

## Effects of an aerobic exercise and milk consumption on vascular inflammation in obese pre-pubertal children

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### Abstract

The aim of present study was to determine the effects of eight weeks of aerobic exercises and milk consumption on vascular inflammation in obese pre-pubertal children. 28 obese pre-pubertal boys (8-10 years) (BMI) were selected based on self nutrition, athletic performance, body mass index, physical health questionnaire among volunteers. Then, subjects were divided into four groups of exercise- supplement group, exercise group, control-supplement group and control group. The training program consisted of 8 weeks, Recipient group of milk supplement consumed 300 ml milk per day. Blood samples were taken before and after 8 weeks and 12 hours from fasting time for analysis of ICAM, VCAM and E-Selectin. Data analysis was performed using factor analysis of variance. Results showed that ICAM and VCAM were reduced significantly after 8 weeks in exercise, exercise-supplement, control and control- supplement groups. The results of variance analysis showed that there is significant difference between four groups, so there is difference between exercise - supplement and control- supplement. Also, E SELECTIN was reduced significantly in exercise and in exercise - supplement, in control and control- supplement groups. The results of variance analysis showed that there is significant difference between four groups so that there is difference between exercise group and control- supplement group and exercise- supplement group and control group and control - supplement group. In general, results showed that aerobic exercise and milk consumption may be considered as a non-pharmacological method for controlling obesity and preventing cardiovascular diseases through reducing endothelial adhesive molecules of obese pre-pubertal children.

**Keywords:** aerobic exercises, obese, vascular inflammatory factors

### INTRODUCTION

Obesity is a metabolic disorder that is characterized by an increase in body fat that is an important risk factor for disease [1]. Also, obesity is a complex reaction among genetic, physiological and social- economic- cultural factors [2].

The prevalence of obesity among 7- 13 years old Canadian children between 1981 and 1996 became threefold and increased from 5 percent to 15 percent. While the results of various studies conducted in Iran showed that the prevalence of obesity among children 6-12 years old was between 7-16 percent [3].

Scientific evidences indicate cardiovascular risk factors during childhood, particularly with respect to the pattern of changes in body mass index (BMI) and lifestyle [4].

There are evidences indicating that some people despite having normal blood cholesterol in high density lipoprotein (HDL) and low density lipoprotein (LDL) are prone to the risk of cardiovascular disease, therefore other sensitive indicators must be evaluated clinically in order to identify clinically individuals who are exposed to risk of Coronary heart disease (CHD) [4]. The plasma level of adhesion molecules and lipid profile are considered as important indicators in estimating risk of cardiovascular disease [5].

Studies show that inter cellular adhesion molecules (ICAMs) increase the activity of vascular endothelial and have more sensitivity and accuracy in predicting cardiovascular disease compared to lipid profile and have been introduced as new inflammatory markers in order to predict cardiovascular disease that play an important role in pathogenesis of Arthritis Sclerosis [6]. Previously, it was assumed that vascular endothelial cells are inactive and act like a semi-permeable barrier between blood and tissue. But now there are growing evidences that focus on role of vascular endothelial in maintaining human homeostasis, for example, endothelial cells in response to their activation produce adhesion molecules such as Inter cellular adhesion molecule (ICAM), Vascular adhesion molecule (VCAM)- Endothelial –selectin molecules (E-Selectin) [7].

Also, due to the increasing epidemic of cardiovascular disease and high financial and economic costs of treating these diseases, development of preventive measures, non-pharmacologic sports and treatment of this disease have always been of interest to researchers in various sciences. The sport physiology researchers attempt to develop the most effective and efficient executive methods of physical practices in order to upgrade and update recommendations for physical activities as an important part of healthy lifestyle and integral part of predicting and treating many diseases associated with lack of movement including cardiovascular disease [6].

Gerey et al (2008), investigating the effects of 12 weeks of aerobic trainings on patients with chronic heart failure observed that aerobic exercise has no effects on ICAM activities. These studies observed the lack of exercise effects on ICAM- 1 inflammatory markers [7]. Loeis et al (1999) reported a significant positive relationship between triglyceride levels and ICMA - 1 in a study on 948 healthy volunteers with no history of heart disease [6].

