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The Effect of Chronic and Acute High-Intensity Training on the Macrophage (CD11b) Expressing NF-κB Due to Malondyaldehide (MDA) Level

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Abstract

Aimed: The purpose of this study was to determine the effect of chronic high-intensity eccentric training of 80%-90% VO2max and Acute high intensity eccentric training on the expression of CD11b and NFkB due to Malondyaldehide level. **Methods**: In this study twenty nine male mus musculus after one week of familiarization with environtment and training were randomly divided into three groups, control (n=10), chronic HIT (n=10) and acute HIT (n=9) were performed a dynamic high intensity training on animal treadmill where speed and endurance are done with a load of 80 - 90% of maximum capacity .The form of exercise used in this study is downhill treadmill training with a negative angle inclination of -15^{o} for 23 minutes for 7 days on the chronic high intensity training group and one day for acute high intensity training group and then termination was carried out to collect the spleen of the mice which were then processed by flowcytometric method to observe the percentage of macrophage cells CD11b expressing NFkB. **Results** : The result of flowcytometry tests obtained that macrophage cells CD11b expressing NFkB showed that the mean of control group was 256.50±129.719, chronic high-intensity eccentric training group 251.10±149.637, acute high-intensity eccentric training group 273.78±101.867.Anova test significant level was 0.925 which mean that there were no significant difference between group. **Conclusion**: The mean of CD11b expressing NFkB of acute HIT group higher than control and chronic HIT group, while Compare Anova test didn't showed significant difference between control, chronic and acute high intensity training group on macrophage cells (CD11b) expressing NFKB.

Keywords: High Intensity Training, Malondyaldehyde (MDA), CD11b, NFkB.

INTRODUCTION

Several studies have confirmed that acute exercise can affect systemic inflammation. One study mentioned that intensive exercise can lead to increased synthesis and release of pro-inflammatory cytokines, with elevated serum levels of anti-inflammatory cytokines (e.g., IL-10 and IL-1ra) observed as secondary phenomena. Serum levels of periostin, a hallmark of type 2 inflammation, do not increase within 1 hour after acute exercise. In a study that assessed serum cytokine responses to treadmill running exercises, resting levels of anti-inflammatory and immunomodulatory cytokines (IL-1ra, IL-10) were higher in URTI-free subjects, conversely acute exercise-induced release of IL-6 was more pronounced in subjects prone to respiratory symptoms. These findings suggest that infection-prone activity may experience some dysregulation in cytokine balance and disruption of anti-inflammatory mechanisms. (Kurowski et al. 2022)

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In general, most studies assessing the influence of exercise on immune and inflammatory parameters at the systemic level are based on acute exercise rather than regular exercise Regularly physical exercise with right frequency, type, intensity and duration may increase immune system.(Budiastuti et al. 2021) (Maulana and Rochmania 2020)

For the immune system haemodynamic stress, hormones, exosomes, and O2 availability are proposed stimuli that mediate their effects by alteration of different signalling processes leading to local and systemic (anti)inflammatory responses. Overall, high-intensity training shows specific molecular signatures that demonstrate its high potential to improve health and physical performance (Wahl, Bloch, and Proschinger 2022)

High Intensity Training with long durations can lead to a decrease in white blood cell function and lead to a decrease in host protection which can increase the risk of infection, this mechanism is called an 'open window' which can interfere with cellular homeostasis processes and increase the stressor response in the body (Rahman and Ilham Setya Budi 2022)

