

The Effect of a Single About of Resistance Exercise with Two Intensities and Different Recovery on Pro-Inflammatory Factor TNF-A, HSP-70, And Anti-Inflammatory Factor BDNF In Female Bodybuilders

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ABSTRACT

Background and purpose: resistance exercises with high loads of HLRT can cause physiological changes, including in the immune system. The purpose and credibility of qualitative research investigated the effect of a resistance activity session with two different intensities and recovery on the expression of TNF- α , HSP-70, and BDNF genes in female bodybuilders.

Methods: In this Quasi-experimental study, twelve healthy female bodybuilders (age 30 \pm 5) volunteered to participate in the research. The samples performed one weekly strength training protocol session designed at two intensities of one maximum repetition in two different recovery times of one and two minutes. 36 hours after the end of the training, sampling was done after ten hours of fasting, and the samples were centrifuged and stored at minus 20°C. At the end of the four stages, the levels of TNF- α , HSP-70, and BDNF were checked in the medical laboratory. A consent form was obtained from all the participants. SPSS version 27 software was used for data analysis at a significance level of $\alpha \leq 0.05$.

Findings: The results showed that HLRT resistance training compared to moderate intensity resistance training, by increasing the training intensity to 85% of a maximum repetition and recovery time of one and two minutes, significantly reduced TNF- α level (with $p < 0.001$ and a significant increase in the serum levels of BDNF and HSP-70 (with $p < 0.0001$).

Conclusion: It seems that increasing the intensity of HLRT resistance training has no negative effect on the immune system, and probably with the decrease of the pro-inflammatory factor TNF- α and HSP-70, the anti-inflammatory factor BDNF also increased reduces the incidence of inflammation in female bodybuilders.

1. Introduction

Today, resistance training is one of the most attractive and effective ways to improve fitness. Many beginners and professional athletes of different disciplines focus on increasing resistance exercises as effective basic exercises to increase muscle capacity and create more strength. However, despite the positive effects of such exercises on the musculoskeletal system, there are concerns about the possibility of damage to some body systems, including the immune system, and the creation of an inflammatory process, which can be a factor in causing inflammatory diseases as well as an obstacle to the implementation of optimal sports performance [[1]].

Performing movements and exercises with weights or body weight makes resistance exercises. On the other hand, endurance athletes usually follow the resistance training approach based on endurance. This includes doing high reps and sets in resistance training. However, in recent years this view has changed. Heavy load resistance training (HLRT) has become more common in training. HLRT involves the use of low-repetition exercises. Loads are usually at an intensity greater than 80% 1RM [[2]]

Resistance training (RT) reduces the risk of low-grade inflammation-related diseases [[3]]. However, the intensity of these exercises, including high-load resistance exercises HLRT, can affect this result [[4]]. Research shows that high-intensity resistance training benefits some inflammatory markers [[5]]. It also did not increase pro-inflammatory factors [[6]]

A similar study in obese individuals at high risk of systemic inflammatory diseases showed that intense resistance exercise can effectively reduce inflammatory factors by the exercise protocol [3].

In meta-analysis research, it has been said that in elderly people, exercise intensity, time under tension, and rest between sets play an important role in the physiological changes of the body. be effective against the progression of the disease [[7]]. In another study, it was shown that resistance training has anti-inflammatory effects depending on the amount of external load used in training [[8]].

This is while in a study, changes in enzyme antioxidant defense and inflammatory markers were expressed after resistance training independent of training intensity [[9]]. In a study on obese men, it was found that resistance training reduces insulin resistance by reducing the pro-inflammatory factor (tumor necrosis factor- α) [[10]]. It can generally lead to an increase in inflammatory mediators and as a result the risk of injury and chronic inflammation increases. Conversely, moderate or vigorous exercise with appropriate rest periods can improve inflammation [[10, 11]]. TNF- α is mainly secreted by macrophages and lymphocytes in response to cell damage caused by infection or inflammation [[12]]. TNF- α is a pro-inflammatory cytokine characterized by a wide range of functions [[13]]. It is one of the important dependent variables with which the possibility of inflammation is checked. Research results showed that different intensities of resistance training do not change the inflammatory factors TNF- α , (hs-CRP, a C-reactive protein with high sensitivity), while the volume of training can affect these factors [16]. On the other hand, a study states that resistance training can be effective in reducing inflammatory factors regardless of their intensity and duration [[14]].

