Effect of Vitamin A Supplementation on IFN-γ, TNF-α, IL-2, and IL-6 Levels in Elite Taekwondo Players

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KEYWORDS Vitamin A, Cytokines, Exercise, Fatigue, Taekwondo

ABSTRACT This study aims to investigate the effects of vitamin A supplementation on cytokine release in 10 male elite taekwondo players. The subjects who were supplemented with 100 mg of vitamin A in daily tablet form for 4 weeks were exercised to exhaustion twice, before and after the supplementation. In the course of the four-week study period, blood samples were collected from the subjects four times in exhaustion and at rest, before and after supplementation. The blood samples were analyzed for IFN-γ, TNF-α, IL-2, and IL-6 levels. Vitamin A supplementation resulted in a significant increase in serum IL-2 levels (p<0.05). However, vitamin A supplementation led to the inhibition of serum TNF-α levels, in comparison to pre-supplementation (exhaustion and resting) values (p<0.05). The results of the study indicate that vitamin A supplementation might cause changes in the release of cytokines independent of exercise.

INTRODUCTION

Vitamins, the primary regulators of metabolic functions, have a critical part to play in physical performance (Patlar et al. 2012). The fact that physical performance was reported to decline due to long-term vitamin deficiency (Patlar et al. 2011a) attests to the importance of vitamins in physical activity (Patlar et al. 2011a,b). Physical activity is enhanced by exercise and athletic performance by optimal nutrition. Therefore, mineral, vitamin and hormone supplementation is crucial for performance (Bicer et al. 2015; Rodriguez et al. 2009). It was reported that in the case of athletes engaged in exercises causing weight loss, fat-soluble vitamins are needed to maintain performance and protect the body weight (Patlar et al. 2011b). It was noted that mineral and vitamin support was necessary for superior performance and that such support was required particularly for delaying fatigue and sustaining performance (Akil et al. 2015; Sobal and Marquart 1994). Thus, it can be said that there has been an increasing interest in the relation between exercise and vitamins. One of the most commonly supplemented vitamins is vitamin A (Patlar et al. 2011a; Briefel et al. 2006). While developing strategies to increase performance with regard to physical training and nutrition, the factors that can have an unfavorable effect on athlete health need to be eliminated (Oberman 1984). Consequently, there has been a remarkable increase in research on the relationship between exercise and immune system (Kara et al. 2011). Vitamin A, known to have important effects on reproduction, embryogenesis, and tissue development, also figures significantly in the immune system and its reactions (Klassert et al. 2014). The effect of vitamin A on immune reactions results especially from vitamin A being a potential mediator of T-helper cells (Mottaghi et al. 2014). The recently shown immune-modulator effect of vitamin A appears to be a triggering factor for research into the relation between vitamin A and the immune system (Harrchian et al. 2014). While mild and moderate regular exercises have a positive impact on the immune system (Mackinnon 1994; Simpson et al. 2015), slow-paced and long-term exercises inhibit the immune system (Malm 2004). Helper T-4 lymphocytes are divided into two as Th1 and Th2. Th1 produces IFN-gamma, IL-2 and TNF alpha, whereas Th2 leads to the production of IL-4, IL-6, and IL-10. Actually, IL-6 and IL-10, cytokines produced by Th2, exercise a negative effect on cellular immunity by inhibiting the gamma interferon synthesis of T cells (Baltaci et al. 2005; Baltaci et al. 2003). Slow-paced exercise increases the Th2-dependent cytokine response, thereby inhibiting the cytokine release of Th1s and causes an unfavorable effect on cellular immunity (Lu et al. 2011).
An overall evaluation of the information provided above suggests that there are considerable relationships between exercise and vitamin A, exercise and immune system, and vitamin A and immune system. However, there are only a limited number of publications about how vitamin A supplementation influences cytokine release in exercise (Vassilakopoulos et al. 2003). Thus, the present study aims to examine the effects of vitamin A supplementation on cytokine release in individuals practicing taekwondo sports.

**MATERIAL AND METHODS**

**Subjects**

The research registered 10 male elite taekwondo players studying at Selcuk University School of Physical Education and Sports with a mean age of 22.06±0.25 years and mean weight of 63.50±2.30 kg.

