# Serum Magnesium and Immune Parameters after Maximal Exercise in Sportsmen. Are they Related?

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## Zusammenfassung

Das Ziel der Studie war die Messung der Änderung des Serum-Magnesiumspiegels bei Sportlern nach maximaler Belastung und deren Einfluß auf Immunglobuline und Lymphozyten (CD + 3T und CD + 19B). Zwölf männliche Probanden, Volleyballspieler in der höchsten Kategorie der offiziellen spanischen Volleyball-Liga, stellten sich freiwillig für diese Studie zur Verfügung, nachdem sie ihr Einverständnis zum Protokoll gegeben hatten. Es wurde Gesamt-Serumprotein (TP) IgE, IgA und IgM bestimmt. Die Gesamtzahl der CD3 + T und CD19 + B Lymphozyten wurde im peripheren Blut gemessen. Der Streßstatus wurde durch analytische Methoden (Cortisol) bestimmt. Gesamtserummagnesium (MgT) und ultrafiltrierbares Serummagnesium (MgU) wurden gemessen. Nach maximaler Belastung stieg MgT signifikant in beiden Gruppen ($p < 0,05$) und ging in der Erholungsphase wieder auf die Ausgangskonzentrationen zurück. In der Kontrollgruppe blieb MgU nach Belastung und in der Erholungsphase unverändert: 62% in Ruhe, 61,5% nach Belastung und 60% in der Erholungsphase. Jedoch fiel bei den Elitesportlern nach Belastung MgU signifikant ab (60,5% in Ruhe, 52% nach Belastung und 56% in Erholungsphase). Bei Anwendung des Wilks Lambda Tests auf die Belastungseffekte auf Immunglobuline und Serummagnesium erhält man ein ähnliches Verhalten der immunologischen Parameter und Serummagnesium in Elitesportlern und in der Kontrollgruppe, jedoch gibt es keine Interaktionen.

## Summary

The aim of this paper was to evaluate the variations of total serum magnesium and its relations with the immunoglobulins and lymphocytes (CD + 3T and CD + 19B) after maximal exercise in sportsmen. Twelve normal male subjects, volleyball players in the maximal category of the official Spanish league of Volleyball, volunteered for this study after having been informed of the protocol involved. Total serum proteins (TP), IgG, IgA, IgM were determined. The total number of CD3 + T lymphocytes and CD19 + B lymphocytes in peripheral blood were measured. The stress level was determined by analytical methods (Cortisol). Total serum magnesium (MgT) and ultrafiltrable serum magnesium (MgU) were determined. After maximal exercise, Mg-T increased significantly in both groups ($p < 0.05$) and returned, in recovery state, to levels of baseline state. In control group Mg-U remained unchanged after maximal exercise and in recovery: 62% at rest, 61.5% after exercise and 60% at recovery period. However in the elite sportsmen Mg-U decreased significantly after exercise (60.5% at rest, 52% after exercise and 56% at recovery). When applying the Wilk's Lambda test to study the effect of exercise on immunoglobulins and on serum magnesium we obtain that the behaviour of immunological parameters and serum Mg is similar in elite sportsmen and in control group, however there are no interactions between them.

## Résumé

Le but du présent travail était l'évaluation des variations du magnésium sérique et leur relation avec les immunoglobulines et les lymphocites (CD + 3T et CD + 19B) après l'exercice maximal des sportifs de haut rendement. Douze personnes volontiers – joueurs de volleyball de la catégorie la plus haute de la Ligue Nationale de Volley-Ball Espanole officielle – se mettaient à la disposition pour cette étude, après avoir consigné leur consentement. Le taux des protéines totales du sérum (TP), IgE, IgA et IgM était déterminé, ainsi que le taux des lymphocites CD3 + T et CD19 + B dans le sang périphérique. L'état du stress était mesuré par des méthodes analythiques (Cortisol), ainsi que le magnésium sérique total (MgT) et le magnésium sérique ultrafiltrable (MgU). Après des exercises maximales, le taux de MgT avait augmenté considérablement dans les deux groupes ($p < 0.05$), tandis que pendant la recuperation il retournait à la base de départ. Chez le groupe de contrôle, le Mg-U restait inchangé après les exercices maximales et pendant la récupération: 62% en repos, 61.5% après les exercises et 60% pendant la récupération. Après avoir appliqué le test Wilk's Lambda pour éprouver les effets de l'exercice sur les immunoglobulines et sur le magnésium sérique, nous avons trouvé un comportement similaire des paramètres immunologiques et du magnésium sérique chez les sportifs d'élite et chez le groupe de contrôle; cependant, il n'y a pas des interactions entre eux.


## Introduction

Magnesium is an essential cofactor in many biochemical reactions [1]. Many studies are communicated that it may play a role in several mechanisms of defense against allergic, anaphylactic and antiinflammatory processes, lymphocyte transformation, immunoglobulin synthesis, activation of the complement system, etc. [2–4].

