Influences of Vitamin A and E Supplementation on Haematological, Functional and Oxidative Balance Parameters on Handball Players

DANA BADAU1, ADELA BADAU4*, FLORIN PARASCHIV1,2, LILIANA MARCELA ROGOZEA1, HAMIDA AL HUSSEIN1, KLARA BRINZANIUC1
1 University of Medicine and Pharmacy, Faculty of Medicine, 38 Gh. Marinescu Str., Tîrgu Mureș, Romania
2 National Olympic Handball Youth Center, 9 Garii Blvd., Brasov, Romania
3 Transilvania University, Faculty of Medicine, 29 Eroilor Blvd., Brasov, Romania
4 University of Transilvania, Interdisciplinary Doctoral School, 29 Eroilor Blvd., Brasov, Romania

The objective of this study was to evaluate the antioxidant effect of the vitamins A and E before and after intensive aerobic exercise effort and to provide pros and cons arguments on the use of both vitamins during the physical training. The research highlighted important adaptive changes of the functional parameters: PMA, VCEE, VO2max, of the oxidative balance: LPx, MDA, as well as the haematological parameters: HGB, CRT, LYM, LAK.

Keywords: vitamins A and E, intensive effort, evaluation of antioxidant effect

The current performance sports target modern dietary strategies, including the use of effective oral supplements to compensate for the loss of vitamins and minerals during exercise. The study of chemical transformations, adaptive functional and movement changes of the body induced by physical effort is part of the current trends of scientific research, from the perspective of interdisciplinary approaches between: chemistry, medicine and sports activity [1-4].

All forms of exercise cause some effects on the oxidative stress [5]. The relationship between exercise and the oxidative stress is extremely complex. While regular moderate physical activity appears to be beneficial for the oxidative stress and health, for different types of sports the oxidative stress could be high [6-8]. Very often, it is ignored that the term vitamin E represents a family of eight natural, structurally related compounds containing a chromanol ring with a phytol side chain, saturated for tocopherols, respectively unsaturated for tocotrienols [9] (fig. 1). Recent studies suggested that tocopherols and tocotrienols are metabolized by hydroxylation and oxidation to 13'-hydroxychromanols and various carboxychromanols, which appear to be more bioactive than tocopherols in anti-inflammatory and anticancer actions [10].

Also, it is thought that Vitamin E have anti-atherogenic, anti-thrombotic, immunomodulatory, neuroprotective and anti-viral effects through multiple mechanisms of action.

The anti-atherogenic effect was explained by the inhibition of low-density lipoprotein (LDL) oxidation and oxLDL accumulation in the arterial walls, which are the first steps in the atherogenic process, as well as the inhibition of protein-kinase C (PKC) which is involved in smooth muscle proliferation.

The anti-thrombotic effect was explained by the downregulation of intracellular cell adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 expressions, as well as the upregulation of the cytosolic phospholipase A2 and cyclooxygenase (COX)-1 expressions which increase the release of prostacyclin, a vasodilator and platelet aggregation inhibitor. α-tocopherol downregulates GPIIb (belongs to the glycoprotein IIb/IIIa) promoter activity which results in reduction of GPIIb protein expression and decreased platelet aggregation. A metabolite of vitamin E, quinone or α-tocopherol quinone (TQ) is a potent anticoagulant. The immunomodulatory effect is not completely understood and has been demonstrated in vitro, α-tocopherol increasing the mitogenic response of T lymphocytes from aged mice. The neuroprotective and antiviral effects of Vitamin E have been explained by its antioxidant effect and the reduction of oxidative stress [13]. A new derivative of α-tocopherol (D,L-α-tocopheryl-β-D-galactofuranoside) was synthesized by the galactofuranosylation of D,L-α-tocopherol, separated and characterized by Iga et al. (2010), they suggesting that the new metabolite could serve as a new drug delivery system for vitamin E in the gastrointestinal tract [14].

Vitamin A plays the role of a pro-oxidant, being a fat-soluble vitamin involved in: toxins and free radicals removal from the body, acceleration of the physical development and increasing the bone strength, inflammations reduction by fighting the free radicals, immunoglobulin biosynthesis, immune system activity stimulation and cell differentiation. Vitamin A mechanism of action in the epithelial differentiation and other physiological processes, as well, consists in the binding to the retinoid nuclear receptors (RARs-Retinoic Acid Receptors and RXRs- Retinoid-X Receptors) [15]. Naturally occurring and synthetic vitamin A (retinol) is important for the human body, the body itself

* email: adela.badau@umftgm.ro
cannot synthesize it. Retinol, a fat-soluble unsaturated isoprenoid-like and its two important metabolites, retinaldehyde and retinoic acid (chemical structures presented in the fig. 2), are essential for growth, differentiation and maintenance of the epithelial tissues; they represent a suitable noninvasive tool for preventing or reducing the oxidative stress during training [7,16,17].

