Original Article

Effects of a Multivitamin/multimineral Supplement on Young Males with Physical Overtraining: A Placebo-controlled, Randomized, Double-blinded Cross-over Trial*

LI Xin, HUANG Wen Xu, LU Ju Ming#, YANG Guang, MA Fang Ling,
LAN Ya Ting, MENG Jun Hua, and DOU Jing Tao

Department of Endocrinology, Chinese PLA General Hospital, Beijing 100853, China

Abstract

Objective To investigate the effects of vitamin-mineral supplement on young males with physical overtraining.

Methods Two hundred and forty male Chinese field artillery personnel who undertook large scale and endurance military training and were on ordinary Chinese diet were randomized to receive a multivitamin/multimineral supplement or a placebo for 1 week. After a 1-week wash-out period, a cross-over with 1 week course of a placebo or multivitamin/multimineral supplement was conducted. Blood and urine samples were analyzed for adrenal, gonadal and thyroid hormones. In addition, cellular immune parameters (CD3+, CD3+CD4+, CD3+CD8+, CD4/CD8, CD3-CD56+, CD3-CD19+) were examined and psychological tests were performed before and after the training program and nutrition intervention.

Results After a large scale and endurance military training, the participants showed significantly increased thyroid function, decreased adrenal cortex, testosterone and immunological function, and significantly increased somatization, anger and tension. Compared to placebo, multivitamin/ multimineral intervention showed significant effects on functional recovery of the pituitary - adrenal axis, pituitary-gonadal axis, pituitary- thyroid axis and immune system as well as psychological parameters.

Conclusion High-intensity military operations have significant impacts on the psychology, physical ability and neuroendocrine-immune system in young males. Appropriate supplementation of multivitamin/multimineral can facilitate the recovery of the psychology, physical ability and neuroendocrine-immune system in young males who take ordinary Chinese diet.

Key words: Overtraining; Vitamins; Intervention; Randomized; Diet supplement

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INTRODUCTION

The demands of military operation, including physical and psychological stresses, may result in overtraining, and in some subjects, lead to "overtraining syndrome", which is presented as persistent performance incompetence, high fatigue ratings, altered mood state, increased risk of infections, and reproductive dysfunction[1]. Many studies showed that long-

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#Correspondence should be addressed to Prof. LU Ju Ming, E-mail: Why46301@163.com

Biographical note of the first author: LI Xin, Female, borin in 1968, MD, majoring in endocrionology, nutrition and immunology.

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duration and high-intensity exercise is associated with immunosuppression, higher susceptibility to infections[2], micro injuries and a local inflammatory reaction in the musculature causing changes in serum markers of inflammation[3-4]. Meanwhile, overtraining can induce the oxidative stress, which is also related to inflammation, leading to an impaired antioxidant defenses and a lack of anticipated adaptations to training, and a bias of the redox balance as well[5]. It is also well recognized that overtraining can decrease the adrenal sensitivity to ACTH (cortisol release) and increase the pituitary sensitivity to GHRH (GH release), which in turn cause a counter-regulatory shift to a more serious endocrine imbalance[6]. Besides physical changes, overtraining may also cause psychological alterations, including depressed mood, general apathy, decreased self-esteem, emotional instability, disturbed sleep and other symptoms[1].

Several previous studies have failed to show that use of vitamin and mineral supplements does not improve measures of performance in physically overtraining people consuming adequate diets[7]. However, The American Dietetic Association, Dietitians of Canada, and the American College of Sports Medicine recommended in groups of physically active people who are at increased risk of nutrient depletion the use of a vitamin and mineral supplement, not exceeding the recommended daily intake, may be consumed as a preventive measure[8]. In this study, we investigate the effects of vitamin-mineral (VM) supplement on physical overtraining young males who were on ordinary Chinese diet.

METHODS

Subjects

Two hundred and forty soldiers who undertook large scale and endurance military training and were on ordinary Chinese diet were enrolled in this study. All subjects were male; the mean age was 19.2±1.6 years. All subjects were screened for contraindicating health problems or pharmaceutical use and were cleared for unrestricted physical activity by a physician. The experimental protocol was approved by the Chinese PLA General Hospital's Ethical Committee, and all subjects gave written informed consent.

