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PITUITARY-GONADAL, PITUITARY-ADRENOCORTICAL HORMONES AND IL-6 LEVELS FOLLOWING LONG-TERM MAGNESIUM SUPPLEMENTATION IN MALE STUDENTS

NIVO HIPOFIZNO-GONADNIH I HIPOFIZNO-ADRENOKORTIKALNIH HORMONA I IL-6 NAKON DUGOTRAJNE SUPLEMENTACIJE MAGNEZIJUMOM KOD STUDENATA

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Summary

Background: Sleep deprivation, malnutrition and lack of physical activity are contemporary stress-related factors present in the student population. Stress activates the HPA and often suppresses the HPG axis, but also influences cytokine synthesis and consequently regulates immune response. Since magnesium deficiency facilitates negative pathophysiological consequences, a reasonable question imposes, whether Mg supplementation might correct the adrenal/gonadal hormone balance and immuno-endocrine function.
Methods: Fifteen male students were given 2 × 250 mg Mg for four weeks. Serum levels of FSH, LH, testosterone (T), ACTH and cortisol (C) were measured before and after supplementation and the T/C ratio was calculated. Furthermore, IL-6, red blood cells (RBC), hemoglobin (Hb), white blood cells (WBC) and the WBC differential were measured.
Results: Despite no change in the serum level of ACTH, a statistically significant ($p<0.05$) decrease in the serum cortisol level appeared, accompanied with an IL-6 level reduction ($p<0.05$) after Mg supplementation. Analysis of the pituitary-gonadal axis hormones showed an increasing trend of the FSH level ($p=0.087$), and a significant increase ($p<0.05$) in the T/C ratio. An RBC count increase ($p<0.001$) was found, along with a decrease in the percentage of neutrophils ($p<0.05$), and a trend toward a lymphocyte percentage increase.
Conclusions: The results suggest that chronic oral magnesium supplementation in male students improves the balance of pituitary-gonadal and pituitary-adrenal hormones and is involved in the regulation of the basal IL-6 level.

Keywords: magnesium, testosterone, cortisol, IL-6

Kratak sadržaj

Uvod: Nedostatak sna, fizičke aktivnosti kao i neadekvatna ishrana su savremeni faktori stresa u studentskoj populaciji. Stres aktivira hormone osovine hipotalamus–hipofiza–nadbubreg i često suprimira aktivnost osovine hipotalamus–hipofiza–gonade, a takođe utiče i na sintezu citokina, posredno regulišući imuni odgovor. Kako nedostatak magnezijuma (Mg) može inicirati patofiziološke posledice, postavlja se pitanje da li njegov dodatak ishrani može povoljno uticati na ravnotežu hormona nadbubrežne žlezde i gonada kao i neuro-endokrinu funkciju.
Metode: U ovoj studiji petnaest studenata muškog pola dobijalo je 2 × 250 mg Mg tokom četiri nedelje a nivoi FSH, LH, testosterona (T), ACTH i kortizola (C) mereni su u krvi pre i nakon suplementacije. Takođe, određivani su i nivoi IL-6, eritrocita, hemoglobina, leukocita kao i leukocitarna formula.
Rezultati: Uprkos tome što nije bilo promene u nivou ACTH, naši rezultati su pokazali statistički značajno smanjenje nivoa kortizola ($p<0,05$), koje je bilo praćeno smanjenjem nivoa IL-6 ($p<0,05$) nakon dodatka Mg. Analiza hipofizno-gonadnih hormona pokazala je trend u povećanju FSH ($p=0,087$) i značajno povećanje T/C količnika. Uočeno je značajno povećanje broja eritrocita ($p<0,001$), smanjenje procenta neutrofila ($p<0,05$) i trend povećanja procenta limfocita.
Zaključak: Rezultati studije sugerišu da dugotrajni dodatak Mg ishrani studenata doprinosi održavanju ravnoteže hipofizno-gonadnih i hipofizno-adrenokortikalnih hormona i uključen je u regulaciju bazalnog nivoa citokina IL-6.

