Effect of 3 types of training on Interleukin 15 and Insulin-like growth factor-1 in Adolescent females
Running Head: Effects of training on IL-15 and IGF-1

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Abstract: Objective: The purpose of this study was to determine the effect of endurance, resistance and concurrent training on the serum levels of interleukin 15(IL-15) and insulin-like growth factor-1(IGF-1) in adolescent females. **Methods:** Twenty seven girls randomly were selected from among 150 adolescent girls. Then, they randomly were divided to four groups of resistance (n=7), endurance (n=7), concurrent (n=7) and control (n=6). The training programs of the groups lasted for 8 weeks, three days per week, which were performed from simple to difficult exercises and from low-intensity to high-intensity considering the principle of overload and increase in exercise intensity. Blood samples were taken before, in the middle and after the training program and IGF-1 and IL-15 variables were measured. Statistical analyses used were ANOVA with repeated measures (split-plot or mixed factorial test). **Results:** The results showed that eight-week resistance training made no Significant difference of IL -15 in the pre, mid and end of study protocol into the control, endurance and concurrent training groups. But was found a significant increase in concentration of IL -15 in three stages (pre-mid, and end test) in the resistance group ($P=0.029$; $f = 7.794$). Not significant difference in serum IGF-1 levels was observed into and between the four study groups. **Conclusions:** The results confirmed that fulfill of different types of physical activity, may improves body composition and VO_{2max} in adolescent girls. Resistance training, especially can improve one of the important immune system indicators (IL-15) in adolescent females.

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Introduction

During exercise, the muscles of the body with many demands facing that cause many physiological changes. In such circumstances, homeostasis must be kept constant for survival. In this relation, the nervous and endocrine systems initiate and control in a coordinated action, Stimulation and the physiological processes involved in. Immune system regulates Activity of the hormones, and pro inflammatory cytokines lead to the insensitivity of IGF-1 (David 2009).

Growth hormone and IGF-1 are the main hormones which control growth after birth. It is believed that, the immune system in forms of pro-inflammatory cytokines reciprocity does with both growth hormone and IGF-1 to stop cell growth and survival. (David 2009). IL-15 cytokine has been recently identified as the growth factor which is expressed in skeletal muscles (Grabstein et al. 1994). IL-15 can cause the accumulation of myosin heavy chain in the transfer tubular in the cultured cells of human skeletal muscles and, in skeletal muscles, the performance of IL-15 differs from that of IGF-1 and inhibits its effects (Quinn et al. 2005). Later studies showed that IL-15 not only has some effects which are independent from IGF-1 but also has completely different effects compared with IGF-1 and IL-15 in muscle fibers (Quinn et al. 2002); IGF-1 only stimulates the protein synthesis and IL-15 inhibits protein analysis in cultured muscle fibers (Tanaka H 1998). The main mechanism involved in the IL-15 anabolic effects is related to the decrease in the level of protein analyzers. While IL-15 has no effect on the total protein synthesis measured by phenylalanine incorporation into muscle protein, it causes muscular hypertrophy in high and low concentrations of IGF-1 (Binder et al. 2005).

On the other hand, the results of some studies have shown that effective training program demands a combination of intensity, duration, number of sessions and type of training in order to impose overload on different body systems and create adaptation (Cullinen; Caldwell 1998). Exercise adaptation is special associated with the applied training program.

Strength training causes increase in the construction of contractile proteins and muscle hypertrophy, especially in fast twitch fibers which may, in parallel, lead to decrease in the mitochondrial volume density (David 2009). Also, strength training causes increase in muscle strength, increase in free fat mass and decrease in body fat percentage (Nichols et al. 1993; Shaw;