Experimental Design

After 1 week of military training, subjects were randomly divided into two groups. In group A, a multivitamin/multimineral pill, which include vitamin D 2.5 μg, vitamin E 8.7 mg, vitamin B1 0.5 mg,vitamin B2 0.5 mg, vitamin B6 0.5 mg, vitamin C 150 mg, folate μg, calcium 200 mg, iron 3 mg, zinc 3 mg, and selenium 12.5 mg, was supplemented daily for a week, while in group B, subjects was given placebo. After a 1-week wash-out period, multivitamin/ multimineral supplement and placebo were cross over in group A and group B. Blood and urine samples analysis, cellular immune parameters examination and psychological tests were performed before the military training, and then after each week for 4 weeks. (Figure 1).

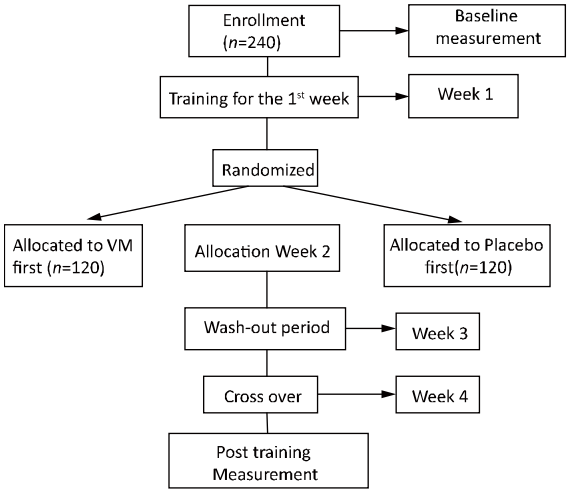

Figure 1. Flow Chart of the trial.

Hematological and Hormonal Analysis

Blood samples were collected from subjects under fasting and proportion of CD3+, CD4+, CD8+, CD4+/CD8+, B cell, NK cell subsets were determined by flow cytometer.

At 8:00 a.m., 15 mL of blood was drawn from the antecubital vein of subjects after a 12-hour fast and collected into sterile vacuum tubes. Once blood was collected, these tubes were immediately placed on ice and later centrifuged at 3000 rpm at room temperature for 10 min, and then the sera were transferred into cryo-freeze tubes and stored frozen at -80 Celsius degree. Levels of Adrenocorticotropin (ACTH), Cortisol (F), Thyrotropic hormone (TSH), 3,5,3-triiiodothyronine (TT3), Thyroxine (TT4), Free 3,5,3-triiiodothyronine (FT3), and Free Thyroxine (FT4) were determined in plasma by IMMULITE2000. At 9:00 a.m. on the same day, urine samples were also collected from subjects after a 12-hout fast.

Urine samples were evaluated for urine free cortisol (UFC), Luteinizing hormone (TH) and Estradiol (E2).

Psychological Tests

We used the Symptom Checklist-90 (SCL-90) to measure the psychological symptoms. The Chinese version of SCL-90 has been validated and widely use in China[9]. Each of the items is rated on a five-point scale of distress ranging from ''not at all'' (1) to ''extremely'' (5). The nine primary symptom dimensions were labeled as: somatization, obsessive-compulsive behavior, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety, paranoid ideation, and psychoticism.

Statistical Analysis

All statistical analyses were performed by using SPSS (version 13.0; SPSS Inc., Chicago, IL, USA). All data are presented as means±SD, and for all tests, the statistical significance was set at $P<0.05$. Compared *t*-test was used to determine whether there were significant differences of individual parameters before and after military training. ANOVA with repeated measures was used to determine the differences between before and after vitamin-mineral interventions.

RESULTS

For participants in both groups, there were no significant of body weight, systolic and diastolic blood pressure. However, after 4 weeks' training, participants had significantly increased rest heart rate (Table 1).

Neuroendocrinological and Immunologic Changes after Overtraining

Table 1 shows the neuroendocrinological and immunologic parameters before and 4 weeks after the large scale and endurance military training. LH, E2, and TT4 did not show any significant differences between the pre- and the post- evaluation, while the differences in ACTH, F, UFC, T, FSH, TT3, FT3, and FT4 reached statistical significance ($P<0.05$). Among them, the ACTH F, UFC, and TSH decreased while TT3, FT3, and FT4 increased. After 4 weeks training, there was a decreased CD4+/CD8+ ratio and an increased NK compartment in these subjects (from 0.24±0.09 to 0.30±0.11). These data suggested that the cellular immunity and human immunity were compromised after high-intensity military operations.

