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Effect of Exercise at Different Times of Day on the Inflammatory markers of cardiovascular disease Risk in Obese Men

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ABSTRACT

The aim of the present research was to determine the effect of exercise at different times of day on the inflammatory markers which make the risk of coronary heart diseases in obese men. 40 non-athletes, obese men (age mean 22.25±2.5 and weight mean 89.74±6.07) were randomly allocated to four groups of morning and evening aerobic training and morning and evening control. Morning and evening training groups performed the aerobic training protocol three sessions per week for 12 weeks while the control groups were denied doing training programs during the research period. Blood samples (5 cc) were taken from the participants at the beginning of the period, week 6 and end of week 12 in order to measure C-reactive protein (CRP), interlukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- α). The results showed that morning and evening aerobic training decreased CRP (p=0.002) and IL-6 (p= 0.001) in the experimental groups compared with the control ones. Also, the amount of produced inflammatory markers was less in the evening than in the morning (in the evening training group, the amount of CRP was 17.1%, 10.5% and 18.87% in week 1, week 6 and week12 and the amount of IL-6 was respectively 21.5%, 16.74% and 25.32% lower than that of the morning training group). According to the results of this study, it was determined that different times of the day influence the compatibility of inflammatory markers caused by aerobic training and these markers show more reduction in the evening. Therefore, it is recommended to young obese men to do exercises in the evening rather than morning.

Key words: Morning training, Evening training, inflammatory markers, Coronary heart disease, obese men.

INTRODUCTION

Coronary Heart Diseases (CHD), especially coronary artery problems, are one of the main causes of mortality in the new century and the first cause of mortality in Iran. High blood pressure, high blood lipoproteins and lipids, age, gender, lifestyle, nutrition, smoking, inactivity, diabetes and obesity are considered among the CHD risk factors [1]. American Heart Society has announced

that CHD spread has an inflammatory root and general inflammation has a main role in atherosclerosis development and progression [2]. In past, lipid profile was considered a CHD marker; however, recently, research results have shown that some people with natural HDL-c and LDL-c are influenced by CHD [2]. In this regard, many studies have found that the relationship between inflammatory markers [like adhesive molecules, Fibrinogen and Interlukine-6(IL-6), C-reactive proteins (CRP) and Tomor Necrosis Factor-α (TNF- α)] and coronary heart diseases can be appropriate for predicting these diseases [3]. On the other hand, there is a relationship between the plasma levels of CRP, IL-6 and TNF- α and obesity, diabetes and the level of physical activity [4] in a way that some studies have reported the relationship between obesity and increase of TNF-α, IL-6 and CRP inflammatory markers and the increased risk of CHD. Also, it has been shown that regular physical activity and exercise lead to the decrease of inflammatory markers and CHD risk [5]. The results of the studies conducted in this field have shown that doing regular exercises significantly decreases TNF-α, IL-6, IL-1β and CPR [6-11] and there is a relationship between higher levels of physical activity and physical fitness and lower levels of these inflammatory markers [2, 12]. At the same time, some researchers like Hammett (2004), Fairey (2005) and Arsenaulet (2009) reported the lack of change in inflammatory markers during prolonged aerobic exercises [1, 13, 14]. On the other hand, different reports have shown that most CHDs occur during daytime and at specific hours, especially early in the morning [15-17]. Therefore, this question can be raised that whether the levels of risk factors related to CHD risk factors are higher at some hours of day. Aldmir (2005) obtained similar results while investigating the effect of one-session aerobic sub-maximal exercise in the morning and evening on the human platelets and concluded that doing exercise in the evening is better than doing it in the morning [17]. Furthermore, Pledge et al (2011) examined the effect of resistance training in the morning and evening on the response of cortisol and interleukin-6 and found a significant relationship between IL-6 and cortisol concentration in the morning compared with the evening [15]. However, most studies conducted in this regard have investigated the acute effect (response) of coronary heart risk factors at different hours of daytime and more studies are required for determining the exact effect of regular prolonged (adaptations) exercises or aerobic activities on the resting levels of inflammatory markers. Thus, the following question can be raised: are the concentration levels of TNF-α, IL-6 and CRP inflammatory markers (like those of platelets) influenced by evening and morning exercises? Considering the importance of preventing from CHDs and the effect of physical training on general health, the present researchers attempt to study the influence of 12-week morning and evening aerobic trainings on some coronary heart inflammatory markers like (CRP،IL-6 و TNF-α) in obese men.