Studies have shown that the anti-inflammatory factor HSP-70 (heat shock proteins weighing 70 kilodaltons) is affected by resistance exercise [[15]]. On the other hand, it was clarified in research that the increase of the HSP-70 factor during exercise affects the immune system [16]]. In another study, it is stated that even in diabetic patients, resistance training with the reduction of the anti-inflammatory factor HSP-70 affects the improvement of the patient's condition[17]]. Of course, the amount of change in the HSP-70 factor depends on the type of exercise as well as the temperature and environmental conditions in addition to the intensity [[18]].

In a study, it is stated that resistance exercise does not affect the level of brain-derived neurotrophic factor

(BDNF) as much as aerobic exercise [[19]]. On the other hand, in another study, the findings showed that both strength and endurance interventions increase BDNF. They have a similar effect [[20]], while it was said in research that resistance training even in diabetic patients increased BDNF levels [[21]].

Men and women show different effects and feedback in response to resistance training in inflammatory responses [[22]]. Because little attention has been paid to the effects of resistance exercise on women, more studies are needed to determine the extent and manner of the physiological response of women to resistance exercise in the inflammatory and immune systems [[23]]. Considering the contradictory results regarding the effect of intensity and duration of resistance training, there is a need for more research in this field. The purpose of this study is to investigate the effect of resistance training with two different intensities (loads) in two different recovery times on pro-inflammatory factor TNF- α , HSP-70, inflammatory factor hs-CRP, and anti-inflammatory factor BDNF in female bodybuilders.

Method:

This semi-experimental study was conducted in one group and as a repeated design. In interventional studies, whenever the possibility of random allocation of samples; If there are no intervention and control groups, we can call it a quasi-experimental or quasi-experimental study. Because this study examines the change in the average scores of more than three (4 values) at different times, repeated design variance analysis was used. The criteria for entering the study were a history of at least two years of continuous and regular exercise, not taking drugs or supplements in the last six months, not smoking or alcohol, with an age range of 30-40 years. If the samples were damaged during the research period or they refused to continue the research, they were removed from the sample size.

Before starting the study, an informed consent form was obtained from all the participants. The subjects of this research were female bodybuilders who had at least two years of experience in bodybuilding and were in perfect health, in the age group of 30 to 40 years, who are most inclined to bodybuilding. They had a body mass index of 23 to 26 and volunteered to participate in the research after the briefing session. After learning the training protocol, the subjects started conducting the research under the supervision of the researcher and the team of coaches of the Mumtaz Rasht Club. The whole research was done in one month with one training session per week. Every Wednesday evening, the exercises were performed with full supervision by the exercise protocol, and thirty-six hours later, in the state of fasting for ten hours, on Thursday morning of the same week, blood sampling was done in the medical laboratory under the supervision of the researcher by laboratory experts. After centrifugation at 4000 revolutions per minute, the blood samples were kept at -20°C, and after finishing the exercises, they were sent to Pasargad Histogenic Research Institute, Tehran, and were analyzed by human kits using the ELISA method. The ELISA method is an immunological method. It is sensitive for detecting and measuring antigen and antibody reactions. This method is used to check the amount of proteins, glycoproteins, and hormones in a sample of tissues, cells, or body secretions. The basis of this test is the binding of specific antibodies to the corresponding antigen. In this research, the blood plasma of the samples was used. In the laboratory, after washing and drying, the required solution was added to the wells. The plate was incubated in the dark at 37 °C for 10 minutes. Then, the stopping solution was added to all the wells until the blue color turned to yellow. Light absorption the well was read at a wavelength of 450 nm by an Elizarider device in Biotech, America. Using the optical absorption of standard samples, the standard curve was drawn and then the values of each sample were calculated using the obtained formula. The training protocol included 4 training sessions with two intensities of 85% maximum one repetition maximum and 75% maximum one repetition maximum, with two recovery times of one and two minutes, which were performed during 4 consecutive weeks. Also, the samples did their usual exercises two days a week. The 4 training protocols are shown in detail in Table 1.

