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The Effect of Low- Intensity Resistance Training with Blood Flow Restriction on Serum Cortisol and Testosterone Levels in Young Men

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Background: Based on the research evidence, the efficiency of resistance exercises depends on the changes in the hormones level to improve the muscle strength and mass.

Objectives: This study aimed to investigate the effect of a short course blood flow restricted resistance training on serum cortisol and testosterone levels in young men.

Patients and Methods: A total of 30 healthy young men (aged 19 - 24 years) were volunteered for this study. Subjects were randomly assigned into three groups: low-intensity blood flow restricted (LIBFR) (n = 15) resistance exercise group (3 sets of 15 repetitions at 20% of 1RM (one repetition maximum)), traditional high-intensity without blood flow restriction (HIWBFR) (n = 12) resistance exercise group (3 sets of 10 repetitions at 80% of 1RM), and a control group (n = 13). Both LIBFR and HIWBFR groups trained for front leg and squat exercises 3 days per week for 3 weeks. Fasting growth hormone cortisol and testosterone levels were measured in the morning before and after exercise sessions. Data were analyzed with paired t-test and One-way ANOVA at the significant level of P < 0.05.

Results: Serum cortisol level significantly increased after the exercise for both protocols compared to baseline (P < 0.05). After 3 weeks, serum cortisol level increased significantly in LIBFR and HIWBFR groups compared to control group (P < 0.001). At the end of the exercise protocols, serum testosterone level was higher in LIBFR group compared to HIWBFR group; however, this difference was not significant (P > 0.49).

Conclusions: Short term blood flow restriction (BFR) exercise stimulates cortisol hormones production in young men. However, this program seems too short to express any difference in testosterone change. Generally, it can be concluded that low-intensity resistance training can increase short-term BFR catabolic-anabolic hormones in young men.

Keywords: Resistance Training; Cortisol; Blood Flow Restriction; Testosteron

1. Background

Based on the research evidence, the efficiency of resistance exercises depends on the changes in the hormones to improve the muscle strength and mass (1, 2). According to the previous studies, intensity of 70% - 80% of 1RM (One repetition maximum) of resistance exercises was reported to be appropriate for increasing muscle strength and size as well as changing in the hormones effective on them (3). However, different results have been reported after various exercise protocols in case of short-term and long-term changes of hormones related to increase the muscle mass and strength. It seems that resistance exercises with high-intensity is accompanied with significant changes in anabolic-catabolic hormones. The balance between cortisol catabolic and testosterone anabolic hormones has important application in the performance and recovery periods after resistance exercises (4, 5).

Cortisol is a catabolic hormone and the most important anti-stress hormone in the body. However, its increase in the long-term causes some problems, the most important one relates to damaging the immune system and proteins (6). The results of the studies show that changes of cortisol depend on intensity and duration of exercise (4). Moreover, the growth rate of muscular cells depends on the activity of sex steroid hormones, especially testosterone (7). The results of the studies about acute hormonal response to resistance exercise show that testosterone level increases during and after resistance exercises (8). In this regard, Kraemer stated that if the rest interval between the periods of resistance exercise (performed with 80% of 1RM in 6 periods and 10 repetitions in each period) is less than 1 minute, it can lead to a significant increase in testosterone secretion (9).

Testosterone is an anabolic hormone, which stimulates protein construction and plays an important role in growth and maintenance of muscular tissue (10). Despite numerous recommendations for performing highintensity resistance exercises (more than 80% of 1RM), for preventing the reduction of muscular mass, these exer-

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cises have some practical limitations for some people, especially the elderly and people with cardiovascular diseases. Recently, the results of studies in the field of physical fitness and rehabilitation have presented a new form of resistance exercises, which have lower administrative limits than the high-intensity resistance ones and at the same time meet the goals expected from high-intensity exercises.

In these exercises, the proximal femoral muscle is closed by a cuff, leading to reduced blood flow and muscle hypoxia. The blood flow restricted (BFR) condition can cause muscle hypertrophy and increase in muscular strength surprisingly in a short-time. This fitness level is equal to changes caused by the high-intensity resistance exercises (11, 12). Based on the available scientific evidence, the blood flow reduction during exercise is one of the essential conditions for creating fitness after the resistance exercises (13). The unique feature of BFR resistance exercises is the ability to perform them with low intensity (usually 20% to 30% of 1RM) (14).