Ključne reči: magnezijum, testosteron, kortizol, IL-6

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Introduction

Contemporary psychosocial environment poses complex tasks before young people who often suffer from lack of sleep, improper diet, unemployment, and lack of resources for forming a family, thereby causing a »stressful way of life«. Students are particularly confronted by demanding responsibilities and an excessive amount of stress, consequently becoming susceptible to development of interpersonal conflicts, low self-esteem, sleeping disorders, decreased attention and alcohol or drug abuse (1).

A prominent neuroendocrine response to stress that promotes survival is the activation of the hypothalamo–pituitary–adrenal (HPA) axis. Stimulation of this axis results in hypothalamic secretion of corticotrophin-releasing factor (CRF) that stimulates the pituitary to secrete adrenocorticotropin (ACTH), which consequently stimulates the adrenal cortex to produce cortisol, the main glucocorticoid form in humans. Plasma levels of these hormones can increase two- to five-fold during stress in humans (2).

Even though it is considered that HPA hormones predominantly manage stress response, other hormones are up- or downregulated during stressful conditions (3). Particularly, hypothalamo–pituitary–gonadal (HPG) axis hormones are influenced by HPA activity and gonadotrophin-releasing hormone drive to the pituitary is decreased, probably due to increased endogenous CRH secretion. It was demonstrated that in stress, suppression of circulating gonadotrophins and gonadal steroid hormones leads to disruption of the normal menstrual cycle (4). Furthermore, prolonged exposure to stress can lead to complete impairment of reproductive function (5, 6). Anticipatory stress has also been shown to decrease testosterone plasma levels (7). On the other hand, it has been reported that androgens have a mild suppressive effect on the HPA axis and the immune actions (8, 9).

Hormone systems are highly interconnected, and it may be important to examine multiple systems simultaneously to gain a clearer picture of how hormones work together in order to predispose for a certain construct. Cortisol (C) is a catabolic hormone secreted from the adrenal cortex in response to physical and psychological stress and testosterone (T) is considered a key anabolic hormone with multiple physiological functions in the human body, which in males is mainly produced and secreted from the Leydig cells of the testes. These steroids are now regarded as a dual system that defines and predicts many pathophysiological processes, so it is considered that the balance between levels of cortisol and testosterone is probably even more important than the absolute level of either hormone, and in many studies the T/C ratio has been explored. Psychological stress of exams increases C and reduces T levels in both male and female students (10). In addition, low T/C increases the risk of heart disease, and is strongly related to insulin resistance (prediabetes) (11).

The HPA axis, apart from interacting with the reproductive system at multiple levels, has potent actions on the immune and inflammatory reaction (8, 9). Cytokine synthesis is one of the possible ways in which the immune system reacts in stressful situations. In humans, acute administration of IL-6 increases ACTH and cortisol concentrations in a dose-dependent manner (12, 13). The HPA axis response to stress requires cytokines (14), including IL-6 which, probably as a mediator, participates in the complex HPA axis response to stress or inflammation.

Beneficial effects of magnesium (Mg) in physiological and pathological conditions have been known for years. This ion is the second most important intracellular cation after potassium and is present in all tissues. Synthesis and utilization of ATP is directly linked to Mg which activates a substantial number of different enzymes involved in diverse functions such as DNA and RNA synthesis, glycolysis, intracellular mineral transport, nerve impulse generation, cell membrane electrical potential, muscle contraction and blood vessel tone. However, refined foods are depleted from magnesium and some therapeutics such as cyclosporine, chemotherapy, fungicides, diuretics, and cardiac glycosides may significantly reduce the Mg level (15). In addition, substantial loss of magnesium can be a consequence of alcohol or opiates abuse, excessive consumption of modern-day beverages, malnutrition, malabsorption, diarrhea and dehydratation. Of significant importance are the findings showing that magnesium excretion is increased during activity of the sympathetic nervous system and the HPA axis (16, 17). Magnesium deficiency may decrease membrane integrity and function and increase the susceptibility to oxidative stress, cardiovascular heart diseases, cancer, as well as accelerated aging (18).