MATERIALS AND METHODS

Participants

After the call for research at Mobarakeh Islamic Azad University, the obese, male, non-athlete students who volunteered to participate in this study received and filled out a questionnaire which included personal characteristics, health, smoking and physical training history. Then, their height and weight were measured and their body mass index (BMI) was calculated by placing the numbers related to weight and height in the equation (weight in kg was divided by the squared height in m). From among 167 volunteers who were qualified for this research, 40 people were chosen using random sampling with replacement. The participants were non-athlete and their age ranged from 20 to 25 years old. Their BMI was between 30 and 33 kg/m^2. Also, they had no smoking and allergy history and took no medicine since at least 2 weeks before the research and during the research period and followed their own normal diet. They first filled out and signed the consent form. Then, they were allocated to 4 groups of morning training, evening training, morning control and evening control with 10 people in each and using simple random sampling.

The participants' general characteristics are given in Table 1.

Table 1: Participants' general characteristics (mean± standard deviation)

Group Variable	Morning training group (N=10)	Evening training (N=10)	Morning Control (N=10)	Evening control (N=10)	F	P
Age (year)	23±2.1	22±2.7	22±2.4	22±2.6	5.461	0.763
Weight (kg)	78.25±5.3	89.7±6.47	92.03±7.19	90±5.34	6.748	0.849
Height (cm)	170.29±4.02	172.31±4.41	175.14±6.1	173±5.2	5.283	0.791
VO_{2max}(ml.kg/min)	35.83±2.3	36.64±1.6	36.84±1.8	37.19±1.9	1.887	0.457
BMI	30.12±2.26	30.04±2.88	30.01±2.45	30.07±2.12	2.06	0.522
Body fat %	23.74±2.85	23.27±2.49	24.36±2.04	22±3.24	3.01	0.641

Physiological Measurements

To measure weight and height, a digital scale and a tape were used, respectively. BMI was also calculated by placing the numbers related to height and weight in the equation (squared height in m / weight in km) and body fat percentage was computed by measuring subcutaneous fat in three areas of chest, abdomen and thigh and placing it in Jackson and Pollock Equation (Williams, 2002) [18].

The Training Program

First, the maximum heart rate was measured for each person using the following formula: 208-(0.7 * age) [19].

In this research, the morning and evening training groups performed a 12-week aerobic training program. The aerobic training program included a 10-min warm-up by fast walking, slow running and stretching. Then, continuous running was done with the intensity between 75 and 85 percent of the maximum heart rate of the participants. The running period was 15 min in the first session and, every two sessions, one min was added to the running period in a stepwise way so that the running period reached 30 min. After that, this period was kept until the last session of the training program (the end of the 12^{th} week). Exercise intensity was controlled using a belt heart rate sensor (polar beat) and, at the end of each session, a cool-down was performed by slow running and stretching for 10 min.

Blood Sampling

To investigate biochemical variables, in the first stage, the participants of each group were required not to do any sport activities until two days before the test and to maintain their normal diet. Then, 5 ml blood was sampled from the participants after 12 h of fasting which was taken from their left-hand antecubital vein while sitting and resting. The blood sampling was conducted for the morning training and morning control groups at 8 a.m. and for the evening training and evening control groups at 18. After this stage, the training groups performed the aerobic training program for 12 weeks. Also, after 6 and 12 weeks of aerobic training and after 48 h of the last training session and 12 h of fasting, the second and third stages of blood sampling from the participants of the control and experimental groups were done similar to the first stage.

Biochemical Measurement

To measure inflammatory markers (serum TNF- α, CRP and IL-6), Elisa method was used and the kits from the French Diaclone Company with the sensitivity of less than 7, 2 and 8 pg/mL were applied, respectively.