There is credible evidence indicating that the degree of hypertrophy and increased muscular strength after resistance exercise intensity (with BFR) of approximately 20% 1RM equals to intense resistance training (intensity of approximately 80% of 1RM) but without the restriction of blood flow (15, 16). In this regard, Takarada et al. showed that low-intensity resistance exercises and restricted blood flow as well as walking with restricted blood flow would dramatically lead to the muscle hypertrophy and increase in the muscular strength compared to high-intensity exercises without vessel restrictions (11). Abe et al. (15) and Fujita et al. (17) also reported that the muscle protein synthesis in BFR resistance exercises with 20% of 1RM will cause hypertrophy. The results showed hypertrophy induced by low-intensity resistance training of 20% to 30% of 1RM with restricted blood flow would be the same as the exercise but without restricted blood flow (18, 19). Research also reported that the mechanism of increasing strength in resistance exercises along with vessel restriction is different from the mechanism of increasing muscular strength due to exercises without vessel restriction. They attributed the strength increase after BFR resistance exercises to the muscular hypertrophy and considered negligible the share of neural adaptation in increasing the strength after such exercises (20). Since the highest consistency in the structure of skeletal muscle happens after the resistance exercises, in rest time after exercise and during recovery (21), it seems that change in muscle structure is more excited after BFR resistance exercises in which the blood flow is resorted after a limited period during exercise. According to studies of the researcher, there are limited studies about long-term adaptations of cortisol and testosterone following BFR resistance exercises. It appears that the level of these hormones change due to the growth of muscular cells, which happens at least after 8 days (15) of low-intensity BFR resistance exercises. Hence, the present study by evaluating the effect of resistance exercise with blood flow restriction on cortisol and testosterone hormone levels, seeks to answer this question that whether the possible increase in muscle strength and size after three weeks of BFR resistance exercises depends on hormonal compatibility.

2. Objectives

The aim of the study was to investigate the effect of a short course blood flow restricted resistance training on serum cortisol and testosterone levels in the paramedical students of AJA University of Medical Sciences.

3. Patients and Methods

The method of the present study is of quasi-experimental and the research design is of pretest-posttest with control group. The research population is consisted of male paramedical students (third year) of AJA University of Medical Sciences. Inclusion criteria were as follows: having no history of smoking, high blood pressure, and overweight; avoiding drugs or performance-enhancing supplements, and other risk factors of cardiovascular diseases according to doctor's diagnosis and medical records in a diagnostic test. Also, the subjects should not have regularly participated in any resistance exercises at least 6 months before the study (22). One month after the call, 40 students who volunteered for cooperating in the research were selected by purposive sampling method, interviews, and checking the inclusion and exclusion criteria.

In the first session, the subjects were introduced with the type of plan, objectives, and method of its implementation in the form of written and oral presentation. They were assured that their information will remain strictly confidential and encoding method will be used to evaluate the data. They were also allowed to withdraw from study if they did not want to continue their cooperation. After approving the study at university research council and ethics committee, the subjects signed a written consent form and filled the questionnaires comprising their personal information as well as medical and sport history. In this study, the principles of the Declaration of Helsinki and medical ethics were completely considered. One week before starting the exercise program, anthropometric parameters, including age, height, weight, Body Mass Index (BMI), and the thigh muscle circumference. Physiological parameters such as systolic and diastolic blood pressure and One Repetition Maximum (1RM) were measured too.

Then the subjects were randomly assigned into three resistance groups: Low Intensity (20% of 1RM) with blood flow restricted (LIBFR), (n = 15); high intensity (80% of 1RM) without blood flow restricted (HIWBFR), (n = 12); and control group without doing exercise and BFR (CON), (n = 13). The subjects were advised to avoid doing other programmed sport activities or changing their diet at the time of study. After three weeks of exercises, all measurements but the height, were repeated. To calculate

1RM, first, the heaviest weight which an individual could move was selected. Then, the subject began to perform the desired movements with the selected weight. If he could perform the movement only for one time, that weight was considered as 1RM. But, if the movement with selected weight was done more than once, 1RM would be determined by putting the number of repetition and amount of weight in the following formula (Equation 1) (23). During the implementation of the protocol, exercise intensity was kept constant. Also 1RM was used to notify the change of power, after 3 weeks of exercise.