Shaw 2006). Although strength training is not considered as a main method for improving the aerobic performance, doing some circular strength training can increase the aerobic power (McArdel WD 2000). Endurance training results in the increase in aerobic power and capacity, increase in capillary density (Stone et al. 1991; Taaffe et al. 1995), change in the ratio of muscle fibers (Carter et al. 2000), decrease of heartbeat, resting (Takeno et al. 2001) and decrease in body fat percentage (Maiorana et al. 2000). In many sports, in order to create optimized adaptation, the types of exercise in the athlete's training program are determined in a way to be more than just an energy production system. Several studies have investigated the effect of concurrent training on endurance and strength adaptation (Leveritt et al. 1999; Sale et al. 1990). Some research findings have demonstrated the detrimental effects of concurrent training on endurance and strength adaptation (Heyward VH 2004; Leveritt et al. 1999). In many cases, endurance adaptation is in contrast with the adaptation caused by strength training (Inder et al. 1998). Most of recent studies have indicated that combining strength and endurance training may interfere with the improvement of physical fitness factors (Nieman et al. 2004). In order to thoroughly understand the biological roles of cytokines and insulin-like growth factor resulted from doing sports, more studies are required since 1) previous results (Balabinis et al. 2003; Leveritt et al. 2003; Raastad et al. 2003) concerning the IL-15 and IGF-1 production while doing sports and their importance during and after sports are contradictory and 2) there are few studies which have measured and compared the effects of different types of training programs (such as resistance, endurance and especially concurrent) with their special adaptation in a definite time and in a controlled way for female adolescents.

Materials and methods

Subjects

One-hundred and fifty students voluntarily filled in the form and questionnaire and 34 of them were randomly selected and randomly allocated to four groups of resistance, endurance, concurrent and control. Five of them were omitted because of lack of regular participation in the training sessions and only 27 were left. The personal characteristics of subjects are given in Table 1.

Physiological assessments

Fat Percentage

Participants' subcutaneous fat was measured in the two-point method in calf and triceps using the Skinfold Caliper Baseline made in Korea in the following way: measurements were done on the right side of the body and repeated for three times with 20-second intervals between each time of measurement in order to return to the initial state. The average of the three times of measurement was recorded and then inserted in the following formula in order to calculate the body fat percentage (Heyward VH 2004):

Body fat percentage= 5.1 sum of subcutaneous fat thickness in calf and triceps+ 0.61

VO2max mesurments

Maximum oxygen uptake of subjects was measured using shuttle run test (Berthoin et al. 1994).

$VO_2max\ (ml/kg/min) = 31.025 + (3.238 \times speed) - (3.248 \times age) \times (0.1536 \times speed \times age)$

The physiological characteristics and body composition of subjects are presented in table 2.

Training Programs

All the training programs lasted for 8 weeks, 3 sessions per week. Subjects started with a warm-up followed by slow running and stretching the body's main muscles for 10 minutes. It ended with the cool-down including stretching and exercising for 10 minutes. To reduce the effects of fatigue, there was a 48-hour resting period after each session. All the protocols were approved by Graduate Council, Faculty of Physical Education and Sports Sciences, Central Tehran Branch, Islamic Azad University.

Resistance Training Program

According to the protocol the weight training program included circle training with 2-minute resting interval after each set and 5-minute resting interval after each circle. The movements included chest press, squat, sit-up, front arm, lounge and opening waist or fillet. The first week of Resistance Training included 2 sets, 10 repetitions(reps)and with intensity of 30% 1RM, the second week 3 sets, 10 reps with 30% 1RM, the third week , 3 sets, 10 reps, and 40% 1RM, fourth week, 2 sets, 10 reps, and 40% 1RM, the fifth week 3 sets, 10 reps 50% 1RM, sixth week 3 sets, 10 reps 60% 1RM, seventh week, 4 sets, 8 60% 1RM reps the eighth week in four sets, 6 reps with 70% of the 1RM.

Endurance training program

The intensity of aerobic training(continuous running) the first and second weeks was 50% maximum heart rate (MHR), week third-four 60% MHR, Week Five-Six 70% MHR in two weeks and finally increased to 80% MHR. In the beginning of endurance training, time of exercise per session was 15 minutes, , then added 5 minutes progressively each week, and finally increased to forty minutes per session in the last weeks.

