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# Impact of Aerobic Exercise Training on Insulin Resistance and Plasma Lipocalin 2 levels in Obese Young Men

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**ABSTRACT**

Obesity has been known as major public health problem, was reported to be associated with insulin resistance, type 2 diabetes mellitus and cardiovascular disorders. Therefore the purpose of this study was to examine the effects of Aerobic exercise training on Insulin Resistance and plasma lipocalin2 (as Lcn2) in obese young men. Thirty healthy young men (aged 27.83 ± 1.69 years, height 1.71± 5.37 cm, BMI 30.1 ± 1.49 kg/$m^2$, mean ± SD) participated as subjects in this study. The subjects were randomly assigned to aerobic training group (n=15) or control group (n= 15). Aerobic training group underwent an 8-week intervention, with a frequency of 3 d/wk at an intensity corresponding to 65 – 80% maximum heart rate for 35 – 55 min. The results showed that body fat percent, WHR, BMI, were decreased ((P<0.05), in the training group compared with control group. Maximum oxygen consumption ($VO_{2max}$) on the other hand, increases significant (P<0.05) in the training group compared with the control group. Adipokin lcn-2, LDL-c, TG, TC, and insulin resistance determined by HOMA-IR decreased (P<0.05) and HDL-c increased (P<0.05). hs-CRP, did not change in the aerobic training group compared with the control group**.** It seems that 8 weeks aerobic training induced change in adipose tissue, decrease plasma Lipocalin2 and improve insulin resistance in young obese men.

**Key words**: Aerobic training, Lipocalin2, Insulin Resistance, obese men.

## INTRODUCTION

One of the most prevalent and well documented health problems in adults of the developed world is obesity. It is a serious health problem for increases the risk of developing cardiovascular diseases, type2 diabetes, hyperlipidemia, hypertension, and increased mortality (Yang, 2005). It's also an increase in fasting glucose, blood pressure, triglycerides and a decrease in high density lipoprotein (HDL) So it's known as metabolic syndrome (Wang, 2007). The increasing prevalence of obesity in individuals is linked with the metabolic syndrome which greatly increases disease risk. Studies have demonstrated close associations between obesity and increased circulating concentrations of proinflammatory molecules, including acute-phase proteins, cytokines, adipokines, and chemokines (Tataranni. 2005; Weisberg, 2003). In obese states, these proinflammatory factors are produced predominantly from enlarged adipocytes and activated macrophages in adipose tissue and liver. Many of these inflammatory factors, such as interleukin-6 (IL-6), tumor necrosis factor-$\alpha$ (TNF-$\alpha$) and hs-CRP, can directly induce glucose intolerance and insulin resistance by antagonizing insulin's metabolic actions at peripheral tissues, especially in liver and skeletal muscle (Fantuzzi, G. 2005). Insulin resistance usually connotes resistance to the effects of insulin on glucose uptake, metabolism, or storage. Insulin resistance in obesity and type2 diabetes is manifested by decreased insulin-stimulated glucose transport and metabolism in adipocytes and skeletal muscle and by impaired suppression of hepatic glucose output. Adipokin Lipocalin2 (Lcn2) also

known as neutrophil gelatinase associated lipocalin, sidrocalin and 24p3, is another member of the lipocalin family recently reported to have possible metabolic roles. (Fantuzzi, G. 2005). Lipocalin2 is expressed in many tissue, including neutrophils, macrophage, kidney, liver, lung, thymus, small intestine mammary tissue as well as adipocytes and is known to play a role in inflammation. Lcn2 has been recognized as an adipocyte drive acute phase protein that is positively correlated with potential effect in obesity inflammation and insulin resistance in mice and humans (Choi. 2009; Wang, 2007). It also has been showing that circulating levels of this adipokin has a strong direct correlation with other inflammation factors as an acute phase protein (Van Dam. 2007). With the increased prevalence of obesity and insulin resistant disorders, research has attempted to elucidate the potential mechanisms driving these disease processes, with the hope of ultimately providing cost effective interventions. Aerobic exercise training has been well documented to improve glucose tolerance and insulin action in patients who are obese (Goodpaster, Kelley, 2003).The lack of physical activity in daily life induces obesity and increases the risk of hypokinetic diseases; diabetes mellitus, hypertension, heart diseases etc. It's also well known as the cornerstone treatment for obesity-related metabolic complications, including insulin resistance, hypertension, impaired glucose tolerance or diabetes, hyperinsulinemia, and dyslipidemia, that are characterized by elevated adipose accumulation (Hu, 2001; Tuomilehto. 2001). Therefore one of the best strategies for preventing obesity and its associated inflammation is participation in regular physical activity (Petersen & Pedersen, 2005). On the other hand, exercise has been shown to have beneficial effects on obesity, type2 diabetes and the metabolic syndrome. Although the changes in Lcn2 levels might be an important clue for understanding the beneficial effects of exercise, data on exercise-induced changes of inflammation factors such as adipokin Lipocalin2, insulin resistance and hs-CRP…is still unclear. Recently, Damirchi (2011) reported that Lcn2 increased after single bout graded exercise in obese and normal weight men. Choi et al (2009) in an only available study, isn't reported that any change in Lcn2 level in obese women after 12 weeks moderate exercise training. The physiological and biochemical responses to resistance exercise are different from those exhibited in response to aerobic exercise (Kraemer, 1994). Despite the numerous studies demonstrating the benefits of exercise training intervention in obesity and/or insulin resistant states, there are no studies to date that have examined the effects of this aerobic exercise protocol on obese young men. In order to develop appropriate treatment programs in obese patients, we need to understand how exercise affects insulin resistance and inflammatory adipokin secretion in this disease process, Therefore the present study was designed to determine the effects of aerobic training on insulin resistance, hs-CRP and Lcn2 concentration in obese young men.