Fig. 2. Retinol and its metabolites chemical structures [18]

Malondialdehyde, is a reactive aldehyde that causes toxic stress in cells, its production being used as a biomarker to measure the level of the oxidative stress in the body [19]. Malondialdehyde is the end product that results from the degradation of polyunsaturated lipids by the reactive oxygen species [20]. Posea et al. (2015) found that even for overweight/obese patients following a low-calories diet for weight loss, the dietary intake of vitamin E and A were lower than the daily recommended intake before and after the nutritional intervention applied to all patients, suggesting the recommendation of oral supplementation during a low calories program [21]. Diet supplementation with the vitamins E and A should be adjusted qualitatively and quantitatively to the athletes peculiarities, in order to have multiple positive effects on the body’s energy and plastic losses, to improve/relieve the health and body performance, helping to compensate peculiarities, and to control water elimination.

The novelty of our research consists in the evaluation of three categories of parameters: hematological, functional and oxidative, being analyzed the interaction between pre- and post-workout supplementation, pre- and post-effort respectively.

The aim of the study was to evaluate the hematological, functional and oxidative balance changes in two stages: pre- and post-workout supplementation with the vitamins A and E before and after intensive aerobic exercise effort amongst top league handball players.

**Experimental part**

**Study design:** The research was conducted between February - April 2017 in the National Olympic Handball Youth Center. The research sample comprised 36 male handball players divided into three groups of 12 subjects each: 2 experimental groups named according to the type of the supplemented vitamins as: vitamin A group and vitamin E group respectively, and the control group, without supplementation, the subjects having the same sporting and age characteristics. The inclusion criteria targeted: active and healthy handball players, aged between 19-21 years old. The exclusion criteria: medical contraindications to the vitamins A, E, supplementation with other doping substances or vitamins. The results of the two experimental groups were compared with those of the control group.

**Organization of the research:** 20-24 February 2017 - pre-testing before supplementation; 25 February - 26 April 2017 - supplementation with the vitamins A and E; 27 -30 April 2017 - post-testing after supplementation. All procedures performed in studies were in accordance with the ethical standards, with the 1964 Helsinki declaration. The ethics aspect are very important in research area [22], and in this regards the study was conducted with the Ethics Committee of Transilvania University of Brasov approval. The subjects included in the study were volunteers, they have expressed their consent for participation by signing the informed consent.

**The haematological and oxidative balance parameters:** Venous blood collection was used for assessments, pre-conditioned in two sessions: pre- and post-effort, respectively in two stages: at the beginning of the experiment before vitamin supplementation and at the end of the research, after supplementation. The targeted haematological parameters were as follows: red blood cells (RBCs - mil/µL), hematocrit (HCT - %), hemoglobin (HGB - g/dL), lymphocytes (LYM - K/mm3), lactate (LAK - mmol/L). The oxidative balance markers were: lipoperoxide (LPx) and free malondialdehyde (MDA - nmol/mL).

**The functional parameters:** - were performed using an Abbott Cell-Dyn 3700 Haematomy analyser: the maximum aerobic power (MAP), expressed in mL/kg, formula: MAP = VO_{max}/kg; the cardiovascular effort-saving (CVEE), expressed in mL/min, formula: CVEE = VO_{max}/Maximum Cardiac Frequency; the maximum O_{2} consumption (VO_{max}), expressed in mL, with the adjusted formula for men [23]:

\[ VO_{max} = \left( \frac{0.00212 \times \text{workload} + 0.399}{0.769 \times HR_{xe} - 48.5} \right) \times 100 \]

where \( HR_{xe} \) is the steady heart rate after 6 min of exercise.

The indirect determination Astrand-Ryhming test was used, consisting of a submaximal exercise of 6 minutes performed on the cycloergometer, with a rotation of 40-90 rot/min and an intensity of 170 W/kg, maintained constant throughout the test [24]; the heart rate was measured in the last 10 seconds of pedaling.

The blood and functional tests were performed in the same day with a break of 30 min between them, in the following order: first blood sampling (before effort), test of 6 min pedaling on the cycloergometer followed by the second blood sampling (after effort).

**Vitamin supplements administration:** the subjects belonging to the Vitamin A group were given 1.2 mg of vitamin A in a single dose/day for 8 weeks, whereas the subjects belonging to the Vitamin E group were given 20 mg of vitamin E, once daily for 8 weeks.