**Method**

**Vitamin A Supplementation**

Vitamin A was supplemented in the form of 100 mg vitamin A (retinol) oral tablet a day at 10:00 am on a full stomach for a period of 4 weeks.

**Taekwondo Exercise**

The athletes were engaged in taekwondo training for 7 days a week for 4 weeks. The training started with a 20-minute warm up exercise. The warm up was followed by individual hand attack training. The athletes practiced all hand attack techniques at the maximal performance level until exhaustion. The same training was repeated for three rounds and the exercise was completed with stretching.

**Strenuous Exercise (Bruce Protocol)**

The subjects performed strenuous exercise twice, before and after the 4-week vitamin supplementation. As a strenuous exercise, the Bruce protocol, the most commonly used clinical exhaustion exercise, was employed. The incline and speed of the treadmill (Cosmed T150) was increased at three-minute intervals until the athletes could no longer continue (Kara et al. 2010).

**Collection of Blood Samples from the Subjects**

Before and after the 4-week supplementation, four rounds of blood samples were collected both at rest and after exercise. From each subject, 4 ml of blood samples were collected from the forearm vein at 9:00 am (on an empty stomach). The samples were centrifuged at 3000 rpm for 10 minutes to separate the serum and then stored at -80°C until the time of analysis.

**Biochemical Analyses**

After the serum was separated, blood samples were analyzed to measure levels of IFN-α (LOT:121101/A) as “IU/ml”, IL-2 (LOT:122301) as “U/ml”, IL-6 (LOT124802) as “U/ml” and TNF-α (LOT:121902/A) as “pg/ml”. Colorimetric kits were used in the measurements carried out with a DIA Source brand ELISA test (Belgium).

**Statistical Evaluations**

The SPSS 16.0 software package was used in the statistical evaluation of data and the arithmetic means and standard deviations of all parameters were calculated. Repeated Measures Analysis of Variance was used to identify the differences between measurements conducted at different times and the Least Significant Difference test was used to determine the time from which the difference arose. Differences for which p<0.05 were accepted as significant.

**RESULTS**

Levels of IFN-γ and IL-6 did not show any significant difference before and after supplementation. IFN-γ levels of group before vitamin A supplementation 0.20 ± 0.19 IU/ml at rest and 0.22 ± 0.21 IU/ml at exhaustion. IFN-γ levels of group after vitamin A supplementation 0.21 ± 0.06 IU/ml at rest and 0.19 ± 0.05 IU/ml at exhaustion. IL-6 levels of mentioned group 18.13 ± 2.23 pg/ml, 20.79±8.25 pg/ml, 19.62 ± 5.10 pg/ml, 17.63 ± 2.56 pg/ml, respectively (Tables 1 and 2). The IL-2 levels of the group were not different before vitamin A supplementation at rest (0.43 ± 0.18 U/ml) at exhaustion (0.45 ± 0.19 U/ml). Similarly IL-2 levels were not different after vitamin A supplementation at rest (0.75 ± 0.08 U/ml) at exhaustion (0.69 ± 0.27 U/ml). However, vitamin A supplementation has increased IL-2 levels at rest and exhaustion compared to before vitamin A supplementation (p<0.05, Table 3). TNF-α lev-
EFFECT OF VITAMIN A SUPPLEMENTATION ON CYTOKINES