Moreover, during the research, their receiving diet was controlled using the 24-h food recall

questionnaire, standardized by the nutrition group, Tehran University of Medical Sciences (in week 0, week 6 and week 12).

Statistical Methods

In order to determine data naturalness and parallelism, Kolmogorove- Smirnov test was used and, to investigate within group differences, repeated measures analysis of variance (ANOVA) was applied considering the Greenhouse Gazer (GG) Amendment and, if significant, a dependent t test was used considering the P. BonFerroni Amendment in order to locate within group differences. Furthermore, one way analysis of variance (ANOVA) was conducted for examining between group differences and, if there was a statistically significant difference, Tukey's post hoc test was used for locating between group differences. This study's statistical process was done using SPSS software, version 15, and the significance level was considered P<0.05.

RESULTS

The obtained results showed that 6- and 12-week aerobic training programs in the morning and evening increase VO_{2max} (p=0.0001), decrease body fat percentage (p=0.001) and decrease body mass index (p=0.001) compared with the morning and evening control groups. Also, serum IL-6 and CRP levels of morning and evening training groups decreased after 6 weeks of training which was not statistically significant (CRP levels of morning and evening groups were p=0.27 and p= 0.54 and IL-6 levels of morning and evening groups were p= 0.36 and p= 0.43, respectively). However, after 12 weeks of exercising, these changes significantly decreased compared with the pre test (before doing exercises) (CRP levels of morning and evening groups were p= 0.002 and p= 0.002 and IL-6 levels of morning and evening groups were p=0.001 and p=0.001, respectively) while serum IL-6 and CPR levels of morning training and evening training groups were not significantly different between the middle test (week 6) and post test (week 12) (CRP levels of the morning and evening training groups were p= 0.091 and p= 0.087 and IL-6 levels of morning and evening training groups were p= 0.065 and p= 0,074, respectively). Moreover, there was no group differences in none of the stages in the morning and evening control groups (P>0.05).

Table 2: Mean and standard deviation changes of inflammatory markers in training and control groups at different test stages

Variable	Groups	Pre test (week 0)	Mid test (week 6)	post test (week 12)
CRP (pg/ml)	Experimental group 1 (morning training)	1.70±0.43	1.43±0.4	1.06±0.46 +–
	Experimental group 2 (evening training)	1.41±0.42	1.28±0.39	0.86±0.34 +–*
	Control group 1 (morning control)	1.68±0.51	1.72±0.45	1.65±0.42
	Control group 2 (evening control)	1.34±0.47	1.39±0.52	1.30±0.5
TNF-α (pg/ml)	Experimental group 1(morning training)	13.32±3.28	12.94±3.11	10.16±3.25
	Experimental group 2 (evening training)	11.15±2.17	9.74±2.06	8.82±2.27
	Control group 1 (morning control)	13.07±2.82	13.57±2.67	12.80±2.95
	Control group 2 (evening control)	11.69±2.15	10.93±2.13	10.18±2.86
IL-6 (pg/ml)	Experimental group 1 (morning training)	7.42±3.21	5.26±3.45	1.93±0.69 +–
	Experimental group 2 (evening training)	5.82±2.5	4.38±2.31	1.48±0.47 +–*
	Control group 1 (morning control)	8.01±3.86	8.62±4.07	8.23±3.34
	Control group 2 (evening control)	6.12±2.18	6.27±2.44	5.96±2.58

*+ Denote significant with pre test (p<0.05) ; - Denote significant between experimental groups and control group1 (p<0.05); * Denote significant between experimental groups and control group2 (p<0.05)*

The results of one way analysis of variance with Tukey's post hoc test showed a significant

difference between morning training and control groups in the amount of serum IL-6 and CRP after 12 weeks of aerobic training. This was in a way that, after 12 weeks of exercising, serum IL-6 and CRP of the morning training group were 76.55% and 46.76% less than those of the morning control group, respectively (p=0.002CRP and p= 0.001 IL-6). Moreover, the obtained results showed a significant difference in the amount of serum IL-6 and CRP in the evening training group compared with morning control and evening control groups after 12 weeks of training (CRP of the evening training group was 44.88% and 35.48% less than that of morning and evening control groups and IL-6 of the evening training group was 82% and 75.17% less than that of morning and evening control groups, respectively). (CRP of the morning and evening training groups (p=0.001 and p=0.002, respectively) and IL-6 of morning and evening training groups (p=0.001 and p=0.002, respectively) in the pre test (week 0) and middle test (week 6) did not demonstrate a significant difference at the levels of research variables between training and control groups (p>0.05).