Different researchers have shown the deleterious effects of magnesium deficiency on lymphocyte proliferation as well as on the immune related functioning of several kinds of T-cells [2, 4]. Also the mononuclear phagocyte system participates in immune mechanisms against inflammation process, and magnesium is involved too in this defensive function [2].

The practice of sport at high level (élite competition) can provoke several neuroendocrine changes associated to alterations on the immune system [5–7]. The elite athletes show decreases


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on performance and endurance levels associated with decreased immune function and increased susceptibility to infections [5, 6, 8]. These alterations are attributed, at least in part, to physiological response to stress [9]. The mechanisms by which physical stress modulates immune competence are complex, involving several neuroendocrine messengers [10, 11].

Various stressors as exercise result in a variation of behaviour of magnesium and the immune system [1, 12–14]. It has also been suggested that some athletes may have suboptimal magnesium status, which could adversely affect their exercise performance [13, 15, 16], and also it has been indicated that the immune system alterations provoke decreases on physical performances [6–8].

In view of these previous investigations, the aim of this paper was to evaluate the variations of total serum magnesium and its relations with the immunoglobulins and lymphocytes (CD 3 + T and CD 19 +B) after maximal exercise in sportsmen.

## Material and Methods

Twelve normal male subjects, volleyball players in the maximal category of the official Spanish league of Volleyball, volunteered for this study after having been informed of the protocol involved. The procedures followed were in accordance with ethical standards of the committe on human experimentation, in accordance with the Helsinki declaration. All these sportsmen completed a medical questionnaire and had a normal cardiopulmonary and electrocardiographic examination. Direct measurements of maximum oxygen uptake ($VO_2$ max) was obtained by progressive exercise. Oxygen uptake was measured continuously using a Jaeger ergo-pneumotest (Eos-Compact). Heart rate was also monitored continuously on the electrocardiogram. In parallel, twelve male volunteer universitary students, that were moderately trained, participated in the study as control group. They did not smoke or consume any alcohol, and they did not take any medication. The test was performed on a cycle-ergometer (Monark). The exercise program consisted in a progressive test (TPT): an initial load of 100 W was increased by 25 W every 2 min. The highest level maintained for 2 or 3 min, referred to as the maximum tolerated power (MTP), is an essential measurement for the experimental tests to be described [17].

The subjects arrived at the laboratory at approximately 08.00 h on the day of the examination, having eaten a non fat breakfast 60 min earlier. They rested on a couch for about 30 min. Blood samples were obtained at rest (before exercise, after about 30 min of rest), immediately after exercise and in recovery period (30 min after the end of exercise). Total white blood cell counts, and hematocrit (Hct) were determined on a Coulter Counter (model MAX-M). The percentage in plasma volume variation (%ΔPV) was calculated using pre- and posthematocrit according to *Van Beaumont* [18]. Total serum proteins (TP) were measured by colorimetric Biuret method in an autoanalyzer aparatus (Hitachi 702).

The serum levels of the IgG, IgA, IgM were determined by nephelometry method. The total number of CD3 + T lymhocytes and CD19 + B lymphocytes in peripheral blood were measured by flow citometry as previously described [19]. The stress level was determined by analytical methods, using a radioimmunoassay Kit (Diagnostic products Corporation).

Total serum magnesium (MgT) and ultrafiltrable serum magnesium (MgU) were calculated by atomic absorption spectrophotometry (Perkin Elmer 272). The MgU were obtained by the Millipore method of ultrafiltration.

### *Statistical Analysis*

Results are represented as means ± SD. To analyze the results, we have used multivariant analysis (MANOVA), and Wilk's Lambda test. Furthermore, in orther to obtain explanation of each phenomena, the analysis of variance (ANOVA) was performed. When the ANOVA indicated signification ($p < 0.05$) the Bonfeoni test was applied.

## Results

Physical characteristics of sportsmen are shown in table 1. The hematocrit, total white blood cells and percentage variation of plasma volume (%ΔPV) after exercise are shown in table 2.

Total serum Mg (Mg-T) and ultrafiltrable serum Mg (Mg-U) were studied in both groups, sportsmen and control (table 3). After maximal exercise, Mg-T increased significantly in both groups ($p < 0.05$) and returned, in recovery state, to levels of baseline state. In the control group Mg-U remained unchanged after maximal exercise and in recovery, without significant differences between the situations studied: 62% at rest, 61.5% after exercise and 60% at recovery period. However in the elite sportsmen Mg-U decreased significantly after exercise (60.5% at rest, 52% after exercise and 56% at recovery). In elite sportsmen, compared with control group (students), both serum Mg-T and Mg-U at rest were significantly different ($p < 0.05$). This tendence also remained after maximal exercise, however in the recovery state, Mg-U decreased in elite sportsmen with respect to the control group.

