heart (8) and secondary Mg^{2+} efflux following ketamine-induced increase in $[Mg^{2+}]_i$ in guinea pig heart (10). Interestingly, blood iCa^{2+} after exercise decreased and showed a negative correlation with blood iMg^{2+} in this study. Since Mg^{2+} is recognized as a physiological Ca^{2+} inhibitor (12), the iMg^{2+} loss from muscle influences harmfully a great number of intracellular processes of the muscle, including adenylate cyclase activity, Na^+ pathways, K^+ pathways, excitation-contraction coupling, Ca^{2+} sensitivity of the myofilaments and the Ca^{2+} release from the sarcoplasma reticulum (4,12,16). Because metabolic acidosis has been shown to cause magnesuria by reducing renal tubular reabsorption of Mg^{2+} (14) that may accelerate Mg^{2+} loss from muscles, the Mg^{2+} loss from cell and subsequent loss may often lead to develop diseases in gastrointestinal, cardiovascular and neuromuscular and that may exacerbate the damage caused by disease (17).

In view of the above arguments and the new data presented

herein, we strongly propose that the acute high-intensive swimming exercise could increase in blood iMg^{2+} via stimulation of Mg^{2+} efflux following increase in intracellular iMg^{2+} from muscle induced by metabolic and respiratory acidosis and subsequent Mg^{2+} intake should be needed after excessive physical performances even though there is hypermagnesemia.

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단기간 고강도의 수영운동이 혈액 이온 및 대사산물에 미치는 영향

김상진 · 박혜민 · 신세린 · 전설희 · 김진상*· · 강형섭*** [1]**

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**전북대학교 생체안전성연구소, ***전북대학교 헬스케어기술개발사업단

요 약 : 마그네슘(Mg^{2+})은 325 개 이상의 생리적 및 생화학적 과정에 필수적인 조효소이며 신경활성, 심장근 감수성, 신경근 전달, 근수축, 혈관운동 긴장과 혈압 등의 신체활동과 관련된 일련의 과정에서 중요한 역할은 한다. 하지만 신체활동과 관련된 혈액 이온화 Mg^{2+} (iMg^{2+})에 관한 보고는 거의 없을 뿐만 아니라 혈액 총 Mg^{2+}을 측정한 연구결과들은 논란의 여지가 있다. 대사적 요구가 증가하는 단기간의 고강도 운동에서 혈액 iMg^{2+}의 변화를 측정하였다. 고강도의 수영운동 후에 혈액 산도, 혈당, 중탄산염, 산소 및 칼슘은 감소한 반면, 젖산, 이산화탄소, iMg^{2+}, 나트륨, 칼륨은 유효한 증가를 보였다. 고강도 수영에서 혈액 iMg^{2+}의 변화는 혈액 산도, 혈당, 중탄산염과 칼슘의 변화와는 역관계의 상관을 보인 반면, 젖산과 음이온차와는 정관계의 유의한 상관을 보였다. 이 결과는 단기간 고강도의 수영운동이 고마그네슘혈증을 야기할 수 있고 이는 대사성 및 호흡성 산증에 의한 근육내 iMg^{2+}의 증가에 수반하는 근육에서의 iMg^{2+} 유출의 증가에 의한 것으로 판단된다.

주요어 : 혈액 이온화 Mg^{2+}, 수영, 산증, 고마그네슘혈증