<i>Protocol</i>		<i>Type of movement</i>	<i>set number</i>	<i>Number of repetitions</i>	<i>Rest between sets</i>	<i>Description</i>
Protocol one	warm up					Fit the muscles with the trainer
	The body of the exercise	front squat	3	12	two minutes	For each movement appropriate to the main target muscle with seventy percent of one repetition maximum (70% 1RM)
		chest press	3	12	two minutes	
		Split squat	3	12	two minutes	
		Military shoulder press	3	12	two minutes	
Cool down						
Protocol 2	warm up					fit the muscles with the trainer
	The body of the exercise	front squat	3	12	one minutes	For each movement appropriate to the main target muscle with seventy percent of one repetition maximum (70% 1RM)
		chest press	3	12	one minutes	
		Split squat	3	12	one minutes	
		Military shoulder press	3	12	one minutes	
Cool down						
Protocol 3	warm up					fit the muscles with the trainer
	The body of the exercise	front squat	3	6	two minutes	For each movement appropriate to the main target muscle with eighty five percent of one repetition maximum (85% 1RM)
		chest press	3	6	two minutes	
		Split squat	3	6	two minutes	
		Military shoulder press	3	6	two minutes	
Cool down						
Protocol 4	warm up					fit the muscles with the trainer
	The body of the exercise	front squat	3	6	one minutes	For each movement appropriate to the main target muscle with eighty five percent of one repetition maximum (85% 1RM)
		chest press	3	6	one minutes	
		Split squat	3	6	one minutes	
		Military shoulder press	3	6	one minutes	
Cool down						

Collection of blood samples:

Each of these four training programs was conducted on Wednesday evening. Thirty-six hours later and following ten hours of fasting, blood samples were taken from the antecubital vein in the medical laboratory, after centrifugation at a speed of three thousand revolutions per minute by a Hitachi device (Germany), the samples were kept at a temperature of minus twenty degrees Celsius. For tumor necrosis factor-alpha (TNF-

α), HSP-70, and BDNF, ZellBio (ZellBio GmbH, Germany) ELISA kits and ELISA READER (DANA-3200, Taiwan) were used (for all cases).

Data analysis method:

Data analysis was done using SPSS software version 27 (IBM SPSS Statistics 27). The normal distribution of the data was checked using the Shapiro-Wilk test, and after determining the normality of the data, the analysis of variance of the repeated design was used for evaluation. Bonferroni's post hoc test between groups was used.

Results

The demographic characteristics of the participants in the study are presented in Table 2. As the results of the table show, the average age of the athletes was 21.5 ± 33.35 years, their experience in bodybuilding was 7.0 ± 5.3 years, and their BMI was $67 \pm 1.65 \pm 25$.

Table two: Demographic characteristics of female bodybuilders participating in the study

Standard deviation \pm mean	variable
33/35 \pm 5/21	(Age (years))
3/58 \pm 0/70	(History of bodybuilding (year)
25/65 \pm 1/67	BMI (kg/m2)

The results of repeated design analysis of variance

The description of changes in the average and standard deviation of the variables was also given in Table number three.

