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INVESTIGATION ON THYROID HORMONES LEVEL IN PROBIOTIC-SUPPLEMENTED TRAINED ATHLETES

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ABSTRACT

With attention to beneficial effects of probiotics on thyroid function, this study is done in order to answer the question of what affect the use of probiotic supplements on thyroid hormone indices in trained athletes. Subjects (athletes) were randomly assigned to two groups taking probiotic and placebo groups, respectively. The procedure has four main steps include: 1-PEPS; pre-exercise and pre-supplementation (onset of experiment), 2- EPS; post-exercise and pre-supplementation (after first time exercise and before supplementation), 3- ES; post-exercise and post-supplementation (after first time exercise and 30day probiotic/ or starch capsule supplementation), 4- ESE; post- supplementation and post-exercise (after 30day probiotic/ or starch capsule supplementation and after second time exercise). Blood samples were taken at each of these four steps. Before probiotic-supplementation, subjects attended the aerobic training. Next, subjects have Bruce test. Probiotics (familac) in capsule was as a combination of several types of probiotics (dosage of 1 capsule per day). Based on laboratory analysis of serums, ES group (probiotic-supplemented) has greater T_4 level ($P < 0.05$) and lower TSH levels in comparison with other groups ($P < 0.01$). There is no significant difference between groups for T_3 , during experimental procedure. It can be concluded that probiotic supplementation for 30-days can stimulate thyroid activity in trained athletes (via increase T_4), but this effect is somewhat instable, and the elevated T_4 levels can be declines to normal range. So, probiotic supplementation can be used in athletes without possible adverse effect on thyroid activity.

Keywords: Dietary Supplements, Exercise, Probiotic, Thyroid Activity, Tradmil-tests

INTRODUCTION

Health effects of probiotics in the prevention and treatment of diseases such as colon cancer, hypercholesterolemia, high blood pressure, constipation, diarrhea, lactose intolerance, irritable bowel syndrome, osteoporosis, allergies and urinary- genital tract infections are documented (Pyne *et al.*, 2013). Nowadays, probiotics are considerable dietary supplements, and probiotic-complexes are produced and purchased continuously. Femilac is a supplement with mixture of probiotics includes 7 strains of bacteria and fructose oligosaccharide prebiotic for improves probiotic activity (Pirota *et al.*, 2004). Currently, following a study on immunological effect of this kind of probiotic supplements, it has been reported that femilac can be a selected probiotic supplement for athletes (Aghaei *et al.*, 2013). In Aghaei *et al.*, (2013), Athletes who received femilac-supplemented after exhaustive exercise (aerobic exercise show high levels of monocytes, granulocytes and lymphocytes, respectively. In this study (Aghaei *et al.*, 2013) and other studies of lactobacillus probiotic effects on the athletes (Gleeson *et al.*, 2011; Lamprecht *et al.*, 2012), hormonal responses and blood biochemical parameters have not been studied. Intensive training and difficult jobs decrease the strength of the immune system and digestive disorders are followed, poor performance can cause chronic fatigue in athletes. Although there is no evidence on the energetic effects of probiotics but the secondary positive effects on athletic performance via reducing fatigue during exercise, improve immune function and maintain a healthy digestive system, are taken into consideration (Nichols, 2007). Studies on species of probiotics in athletes are limited to *L. casei* *L.Fermentus*, *L.Acidophilus* and *L.rhamnosus* (Nichols, 2007; West *et al.*, 2009). Given the beneficial effects of probiotics on thyroid function (Awaisheh *et al.*, 2013), this study is done in order to answer the question of what affect the use of probiotic supplements on thyroid hormone indices in athletes.