Since it was shown that intense mental and physical stress leads to magnesium depletion and that hypomagnesemia may be associated with anxiety-like states, it could be assumed that Mg supplementation in persons frequently exposed to stress, such as students, could reduce harmful stress effects. Furthermore, the relationship between magnesium and anabolic hormones in men has not been much examined. The aim of this study was to investigate whether long-term Mg supplementation leads to changes in pituitary-gonadal and pituitary-adrenocortical hormonal status as well as IL-6 levels in young men. Since even low intensity exercise if prolonged enough in duration can result in significant elevations of testosterone and other parameters of interest for this study, only sedentary students who had not been involved in any regular exercise for at least six months were included in the study.

Materials and Methods

Subjects

Fifteen healthy young men (age 22.3 ± 0.22 years, height 185.8 ± 1.60 cm, body mass 82.8 ± 3.04 kg, and BMI=23.4 ± 0.75 (mean ± SD)) participated in the study. Subjects were students of the University of Belgrade and had not been involved in any regular exercise for at least six months. They remained sedentary for the duration of the study. All participants gave their written consent to participate after being fully informed of all experimental procedures. The investigation was carried out according to the guidelines and study protocol that has been approved by the Ethical Committee for Clinical Trials of the University of Belgrade, Faculty of Pharmacy, No. 199/2.

All test subjects received 500 mg of Mg in the form of magnesium oxide for 4 weeks, one tablet of 250 mg 2 times per day (Magnesium 250 mg ® Natural Wealth® NBTY Inc.). Three months before and during the study none of the test subjects was taking other vitamin or mineral supplements.

Blood collection, hormonal and hematologic analysis

Blood samples for hematologic and hormonal analyses were collected in the morning (09 h), before supplementation, and on the 29th day of experiment (first day after 4 weeks of supplementation). After a 12h fasting period, blood was drawn from an antecubital vein in a sitting position into 2 mL EDTA Vacutainer tubes (for plasma and hematologic parameters) and 10 mL Vacutainer tubes with gel with intermediate density between blood cells and serum (for serum), according to a standardized protocol. EDTA tubes for plasma were centrifuged at 3000 rpm for 15 min at 4 °C. Serum tubes were centrifuged at 3000 rpm for 15 min at room temperature. Plasma and serum were stored in duplicate at –80 °C until analyzed.

Serum levels of testosterone and cortisol were measured on an Access® 2 analyser (Beckman Coulter, Inc., Brea, USA) using a chemiluminescent immunoassay with the paramagnetic particle (CLIA) method, with Beckman Coulter immunoassays Access Testosterone and Cortisol assay comercial tests, while levels of FSH and LH were measured using the Chemiluminescent Microparticle Immunoassay on an Architect ci8200 (Abbott Laboratories, Wiesbaden, Germany). IL-6 serum concentrations were determined using a high-sensitivity ELISA method (Quantikine HS ELIS, Human IL-6 Immunoassay, R&D Systems, Abingdon, UK) with sensitivity: 0–10 pg/mL, on Elecsys (Roche Diagnostics GmbH, Mannheim, Germany). ACTH was analysed in plasma samples using the ECLIA method (electrochemiluminescence immunoassay), on Elecsys (Roche Diagnostics GmbH, Mannheim, Germany). Hematological parameters: total red blood cells (RBC), hemoglobin (Hb), white blood cells (WBC) and WBC differential, were determined using Beckman Coulter® LH750.

Statistical analysis

Basal values of hormones, IL-6 and hematological parameters prior to and after magnesium supplementation were compared using paired Student's t-test. The statistical analysis was performed with the SPSS 17.0 statistical program and P-values less than 0.05 were considered significant.