Acute high-intensity training has been shown to have negative effects on health but on the contrary Long-term physical training has the potential to increase maximum aerobic velocity (MAV), boost immunity and reduce inflammation. Training intensity is a key determinant of its positive and negative effects, some studies show a significant increase in MAV after 3.5 to 7 weeks of training. High-intensity training can induce oxidative stress and inflammation, which can have detrimental effects on the body. This oxidative stress is often characterized by increased levels of malondialdehyde, a marker indicative of lipid peroxidation, which can further trigger inflammatory pathways such as the upregulation of CD11b and NF-κB signaling (Rhibi et al. 2022) However, exhaustion can destroy the useful effects of exercise. During exhaustive exercise, the body's oxygen consumption and oxygen uptake markedly increase through the muscles, inducing harmful effects on health. This causes an accelerated generation of reactive oxygen species (ROS) to exceed the capacity of body antioxidant defenses known as oxidative stress. Extra accumulation of ROS results in structural damage to the contractile tissue, especially skeletal muscles, by oxidizing cellular components such as membrane lipids, proteins, carbohydrates and DNA. Previous studies have reported that by-products of lipid peroxidation, especially malondyaldehyde (MDA)

MATERIAL AND METHOD

In this study twenty nine male mus musculus after one week of familiarization with environtment and training were randomly divided into three groups, control (n=10), chronic HIT (n=10) and acute HIT (n=9) were performed a dynamic high intensity training where speed and endurance are done with a load of 80 - 90% of maximum capacity .The form of training used in this study was downhill treadmill training with a negative angle inclination of -15^{o} for 23 minutes, consisting of 5 minutes of running adaptation and 18 minutes of actual running load at a speed of 30 cm/second for 7 days. Before the actual training, the mice were given 5 minutes to adapt to downhill running, which was done gradually and included the following stages: the first 1 minute at a speed of 0 cm/second, the next 2 minutes at a speed of 14 cm/sec and the next 2 minutes at a speed of 21 cm/second. The chronic High Intensity Training group were given training for 7 days and on the 8th day, termination was carried out to collect the spleen of the mice while The acute high intensity training group were given treatment one time and after 2 hours recovery got termination to collect the spleen which were then processed by flowcytometric method to observe the percentage of Macrophage cells (CD11b) that

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expressing NFkB. The antibodies used were conjugated FITC anti-mouse CD11b, PE anti-mouse NFkB antibody.

RESULTS

Based on the mice spleen flowcytometry test on the percentage of T lymphocyte cells expressing CD11b expressing NFkB the results given in Tables 1 and Fig. 1, Fig 2