(1) $1RM = Weight \times [1 + (0.033 \times number of repetition)]$

We used powerlifting rubber band to restrict the blood flow. Doppler ultrasound device was used to calibrate and determine the amount of applied force and blood flow restriction of some subjects with different thigh size and phenotypes; so that the rubber band was closed around the thigh with different tensions and the femoral artery blood flow was measured with the device. The amount of band tension was enough to block the venous blood flow and restrict the hip artery.

Doppler ultrasound was used to measure blood flow and blood pressure. High-frequency sound waves were used to hit the red blood cells and after returning show it as Doppler ultrasound. It can measure the speed of blood flow (24). BMI was calculated via dividing body weight (kg) over square of height (m²). Thigh circumference was measured from the center of thigh by a tape meter. Blood pressure was measured by digital sphygmomanometer (Accumed® model CG175f, by USA).

Blood samples were taken before and 3 weeks after the exercises, at 8 am (12 hours fasting). In the first round of blood sampling, the subjects were asked to avoid doing any type of exercise 24 hours before the blood test. The second round of blood sampling (3 weeks after doing exercises) was done 24 hours after the last session of exercise. In each round of blood sampling, a laboratory specialist took 2 mL of blood from the brachial vein of each subject in sitting position. Then, the blood sample was centrifuged for 15 minutes at 3000 rpm. Isolated serums were stored at -70 °C until the time of testing. Serum level of cortisol and testosterone was measured by hormonal kit for human (Monobind®, USA) using EIISA (The Enzyme-Linked Immunosorbent *Assay*) method.

Program of BFR resistance exercise was performed under the bodybuilding coach campus for 3 weeks, 3 sessions per week and in each session two front leg and leg squat. Each exercise session was consisted of 15 minutes warming (stretching and jogging), performing front leg and leg squat movements with 3 sets of 15 repetitions at 20% of 1RM and 15 minutes cooling. In each session, the exercises were performed in 3 sets with 30 seconds rest between the sets and also 30 seconds rest between exercises (Table 1). The exercise intensity was constant during the exercise period. Powerlifting rubber band with 80% elastic property was used for restricting the blood flow. Doppler test was used to ensure the blood flow restriction. In the first week of exercise program, the subjects were trained about correct techniques of closing powerlifting band to the end of hip proximal and correct performing the movements with front leg and leg squat devices (Table 2).

Program of low-intensity BFR resistance exercise in the present study was taken from the studies of Abe et al. (2005) and Yasuda et al. (2005) (15, 25) with slight modifications. Program of resistance exercises without BFR was consisted of front leg and leg squat movements with 80% of 1RM in three sets with 10 repetitions. The break between each set and also between the exercises was 30 seconds. Each exercise session was consisted of 15 minutes warming and 15 minutes cooling with stretching movements. To ensure the well-being of the subjects, blood pressure was measured by a physician before and after each exercise session and 24 hours after the end of exercise.

Paired t-test was used to compare the differences before and after the exercise; ANOVA and Tukey tests were used to compare the means differences at the end of exercise among the three groups. In all tests, the significant level was considered P < 0.05 and SPSS software version 18 was used for data analyzing. The sample size was set to guarantee at least 80% power.

Table 1. Low-Intensity Resistance Training Program							
Week							
1		2		3			
Set	Repeat	Set	Repeat	Set	Repeat		
3	15	3	15	3	15		
3	15	3	15	3	15		
	Set	1 Set Repeat 3 15	I N Set Repeat Set 3 15 3	Week12SetRepeatSetRepeat315315	Week12SetRepeatSetRepeatSet3153153		

Table 2. High-Intensity Resistance Training Program (HighIntensity Resistance Exercise Without Blood Flow Restriction(HIWBFR))

Training	Week					
	1		2		3	
	Set	Repeat	Set	Repeat	Set	Repeat
Front leg	3	10	3	10	3	10
leg squat	3	10	3	10	3	10