Results

Levels of ACTH, cortisol and IL-6 are shown in *Figure 1*. Results presented in this figure reveal that

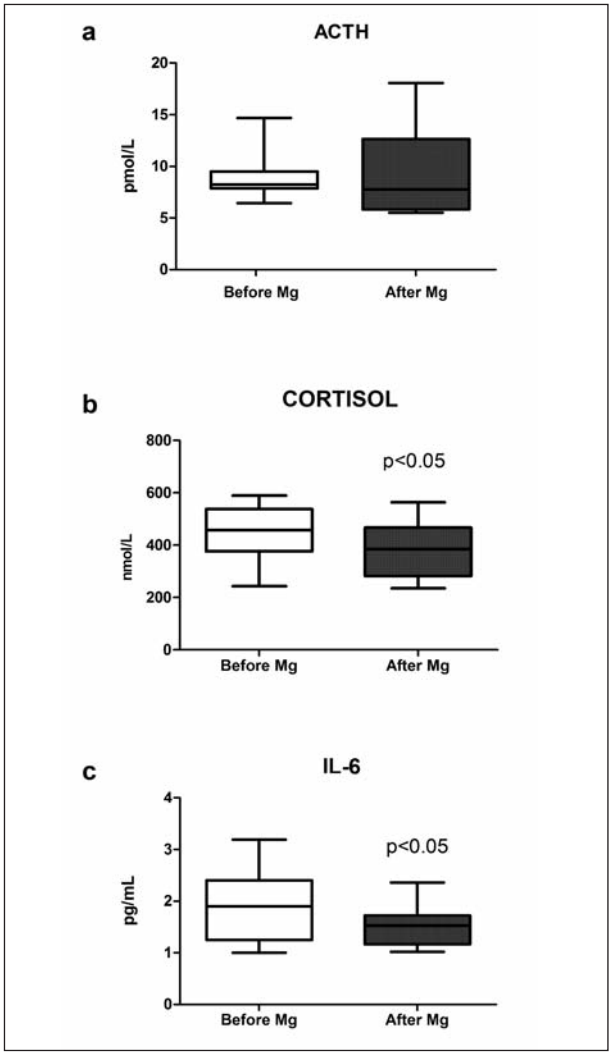

Figure 1 Changes in basal plasma levels of ACTH (a), Cortisol (b) and IL-6 (c) after four-week-long magnesium supplementation in male students.