Table 1. Physiological Parameters before and 4 Weeks after the Military Training

Parameters	Before Training	4 Weeks After Training
General (*n*=240)		
Body weight (kg)	63.7±7.3	63.9±7.0
Rest heart rate (beats/min)	68.9±7.7	75.7±8.5*
Systolic blood pressure (mmHg)	111.9±10.0	110.0±11.0
Diastolic blood pressure (mmHg)	72.7±9.6	72.4±8.6
Adrenal cortex function (*n*=235)		
ACTH (pmol/L)	6.6±3.5	6.1±3.4*
F (nmol/L)	337.0±105.2	283.9±84.6†
UFC (nmol/24 h)	426.4±162.3	371.8±155.5†
Gonadal function (*n*=235)		
LH (mIU/L)	3.6±2.8	3.4±3.0
T (nmol/L)	23.5±6.5	18.9±5.9†
E2 (pmol/L)	106.9±38.0	111.9±61.9
Thyroid function (*n*=239)		
TSH (mU/L)	2.9±1.5	2.5±1.4†
TT3 (nmol/L)	2.1±0.3	2.7±2.3*
TT4 (nmol/L)	94.4±15.0	94.9±15.0
FT3 (pmol/L)	5.2±0.4	5.6±0.6†
FT4 (pmol/L)	13.5±1.5	15.9±2.0†
Immunity (*n*=226)		
CD3	0.6±0.1	0.6±0.1
CD4/CD8	1.2±0.4	1.0±0.4*
B cells	0.1±0.04	0.1±0.04
NK cell	0.2±0.1	0.3±0.1†

Note. $^{*}P<0.05$, $^{\dagger}P<0.01$. ACTH: Adrenocorticotropin, F: Cortisol, UFC: Urine free cortisol, LH: Luteinizing hormone, T: Testosterone, E2: Estradiol, TSH: Thyrotropic hormone, TT3: 3,5,3-triiiodo thyronine, TT4: Thyroxine, FT3: Free 3,5,3-triiiodot hyronine, FT4: Free Thyroxine.

Psychological Changes after Overtraining

Table 2 shows stress related psychological scales measured pre and post training. After the high-intensity military operations, psychological scales demonstrated that feeling of anger, tension, and compulsive of the soldiers significantly increased ($P<0.05$).

Effects of Vitamin-mineral Supplements Intervention on Endocrine and Immunity and Psychological Function Recovery

Table 3 and Table 4 show the changes of hormones, hematological parameter and psychological status after VM intervention for the two

Table 2. Psychological Assessment before and 4 Weeks after the Military Training

	Before Training	4 Weeks after Training
Sleepiness	9.8±3.5	9.3±3.5
Total mood	7.9±3.1	8.0±2.9
Anger	7.5±2.6	8.0±3.1*
Tiredness	8.9±2.9	9.3±2.9
Visual fatigue	8.0±3.2	8.3±3.4
Total stress	131.0±32.8	131.8±37.6
Somatization	1.5±0.4	1.6±0.5*
Compulsive	1.5±0.5	1.6±0.5*
Interpersonal sensitivity	1.6±0.5	1.6±0.5
Depression	1.4±0.4	1.4±0.5
Anxiety	1.3±0.4	1.3±0.4
Hostility	1.6±0.5	1.6±0.6
Terror	1.2±0.3	1.2±0.3
Paranoid	1.5±0.5	1.5±0.5
Psychoticism	1.5±0.4	1.4±0.5

Note. *P<0.05.

sessions. As shown in Table 3 and Table 4, the functional recovery of the pituitary-adrenal axis was observed in groups with VM intervention in a time dependent manner, whereas pituitary - gonadal axis, pituitary-thyroid axis, and immune system of subjects were also observed in groups with VM intervention, but in a time independent manner.

DISCUSSION

The main finding of the present study is that large scale and endurance military training has significant impact on the participant's psychology and neuroendocrine-immune system, including increased thyroid function, decreased adrenal cortex, testosterone and immunological function, and increased somatization, anger and tension. For these participants on ordinary Chinese diet, multivitamin/ multimineral intervention showed significant beneficial effects on functional recovery of the pituitary-adrenal axis, pituitary-gonadal axis, pituitary- thyroid axis and immune system as well as psychological parameters as compared to placebo.