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MATERIALS AND METHODS

In present study, effect of oral probiotic supplement on serum parameters, after exhaustive exercise (two time exercise) in male athletes has been examined. The procedure has four main steps include: 1- pre-exercise and pre-supplementation (onset of experiment), 2- post-exercise and pre-supplementation (after first time exercise and before supplementation), 3- post-exercise and post-supplementation (after first time exercise and 30day probiotic/ or starch capsule supplementation), 4- post- supplementation and post-exercise (after 30day probiotic/ or starch capsule supplementation and after second time exercise)

The populations of groups are professional bodybuilding athletes from East Azerbaijan province – Iran, and the sample included 14 male bodybuilders (three days a week, regular physical activity and exercise at least 5 years) in the age range 55-20 years.

Subjects were randomly assigned to two groups taking probiotic and placebo groups. Participants prior to the implementation of the exercise program to initial measurements, such as measurement of anthropometry, such as height, body mass and heart rate at rest and adaptation with the treadmill and standardized test to determine HR_{max} and VO_{2max} in sport location protocol attended.

- Bruce Test

Before supplementation, subjects attended the afternoon for aerobic training. Subjects after 5 min exercise (a walk on the treadmill), have Bruce test (Bruce *et al.*, 1949) (table 1). In the end, Bruce-subjects cooled down for 5 minutes to warm up the same way will do. The second phase of work was completed after 28 days, so the first step was repeated. Place in both the pre-and post-supplementation, as well as pre-and post-exercise blood samples from the subjects of the study were to measure the variables.

Table 1: Bruce test procedure

Stage	Speed (km/hr)	Speed (mph)	Gradient
1	2.74	1.7	10
2	4.02	2.5	12
3	5.47	3.4	14
4	6.76	4.2	16
5	8.05	5.0	18
6	8.85	5.5	20
7	9.65	6.0	22
8	10.46	6.5	24
9	11.26	7.0	26
10	12.07	7.5	28

Aerobic power or maximal oxygen consumption using exhaustive testing (treadmill running) and it was calculated by the formula of the first-stage test speed of 1.7 miles per hour (2.47 miles per hour) and began a steep 10 per cent each phase velocity of 1.3 km on the clock and 2% was added to the gradient system. Lag time when subjects were able to continue (table1)

- Probiotic Supplementation

Probiotics in capsule form used in this study as a combination of several types of probiotics and Familac dosage of 1 capsule per day. After the first bout of exercise protocol 30 capsules of probiotics or placebo were consumed (1 per day for 28 days).

Multiple oral probiotic bacteria lactate was used as a product of the Iranian Zist Takhmir Company, Iran. Probiotic strains used in this product are: L. Casei (CFU/g 109×5.1), L. Acidophilus (CFU/g 109×2), Lactobacillus C. (CFU/g 109×5.1), L. Bulgaricus (CFU/g 108×2) , B. Breve (CFU/g 1010× 2), B. Longum (CFU/g 109× 7), S. Thermophilus (CFU/g 109× 5.1). In addition fructo-oligosaccharide as prebiotic products was used for growth and activity of probiotics.

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About when and how to use (best time 1 hour after the meal with water) and avoid eating yogurt or other supplements affect the research process was presented. Placebo capsules contents starch only.

- Laboratory and Statistical Analysis

Laboratory analyses were conducted by auto-analyser using special Elisa kits for TSH and thyroid hormones, in a biomedical laboratory.

Collected data were analyzed by SAS software Ver. 9.1 and comparisons between variables was done via Unpaired t-test, (P<0.01, and 0.05).

RESULTS AND DISCUSSION

TSH, T₃ and T₄ levels of two experimental groups at different step of experimental sport protocol are presented in table 2.