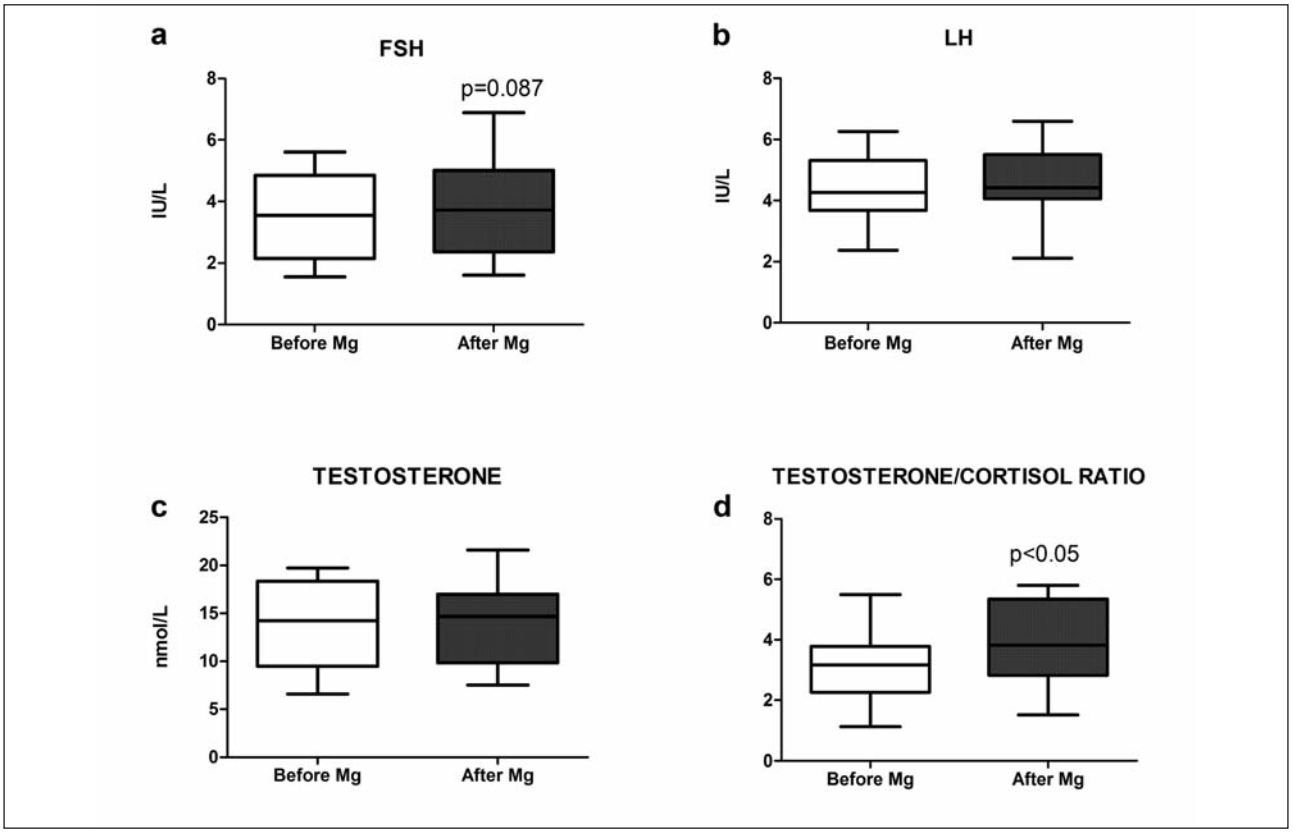

Figure 2 Basal levels of FSH (a), LH (b) and Testosterone (c) in plasma of male students supplemented with magnesium for four weeks. Calculated plasma Testosterone/Cortisol ratio (d).

Table I Concentration of main hematological parameters – red blood cells (RBC), hemoglobin (Hb) and white blood cells (WBC) before and after four-week-long magnesium supplementation.

	RBC ($x10^{12}$/L)	Hb (g/L)	WBC ($\times10^9$/L)
Before Mg	5.08±0.098	157.1±1.89	6.77±0.371
After Mg	5.34±0.109***	157.4±2.57	6.98±0.473

Data are expressed as mean ± SEM (n=15). ***p<0.001

despite the fact that there was no significant change in the plasma level of ACTH, a statistically significant ($p<0.05$) decrease in serum cortisol level after Mg supplementation was observed. High-sensitivity IL-6 test showed that the basal level of this cytokine was significantly decreased in male students ($p<0.05$) after long-term application of Mg.

In *Figure 2*, results of the analysis of pituitary-gonadal axis hormones are presented, and our results did not show any statistically significant change apart from an increasing trend of the FSH level ($p=0.087$) after four-week-long Mg treatment. However, when the T/C ratio was calculated, paired t-test revealed a significant increase ($p<0.05$) in this parameter (*Figure 2*).

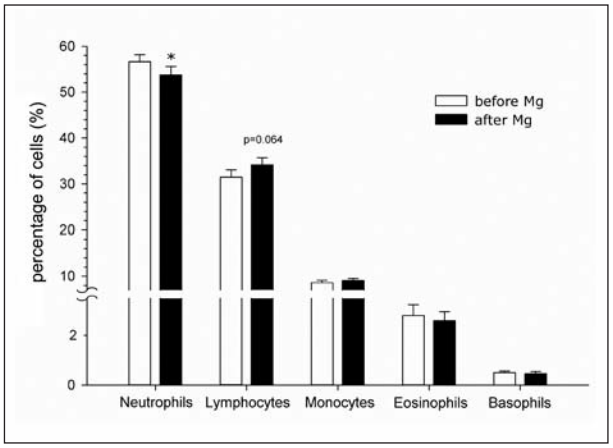

Figure 3 White blood cells differential in blood of male students before and after four-week-long magnesium supplementation.