4. Results

The mean and standard deviation of anthropometric parameters and 1RM before and after exercise are presented in Table 3. The analyzed data is related to 30 subjects who have exercised with the researcher regularly until the end of the study. Five subjects from LIBFR group and 5 subjects from resistance group HIWBFR withdrew from the study because of severe muscular pain or injury. Before the exercise, there was no significant difference among the three groups with regard to body weight, BMI, hip circumference, and 1RM (P > 0.05). Data analysis showed that BFR resistance exercise led to significant increase in 1RM of squat (P = 0.001), 1RM of front leg (P = 0.001), size of the hip circumference (P= 0.007), and significant reduction of diastolic blood pressure (P = 0.04) compared to the corresponding values before the exercise. However, the BFR resistance exercise did not cause significant changes in body weight (P = 0.19) and systolic blood pressure (P = 0.61) in the participants. Resistance exercise without BFR caused significant increase in body weight (P = 0.001), 1RM of squat (P = 0.006), 1RM of front leg (P = 0.001) and the size of thigh circumference (P = 0.04) compared with the values before the exercise. The results also showed that there is a significant difference among the three groups with regard to 1RM of squat (P = 0.03) and 1RM of front leg (P = 0.04) at the end of the study. However, there was no significant difference among three groups with respect to body weight (P = 0.68) and the size of thigh circumference (P = 0.74) at the end of the study. The results of Tukey test indicated that there was a significant difference between the value of 1RM of squat (P = 0.03) and 1RM of front leg (P = 0.04) of BFR exercise group and control group; but the difference was not significant among the two exercise groups.

The mean and standard deviation of cortisol and testosterone serum level in experimental and control groups before and after exercise are shown in Table 4. According to the information in Table 4, there was no significant difference between serum level of cortisol and testosterone of the subjects at the beginning of the study and before performing the exercise program (P > 0.05). Data analysis showed that cortisol serum level in each of the two resistance groups with and without BFR increased significantly (P < 0.05) compared to the corresponding values before the exercise. Furthermore, after three weeks of exercise, there was a significant difference between cortisol serum level of LIBFR group (P = 0.02) and HIWBFR (P= 0.008) compared to control group. However, the difference between the two exercise groups was not significant at the end of exercise (P > 0.05).

The results also showed that there was no significant difference between testosterone serum level of resistance exercise groups with and without BFR before and after the exercise. In addition, the difference between serum levels of testosterone in different groups was not significant at the end of the exercise.

Table 3. Comparison of Anthropometric and Physiological Parameters in Different Groups of Before and After the Exercise ^{a,b}								
Variable	ariable Group							
	LIBFR(n=11)		HIWBFR $(n = 7)$		Control(n=12)			
	Before	After	Before	After	Before	After		
Weight, kg	6.54 ± 75.63	6.59 ± 75.90	5.45 ± 71.14 ^C	5.62 ± 72.42	12.08±73.46	12.36±73.53		
Muscles around the thigh, cm	2.97 ± 54.55 ^c	2.73 ± 57.09	$3.68 \pm 54.71^{\mathrm{d}}$	3.93 ± 55.21	3.67 ± 53.77	3.68 ± 53.54		
Squat 1RM, kg	5.82 ± 72.52 ^c	8.97 ± 84.33 ^e	10.82 ± 65.12 ^c	10.90 ± 71.52	10.49 ± 68.92	10.46 ± 68.85		
Knee Extension 1RM, kg	56/2±29.00 ^c	2.46±35.90 ^e	1.25 ± 28.29 ^C	1.57 ± 31.85	4.03 ± 29.53	4.12 ± 29.57		

^a Abbreviations: HIWBFR, high intensity resistance exercise without blood flow restriction; LIBFR, low intensity blood flow restricted resistance exercise.

Data are presented as Mean \pm SD.

^C Significant difference between the groups before and after training P \leq 0.01.

d Significant differences among the groups before and after training P \leq 0.05.

^e Significant difference between LIBFR and control after exercise P ≤ 0.05 .