**The training program of the three groups during the experiment:** Monday - a 2 h training/day, with submaximal intensity effort; each Tuesday and Thursday, a 2 h training/day with maximum intensity effort; each Wednesday and Friday a 2 h training/day with submaximal intensity effort/technical training.

**Statistical analysis:** The data collected were presented as means ± standard deviation (SD). ANOVA two-way analysis of variance for repeated measures on one variable (pre- and post-test values) was initially performed to compare the differences between the analyzed groups. Three follow-up analyses were carried out to determine the differences and possible significant interactions between the three groups. To prevent an inflation of the Type I error rate, the alpha level was adjusted with the Bonferroni method by dividing 0.05 by the number of t tests performed (three). Were calculated: estimated power at 5% probability, partial eta squared (η_{p}^2), Bonferroni post-hoc tests. The paired t Student test was calculated on the
pre- to post-test change for each group before and after supplementation, for all parameters. The threshold of significance \( p<0.05 \) was considered statistically significant for research.

Results and discussions

The hematological parameters values, the statistical descriptors of the functional parameters and the oxidative balance markers values were presented in the tables 1, 2 and 3.

Vitamins A and E supplementation’s influence on the haematological parameters: Significant influence of supplementation was found between pre- and post-supplementation values within both groups, Vitamin A group as well as the Vitamin E group, for the following haematological parameters: HGB, \( F(1,33) = 128.13, p=0.0001 \), effect size \( \eta^2 = 0.79 \), estimated power at 5% probability = 1.00; HCT \( F(1,33) = 23.21, p=0.0001 \); effect size \( \eta^2 = 0.41 \), estimated power at 5% probability = 0.99; LYM \( F(1,33) = 371.69, p=0.0001 \); effect size \( \eta^2 = 0.91 \), estimated power at 5% probability = 1.00), highlighting the importance of vitamin supplementation. No significant influence was noted for RBC values between pre- and post-supplementation with the studied vitamins. A statistically significant difference was also observed between the three experimental groups \( F(2,33) = 26.04, p=0.0001 \); effect size \( \eta^2 = 0.62 \), estimated power at 5% probability = 1.00. Bonferroni post-hoc tests indicated that there were significant differences between pre- and post-supplementation values of LYM and LAK between the Vitamin A group compared with Vitamin E group (\( p<0.001 \)). Vitamins A and E supplementation’s influence on the functional parameters is presented in table 2.

A statistically significant influence of supplementation for both vitamins, A and E respectively, was found between pre- and post-supplementation values for the following functional parameters: PMA \( F(1,33) = 276.35, p=0.0001 \); effect size \( \eta^2 = 0.89 \), estimated power at 5% probability = 1.00; CVEE \( F(1,33) = 901.169, p=0.0001 \); effect size \( \eta^2 = 0.96 \), estimated power at 5% probability = 1.00; VO2max \( F(1,33) = 15.28, p=0.0001 \); effect size \( \eta^2 = 0.32 \), estimated power at 5% probability = 0.96), suggesting the beneficial effects of supplementation with the studied vitamins. In addition, a significant difference was observed between

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Vitamin A Group</th>
<th>Vitamin E Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (mL/dL)</td>
<td>T1</td>
<td>5.16±0.12</td>
<td>17.50</td>
<td>0.0001</td>
</tr>
<tr>
<td>HGB (g/dL)</td>
<td>T1</td>
<td>14.22±0.53</td>
<td>11.82</td>
<td>0.0001</td>
</tr>
<tr>
<td>LYM (K/mm³)</td>
<td>T1</td>
<td>15.40±0.47</td>
<td>15.03</td>
<td>0.0001</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>T1</td>
<td>40.54±1.97</td>
<td>16.16</td>
<td>0.0001</td>
</tr>
<tr>
<td>LAK (mmol/L)</td>
<td>T1</td>
<td>2.26±0.11</td>
<td>11.76</td>
<td>0.0001</td>
</tr>
<tr>
<td>PMA</td>
<td>T1</td>
<td>64.08±1.16</td>
<td>17.35</td>
<td>0.0001</td>
</tr>
<tr>
<td>CVEE</td>
<td>T1</td>
<td>33.10±0.64</td>
<td>44.59</td>
<td>0.0001</td>
</tr>
<tr>
<td>VO2max</td>
<td>T1</td>
<td>4306.41±40.38</td>
<td>78.81</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Table 1: DESCRIPTIVE STATISTIC OF THE HAEMATOLOGICAL PARAMETERS