The measurements carried out before and after supplementation at rest and exhaustion did not show any significant difference in the IFN-γ and IL-6 levels of the subjects. It was demonstrated that an 8-week endurance exercise did not affect TNF-α levels, but reduced IFN-γ levels in sedentary males (Jahromi et al. 2014). In another study, which included nine healthy young males and conducted in normal room temperature, 60-minute low and moderate intensity exercises were reported to elevate IL-2, IL-6 and IFN-γ levels (Gagnon et al. 2014). Marques and colleagues (2013) noted that a 32-week exercise program did not alter TNF-α levels in old subjects. While a swimming exercise did not change IL-6 and IFN-γ levels in young rats, it reduced IL-6 and IFN-γ levels in middle-aged rats and elevated all three parameters in old rats (Leite et al. 2014). Consequently, it is seen that the results of the studies about the relationship between exercise and cytokines are inconsistent (Allen et al. 2015). These inconsistencies may be accounted for by the differences between exercise programs, duration intensity of exercises, subjects, and supplementation regimes (Dorneres et al. 2015). In this study, the measurements at rest and exhaustion before and after vitamin A supplementation did not show any significant difference between serum IFN-γ and IL-6 levels of the subjects. This result is consistent with the results of Marques et al. (2013), LaVoy et al. (2013) and Touva et al. (2011) who established that the concerned cytokines were not affected by exercise. However, TNF-α and IL-6 levels remained unaffected not only by exercise, but also by the 4-week vitamin A supplementation. The decrease found in TNF-α and IL-6 levels after vitamin A supplementation, which had been elevated in fungus infections, suggests that the concerned vitamin might be significantly involved in the regulation of these cytokines (Klassert et al. 2014). The study of Farhangi et al. (2013) who showed that vitamin A supplementation did not alter TNF-α levels in obese women is consistent with the TNF-α levels, which the researchers found to stay unchanged not only after exercise, but also after vitamin A supplementation. Similarly, the results obtained by Jensen et al. (2014) showing that vitamin A did not change IL-6 levels in children lend strong support to the results, which did

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### Table 1: Evaluation of Serum IFN-γ levels in subjects

<table>
<thead>
<tr>
<th>Measurement times</th>
<th>n</th>
<th>IFN-γ (IU/ml) Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before vitamin A supplementation</td>
<td>10</td>
<td>0.20 ± 0.19</td>
</tr>
<tr>
<td>A supplementation</td>
<td>10</td>
<td>0.22 ± 0.21</td>
</tr>
<tr>
<td>After vitamin A supplementation</td>
<td>10</td>
<td>0.21 ± 0.06</td>
</tr>
<tr>
<td>A supplementation</td>
<td>10</td>
<td>0.19 ± 0.05</td>
</tr>
</tbody>
</table>

* A, B: Means with different superscripts in the same column are statistically significant (p<0.05).

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### Table 2: Evaluation of Serum IL-6 levels in subjects

<table>
<thead>
<tr>
<th>Measurement times</th>
<th>n</th>
<th>IL-6 Levels (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before vitamin A supplementation</td>
<td>10</td>
<td>18.13 ± 2.23</td>
</tr>
<tr>
<td>A supplementation</td>
<td>10</td>
<td>20.79 ± 8.25</td>
</tr>
<tr>
<td>After vitamin A supplementation</td>
<td>10</td>
<td>19.62 ± 5.10</td>
</tr>
<tr>
<td>A supplementation</td>
<td>10</td>
<td>17.63 ± 2.56</td>
</tr>
</tbody>
</table>

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### Table 3: Evaluation of Serum IL-2 levels in subjects

<table>
<thead>
<tr>
<th>Measurement times</th>
<th>n</th>
<th>IL-2 Levels (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before vitamin A supplementation</td>
<td>10</td>
<td>0.43 ± 0.18</td>
</tr>
<tr>
<td>A supplementation</td>
<td>10</td>
<td>0.45 ± 0.19</td>
</tr>
<tr>
<td>After vitamin A supplementation</td>
<td>10</td>
<td>0.75 ± 0.08</td>
</tr>
<tr>
<td>A supplementation</td>
<td>10</td>
<td>0.69 ± 0.27</td>
</tr>
</tbody>
</table>

* A, B: Means with different superscripts in the same column are statistically significant (p<0.05).

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### Table 4: Evaluation of Serum TNF-α levels in subjects

<table>
<thead>
<tr>
<th>Measurement times</th>
<th>n</th>
<th>IFN-γ levels (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before vitamin A supplementation</td>
<td>10</td>
<td>5.27 ± 3.12</td>
</tr>
<tr>
<td>A supplementation</td>
<td>10</td>
<td>5.19 ± 3.06</td>
</tr>
<tr>
<td>After vitamin A supplementation</td>
<td>10</td>
<td>3.92 ± 0.93</td>
</tr>
<tr>
<td>A supplementation</td>
<td>10</td>
<td>4.05 ± 1.58</td>
</tr>
</tbody>
</table>

* A, B: Means with different superscripts in the same column are statistically significant (p<0.05).
not reveal any vitamin A supplementation-associated change in IL-6 levels.