Table 3. Effects of Vitamin-mineral Supplements Intervention on Endocrine and Immunity Parameters

	Baseline	Group A 1st VM	2nd Placebo	Baseline	Group B 1st Placebo	2nd VM
ACTH (pmol/L)	6.3±3.3	7.1±5.4*	8.7±4.2†&	6.0±3.5	6.4±3.4	7.4±4.5†&
F (nmol/L)	302.1±83.3	359.6±101.7*	379.7±112.8†&	263.4±80.8	287.6±73.2*	363.3±70.1†&
UFC (nmol/24h)	381.8±110.9	426.6±109.9*	438.6±1.0†	365.6 ±120.8	390.6 ±105.8*	421.4±0.7†&
LH (mIU/L)	3.4±1.9	3.6±1.7	3.9±1.6†	3.5±3.8	3.8±3.4	4.0±3.5*
T (nmol/L)	19.9±6.0	21.3±5.6*	21.0±5.6†	18.7±5.9	21.6±5.1*	20.2±5.2†&
E2 (pmol/L)	115.8±78.4	156.3±55.6*	130.8±37.0†&	108.2±39.0	151.7±34.9*	123.6±38.6†&
TSH (mU/L)	2.3±1.1	2.4±1.2	2.1±1.2†	2.2±1.6	2.7±3.5	2.7±1.6
TT3 (nmol/L)	2.6±0.4	2.3±0.5*	2.1±0.4†&	2.9±1.8	2.2±0.3*	2.1±0.3†
TT4 (nmol/L)	95.8±17.5	101.0±21.5*	99.9±16.7†&	93.7±13.4	99.4±15.4*	98.5±14.1†&
FT3 (pmol/L)	5.8±1.0	5.7±0.6*	5.7±0.7	6.1±4.2	5.9±0.4*	5.6±0.4
FT4 (pmol/L)	17.5±2.7	16.2±2.1*	15.6±1.9†&	16.7±2.0	15.6±1.7*	15.4±2.0†&
CD3+	0.6±0.1	0.6±0.1*	0.6±0.1†&	0.6±0.1	0.5±0.1*	0.6±0.1†&
CD4+/CD8+	1.0±0.4	1.0±0.4	1.1±0.4†&	1.0±0.4	1.0±0.5	1.1±0.4
B	0.1±0.04	0.1±0.1	0.1±0.03	0.1±0.04	0.1±0.04	0.1±0.04
NK	0.3±0.1	0.3±0.1*	0.2±0.1†&	0.3±0.1	0.3±0.2	0.3±0.1†&

Note. Significantly different from baseline and post 1st session were shown as *P<0.05. Significantly different from baseline and post 2nd session were shown as †P<0.05. And significantly different from post 1st session and 2nd session were shown as &P<0.05.

Table 4. Effects of Vitamin-mineral Supplements Intervention on Psychological Symptoms

	Baseline	Group A 1st VM	2nd Placebo	Baseline	Group B 1st Placebo	2nd VM
Sleepiness	9.5±3.4	8.5±3.6*	7.6±2.9$^{\dagger\&}$	9.1±3.6	7.9±3.5*	7.2±2.9$^{\dagger\&}$
Total mood	8.0±3.1	7.6±3.6*	6.6±2.6$^{\dagger\&}$	8.0±2.6	6.8±2.7	6.1±2.1$^{\dagger\&}$
Anger	8.0±3.2	7.4±3.1	6.6±2.6†	8.0±3.1	7.0±2.6	6.4±2.3$^{\dagger\&}$
Tiredness	9.0±2.9	8.7±3.2*	7.9±2.5$^{\dagger\&}$	9.6±2.8	7.9±2.9	7.6±2.4†
Visual fatigue	8.2±3.5	7.7±3.5*	6.9±2.8$^{\dagger\&}$	8.5±3.4	7.4±3.4	6.2±2.4†
Total stress	127.6±33.0	116.7±30.4*	106.7±26.1$^{\dagger\&}$	134.8±41.6	126.4±43.8*	118.4±41.4$^{\dagger\&}$
Somatization	1.6±0.5	1.3±0.4*	1.2±0. 4$^{\dagger\&}$	1.6±0.6	1.4±0.5*	1.4±0.5$^{\dagger\&}$
Compulsive	1.5±0.5	1.3±0.4*	1.2±0.4$^{\dagger\&}$	1.6±0.5	1.5±0.5*	1.4±0.5$^{\dagger\&}$
Interpersonal Sensitivity	1.5±0.5	1.4±0.5*	1.2±0.4$^{\dagger\&}$	1.6±0.6	1.5±0.6*	1.4±0.6$^{\dagger\&}$
Depression	1.4±0.5	1.3±0.4*	1.2±0.4$^{\dagger\&}$	1.5±0.5	1.4±0.5	1.3±0.5$^{\dagger\&}$
Anxiety	1.3±0.4	1.2±0.3*	1.1±0.3	1.4±0.5	1.3±0.5*	1.3±0.5$^{\dagger\&}$
Hostility	1.5±0.5	1.4±0.5*	1.2±0.3$^{\dagger\&}$	1.7±0.7	1.5±0.7*	1.4±0.6$^{\dagger\&}$
Terror	1.2±0.3	1.1±0.2*	1.1±0.2$^{\dagger\&}$	1.2±0.4	1.2±0.4*	1.2±0.4$^{\dagger\&}$
Paranoid	1.4±0.5	1.3±0.5*	1.2±0.5$^{\dagger\&}$	1.5±0.6	1.4±0.6*	1.3±0.5$^{\dagger\&}$
Psychotism	1.4±0.4	1.2±0.4*	1.1±0.3$^{\dagger\&}$	1.4±0.5	1.4±0.5*	1.3±0.5$^{\dagger\&}$