Furthermore, our study showed a noteworthy increase in the total RBC count ($p<0.001$), which was not followed by a change in the Hb concentration (*Table I*). Although the total WBC count was unchanged, a statistically significant decrease in the percentage of neutrophil granulocytes ($p<0.05$), and an increasing trend in lymphocytes percentage ($p=0.064$) were observed (*Figure 3*).

Discussion

In this study, we showed that four-week-long magnesium supplementation in male students produced discreet effects on the pituitary-gonadal axis hormone basal levels with a noteworthy augmentation of the T/C ratio, followed by an increase in erythrocyte concentration. Magnesium supplementation, furthermore, caused a considerable reduction of serum cortisol and IL-6 level and a minor switch in the neutrophil/lymphocyte ratio.

The literature data on Mg effects on the HPA axis function are somehow controversial. It has been shown that acute i.v. administration of Mg decreased ACTH level in healthy subjects (19), without affecting cortisol secretion (19, 20). On the other hand, subchronic supplementation with Mg in elderly subjects reduced cortisol concentration without a change in ACTH level (21). Furthermore, in the study on pigs where a Mg-rich marine extract-based supplement was added for four weeks, O'Driscoll et al. (22) showed that supplemented pigs had significantly lower salivary cortisol accompanied with reduction in aggressive behavior. On the contrary, Cinar et al. (23) showed an increase in ACTH after one-month-long supplementation with magnesium. Our results are in agreement with the studies in which subchronic or chronic magnesium administration reduced the cortisol level without affecting the ACTH level. There are a few possibilities that could contribute to such results. One is that acute Mg administration has central effects, via the hypothalamus, hippocampus or amygdala, with the overall result being decreased CRH synthesis in the paraventricular nucleus and consequently a reduced cortisol level. Another step that should be considered is the Mg influence on cortisol crossing of the blood–brain barrier via a p-glycoprotein-dependent (p-gp) pathway, as Mg is a co-activator of p-gp and helps to transport cortisol out of the brain. Acute Mg administration would be expected to reduce the central negative feedback effect, but the outcome of long-term supplementation and p-gp expression deserves more profound studies. Nevertheless, it is not very likely that reduced central negative feedback has a crucial role in regulating cortisol blood level after long-term Mg supplementation. Another possibility which seems rather reasonable is a direct inhibitory effect of increased Mg after long-term supplementation on adrenocortical cells and synthesis/secretion of glucocorticoids, which might be the consequence of reduced sensitivity of the adrenal to ACTH, as proposed by Murck (16). Further investigations on possible molecular mechanisms involved in this proposed effect on adrenocortical cells are necessary. Nevertheless, from up-to-now published data it is clear that Mg influences on HPA activity are multileveled, multifaceted and exceedingly complex.

The immune-neuroendocrine systems have an intimate cross-over communication through cytokines and steroid hormones making possible a satisfactory response to environmental changes (24). It is well known that IL-6 has the ability to activate the HPA axis as it can stimulate CRH release (25), and increase ACTH and cortisol level (12, 13). Nevertheless, impact operates and *vice versa*, as it has been shown that cortisol response to ACTH stimulation correlates with blood IL-6 concentration (26). According to our results, since the IL-6 basal level was significantly reduced after supplementation, we might suppose that Mg could be included in the HPA–IL-6 relation and more studies should be conducted to explore this communication. A group of researchers have been on the track of a possible mechanism of magnesium action on cytokine release, as they showed that $MgSO_4$ attenuates cytokine production by lipopolysaccharide (LPS)-stimulated endothelial cells and placental explants (27–29). These research groups proved that treatment of human umbilical vein endothelial cells and human placental explants with $MgSO_4$ prior to LPS stimulation inhibited inflammatory mediator production by suppressing the activation of the transcription factor, nuclear factor-kappa B (NFκB). Some earlier studies showed that *in vitro* and *in vivo* Mg supplementation increased basal IκBα levels and reduced NFκB activation, thereby decreasing production of cytokines and chemokines (27, 28, 30). Furthermore, it was shown that short-term exposure to a clinically effective $MgSO_4$ concentration *in vitro* reduced the frequency of neonatal monocytes producing TNF-α and IL-6, decreasing cytokine gene and protein expression (30). Even though monocytes are a major source of cytokine secretion, our study did not show a change in their percentage, but a slight switch in the neutrophil/lymphocytes percentage after four-week-long Mg supplementation was noted. Reduced percentage of neutrophil leukocytes is in accordance with the proposed anti-inflammatory effects of magnesium (31).