Vitamin A supplementation in this study brought about an increase in serum IL-2 levels and a decrease in serum TNF-α levels. Exercise performed in a cold environment was shown to elevate IL-2 levels (Gagnon et al. 2014). However, it was also reported that Polygonatum sibiricum supplementation to iron deficient rats subjected to an extended swimming exercise led to a decrease in IL-2 levels (Wu et al. 2014). The results of neither of the studies cited above (Gagnon et al. 2014; Wu et al. 2014) are consistent with the elevated IL-2 levels the researchers found after vitamin A supplementation in the study. In fact, although IL-2 levels did not change in relation to exercise before supplementation in the athletes, vitamin A supplementation caused an increase in this parameter. Kara et al. (2011) established that exercise did not affect IL-2 levels in individuals engaged in wrestling as a sport and this result is in harmony with the pre-supplementation IL-2 levels the researchers found in the present study. Actually, elevated IL-2 levels the researchers observed after vitamin A supplementation can be considered a result independent of exercise. That IL-2 levels were found even higher after combined supplementation of iron and vitamin A in comparison to iron supplementation alone in anemic women (Sun et al. 2010) and the possibility of vitamin A supplementation having a stimulating effect on IL-2-releasing cells (Bessler et al. 2007) are both consistent with elevated IL-2 levels found in this study and this result is independent of exercise.

In the same vein, TNF-α levels of the subjects were not affected by exercise, while vitamin A supplementation reduced TNF-α levels in this study. It was reported that TNF-α levels in diabetic rats increased, whereas the levels in exercised diabetic rats decreased (Kim et al. 2014). In football players, mid-season and post-season measurements showed significantly lower TNF-α levels than pre-season measurements (Del Giacco 2014), and it was reported that exercise reduced TNF-α levels in sedentary individuals who performed endurance exercise (Jahromi et al. 2014). The results of these researchers are not consistent with the TNF-α levels, which remained unchanged after exercise in the present study. On the other hand, Leite et al. (2014) demonstrated, parallel to this study, that TNF-α levels did not change in young rats, which were subjected to a swimming exercise. A similar result that strongly supports the finding comes from the study of Kara et al. (2011) who found that exercise did not change TNF-α levels in individuals engaged in wrestling. Vitamin A supplementation in this study was seen to inhibit TNF-α levels. This result can be attributed more to vitamin A supplementation than exercise. In a study of children, it was reported that vitamin A supplementation significantly reduced TNF-α levels (Jørgensen et al. 2013) and similarly, it was shown in blood culture samples of 291 children that vitamin A supplementation had a diminutive effect on TNF-α levels (Jørgensen et al. 2012). The results of both studies are in harmony with the reduced TNF-α levels the researchers found after vitamin A supplementation.

**CONCLUSION**

1. Serum IFN-γ and IL-6 levels in the athletes were not affected by either exercise or vitamin A supplementation.
2. Serum IL-2 levels of the subjects increased in response to vitamin A supplementation independent of exercise.
3. Vitamin A supplementation reduced TNF-α levels independent of exercise.

The results of this study suggest that exercise did not bring about any change in IFN-γ, IL-6, IL-2, or TNF-α levels in individuals practicing taekwondo. Supplementation of vitamin A, known to be a potential mediator of immune reactions, on the other hand, was seen to cause an increase in levels of IL-2, a cytokine significantly involved in cellular immunity, and a decrease in TNF-α levels.

**RECOMMENDATIONS**

The results of present study show that vitamin A supplementation has affected IL-2 levels. However, the limitation of study is that different doses of vitamin A supplementation and different sport groups may be research to determine the effects of vitamin A and cytokines levels.

**REFERENCES**

EFFECT OF VITAMIN A SUPPLEMENTATION ON CYTOKINES


Paper received for publication on March 2015
Paper accepted for publication on November 2015