Note. Significantly different from baseline and post 1st session were shown as $^{*}P<0.05$. Significantly different from baseline and post 2nd session were shown as $^{\dagger}P<0.05$. And significantly different from post 1st session and 2nd session were shown as $^{\&}P<0.05$.

Although the exact underlying mechanism of overtraining syndromes are still unclear, it is known that increased levels of endure training and stress can induce an unstable immunological and neuroendocrine status, and that such imbalances are associated with the psychopathology[10]. In lines with previous studies, the results of present study indicated that large-scale, high-intensity military training could also cause compromised cellular immune system, and produce negative psychological consequences such as anger, tension, and compel at the same time.

Previous studies with a variety of vitamins or minerals supplementation did not prove their efficacy in improving performance in overtraining population. However, most of these studies were on professional athletes who might have well adapted to intensive physical activity and have specifically prepared adequate diet[7]. In the present study, we investigate the effects of multi-vitamin-mineral supplement on the immunological, neuroendocrine, and psychological status in participants who were freshly enrolled in military and on ordinary Chinese diet. For these participants, their diet may provide enough calories, as indicated by the unchanged body weight before and after the training program, but not adequate micronutrients.

The consequences of providing a nutritionally inadequate diet are well studied. Dietary insufficiency can cause immunological dysfunction[11], prolonged recovery from illness and injury[12-13] and compromised physical performance[14]. There are lines of studies investigated the relationship between decreased levels of micronutrients and overtraining syndromes. The evidence linking cellular metabolism to the changes of calcium regulation that occur during fatigue was well documented[15]. Calcium plays a crucial role in signal transduction pathways, where it acts as a second messenger, in neurotransmitters and release from neurons. Furthermore, many enzymes require calcium ions as a cofactor. Calcium deficiency can lead to muscle spasm, muscle twitching, osteoporosis and poor blood clotting. Iron-containing enzymes and proteins, often contain hemeprosthetic substances, participate in many biological oxidations and other activities. On the other hand, the military training, especially with high intensity and endurance, tends to decrease the serum ferritin level, which causes athletes are likely to have lower hemoglobin concentrations. Continued iron deficiency could accelerate to anaemia and worsen fatigue[16]. The reason why zinc has received attentions is that it serves as components of more than one hundred types of enzyme, such as carbonic anhydrase, lactate dehydrogenase, and as structural ions in

transcription factors. Zinc deficiency always causes delayed growth and sexual maturation, impotence, altered cognition, impaired host defense consciousness, defects in carbohydrate utilization, and depressed immunity[17]. There are more than 30 proteins and enzymes consisting Cu^{2+}, which is the essential component of tyrosinase, ascorbate oxidase, cytochrome oxidase enzymesoxidase. Cu^{2+} is also involved in hematopoiesis and copper deficiency causes the hypo-chromic small cell anemia and the synthesis of norepinephrine, adrenal corticosteroids and progesterone[18]. Vitamin supplements, including vitamin C, B-complex vitamins, and vitamin E are frequently used by athletes to improve sports performance and enhance physical recovery[19]. In humans, vitamin C is essential to the effective antioxidant, acting to lessen oxidative stress and protect DNA, proteins and membrane structures from free radical damage. The B vitamins are necessary to support and increase the rate of metabolism, to enhance immune and nervous system function, to promote cell growth and division. Clearance of peroxide produced by immune cells metabolism, vitamin E can protect cell membranes from oxidative damage, to maintain the integrity and stability of cells and organelles, to guarantee the function of immune cells.