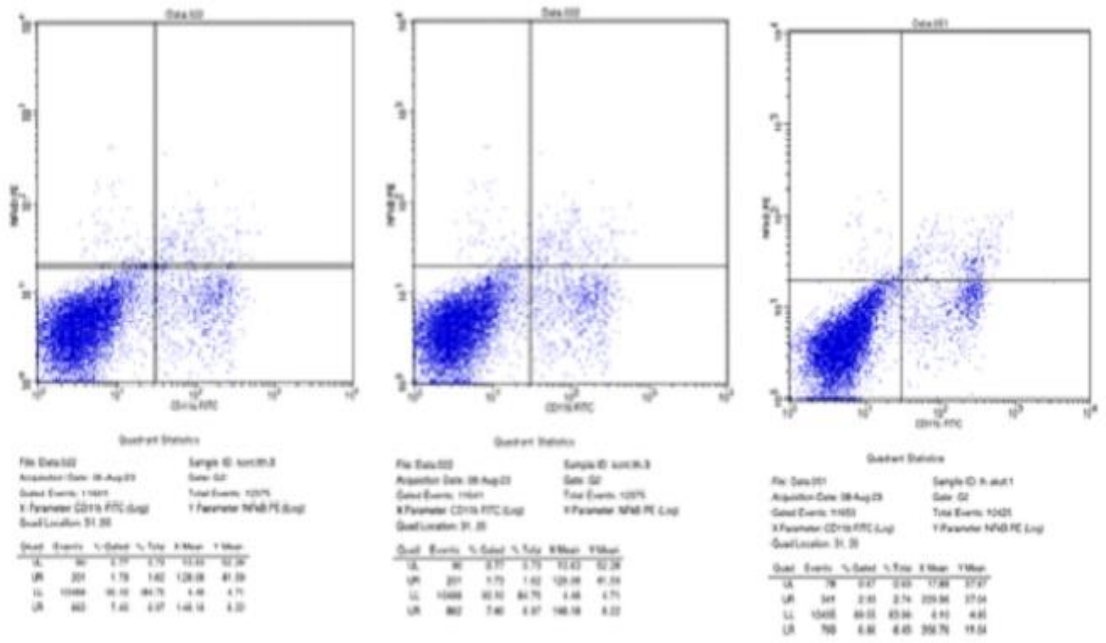

Figure 1: Relative count of Macrophage (CD11b) expressing NFkB based on flowcytometry

This study aimed to determine the effect of chronic high-intensity eccentric training of 80%-90% VO2max and Acute high intensity training to immune response. The result of flowcytometry tests obtained that Macrophage cells expressing CD11b NFkB show the mean of control group was 256.50±129.719, chronic high-intensity eccentric training group 251.10±149.637, acute high-intensity eccentric training group 273.78±101.867.

Table 1: Relative count of Macrophage (CD11b) expressing NFkB

Descriptives								
CD11bNFKB								
	N	**Mean**	**Std. Deviation**	**Std. Error**	**95% Confidence Interval for Mean**		**Minimum**	**Maximum**
					Lower Bound	**Upper Bound**		
Control	10	256.50	129.719	41.021	163.70	349.30	61	468
chronic high intensity training	10	251.10	149.637	47.319	144.06	358.14	73	537
Acute High Intensity Training	9	273.78	101.867	33.956	195.48	352.08	183	508
Total	29	260.00	125.157	23.241	212.39	307.61	61	537

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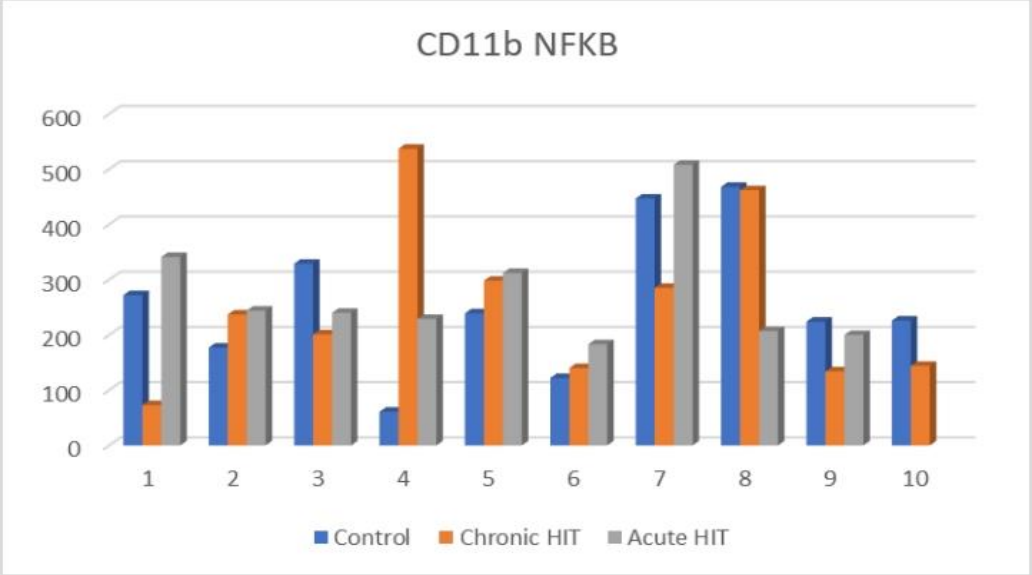

Fig 2: Comparison of Macrophage cell (CD11b) expressing NFkB between control, chronic HIT and acute HIT group

Table 1 and figure 1 show than the mean of relative count on macrophage (CD11b) expressing NFkB on acute HIT group (273, 78) was higher than chronic HIT (251.10) and control group (256.50)

Table 2: Test of normality between control, chronic and acute HIT group

Tests of Normality							
	group	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
CD11b NFKB	Control	.154	10	.200*	.951	10	.675
	chronic high intensity training	.177	10	.200*	.904	10	.240
	Acute High Intensity Training	.282	9	.038	.810	9	.026

*. This is a lower bound of the true significance.