As far as TNF-α is concerned, the results of analysis of variance with repeated measures did not indicate a within group significant difference in the control and training groups. Although its amount decreased in morning and evening groups after 6 and 12 weeks of training, this decrease was not significant. Furthermore, the results of one way analysis of variance showed a significant between group difference between training and control groups at three test stages (p>0.05).

DISCUSSION

The results of the present research showed that 6 to 12 weeks of aerobic training with medium intensity in obese men increased VO2max, decreased body fat percentage and BMI in morning and evening training groups. Moreover, a decrease in the CRP and IL-6 inflammatory markers was observed as a result of 12 weeks of aerobic training with medium intensity in obese men. This was in a way that, after 12 weeks of aerobic training, the difference in the amount of serum IL-6 and CRP between the morning training and control groups and in the evening training group compared with morning and evening control groups significantly decreased. These results were in line with previous reports showing that aerobic training decreases serum IL-6 and CRP [4, 5]. In the first 6 weeks of aerobic training in morning and evening training groups, the changing rates of CRP and IL-6 inflammatory markers were not noticeable, which probably showed the effectiveness of exercise duration, exercise period and intensity on these markers; an increase in exercise duration caused a significant decrease in the amount of CRP and IL-6 of training groups after 12 weeks, which was in line with the findings of Kohut et al (2006), Kadoglou et al (2007), Walther et al (2008), Nicklas et al (2008), Campbell et al (2009) and Christiansen et al (2010)[6-11]. Nevertheless, there was a difference between the findings of the current research and those of Hammett (2004), Fairey (2005) and Arsenaulet (2009) [1, 13, 14] which can be attributed to the differences between the studied groups, their race [20], exercise duration, exercise intensity, period and type [4]. In some studies, more decrease in the amount of CRP has been reported for men than for women. Also, a study has stated that IL-6, TNF-α and their receptors are higher among African Americans than among the white [21]. Evidence has shown than the more the basic amount of inflammatory markers, the more evident the effect of exercise on these markers [12].

Furthermore, the results obtained from this study showed a decrease in the level of CRP and IL-6 in the evening compared with the morning. Although this decrease was not statistically significant in the evening training group in the first, sixth and twelfth weeks, the amount of CRP was 17.1%, 10.5% and 18.87% and the amount of IL-6 was 21.5%, 16.74% and 25.32% less than those of the morning training group. Also, the evening control group showed less inflammatory

markers compared with the morning control group which was not statistically significant ($p>0.05$). These results were in line with the findings of Piccionea, Aldmir and Pledge [15-17].

As far as TNF-α is concerned, the results obtained from this research did not show a significant within group difference in the amount of serum TNF-α. In the morning and evening training groups, TNF-α decreased 2.68% and 12.65% after 6 weeks of aerobic training and 20.90 and 23.76% after 12 weeks of training, respectively, and these results were in line with the findings of Fairey (2005) and Arsenaulet (2009) [13, 14]. Also, the amount of serum TNF-α in the evening was less than its amount in the morning; the amount of TNF-α in the evening training group in pre test, middle test and post test stages was 13.77%, 22.95% and 13.19% less than its amount in the morning training group, which was not significant between the two groups in none of the stages.

Therefore, the results obtained from this research showed a significant decrease in the amount of CRP ($p=0.002$) and IL-6 ($p=0.002$) after 12 weeks of aerobic training in the morning and evening while it was not significant for the TNF-α.