Based on these studies, vitamin-mineral supplements (VM) intervention systems were utilized in the present study to provide military personnel with desirable nutrition to have them recovered from overtraining syndrome caused by large scale and endurance military training. After VM intervention, the subjects in this study showed significant recovery of pituitary-adrenal axis function in a time dependent manner, and the thyroid axis, immune system as well as psychological status recovered in a time independent manner.

In conclusion, this study provides evidences that VM intervention could be utilized to restore the impaired adrenal cortical, thyroid and immunological function as well as psychological conditions in over-trained military personnel.

LIMITATIONS

Cautions in interpreting this study relate to the restricted population of subjects artillery, giving a small sample for the other military personnel. Clearly, additional studies of other military personnel responding to treatment are required.

An additional consideration is the time point for MV intervention. In this study, we evaluated the effect of MV intervention only after military training, due to adherence concerns. The evaluation of the effects of MV intervention during, even before the military training is needed.

REFERENCES

1. Lehmann MJ, Lormes W, Opitz-Gress A, et al. Training and overtraining: an overview and experimental results in endurance sports. J Sports Med Phys Fitness, 1997; 37, 7-17.
2. Radak Z, Chung HY, Goto S. Systemic adaptation to oxidative challenge induced by regular exercise. Free Radic Biol Med, 2008; 44, 153-9.
3. Bresciani G, Cuevas MJ, Garatachea N, et al. Monitoring biological and psychological measures during an entire season in male handball players. Eur J Sport Sci, 2010; 6, 377-84.
4. Main LC, Dawson B, Heel K, et al. Relationship between inflammatory cytokines and self-report measures of training overload. Res Sports Med, 2010; 18, 127-39.
5. Tanskanen M, Atalay M, Uusitalo A. Altered oxidative stress in overtrained athletes. J Sports Sci, 2010; 28, 309-17.
6. Urhausen A, Gabriel H, Kindermann W. Blood hormones as markers of training stress and overtraining. Sports Med, 1995; 20, 251-76.
7. Lukaski HC. Vitamin and mineral status: effects on physical performance. Nutrition, 2004; 20, 632-44.
8. American Dietetic Association. Position of the American Dietetic Association, Dietitians of Canada, and the American College of Sports Medicine: nutrition and athletic performance. J Am Diet Assoc, 2000; 100, 1543.
9. Wang ZY. The self-report symptom inventory (SCL-90). Shanghai Arch Psychiatry, 1984; 2, 68-70.
10. González-Boto R, Molinero O, Márquez S. Psychological models of overtraining: a perspective and a new model. AnsiedadEstres, 2008; 14, 221-37.
11. Arnason BG. Nervous system-immune system communication. Reviews of Infectious Diseases, 1991; 13, S134-7.
12. Keusch GT. The history of nutrition, infection, and immunity. Journal of Nutrition, 2003; 133, S336-40.
13. Brown KH. Dietary management of acute diarrheal disease: contemporary scientific issues. Journal of Nutrition, 1994; 124, 1455S-60S.
14. Kinney JM, Tucker HN. Shortened length of stay is an outcome benefit of early nutritional intervention Physiology, stress, and malnutrition: functional correlates, Lippincott-Raven Press, Philadelphia, PA (1997), pp. 607-27.
15. Jeukenendrup AE, Brouns F, Wagenmakers AJ, Saris WH. Carbohydrate-electrolyte feedings improve 1 h time trial cycling performance. International Journal of Sports Medicine, 1997; 18, 125-8.
16. Steele DS, DukeAM. Metabolic factors contributing to altered Ca^{2+} regulation in sketelal muscle fatigue. Acta Physiol Scand, 2003; 179, 39
17. Umbreit J. Iron Deficiency: A Concise Review. American Journal of Hematology, 2005; 78, 225-31.
18. Diaz Romero C, Henriquez Sanchez P, Lopez Blanca F, et al. Serum copper and zinc concentrations in a representative sample of the Canadian population. J Trace Elem Med Biol, 2002; 16, 75.
19. Marvin LE, Kirby DF. Nutrition and Sports Supplements, Fact or Fiction. Journal of Clinical Gastroenterology, 2002; 35, 299-306.