Concurrent Training Program

This program was composed of both resistance and endurance training program and was exactly the same as those.

Control Group:

This group had no training.

Statistical Methods

To ensure normal distribution of data, Kolmogorov-Smirnov test was used. Since the research design included (within-group factors) three measurement stages in pre, middle and post test and four groups of control, strength, endurance and concurrent training, (between-group factors) ANOVA was run with repeated measures (split-plot or mixed factorial test). In case of observing significant difference, in order to determine the difference location and reduce the error paired-samples t-test with Bonferony amendment was also utilized. The significance level was set at $p<0.05$ using SPSS software, version 16.

Results

The inflammatory and blood indices of subjects in three groups are presented in table3.

The results of this study showed that, the Levels of IL-15 increased in all groups which were not statistically significant. In the control, endurance and concurrent groups the levels of IL-15 in three stages (pre, mid and post test) was not significantly difference. However, significant differences between the three stage in the levels of IL -15 observed in the resistance group [$f(2, 5) = 7.794$; $p=0.029$]. Bonferroni test also showed that the difference in 3 stage and in the resistance group was because of pre and post test levels ($p=0.019$). Additionally, the changes of IL -15 levels was not significantly different between the 4 groups ($P=0.682$).

IGF-1 levels increased in controls and endurance groups and decreased in resistance and concurrent groups, which was not statistically significant. There was no significant difference in IGF-1 levels within a three-phase (pre, mid and post test) measurements (**f = 0.551 ; P=0.584)** . Also, no significant difference in IGF-1 levels were observed between the four study groups **(P=0.872).**

Discussion

The results of the present study showed that the levels of IL-15 had an ascending trend in all the groups and increased after the 8-week training. Also, the significant difference between the measurements was only between the samples of the endurance training group and in other groups, the levels of IL-15 showed no significant difference in the three measurements. Therefore, it can be concluded that the 8-week endurance training caused a significant increase in the levels of IL-15 among adolescent girls (P=0.029). In the study conducted by Riechman et al. (2004), IL-15 plasma levels significantly increased just after acute resistance exercises after 10-week resistance training; however, after the long-term exercises, no change was shown in the muscle responses to the training. Also, he reported a noticeable increase in the IL-15 plasma level after endurance exercises (Riechman et al. 2004). These studies measured IL-15 plasma levels, immediately after the training and performed resistance exercises using multi-purpose machines, which is different from what happened in the present study. In the research done by Nielsen et al. (2007), after doing resistance exercises, IL-15 gene expression (MRNA) in 14 young men doubled after recovery without any changes in the IL-15 protein content and IL-15 plasma content (Nielsen et al. 2007).Nieman (2004) showed no changes in the IL-15 MRNA level after resistance or endurance exercises (Nieman et al. 2004). Acute changes IL-15 with sports may be related to the changes in the situation of blood requirements and formation of new vessels, which happens as a result of exercises. Thus, investigating the physiologic role of IL-15 in the reaction of skeletal muscle to acute sports requires direct and intramuscular evaluation of IL-15 levels. These results suggest that, IL-15 is an important mediator in the response of muscle mass to the resistance training in human and genetic variety in IL-15 holds an important part of variety in these responses (Riechman et al. 2004). Since IL-15 has an anabolic effect on the muscle cells and decreases muscular atrophy, it is likely that IL-15 acts on the adjacent muscle cells by the paracrine method and has a noticeable effect on the protein concentration and adjacent muscle cells' growth. At the end, IL-15 gene expression is reinforced in the type II skeletal muscle fibers and resistance exercises lead to the increase in IL-15 gene expression 24 hours after the training (Nielsen et al. 2007).