On the other hand, it was determined that inflammation mechanisms have a key role in pathological processes of several chronic diseases like coronary heart diseases, cancer, diabetes type 2 and chronic obstructive lung. It seems that Chronoc low- grade inflammation is specified with high levels of CRP, IL-6 and TNF-α [4, 22] and the protective mechanism of heart can be proposed for the relationship between physical activity and lower levels of inflammation. A common concept in terms of pathophysiological mechanisms of inflammation associated with atherosclerosis is the production of cytokines associated with inflammation in response to the oxidized LDL stimulus and macrophages associated with atherosclerotic plaque [5, 12]. The cytokines associated with inflammation which are produced during this process include IL-1B, IL-6 and TNF-α. It was determined in laboratory experiments that different combinations of cytokines stimulate the production of CRP and Leukocytosis [23]. Research has shown that regular sport activities decrease oxidized LDL and serum levels of IL-6 and CRP [24, 25]. Therefore, the influence of regular exercise on IL-6 levels can be responsible for the decrease of CRP in the experimental groups. On the other hand, the relationship between physical activities and lower levels of inflammation can be created by the relationship between endurance training and lower degrees of general and abdominal obesity. It has been shown that obese people produce higher levels of inflammatory markers like IL-6, IL-8 and TNF-α compared with thin control people [26]. Endurance training can decrease the production of inflammatory mediators from fat tissues and increase the production of anti-inflammatory mediators like IL-10 from the fat tissue by directly influencing the fat tissues and increasing lipolysis (through increasing the activity of hormone-sensitive lipase) [22, 27]. The result of these changes is that aerobic training can decrease the amount of circulating inflammatory markers (CRP) by decreasing inflammation resources. Also, fat tissues are considered as endocrine organs due to secretion of different materials like IL-6 and TNF-α [28]. It is likely that TNF-α stimulates the production of IL-6, which is a strong stimulus of producing liver CRP [29]. Therefore, more fat tissues in obese men lead to higher serum CRP levels (in a cascade way). Moreover, TNF-α is one of the most basic and most original mediators of inflammatory processes which is mostly stated in fat tissues (especially visceral fat) and its levels in blood circulation indicate its production in body tissues [22]. There are different findings on the influence of sports on TNF-α level in a way that some have reported its decrease [27] and some others its lack of change [30] in response to sport exercises. In the current study, it was determined that there was no change in the level of serum TNF-α in response to the 12-week evening and morning aerobic training because the half-life of TNF-α is low in blood [21]. Therefore, according to the current findings, TNF-α cannot be

considered a stable marker for the inflammatory condition. So, it is recommended that CRP be used which, to some extent, show a systematic inflammation condition [31]. CRP is an inflammatory indicator which is made by liver cells and in response to inflammatory factors and is secreted from the liver [32]. Thus, the present study is in line with the investigations which have shown that there is a negative correlation between physical fitness and chronic inflammation, and sport activities decrease the inflammatory condition, CRP and IL-6 [13]. So, increase of inflammatory markers as a result of obesity can be related to atherosclerosis [27, 33].
In addition to this, the lower amount of inflammation caused by compatibility with sport activities can be attributed to the anti-oxidative effects of sport activities. Although, in this study, the amount of anti-oxidants was not measured, research evidence showed that aerobic training considerably decreases oxidative stress by increasing the body's anti-oxidant capacity [34]. Probably, regular sport exercises inhibit the release of TNF-α, IL-6 and IL-1β inflammatory mediators from fat tissues by decreasing the stimulation of sympathetic system and increasing the anti-inflammatory cytokines and, after that, the concentration of cell adhesive molecules decreases [34, 35].

In general, considering the findings of this research, it was found that regular and prolonged aerobic physical activities lead to a significant decrease of CRP and IL-6 as new predictor indicators of coronary heart events in the morning and evening and, finally, cause a decrease in the body's general inflammation. Moreover, with regard to the findings of the current study, performing exercises in the evening can be considered more appropriate than doing them in the morning because the level of inflammatory markers is lower in the evening. Therefore, it is recommended to obese people to exercise in the evening in order to decrease the risk of coronary heart attacks to the minimum possible level.

CONCLUSION

In sum, it can be stated that doing aerobic training in the morning and evening can decrease inflammatory markers and probably decrease the risk of future coronary heart events in obese men. Moreover, considering these results, doing exercises in the evening can be considered more appropriate. Therefore, it is recommended to young obese men to do aerobic exercises in the evening.

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