The immunological parameters variations are reflected in table 4. Serum levels of IgG and IgA and in blood the absolute number of CD3 + T lymphocytes, did not differ significantly at rest between the control and sportsmen groups. The serum levels of IgM and blood CD19 + B lymphocytes decreased significantly. After maximal exercise serum IgG, IgA and IgM and CD3 + T and CD19 + B lymphocytes levels were significantly increased and diminished to basal levels with the recovery, in both groups.

Tab. 1: Physical characteristics of elite sportsmen and control subjects.

| | ELITE | CONTROL |
|---|---|---|
| Age (years) | 25.9 ± 2.6 | 22.3 ± 1.2 |
| Weight (Kg) | 86.8 ± 5.1 | 71.3 ± 4.0 |
| Height (cm) | 189.8 ± 7.9 | 176.2 ± 5.1 |
| $VO_2$ max ($ml.Kg.min^{-1}$) | 64.9 ± 4.7 | 46.5 ± 6.3 |

Tab. 2: Hematocrit (Hct), Hemoglobin (Hb), Leucocytes (LEU), total protein (TP), percentage variation of plasma volume (%ΔPV) and Cortisol at rest (R), after exercise (E) and at recovery (RC) in the sportsmen (S) and control (C) group.

| | SPORTSMEN (S) | | | CONTROL (C) | | |
|---|---|---|---|---|---|---|
| | R | E | Rc | R | E | Rc |
| Hct (%) | 44.9±2.5 | 48.5±2.8* | 44.7±2.7 | 43.4±2.7 | 47.8±2.8* | 43.4±1.8 |
| Hb (g/dl) | 14.7±2.3 | 16.8±2.2* | 15.3±2.3 | 14.5±2.4 | 16.3±2.7* | 14.8±2.6 |
| LEU ($\times 10^3/mm^3$) | 5.8±0.9 | 10.3±2.1* | 7.8±2.3 | 5.7±1.2 | 7.9±2.0* | 6.5±1.6 |
| TP (g/dl) | 7.6±0.4 | 8.4±0.6* | 7.4±0.5 | 7.1±0.3 | 7.9±0.5* | 7.2±0.6 |
| Cortisol ($\mu$g/dl) | 18.9±3.9 | 25.4±5.6* | 21.1±6.1 | 9.9±4.4 | 14.9±5.1 | 16.2±5.6* |
| ΔPV (%) | | -13.1 | | | -12.9 | |

Tab. 3: Serum magnesium (total MgT, and ultrafiltrable MgU) variations, and percentage of MgU in both groups (control (C) and sportsmen (S)) at rest (R), after exercise (E) and at recovery (Rc) periods. Data as means ± SD. Significant differences ($p < 0.05$) *S vs C at each state.

| | R | E | Rc |
|---|---|---|---|
| MgT (C) (mg/dl) | 1.95±0.09 | 2.21±0.13* | 1.93±0.11 |
| MgU (C) (mg/dl) | 1.21±0.07 | 1.36±0.10* | 1.16±0.08 |
| % of MgU | 62 | 61.5 | 60 |
| MgT (S) (mg/dl) | 2.01±0.12 | 2.23±0.15* | 1.97±0.13 |
| MgU (S) (mg/dl) | 1.22±0.10 | 1.16±0.09 | 1.11±0.10* |
| % of MgU | 60.5 | 52.1 | 56.2 |

Tab. 4: Variations of immune parameters in both groups (control (C) and sportsmen (S)) at rest (R), after exercise (E) and at recovery (Rc) periods. Data as means ± SD. Significant differences ($p < 0.05$) *S vs C at each state.

| | SPORTSMEN (S) | | | CONTROL (C) | | |
|---|---|---|---|---|---|---|
| | R | E | Rc | R | E | Rc |
| CD3 + LT ($\times 10^3/mm^3$) | 1.58±0.43 | 2.10±0.80* | 1.90±0.64* | 1.70±0.41 | 2.50±0.65* | 2.11±0.20* |
| CD19 + LB ($\times 10^3/mm^3$) | 0.21±0.08 | 0.31±0.13* | 0.24±0.12* | 0.28±0.09 | 0.48±0.11* | 0.37±0.14* |
| IgG (g/dl) | 1.12±0.13 | 1.27±0.15* | 1.20±0.13 | 1.19±0.17 | 1.43±0.21* | 1.33±0.20 |
| IgA (mg/dl) | 0.22±0.07 | 0.25±0.08* | 0.24±0.06 | 0.23±0.06 | 0.28±0.07* | 0.26±0.07* |
| IgM (mg/dl) | 0.17±0.04 | 0.20±0.06 | 0.18±0.05 | 0.19±0.05 | 0.22±0.06 | 0.20±0.06 |

To study the influence of exercise on immunoglobulins and on serum magnesium we made a multivariant analysis (MANOVA). As explanatory variable, we used the exercise creating a model with possible interactions. When applying the Wilk's Lambda test to study the effect of exercise on immune parameters and on serum magnesium we obtain that the behaviour of immunological parameters and serum Mg is similar in elite sportsmen and in control group, however there is no interaction between them.