The results of other studies have shown that variable IGF-1 has increased in the endurance group and has decreased in the resistance and concurrent groups; and these changes were not significant. In the present study, the 8-week training with an imposed training program did not have a significant effect on IGF-1 in adolescent girls (P=0.406). Eliakim et al. (2005) investigated 5-week endurance training (aerobic dance, running and team sports) on the GH-IGF-1 axis of 38 boys aged 15 to 17 years old and reported the significant decrease of circulating IGF-1 despite local anabolic changes(Eliakim et al. 1998). In that study, the type of training program were used was somehow similar to the present study; however, gender of participants and results were different. A potential mechanism for these results may be a fundamental decrease in the circulating growth hormone binding protein (GHBP) in untrained people. GHBP is identified as an extracellular protein of GH cellular

Table 1: subjects personal characteristics
Data presented mean ± SE

	Endurance (N=7)	**Resistance (N=7)**	**Concurrent(N=7)**	**Control(N=6)**
Age (year)	16.87±0.39	16.87±0.39	17.21±0.39	17.20±0.27
Height (cm)	160.71±2.75	159.71±9.46	159.0±3.60	160.66±5.20
Weight (kg)	52.57±7.82	54.42±8.20	47.85±3.89	60.18±12.20

Table 2: The physiological characteristics and body composition of the subjects
Data presented mean ± SE

Groups		Control(**N=6**)	Concurrent(**N=7**)	Resistance(**N=7**)	Endurance(**N=7**)
Fat percentage %BF	Before	35.396±3.971	32.506±3.971	37.935±7.438	35.712±7.804
	Middle	36.723±2.632	31.417±2.997	35.251±7.609	31.940±5.103
	After	35.705±3.549	33.029±2.502	33.595±7.624	32.637±7.646
Weight (kg)	Before	60.167±12.205	48.857±3.891	54.500±8.088	52.571±7.828
	Middle	61.333±10.893	48.714±3.874	57.571±9.162	55.428±8.017
	after	61.16±12.512	49.786±3.871	55. 00±9.092	54.00±7.594
BMI (kg/m2)	Before	22.773±3.624	18.874±1.229	20.637±2.707	19.641±3.933
	Middle	23.235±3.042	19.625±3.042	21.849±3.174	21.449±2.792
	after	23.265±3.483	19.217±1.242	20.97±2.912	20.900±2.642
Waist-hip ratio WHR	Before	0.747±0.008	0.775±0.101	0.769±0.415	0.839±0.097
	Middle	0.774±0.026	0.768±0.067	0.786±0.047	0.774±0.082
	after	0.772±0.025	0.760±0.041	0.770±0.046	0.799±0.074
VO2max (ml/kg/min)	Before	-	33.301±1.598	33.870±3.255	35.985±2.180
	Middle	-	37.223±2.439	35.587±1.939	35.587±1.939
	after	-	35.967±2.356	37.376±2.639	37.945±3.470

Table 3: The inflammatory and blood indices of subjects in three groups
Data presented mean ± SE

Groups		Control(**N=6**)	Concurrent(**N=7**)	Resistance(**N=7**)	Endurance(**N=7**)
IGF-1(ng/ml)	Before	339.16±84.96	344.71±24.41	311.43±75.55	323.14±50.62
	Middle	328.16±65.67	351.71±45.73	347.0±75.59	320.14±40.16
	After	356.0±117.73	335.71±43.20	306.86±49.32	355.71±48.99
IL-15(pg/ml)	Before	3.033±2.334	2.400±2.229	2.428±2.229	2.557±2.356
	Middle	2.117±0.749	3.586±2.379	3.742±1.792	4.371±1.864
	after	4.15±2.569	8.286±8.809	5.528±1.437	7.029±5.591
Hemoglobin(g/dl)	Before	14.133±1.532	14.528±0.801	15.185±0.318	13.871±0.475
	Middle	13.883±0.893	14.442±0.553	14.800±0.369	13.542±0.789
	after	13.100±0.678	14.271±0.553	14.742±0.486	13.442±0.741
Hematocrit(Precent)	Before	43.017±1.532	43.557±2.499	44.871±1.460	41.543±1.690
	Middle	41.933±2.674	43.386±1.642	43.657±1.148	40.600±2.247
	after	41.883±2.163	43.428±1.338	44.342±1.316	41.200±2.318