Normality test showed that significant level of control group was 0.675 and chronic HIT group was 0.240 which mean that distribution of the data between the groups were normal while the acute HIT group significant level was 0.026 which mean that the distribution of the data were unnormal

Table 3: Homogenity test between control, chronic and acute HIT group

Test of Homogeneity of Variances			
CD11bNFKB			
Levene Statistic	**df1**	**df2**	**Sig.**
.637	2	26	.537

The levene test of homogenity between groups showed that significant level was 0.537 which mean that the data between control, chronic and acute HIT group were homogen.

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Table 4: Comparison Anova test

ANOVA					
CD11bNFKB					
	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	2623.044	2	1311.522	.078	.925
Within Groups	435978.956	26	16768.421		
Total	438602.000	28			

Table 5: Multiple Comparison test

Multiple Comparisons						
Dependent Variable: CD11bNFKB						
Bonferroni						
(I) group	**(J) group**	**Mean Difference (I-J)**	**Std. Error**	**Sig.**	**95% Confidence Interval**	
					Lower Bound	**Upper Bound**
Control	chronic high intensity training	5.400	57.911	1.000	-142.79	153.59
	Acute High Intensity Training	-17.278	59.498	1.000	-169.53	134.97
chronic high intensity training	Control	-5.400	57.911	1.000	-153.59	142.79
	Acute High Intensity Training	-22.678	59.498	1.000	-174.93	129.57
Acute High Intensity Training	Control	17.278	59.498	1.000	-134.97	169.53
	chronic high intensity training	22.678	59.498	1.000	-129.57	174.93

Table 4 and 5 showed that significant level of anova test between groups was 0.925 which mean that there was no difference between the control, chronic and acute group. Bonferoni comparison multiple test also showed that there were no difference between control, chronic and acute group on macrophage cell (CD11b) expressing NFkB due to high intensity training.

DISCUSSION

Incremental HIIT training and resistance training on rats for 10 weeks 5 sessions perweek show the significant increase of MDA in serum which represents high -level physiological stress induced by this type of training (Rahmani, Gorzi, and Ghanbari 2019). Exercise preferentially biased with forced-lengthening (eccentric contractions) causes muscle damage, which is a manifestation of ultra-structural changes and protein degradation, and is associated with the development of an acute phase inflammatory response.[1]

This inflammatory response is a microvasculature-induced reaction characterized by the migration of serum proteins and leukocytes from the blood to the skeletal muscle. Involved in this migration are pro- inflammatory cytokines, most notably tumor necrosis factor alpha (TNF-α) which binds its trans-membrane receptor in skeletal muscle and activates a transcription factor known as nuclear factor kappa B (NF-κB).

The activation of NF-κB has been found to be a critical factor involved in the multifaceted homeostasis of muscle. This becomes important as the development and growth of skeletal muscle requires a complicated series of events that are regulated through various pathways. Furthermore, exercise-induced muscle damage is also associated with oxidative stress, a process that not only directly causes cellular damage by oxidation of cellular components such as lipids, protein, and DNA, but also acts as a regulator of the acute phase inflammatory response (Willoughby 2014)

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Physical exercise is a complex biological activity that constantly challenges the oxidation–antioxidant balance of the body (cells, tissues, organs, etc.) while maintaining biological balance. The adjustment of exercise on oxidative stress can be acute or long-term. Acute adjustment is an incomplete adaptation that can easily lead to oxidative damage, so it is important to give the body sufficient rest following exercise to restore balance. The process of balancing—breaking the balance—restoring the balance helps improve the body's ability to cope with oxidative stress. In fact, regular exercise can fundamentally upregulate the body's endogenous antioxidant system (Lu et al. 2021)