The serum levels of cortisol, in basal conditions, are significantly increased in elite sportsmen with respect to control group. After maximal exercise test, a significant elevation was observed in elite sportsmen and in control group, however at the recovery, in elite sportsmen, the cortisol serum level was normalised at similar levels than rest state, whereas in the control group they remained increased.

## Discussion

Magnesium and immunological system are implicated in the response to exercise. Abnormalities of Mg metabolism during exercise affect the endurance and performance of athletes [13, 15, 16, 20, 21]. Also it has been indicated that the immune system alterations provoke decreases on physical performances [6–8]. However, although both Mg and immune parameters show similar behaviour after maximal exercise, in this paper we have not found correlations between serum Mg and the immunological parameters studied.

Changes in plasma Mg after exercise have been investigated in several studies and data showed that variations of Mg levels during and after exercise are inconsistent. In this work we have found that maximal exercise induces an increase in total serum magnesium (Mg-T), that in recovery (30 minutes) returns to similar levels than basal conditions. These results are in agreement with those that we reported previously in humans [17, 20] and in rats [21]. Other authors [13, 22–24] found results in the same way, and these variations appear to be due in part to the percent change in plasma volume and to the redistribution of Mg provoked by the exercise.

The ultrafiltrable serum Mg (Mg-U), active fraction of magnesium in plasma, was significantly minor in the recovery period in elite athletes with respect to control men. This phenomenon could be related to increases in protein turnover and in sweat losses and/or a redistribution of Mg in the organism. Previously, we have indicated [12] that the Mg behaviour after exercise is due to an internal redistribution. Other authors [22, 25] confirm the redistribution phenomenon. This behaviour of Mg could indicate that the stress associated with the exercise is the responsible of this response. In this way the stress level found after maximal exercise, measured by the cortisol levels, was major in elite sportsmen than in the control group. Consequently, as the season advance in elite athletes, daily training might provoke an increase in redistribution and excretion of Mg after maximal exercise.

With respect to immune status, in baseline state both, elite sportsmen and control subjects, are in similar levels considered as normal range. After maximal exercise or after a short period

of training, several alterations have been found on the immune system [1, 6, 7]. The changes associated to the maximal exercise, are justified by the influence of different factors in the distribution and in the function of the immune components during and subsequent to exercise. *Nieman* et al. [7, 8] have suggested that stress of competitive exercise training may be associated with immunological alterations. The mechanisms by which physical stress modulates immune competence are complex, involving both several neuroendocrine messengers that can interact with the immune system [9, 11, 26, 27]. During exercise, increased blood levels of adrenaline and cortisol have been shown to have an immunomodulating effect [5, 7]. The cortisol levels increase in response to the stress of the exercise workload, and have been demonstrated to be related to suppression of several components of the immune system [7, 10]. In the present study, the serum cortisol concentrations have a different pattern of response to maximal exercise. In elite sportsmen the maximal serum levels of cortisol were early reached, during the maximal exercise, with respect to those found in control groups.

Recently, *Reighlin* [9] in a review article summarised the interactions between neural, endocrine and immune system and its adaptation to infection, inflammation and tissue injury. In this way the long term sport training provokes tissue damage and inflammation with activation of immune cells. Severe exertion or heavy exercise training is associated with muscle cell damage and local inflammation [28]. Chronic inflammatory diseases are associated to immune abnormalities that can be involved in the pathogenesis of the disease as a cause and/or a consequence of the tissular damage [29]. Furthermore, the magnesium depletion leads to a complex array of biochemical, electrophysiologic and morphologic abnormalities in skeletal muscle [30]. Rhabdomyolysis, which is the physiological destruction of certain percentage of cells is one of the limiting factors in prolonged exercise. This phenomenon is in part linked to membrane function disorder during physical exercise. Mg plays an important role in muscle, and the activity of Ca transport system in the sarcoplasmic reticulum membranes depends on the presence of Mg ions [31].

In conclusion, although serum magnesium and immunoglobulin levels are altered after maximal exercise both in elite sportsmen and in normal subjects, there are no relation between the immune parameters and magnesium. Therefore as the immunological system is implicated in the response to exercise, and magnesium is also fundamental in many physiological processes during exercise it is possible to hypothesise that the immune system abnormalities and Mg variations found after intensive exercise may be involved in the decreases on performance and endurance of the athletes along the season player.

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