Fat tissues cause cytokine secretion in blood. Cytokine effects on endothelial function and stimulate the production of adhesion molecules. These molecules also play role in cardiovascular and atherosclerosis disease [8].

The results focus on importance of creating simple solutions in order to increase to and maintain milk consumption among children. Creating food habits that includes frequent consumption of milk may result in increased intake of vitamin D and calcium in subsequent years that in turn will reduce risk of chronic diseases such as osteoporosis, obesity, high blood pressure and cancer [9].

In recent years, milk consumption has declined among children; this may play an important role in childhood obesity, because clinical studies show a beneficial effect of milk on weight control [10].

On the other hand, exercises theoretically effect on fatty tissues and reduce the storage of fat cells, reduced fat tissue decreases the secretion of cytokine and as a result the production of adhesion molecules will be reduced [8].

According to the fact that most of researches are done separately in the field of exercise and supplement and their results are inconsistent and also given that no researches have been simultaneously done on effect of exercise and supplement consumption on vascular inflammation, especially in obese children, this study intends to investigate simultaneously effects of exercise and consumption of high-fat dairy products on vascular inflammatory markers in obese pre-pubertal children.

## MATERIALS AND METHODS

The present study is a quasi-experimental research, using pretest-posttest design with a control group that was done in Gonbad city during year 2011. 45 male students voluntarily declared their readiness to participate in this study and subjects were selected according to BMI. Statistical sample of study included 28 obese pre-pubertal between 8 to 10 years old with a body mass index greater than 95 percent. Subjects were examined medically in order to study their physical health and personal and family history, medications, exercise levels and dietary intakes were received through interviews and completing questionnaires. After necessary studies, participants and one of their parents signed consent form including necessary comments and awareness of exercise, nutritional programs, drinking milk

supplements and possible risks and discomforts and then exercise, nutritional programs and dietary recommendations were provided to subjects during a training session in verbal and written form. They were randomly divided into four groups including exercise, milk, exercise+ milk and control (each group constituted of 7 patients).

#### **Aerobic exercise program**

The exercise program consisted of three sessions per week for 8 weeks. Each session lasted 90 minutes, including 20-minute for warm-up through performing mild running and static and dynamic stretching and 60 minutes rhythmic movements associated with Karate sport and 10 minutes for cooling the body. The exercise protocol was conducted under the supervision of researcher and colleagues. Exercise program was began with intensity of 45% maximum heart rate based on formula of  $(220 - \text{age} = \text{maximum heart rate})$  and 5% of intensity was added every two weeks until it rose to 60 percent of maximum heart rate last week. Exercise intensity was controlled using a digital wrist sphygmomanometers made in Japan.

#### **Nutrition and supplementation of milk plan**

The initial information are received based on anthropometric results during first session and competing nutrition questionnaires and daily physical activity and nutrition plan was prepared in a surveys, benchmarking nutritional protocols that are used in a similar study by Avnchy et al in 1990 [11].

In this plan, food is divided into three main categories, first category includes foods that are unlimited in amount and time of food such as fruits and low-fat vegetables, the second category includes low-fat protein material (Fat <5%), low-fat milk and dairy products (Fat <5%), low fat cereal (Fat <3%), vegetable and starchy grains (Fat <3%) with limitation in energy production and consumption as five times a week and three main meal times in day and 2 to 3 snacks a day, third category includes fatty protein (fat > 5%), fats, vegetables and cereals (fat <3%) and other similar energetic materials with the limitation of one times a day or dividing the meal in different times a day or dividing consumption over a week. Holding briefings and training sessions, all participants were taught how to read food labels, identify food materials and nutrition one week before the study began and at least one parent of boys attended during the training and orientation programs. Completing the present plan, each group program was set separately to use drink supplements in combination of basic and daily foods so that both groups of milk supplement consumers receive energy equally through using supplemental drinks during a week. In this program, milk consumer groups received 300 ml of fatty milk 3 times a day. All groups were monitored and observed by expert doctors during eight-week program.

Dietary program of control group was 3000 calories and of exercise group was 3000 calories per day: exercise group: 714 g carbohydrate, 76 g protein and 5 g fat, control group: 469 g carbohydrate, 245 g protein and 7 g fat.

#### **Blood sampling method**

Blood sampling was done in a state of rest with a specialist to assess and measure the variables of ICAM, VCAM, E Selectin.