Table three: Average changes and standard deviation of variables during four training protocols

Analysis of variance		HLRT2	HLRT1	MLRT2	MLRT1	Exercise protocol dependent variable
P value	F	Standard deviation \pm mean	Standard deviation \pm mean	Standard deviation \pm mean	Standard deviation \pm mean	
0/0001 *	67/63	95/34 \pm 21/67	133/8 \pm 27/17	143/9 \pm 27/55	245/1 \pm 30/53	TNF- α
0/025*	5/04	0/341 \pm 0 /341	0/687 \pm 0 /687	0/828 \pm 0 /517	0/7908 \pm 0 /53	HS-CRP
0/001*	49/99	0/486 \pm 0 /103	0/662 \pm 0/05	0/7454 \pm 0/102	0/8560 \pm 0 /05	HSP-70
0/001*	120/66	0/702 \pm 0/078	0/506 \pm 0/035	0/4524 \pm 0/041	0/2766 \pm 0 /042	BDNF

*p<0.05

MLRT-1:70% 1RM,Rest :2min ; MLRT-2: :70% 1RM,Rest:1min ; HLRT-1: :85% 1RM Rest:,2min; HLRT-2: 85% 1RM, Rest:1min

Based on the results of the analysis of variance and Tukey's post hoc test, according to the amount of the pro-inflammatory factor TNF- α , it significantly decreased with increasing load intensity and decreasing recovery time. In the exercise protocol, 70% maximum repetitions with two minutes recovery (MLRT1) compared to 70% of a maximum repetition and one-minute recovery (MLRT2) compared to the protocol of 85% of a maximum repetition with two minutes recovery (HLRT1) and compared to the exercise protocol of 85% of one maximum repetition with one-minute recovery (HLRT2) also reduced the significance There was a difference in the level of TNF- α ($P\leq 0.0001$).

In the comparison of the 70% one-repetition maximum exercise protocol with one-minute recovery with the 85% one-maximum repetition exercise protocol and two-minute recovery, although there was a decrease in TNF- α , this decrease was not significant ($P=0.7927$). 70% of a maximum repetition and one-minute recovery with 85% of a maximum repetition with one-minute recovery, this reduction was significant ($P\leq 0.0004$). Also, comparing 85% of one repetition maximum exercise with two minutes recovery with 85% of one repetition maximum exercise with one-minute recovery, this reduction was significant ($P\leq 0.0058$). The results are given in Figure 1.

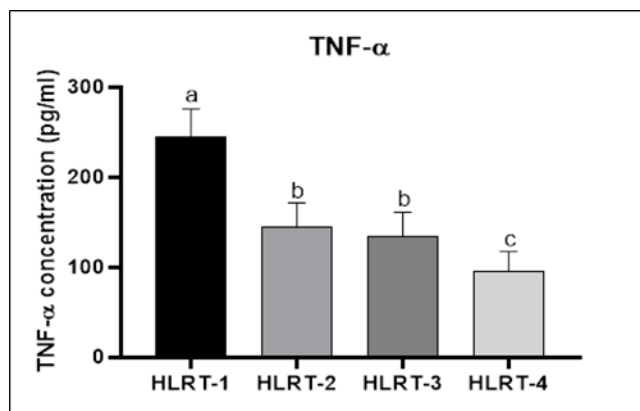


Figure 1: TNF- α average after performing each training session

a significant difference between the effect of training one and the other three training stages

b Significant difference between stage two and three exercises with stage 1 exercise

c significant difference between the fourth exercise with all the steps of the previous exercise

MLRT-1:70% 1RM,2min; MLRT-2: :70% 1RM,1min; HLRT-1: :85% 1RM,2min; HLRT-2: 85% 1RM,1min

Based on the results of the analysis of variance and Tukey's post hoc test, according to the amount of HSP-70 factor, there was a significant decrease with increasing load intensity and decreasing recovery time ($P\leq 0.0001$). In comparing exercise two with exercise three, this decrease was not significant ($P\leq 0.736$). But training one MLRT1 with two MLRT2, one MLRT1 with three HLRT1, one MLRT1 with four MLRT2, two MLRT2 with four MLRT2, and three HLRT1 with four HLRT2, this reduction was significant with ($P\leq 0.0001$).

Figure 2 shows this comparison.