Table 4. Comparison of Serum Cortisol and	Festosterone Levels Among Different Groups Before and After the Exercise ^{a,D}

Variable	Group					
	LIBFR(n=11)		HIWBFR $(n=7)$		Control (n = 12)	
	Before	After	Before	After	Before	After
Cortisol, µ g/dL	$2.02\pm5.13^{\text{ C}}$	1.67 ± 8.06 d	$1.58\pm4.50~^{\rm C}$	$1.34 \pm 8.88 \ ^{e}$	1.60 ± 4.69	2.90 ± 5.41
Testosterone, µg/mL	3.75 ± 8.64	2.66 ± 8.76	2.28 ± 8.43	2.29 ± 8.55	3.41 ± 8.53	4.34 ± 8.55

^a Abbreviations: HIWBFR, high intensity resistance exercise without blood flow restriction; LIBFR, low intensity blood flow restricted resistance exercise.

Data are presented as Mean \pm SD.

^C Significant difference among the groups before and after training $P \leq 0.05$.

^d Significant difference between LIBFR and control after exercise P \leq 0.05.

^e Significant difference between the HIWBFR and control after training BFR P \leq 0.01.

5. Discussion

One of the findings of the present study was the average increase in the muscular strength of the LIBFR group (20% 1RM) and almost the HIWBFR group (80% 1RM). The results showed that the quadriceps muscle 1RM (front leg and leg squat) LIBFR in young men after 3 weeks (with an intensity of 20% of 1RM) significantly increased compared to the control group and HIWBFR. However, the difference between the muscular strength of HIWBFR and control groups was not statistically significant. This finding was consistent with the study results of Moore et al. Takarada et al., Yasuda et al. and Abe et al. (11, 12, 25-27). The exercise duration in these studies varied from 2 to 16 weeks. Takarada et al. showed that 1RM of knee extensors increased about 50% after 8 weeks of LIBFR (11). In this regard, they showed that 2 weeks LIBFR (2 days per week) increased the muscle strength about 14% (25). The exact mechanism involved in the adaptation of muscle strength after the LIBFR is not completely known. Although the focus of studies is more on environmental compatibilities such as hypertrophy, probably the neural compatibilities can also play an important role in increasing muscle strength. According to the literature, increasing the use of fast-twitch muscle fibers is the strongest possible known mechanism in LIBFR. Low and non-significant increase in thigh circumference size in LIBFR group indicates the increase in muscular hypertrophy compared to the HIWBFR group. One limitation of this study is the lack of accurate measurement of muscular hypertrophy. Based on the results of previous studies, at least 4 weeks are required for some forms of hypertrophy to happen after heavy resistance exercises (approximately with the intensity of 80% 1RM). Meanwhile, the result of a case study showed that 1 week of LIBFR with intensity of 20% of 1RM has led to 3% increase in muscle cross-sectional area (9). Growing evidence indicates that muscular hypertrophy happens after LIBFR (10, 11, 21, 24, 28). However, the real reasons of increasing the muscle size after these exercises are not completely known yet. Increasing the muscle protein synthesis is essential for creating muscular hypertrophy. Severe resistance exercise with high-intensity leads to increase in the muscle protein synthesis in the first hours after exercise and it remains at the same level 48 hours after that (29, 30).

Results of this study showed that serum level of cortisol in both groups of LIBFR and HIWBFR increased significantly compared to the corresponding values before the exercise. Moreover, after three weeks of exercises, there was a significant difference between cortisol serum level in exercise groups (LIBFR and HIWBFR groups) and this level in the control group. However, the difference between the two exercise groups was not significant at the end of exercises. In other words, in the present study, pattern of cortisol changes was almost similar after LIBFR and HIWBFR. Based on the results of previous studies, cortisol serum level remarkably increases after resistance exercises (31, 32).