Blood samples were taken from subjects in fasting form, 12 to 14 hours after the last meal in the morning, and similarly, the research program was repeated 48 hours after the last session and samples were transported to the laboratory at -70 ° C under standard conditions.

#### *Measurement of biochemical parameters*

Measurement of plasma levels and biochemical analysis of ICAM- VCAM- E-Selectine amounts were done using commercial ELISA kits and according to the manufacturer's instructions (Rotary Hetich made in Germany) and sensitivity of analyzing above variables was 0.2- 0.6- 0.5 ng/ mL, respectively.

#### **Statistical Methods**

Descriptive statistics including mean, standard deviation and inferential statistics were used for statistical analysis of this study.

Kolmogorov- Smirnov test was used to determine the normal distribution of data distribution and Levine test was used to investigate the homogeneity of variances. T-test and two-factor variance analysis (2factor  $\times$  4) were used in order to study the intra-group changes and intergroup differences of variables and in the case of statistically

significant difference, Tukey Post Hoc test was used to determine intergroup differences. Also, Pierson correlation test was performed to evaluate the correlation between the changes of vascular inflammation factors. Statistical operations was performed by Spss software version 16 and significance level of test was considered as ( $p < 0.05$ ).

**RESULTS**

ICAM (figure1) showed a significant reduction after eight weeks exercise in exercise group ( $p = 0/002$ ,  $t = 5/09$ ) and exercise-supplement group ( $p = 0/000$ ,  $t = 7/25$ ), control group ( $p = 0/012$ ,  $t = 3/ 54$ ) and control- supplement group ( $p = 0/0001$ ,  $t = 5/55$ ). Also, results of variance analysis showed that there is significant difference among four groups ( $p = 0/02$ ), so that the difference between exercise- supplement and control- supplement ( $p = 0/013$ ).

Vcam (figure 2) was reduced significantly in exercise group ( $p = 0/001$ ,  $t = 2/44$ ), exercise-supplement ( $p = / 000$ ,  $t = 2/44$ ), control group ( $p = 0/017$ ,  $t = 2/44$ ) and control - supplement ( $p = 0/001$ ,  $t = 2/44$ ) groups. The results of variance analysis showed that there is significant differences among 4 groups ( $p = 0/26$ ) so that there is difference between exercise group and exercise- supplement group ( $p = 0/22$ ).

E selectin (figure3) rate was significantly reduced in exercise group ( $p = 0/001$ ,  $t = 2/44$ ), exercise- supplement group ( $p = 0/000$ ,  $t = 2/44$ ), control group ( $p = 0/012$ ,  $t = 2/ 44$ ) and control- supplement group ( $p = 0/000$ ,  $t = 2/44$ ). The results of variance analysis showed that there is significant differences among four groups ( $p = 0/000$ ) so that there is difference between exercise group and control- supplement group ( $p = 0/007$ ) and exercise- supplement group and control group ( $p = 0/004$ ) and control- supplement group ( $p = 0/000$ ).