Table 2: TSH, T₃ and T₄ levels of probiotic (femilac)-supplemented athletes during 4 steps of experimental procedure

Treatments ¹	TSH (μIU/ ml)				T ₃ (ng/dl)				T ₄ (μg/dl)			
	PEP S	EPS	ES	ESE	PEP S	EPS	ES	ESE	PEP S	EPS	ES	ESE
Control (placebo)	2.47	3.05	2.47 ^a	2.92	121.8 ³	121.7 ⁸	109.2 ³	107.2 ⁸	6.77	7.50	6.20 ^b	6.72
Probiotic (femilac supplemente d)	2.27	2.40	1.62 ^b	2.35	104.6 ⁵	101.5 ⁵	101.9 ⁵	104.7 ⁸	7.65	7.95	9.60 ^a	9.17
P value ²	0.62 ⁹	0.14 ⁹	0.00 ⁸	0.26 ⁹	0.187	0.311	0.667	0.862	0.53 ¹	0.76 ⁹	0.04 ⁸	0.15 ⁷
SEM ³	0.24 ⁹	0.24 ⁸	0.14 ¹	0.29 ⁸	7.292	11.57 ²	10.17 ²	8.701	0.83 ⁵	0.92 ⁸	0.87 ¹	0.96 ⁰

¹PEPS: pre-exercise and pre-supplementation (onset of experiment), EPS: post-exercise and pre-supplementation (after first time exercise and before supplementation), ES: post-exercise and post-supplementation (after first time exercise and 30day probiotic/ or starch capsule supplementation), ESE: post-supplementation and post-exercise (after 30day probiotic/ or starch capsule supplementation and after second time exercise)

²Significant difference between means shown by different letters; a and b

³Significant error of means

In table2, ES group (probiotic- supplemented) has greater T₄ level (P< 0.05) and lower TSH levels in comparison with other groups (P<0.01). There is no significant difference between groups for T₃, during experimental procedure.

Many studies have been conducted on effects of probiotics on thyroid hormones (Awaisheh et al., 2013, Soheilian et al., 2014; Varian et al., 2014). Also, numerous research works are published in related to probiotics effects on athletes (Lamprecht et al., 2012; Gleeson et al., 2011; Grobbelaar et al., 2012; Aghaee, 2013).

In Awaisheh et al., (2013), dietary combination with probiotic induced the increased activity of thyroid glands, as evident by elevated levels of serum total thyroxine, total triiodothyronine, and free triiodothyronine in hypercholesteromic rats. They suggested that combinations of Lactobacillus-based probiotics and phytosterols in functional foods can be useful in prevention of cardiovascular diseases. Also, Varian et al.,(2014) had stated that probiotic-microbe supplementation may be used to stimulate

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beneficial host immune interactions with improved thyroid function and more healthful aging. In a Soheilian *et al.*, (2014), the supplementation of probiotic yoghurt caused temperate increase in T₃ and T₄ from day8 to day 23 of study, but from day 23 to day 32 it has declines to control levels. In their study, T₄ did not differ significantly between two groups throughout the 32 day study. In the probiotic yoghurt group, mean T₃ (ng/dl) increased significantly on day 24 compared day 16 (P: 0.04) but no significant difference in mean weight and T₄ (mg/dl) was observed between days of sampling.

In present study (table 2), probiotic supplementation in athletes (group ES) cause a temperate elevation in T₄ and decline in TSH levels (ES in comparison with PEPS, EPS and ESE), whereas there is no significant change in T₃ (table 2). After second exercise (ESE), the differences between two groups were not significant (table 2), whereas the level of T₄ is somewhat in higher level (not significant), when compared with control. Present findings for athletes (table 3) are in according to Soheilian *et al.*, (2014) who stated that probiotics effect on thyroid hormones is temperate and not stable. Also, present study is in agreement with Varan *et al.*, (2014) for possible effect of probiotics in thyroid stimulation.

Conclusion

It can be concluded that probiotic supplementation for 30-days can stimulate thyroid activity in trained athletes (via increase T₄), but this effect is somewhat instable and the elevated T₄ levels can be declines to normal range. So, probiotic supplementation can be used in athletes without possible adverse effect on thyroid activity. Further studies on effects of various exercise with different dietary supplements on thyroid activity is seem to be necessary.

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