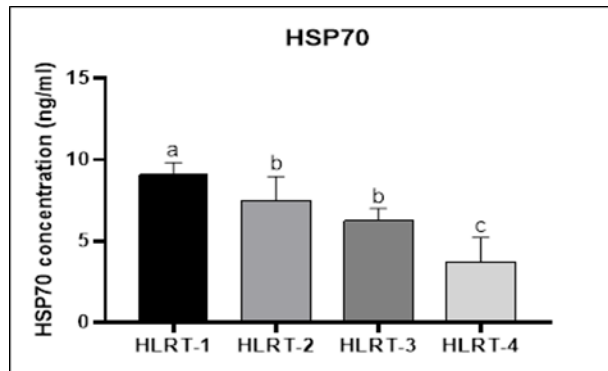


Figure 2: Comparison of average HSP-70 after performing each part of the exercise

b. In this form, the significance of the two-by-three exercise determines that the decrease is not significant

MLRT-1:70% 1RM,2min ;MLRT-2: :70% 1RM,1min ;HLRT-1: :85% 1RM,2min;HLRT-2: 85% 1RM,1min

Based on the results of the analysis of variance and Tukey's post hoc test, according to the amount of BDNF factor, there was a significant increase with increasing load intensity and decreasing recovery time ($P \leq 0.0001$). In training two with three, there was no significant increase with ($P \leq 0.0676$)

But training one (MLRT1) with two (MLRT2), one (MLRT1) with three (HLRT1), one (MLRT1) with four (HLRT2), two (MLRT2) with four (MLRT2), and three (HLRT1) with four (HLRT2)) this increase was significant with ($P \leq 0.0001$). Figure 3 shows this comparison.

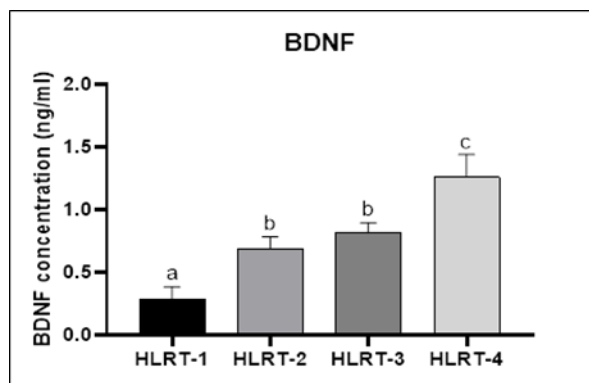


Figure 3: Comparison of average BDNF after performing each training session

b. In this form, the significance of the two-by-three exercise indicates that the increase isn't significant.

MLRT-1:70% 1RM,2min ;MLRT-2: :70% 1RM,1min ;HLRT-1: :85% 1RM,2min;HLRT-2: 85% 1RM,1min

Discussion:

In this research, a significant decrease in the expression of TNF- α and HSP-70 pro-inflammatory factor genes was observed between a bout of resistance training with moderate load intensity (1RM) of seventy percent and HLRT exercises with high load intensity (1RM) of eighty-five percent. There was also a significant increase in the anti-inflammatory factor BDNF.

Mariko et al. (2022) showed in a study that HLRT exercises, like low-load exercises (LLRT), reduce circulating inflammatory biomarkers [[24]]. Also, Sabouri et al. (2021) showed the improvement of inflammatory factors as a result of resistance training similar to endurance training [[25]]. Negoria et al also stated that with resistance training, the level of TNF- α decreased and the athlete improved in performance [[26]]. The heart helped [[26]]. In 2013, after a study of 40 young men, Rodríguez et al. stated that plasma levels of TNF- α decreased significantly after completing an exercise program, and no sports injuries were reported [[27]].

On the other hand, it was shown in another review study that long-term and intense exercises can lead to an increase in inflammatory mediators, while moderate and intense exercises with appropriate rest periods can have the maximum improvement in this field [[11]]. In another study Also, there was a direct relationship between the level of exercise intensity and the increase of inflammatory factors, which requires more research focusing on different exercise intensities [[8]]. α and as a result increase in the level of inflammation [[28]]. Among the possible reasons for the contradictory results in the above research, we can mention the type and intensity of the exercises as well as the gender of the samples.