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Vitamin A Group</th>
<th>Vitamin E Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMA</td>
<td>T1</td>
<td>64.08±1.16</td>
<td>17.35</td>
<td>0.0001</td>
</tr>
<tr>
<td>CVEE</td>
<td>T1</td>
<td>33.10±0.64</td>
<td>44.59</td>
<td>0.0001</td>
</tr>
<tr>
<td>VO2max</td>
<td>T1</td>
<td>4306.41±40.38</td>
<td>78.81</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Table 2: DESCRIPTIVE STATISTIC OF THE FUNCTIONAL PARAMETERS
the three analyzed groups, for all functional parameters, as follows: PMA (F\(_{2,33}\) = 32.37, p=0.0001; effect size \(\eta^2 = 0.66\), estimated power at 5% probability = 0.99), CVEE (F\(_{2,33}\) = 94.59, p=0.0001; effect size \(\eta^2 = 0.85\), estimated power at 5% probability = 1.00), VO\(_{2}\)max (F\(_{2,33}\) = 7.33, p=0.002; effect size \(\eta^2 = 0.31\), estimated power at 5% probability = 0.91). Bonferroni post-hoc pre- and post-supplementation tests also showed a statistically significant difference between vitamin A, vitamin E and control group, for all functional parameters (p<0.05). Vitamins A and E supplementation’s influence on the oxidative parameters is presented in Table 3.

Similarly to the previous analyzed parameters, a statistically significant influence of supplementation with both vitamins, A and E respectively, was found between pre- and post-supplementation values for the oxidative parameters: LPx (F\(_{2,33}\) = 227.80, p=0.0001; effect size \(\eta^2 = 0.87\), estimated power at 5% probability = 1.00) and MDA (F\(_{2,33}\) = 37.12, p=0.0001; effect size \(\eta^2 = 0.52\), estimated power at 5% probability = 1.00). A significant difference was found between the experimental groups regarding both oxidative parameters: LPx (F\(_{2,33}\) = 12.35, p=0.0001; effect size \(\eta^2 = 0.42\), estimated power at 5% probability = 0.99) and MDA (F\(_{2,33}\) = 4.86, p=0.014; effect size \(\eta^2 = 0.22\), estimated power at 5% probability = 0.764), indicating the importance of vitamin supplementation. Bonferroni post-hoc pre- and post-supplementation tests indicated significant differences between the results obtained for the subjects belonging to the vitamin A, E and control group respectively, for all the oxidative parameters.

The results of our research highlighted significant differences of the pre- and post-supplementation values between the two experimental groups (vitamin A and E groups) compared to the control group, especially for the post-effort tests. Our study results are in accordance with the data reported by other authors, regarding the effects of oral supplementation with vitamin A and E in athletes, both on the haematological and functional parameters, as well as on the oxidative balance [25-28].

Rejlík et al. (2015) in their research conducted on 17 elite boxers on dietary intake, evaluated the vitamin status and the oxidative stress in the pre- and post-competition week, highlighting that in both groups, the intakes of vitamins A, E and folate were below recommended values throughout the periods; however, blood vitamin and plasma glutathione levels did not change significantly [29]. Tauler et al (2006) investigated the influence of Vitamin E (500mg/day), beta-carotene (30 mg/day) and Vitamin C (1 g/day) supplementation administered to 15 amateur trained male athletes, through maximal and submaximal exercise tests, founding significantly higher plasmatic final levels of vitamin C, vitamin E and beta-carotene after three months of supplementation [30]. Bloomer et al. (2007) found that six weeks of vitamin E and C supplementation prevented endurance exercise-induced lipid peroxidation, without any effect on the inflammatory markers [31]. Tsakiris et al. (2009) reported that vitamin E and vitamin A supplementation in a group of 10 basketball players caused lower increases in blood lactate levels after exercise [32]. Goroku (2017) investigated the effects of vitamin E and A supplementation on some haematological parameters in a group of taekwondo elite players on a period of 6 weeks, suggesting positive effects of the mentioned vitamins on hemocrit (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), mean platelet volume (MPV), procalcitonin (PCT) and lactate levels [33].

Conclusions

The vitamin A and E supplementations administered to performance athletes have positive effects, increasing the aerobic exercise capacity and preventing the exercise-induced increase in oxidative stress of hematomal parameters.

Our results suggest important adaptive changes in the functional parameters: PMA, CVEE, VO\(_{2}\)max, the oxidative balance: LPx, MDA, as well as the haematological parameters: HGB, CRT, LYM, LAK, showing relevant, important adaptive modifications to antioxidant vitamins supplementation between the experimental groups and the control group.

References

33. GORUCU A., Turk J Sport Exe., 19, no. 1, 2017, p.18

Manuscript received: 20.09.2017

http://www.revistadechimie.ro REV.CHIM.(Bucharest) ● 69 ● No. 2 ● 2018