NF-κB is a ubiquitously expressed transcription factor that is considered vital to numerous cellular processes. It has been suggested that NF-κB directly modifies hundreds of gene products, including genes that encode cytokines, chemokines, cell adhesion molecules, growth factors, immunoregulatory receptors, acute-phase and stress response proteins, cell surface receptors, transcription factors, and several enzymes involved in protein degradation by the ubiquitin-proteasome system, as well as regulators of redox status, apoptosis, disuse atrophy, and host defense (Willoughby 2014) NF-κB is recognized as a crucial component of many immune responses. Innate immune cells, such as macrophages and dendritic cells (DCs), rely on NF-κB for the secretion of pro-inflammatory cytokines after pattern recognition receptor (PRR) activation.

There is a significant increase in oxidative stress markers following exercise at TP0 (0 h) when compared to rest. To investigate oxidative damage after exercise at multiple TP, data were available from 13 studies. This review demonstrates the acute effect on oxidative stress following high-intensity exercise often occurs within 5 min at the end of the exercise, remaining elevated within 30 min following exercise. Moreover, several studies reported that oxidative stress peaked at 5 min post exercise. Most of the increased oxidative damage returned to basal level within 24 h following exercise cessation (Lu et al. 2021).

Single session of HIT can induce oxidative stress; however, a HIT protocol lasting a longer period will attenuate oxidative stress. This statement agrees with the findings that regular HIT could keep oxidative DNA damage at a lower level for a long period and consecutive days of high-intensity exercise improved endogenous antioxidant capacity and reduced exercise-induced oxidative stress (Lu et al. 2021)

The neutrophil concentration in circulation increases during exercise and remains elevated for several hours post-exercise, but the degranulation and oxidative burst response to bacteria are reduced. Conversely, lymphocytes in circulation increase during exercise but may drop below the baseline during the recovery phase for 2–4 h. During the recovery period after heavy exercise, lymphocyte subpopulations such as CD4+ T cells, CD8+ T cells, CD19+ B cells, CD16+ natural killer (NK) cells, and CD56+ NK cells may decrease, and the salivary secretion rate of immunoglobulin A (IgA) may also temporarily decrease. Overtraining or training without sufficient recovery can further exacerbate these effects. The exercise-induced immunodepression may persist for a few hours or a few days after strenuous exercise or training (Lee et al. 2023).

A significant time effect was study was to compare changes in markers of monocyte recruitment following an acute bout of high-intensity (HVY), versus high-volume (VOL) lower-body resistance exercise. Ten resistance-trained men (24.7±3.4y; 90.1±11.3kg; 176.0±4.9cm) performed each protocol in a randomized, counterbalanced order. Blood samples were obtained at baseline, immediately (IP), 30-minutes (30P), 1-hour (1H), 2-hours (2H), and

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5-hours (5H) post-exercise.s observed for CD11b (F = 7.1; p < 0.001; p = 0.44); however, no significant main effect for group, or time x group interaction (p >0.05) was noted. With both trials combined, CD11b was significantly elevated at IP (d = 0.86; p = 0.014), and 1H (d = 1.04; p = 0.009). A trend towards a decrease was in CD11b receptor expression relative to BL was observed at 5H (d = 0.59; p = 0.095). CD11b receptor expression was positively correlated with circulating MCP-1 at IP (r = .576, p = 0.008), and 1H (r = .706, p = 0.001), and with circulating TNFα at 1H (r = .666, p = 0.001)(Wells et al. 2016)

CONCLUSION

The mean of CD11b expressing NFkB of acute HIT group higher than control and chronic HIT group, while Compare Anova test didn't showed significant difference between control, chronic and acute high intensity training group on macrophage cells (CD11b) expressing NFKB.

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