Regarding the effect of resistance training on hs-CRP values, in a study conducted on nine healthy women, it was found that resistance and endurance training significantly reduced the levels of this inflammatory protein [35]. Also, another study was conducted on healthy middle-aged women. showed a significant decrease in hs-CRP after resistance training [[29]]. Ishikaki et al believe that low-intensity resistance training has a direct relationship with reducing inflammation [[30] A very important part of the cellular tools for proper folding is the HSP-70 protein, which protects the cell against stress and pressure [[31]]. Inconsistent studies, it has been said that exercise training by affecting the HSP-70 factor reduces the possibility of inflammation. [[16]]. Also, in research conducted on women, it was shown that HSP-70 is a factor in creating adaptation to sports training [[32]]. On the other hand, in research, an increase in HSP-70 values was observed following sports training [[15]]. In another study, it was stated that high exercise stress increases the level of HSP-70 [[32]] and the change in HSP-70 level is proportional to damage to Muscles that occurs in exercise [[32]]. which shows the effect of the type and intensity of exercise on the amount of this protein. Another result of this research was the increase in BDNF levels following HLRT exercises with a one-minute recovery between sets. In a study that compared the intensity and volume of resistance training on BDNF, it was clarified that BDNF levels increased in acute resistance training regardless of the training pattern [45]. Health promotion is effective [46]. In another study, which was a comparison between resistance and endurance training on elderly people, it was found that both training methods increase BDNF levels [25, 47]. The studies that are in the review article revealed that resistance training can significantly increase BDNF levels and reduce depressive symptoms among older adults aged 60 years or older [47].

The review of past studies shows the effect of adaptation of the inflammatory system the increase in the production of anti-inflammatory factors and the reduction of pro-inflammatory and inflammatory factors. The findings of our research show that the inflammation decreases in proportion to the increase in load intensity in resistance activity in female bodybuilders. However, in some cases, this decrease is not related to the intensity of the exercise, which requires more research. Regarding the intensity of resistance training and recovery time between sets, studies have many contradictions. At one point, resistance training shows a reduction in inflammation [48]. It has been found that TNF- α gene transcription is related to the quality of skeletal muscle contraction [49]. Resistance training can cause muscle damage, for which inflammation is a physiological response and includes the production of cytokines. However, the response of cytokines may differ between training periods based on the type, intensity, duration, and recovery time. Resistance

training reduces the levels of Cytokines that affect the path of inflammation [5]. The anti-inflammatory effects of resistance training along with the reduction of CRP and TNF- α factor can be related to the increase of muscle mass. There is also evidence that a higher volume of resistance training (number of exercises, frequency, and duration of exercise) causes anti-inflammatory effects that may be related to changes in body composition [50]. Resistance exercises with greater intensity and volume, which are created by increasing the load of exercises and reducing the rest time between sets, can reduce the activity of the immune system and, as a result, reduce the possibility of inflammation. It is suggested that high load resistance training (HLRT) can be used in many cases where the athlete's body needs to adjust the activity of the immune system. Among the limitations of this research, we can mention the age of women, the effect of menopause, and the field of sports. Also, comparing the effects of endurance and strength training with high intensity and volume on inflammatory and pro-inflammatory factors is one of the suggestions of this research.

Conclusion

In general, the results of this study showed that resistance training with high load compared to resistance training with moderate load, reduces the plasma level of pro-inflammatory factor TNF- α and also the factor HSP-70. The results also showed that the increase in the anti-inflammatory factor BDNF was also significant. As seen in the results, the changes after the implementation of the research caused a significant decrease in inflammatory factors and markers compared to the severity (burden). According to these findings, it can be said that high-load resistance training (HLRT) can reduce pro-inflammatory factors in female bodybuilders.

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Conflict of interest

The authors declare that there is no conflict of interest in the present study.