Figure 1

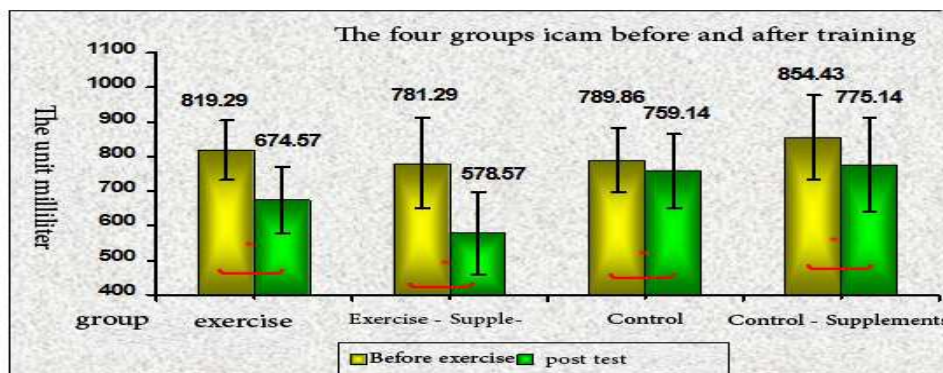


Figure 2

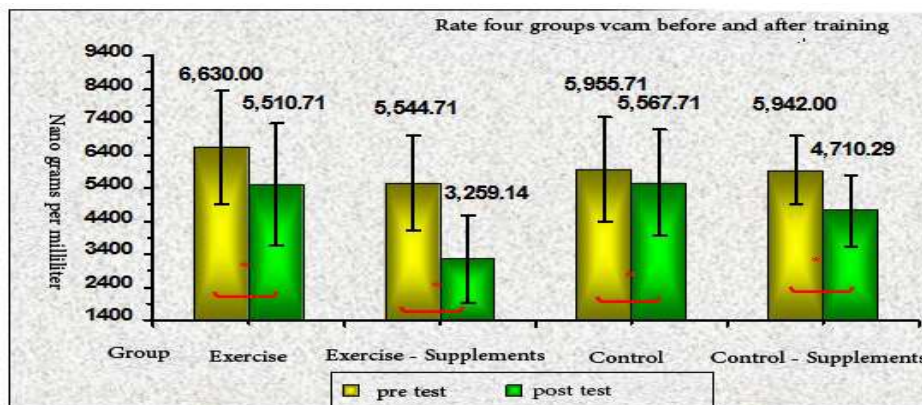
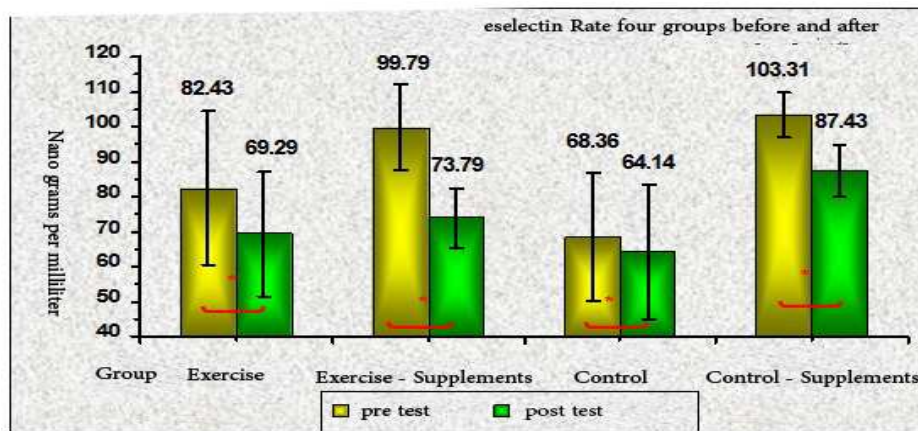


Figure 3



### DISCUSSION AND CONCLUSION

Today, obesity is a major concern for governments and major challenge for public health and simultaneous use of two terms of epidemic and obesity in scientific literature reflects this fact [12].

Results of present study is consistent with results of Moqdasani (2007) study based on reduced amount of ICAM-1 through aerobic exercise, it seems that uniqueness of results is due to gradual increase of exercise load and compatibility with exercise [13].

Also, these results are consistent with results of Ito (2002) and Pontiroly (2004) study, it seems that similarity is due to selecting homogenous groups in terms of age and body type and emphasizing this point can be as an appropriate method to resolve inflammation and to reduce the risk of cardiovascular disease. Citro et al (2011) [10] reported decrease of VCAM-1, ICAM-1 and E-selectin in another study after aerobic exercise in patients with (PAD). In all types of studies, exercises are aerobic that are similar to type of study exercises and type of exercises may lead to same results in all of these studies.

Khalesi and colleagues achieved similar results to previous results in their study on male non-athlete students, and decreased ICAM-1 and lipid profile were reported following 8-week aerobic exercises [14]

Increased fat mass results in increased production of pro-inflammatory factors (adiponectin, leptin, lipoprotein Colin, IL-6, IL-18, TNF  $\alpha$ ) and reduced secretion of anti-inflammatory factors [11,15]

Exercise reduces the risks of obesity, increases secretion of anti-inflammatory cytokines from skeletal muscles (Wood, 2012) and reduces gene expression of monocytes and macrophages through increasing energy consumption and burning body fat [15].