receptors and may decrease the lower level circulating tissue receptors and tissue response to GH in most of the tissues. As a result, the lower level of circulating IGF-1 in the training group may be a consequence of GH decrease produced in the liver. In fact, training decreases GHBP and increases IGFBP-2. In general, training increases muscle volume in adolescents with the fixed weight and, in the situation of energy shortage, noticeably affects IGFBP-2, GHBP and IEF-1 (Eliakim et al. 1998). In a study done by Nemet et al. (2009), the effect of training intensity on the inflammatory cytokines and growth mediators of adolescent boys was investigated. Like the present study, that study was conducted on adolescents, investigated simultaneous anabolic and catabolic responses to training and reported a significant decrease in anabolic mediators, bound IGF-1 and insulin although free IGF-1 did not change. Probably, the reason of the difference between these results and that of the present research is due to gender, type and intensity of applied training in this research. These researchers proposed that the mechanism which maintains the free IGF-1 concentration during the training leads to the IGFBP-3 proteolysis which causes an increase in the circulating IGF-1. These results demonstrate that an acute exercise in adolescents leads to the decrease in anabolic mediators and deep increase of inflammatory cytokines (Nemet et al. 2002). Raastad et al (2002) investigated the effect of 2-week intense resistance training on the hormonal responses and muscle contraction in 17 untrained students' men. The IGF-1 concentration decreased 22 hours after acute exercises in the study group, which may be a natural response to the type of resistance training. Increasing the volume of strength exercises leads to two environmental and systemic changes and the systemic changes decrease the testosterone and IGF-1 concentration. As a result, 2-week endurance training with high volume creates minor changes in the hormone's response to the sports (Raastad et al. 2003). In contrast to the findings by Raastad et al., the present study had a significant decrease in the IGF-1 of the resistance group which could be due to the differences in the training intensity. Rodigo et al. (2008) investigated the Effects of muscle strength and aerobic training (8 weeks, 2 sets with 8 to 10 repetitions and 75% to 80% IRM) on basal serum levels of IGF-1 and cortisol. The resistance group showed an increase in IGF-1, and there was no difference in the cortisol levels (Rodrigo Gomes de Souza Vale 2009). Which is in contrast to the findings of the present study, probably because of the subjects of that study were elderly women. Also in that study the intensity of exercise applied in the aerobic group was very low for the IGF-1 changes although it was sufficient for increasing the strength of the leg muscle. High intensity exercises lead to the change in the level of IGF-1. Finally Rodigo et al. suggested that strength exercises lead to the stimulation of anabolic effects in the elderly women and increase the mobility of fatty acids (Rodrigo Gomes de Souza Vale 2009). Jennifer et al. (2002) investigated the hormonal responses to endurance training (39-minute bicycle riding in 75% of maximum heart beat) and resistance training (3 sets with 8 to 10 times repetition) in 30 women aged 19 to 69 years old. The effect of training on the IGF-1 was not significant and did not change in response to each training protocol. Testosterone levels in resistance and endurance groups were significant, and cortisol decreased in all groups (Jennifer L. Copeland 2002). Although the results were similar to the results of the present study, but age range and training type were different. Levels of anabolic hormones like growth hormone decreases with aging and this decrease is attributed to many changes of body combination and functional decline. Finally, this study could not show whether chronological age has an important effect on the hormonal changes caused by training, which needs more investigations. In fact, increase in the level of anabolic hormones may be useful for maintaining lean body mass and, as a result, maintaining performance capacity (Jennifer L. Copeland 2002).

Conclusion

The results of this study confirmed that fulfill of different types of physical activity, may improves body composition and VO2max in adolescent girls. The results also showed, each three types of training program in Adolescent females is not a detrimental factor for the performance of theIL-15 and it can even efficiently and effectively improve it.

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