According to this study, it seems that Mg has more delicate effects on pituitary-gonadal hormones than pituitary-adrenal components. Even though our study showed an increase in the level of all three examined hormones (FSH +11%, LH +6% and T+7%), these changes were not statistically significant apart from a trend in FSH rise. However, the T/C ratio was significantly higher after Mg treatment, and this outcome is rather important, since it is reckoned that the balance between the levels of cortisol and testosterone is probably even more important than the absolute level of either hormone. This result suggests that Mg could modify the anabolic/catabolic equilibrium in situations where this equilibrium is disrupted. Nevertheless, magnesium relationship with male gonadal hormones has not been extensively explored and data are scarce and contradictory. In the

study of Zofkova et al. (20) done in 10 healthy male subjects, moderate reduction of T serum level was noted after intravenous infusion of $MgSO_4$, without affecting FSH and LH secretion. A study on athletes and sedentary subjects (32) showed that long-term Mg supplementation caused a higher increase in T level after exhaustion in persons who regularly exercised compared to sedentary individuals. Furthermore, the same author showed (33, 34) that after Mg supplementation leukocyte, erythrocyte, Hb and thrombocyte levels increased significantly. In these studies the dose of Mg that was applied was significantly higher (10 mg/kg BW which makes 800–900 mg Mg/day) than the one we used in our study, in which we noticed only an increase in red blood cell concentration. This discrepancy may in part be explained by the dose applied, and it could be concluded that 500 mg of Mg per day for a month has a mild stimulatory effect on erythropoiesis in young men. Furthermore, since the stimulatory effect of testosterone on erythropoietin synthesis and the consequent increase in red blood cells concentration are well established, this rise in erythrocyte number could be explained by the increased bioavailability of testosterone, even though its level is not significantly changed after Mg treatment. Namely, Maggio et al. (35), in their study on magnesium and anabolic hormones in elderly men, showed that magnesium intake increased testosterone bioactivity. In support of this notion, one study clearly demonstrated that there was an uncompetitive inhibition of Mg^{2+} on testosterone-SHBG (Sex Hormone-Binding Globulin) binding, which led to an enhancement of bioavailable testosterone (36). Another promising study that is in line with the abovementioned effects of magnesium intake on male gonadal function is a recent study in male Wistar rats that showed that administration of Mg increased the activity of androgenic enzymes and serum T level (37). The results were more pronounced in groups treated for a longer period of time and the author considered that it was a consequence of the direct action of excess magnesium on the male gonads. Further investigations in this area are necessary, and the role of Mg in modulating the function of the male gonadal/reproductive system remains to be elucidated in the future.

Conclusion

Results of this study support the assumption that, even in the rather low dose of 500 mg Mg per day, after one-month-long supplementation in young men, magnesium reduces the main stress hormone level, and possibly has some mild stimulatory effect on the male pituitary-gonadal system. Furthermore, there are indications that this essential cation participates in the anabolic/catabolic equilibrium regulation and might play a role in immunoregulation, having an anti-inflammatory potential. The data obtained in this study reveal new evidence in consideration of a magnesium supplementation approach to improve certain important physiological parameters impaired in the young male population facing challenged life quality.

Limitation of the study

This was not a randomized placebo controlled study and the data obtained in the study are preliminary, thus, further investigations of this topic on a higher number of participants are needed. During this study, factors that may be unknown or difficult to measure may have occurred that confound the data, such as changes in living conditions, sudden stress in personal life or illness or physical injury that was not of such importance that it would be reported by the subject.

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Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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