Another anti-inflammatory effect of exercise is through increasing the secretion of IL -10 as anti-inflammatory cytokines from adipose tissue. It is reported that exercise decreases the production of TNF  $\alpha$  with intensity of 75%  $VO_2^{max}$ . According to the fact that adhesion molecules secretion will be more significant by increased inflammatory cytokine secretion, in their study, the lipid profile of individuals was normal from the beginning, ICAM-1 as well as lipid profile are influenced by intensity of exercise. Plasma levels of this factor was at normal levels from the beginning, so it was not affected by exercise, however, initial levels of inflammatory factors in obese children who we have examined was high from the beginning and have been affected by intensity and type of exercise and reduce inflammation was observed after exercise [16].

Sabatir et al (2008) reported that 14 weeks of intensive aerobic activity did not result in significant changes in ICAM-1 in healthy women; it is noteworthy that in their study, resistance to blood flow was reduced by 28% and femoral artery

diameter showed an increase of 12 percent. This result is inconsistent with previous findings based on decline in levels of adhesion molecules and reduced the shear stress induced by exercise [17].

Increased shear stress increases the expression of adhesion molecules and releasing them in endothelial lining of vein, the reason for this contradiction may be due to increase of other factors such as, IL-1 $\beta$  and TNF $\alpha$  that will increase and release adhesion molecules [18].

Birc jeland et al (2011) suggested that exercise for 12 months (work with ergometer bicycle) caused no significant increase in ICAM-1 and CRP in patients with coronary artery disease [19].

Singorle et al (2003) reported that aerobic exercises (walking on a treadmill) increases E- selectin, ICAM-1, TNF, VCAM-1, L- selectin in patients with CAD compared to resting state, he stated in his research that vascular damage has been likely present in these patients and has affected negatively on vascular function and hemodynamic and leads to changes in macrophage function in these patients. Previous studies have shown that exercise balances the interaction between blood cells and endothelial, but in this research, exercise on treadmill results in acute ischemia and pain of individuals [20].

Mogharnasi et al (2010) in a study on rats reported the reduced lipid profile, TNF  $\alpha$  and ICAM-1 in these rats after 36 weeks of endurance exercise; it seems that exercise can increase C- HDL of plasma, in addition to the mechanisms mentioned above [21]. This substance causes the prostacyclin secretion from vessel wall or smooth muscle cells and inhibits platelet aggregation and reduces adhesion molecules [22].

In addition, exercise results in changes in endocrine system secretion including Cortisol, growth hormone, epinephrine and nor-epinephrine, the most important consequence of this change is increased lipolysis and using fatty acid as an energy source for body, reducing fat and working mass in order to reduce cytokine and adhesion molecules. Also, cortisol plays role as anti-inflammatory hormone as [11, 23].

reported that exercise and low-fat diet significantly decrease body mass and factors of VCAM-1, E- selectin and ICAM-1 in these individuals after study on diabetic patients. They stated in their study that oxygen free radicals induce inflammatory mediators and adhesion cell molecules. Exercise with increased antioxidant defense results in decreased oxygen free radicals and subsequently reduced rate of adhesion cells [24].

Obtained results similar to previous study results, after studying effects of aerobic exercises on inflammatory factors in patients with myocardial infarction [25].

reported the increased levels of adhesion molecules after severe exercises, they explained the reasons of contradictions in this way that severe exercises result in damages of muscle and connective tissue physiologically, that in turn leads to increased gene expression of adhesion molecules such as Selectin and other inflammatory factors [26]. The exercise type of our study is moderate-intensity aerobic exercise; long term exercise reduces inflammation, while short-term exercises exacerbate inflammation.

stated that participation in endurance exercises is associated with no changes in E- selectin, ICAM-1, VCAM-1, TNF  $\alpha$  in obese individuals, this is due to the low number of subjects participating in study because finding obese people who are able to perform endurance exercises is difficult, in contrast, type and intensity of our study aerobic exercises are based on individuals' power [27].

Research results showed that VCAM-1, ICAM-1, E selectin in patients with cardiovascular problems is higher than healthy men [28].

In current study, the rate of these factors in control group is higher than exercise group. According to research results, regular physical activities are inversely related to inflammatory markers and suppress low-grade inflammation [28].

According to the findings of this research, 8 weeks of increasing aerobic exercises in addition to consuming milk leads to significant reduction in concentration of ICAM-1, VCAM-1 and E - Selectin of obese pre-pubertal children.

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