There are many mechanisms in body that cause changes in the concentration of cortisol hormone; intensity of sport activities and mental stresses are some of the most powerful and influential stimulants on the secretion rate of this hormone (33). Also, physiological factors and circadian changes and changes related to eating and temperature affect the secretion of cortisol hormone (34). It seems that the reason of increasing cortisol concentration in stressing conditions and physical pressures is change in the activity of hypothalamus-pituitary-adrenal axis (33, 35). However, the main reason of increasing cortisol hormone after LIBFR is not completely known. In the present study, changes in cortisol level after LIBFR are consistent with the study results of Fujita et al. (17), Madaram et al. (36), Fry et al. (37), and Kim et al. (38). They showed that LIBFR increases cortisol hormone level, which is equal to the increase due to HIWBFR. Researchers believe that closing cuff and restricting blood pressure in BFR exercises will increase secretion of cortisol hormone by creating hypoxia and acidosis and increasing the lactate levels of blood. Hence, increasing anaerobic glycolysis is a reason for increasing cortisol level in LIBFR due to restricting the blood pressure and in HIWBFR due to high intensity exercises.

Cortisol as a catabolic hormone increases the lipolysis in adipose tissue and breakdown of proteins and reduces the protein synthesis in muscular cells; it also increases the release of lipids and amino acids into the blood flow (39). This hormone has catabolic effect on myofibril proteins and inhibits protein synthesis (7). In addition, catabolic effects of cortisol on muscular fibers of the second type are more and longer than the first type (40). Thus, stimulating the peripheral nerves of fast-twitch muscle fibers during the low-intensity BFR resistance exercise (41) can be one of the reasons for increasing cortisol after these exercises (26). Also, cortisol, in the stage between entering the glucose and its final analysis, directly delays the consumption of glucose and thereby reduces the amount of glucose consumption by the cells throughout the body (33, 42, 43).

Findings of this study are inconsistent with the study results of Abe et al. (2005) and Reeves et al. (2006) which have reported no change in cortisol hormone after LIBFR (27, 44). Since the exercise intensity in those studies is almost similar, the rate of tourniquet pressure is probably the main reason of different results among studies. The other possible reason is the measuring time of cortisol serum level at the end of the exercise. Exercise program of most studies about the effect of BFR exercises on serum level of cortisol is a single session exercise in which, the serum level of blood variables is measured immediately after finishing the exercise; while in present study and other short-term and long-term exercises which aim to evaluate long-term compatibility of serum hormones level to the exercise, blood samples will be measured 24 or 48 hours after the last session of exercise.

According to the results of this study, there was no significant difference between serum level of testosterone in HIWBFR before and after the exercise. Moreover, the difference between serum levels of testosterone in different groups was not significant at the end of exercise. This finding is consistent with the study results of Reeves et al. (44) and Fujita et al. (17), who found no significant difference after LIBFR single session resistance exercises and also study results of Abe et al. (2006) and Yasuda et al, (2010) which did not find significant difference in serum level of testosterone after LIBFR long-term exercises (27, 39). The mechanism of the effect of LIBFR single session and long-term exercises on testosterone level has not been known completely yet (45). Since the exercise programs offered in this study were anaerobic, it was expected that these exercise programs could increase testosterone level, as lactate produced in anaerobic exercises stimulates directly testosterone secretion in Leydig cells (45). However, it seems that the volume of provided exercises and even restricting the muscle blood flow was not enough to significantly increase testosterone level. To support this claim, we can point out the volume of exercise provided in the research of Kraemer. In this regard, Kraemer stated that if the rest interval between periods of resistance exercise (performed with 80% of 1RM in 6 periods and 10 repetitions in each period) is less than 1 minute, it can cause a significant increase in the secretion of growth and testosterone hormones (9). Testosterone is an anabolic hormone, which stimulates protein synthesis and plays an important role in the growth and maintenance of muscle (10).

Generally and according to the results of present study, although the pattern of changing serum level of cortisol and testosterone hormones in both exercise groups is similar, in short-term protocols, LIBFR can increase catabolic-anabolic hormones greater than that of HIWBFR in young males. Hence, restricting blood flow during exercise is an important factor in making hormonal responses. However, because of the limited number of studies of this kind, further studies are required for more precise understanding of the mechanisms involved in the hormonal responses to BFR exercises.

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Authors' Contributions

Alireza Khoshdel developed the original idea and the protocol, abstracted and analyzed the data, wrote the manuscript, and they are the guarantors too. Shahram Mohamadi, Faezeh Naserkhani and Rahimeh Mehdizadeh contributed to the development of the protocol, abstracted data, and prepared the manuscript.

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