## MATERIAL AND METHODS

### Subjects

Thirty healthy and university students aged (aged 27.83 ± 1.69years, mean±SD) enrolled in this study. The inclusion criteria were men who had body mass index (BMI) e"29.9 kg/m$^2$ did not engage in regular exercise training at the time of their enrolment. Student who were afflicted with heart diseases, hypertension, pulmonary diseases and diabetes, who needed orthopedic treatment, and who had neurological limitations to physical exercise were excluded. All the subjects were asked to complete a personal health and medical history questionnaire, which served as a screening tool. The subjects were given both verbal and written instruction outlining the experimental procedure, and written informed consent was obtained. All the subjects completed the 3-day diet recall forms and were instructed to maintain their normal physical activity and dietary habits throughout the study. The subjects were randomly assigned to one of the experience group (n=15) and control group (n=15)

### Exercise training

The participant's of experience group (aerobic training) underwent three exercise training sessions per week for 8weeks. The training exercise consisted of a 10-minute warm-up period, as well as muscle stretches. It's also consisted of walking and running at 65-80% of maximal heart rate ($HR_{max}$) for 35-55 min per day, 3 days per week, for 8 weeks. The programme started with 30 min running for the first few sessions, and this was then changed to 45 min per session until the end of training. Each training

session finished with a cool down. The exercise intensity was controlled by the authors, using a hear rate monitor, who ensured that it was between 65 and 80% of $HR_{max}$ throughout the trial.

**Measurements: (Anthropometric and body composition measurements)**

Height and body weight were measured, and body mass index (BMI; kg/m2) was calculated from height and weight of each subject. Waist circumference was determined by obtaining the minimum circumference (narrowest part of the torso, above the umbilicus) and the maximum hip circumference while standing with their heels together. The waist to hip ratio (WHR) was calculated by dividing waist by hip circumference (cm) (ACSM, 2005). Subcutaneous body fat was measured at 3 sites (chest, abdominal, and thigh) with a Lafayette caliper. Body fat percent was calculated from the formula developed by Jackson and Pollock (1985) .$VO_{2max}$ was determined by Rockport One-Mile fitness walking test. In this test, an individual walked 1 mile (1.6 km) as fast as possible on a track surface. Total time was recorded and HR was obtained in the final minute (ACSM, 2005) $VO_{2max}$ was calculated by following formula:

$VO_{2max =}$ [139.68-(0.388×age (year))] - [0.077× body mass (pb)] - [3.265×time (min)] - [0.156× HR].

**Biochemical analyses**

Approximately 10 milliliters of blood was collected into plain and EDTA filled vacutainer tubes after an overnight fast of at least 12 hours at the same time before and after 8 weeks intervention. The tubes were then centrifuged and serum and plasma were drawn off and stored at -80°C until analysis. Plasma glucose was determined by the enzymatic (GOD-PAP, Glucose Oxidase-Amino Antipyrine) colorimetric method (Pars Azmoun, Tehran, Iran). The intra and inter-assay coefficients of variation for glucose were<1.3% and a sensitivity of 1 mg/dl. The serum insulin level was measured by a radioimmunoassay (RIA) and the insulin resistance index was calculated according to the homeostasis model assessment (HOMA-IR) which correlates well with the euglycemic hyperinsulinemic clamp in people with diabetes (Matthews, 1985). hs-CRP levels were determined in duplicate via an ELISA kits (Diagnostics Biochem Inc, Canada). The intra and inter-assay coefficients of variation for hs-CRP were <5.7% and a sensitivity of 10 ng/ml. The adipokin Lcn2 level was measured in duplicate using an enzyme-linked immunosorbent assay (ELISA) kits (Uscn Life Science Inc, Wuhan, China). The sensitivity of kit was 0.12 ng/ml. Serum cholesterol, triglycerides, HDL-c and LDL-c were assayed with automated techniques.

**Statistical Analysis**

Statistical analyses were performed with SPSS program (version 16, SPSS, Inc., Chicago, IL). Values were expressed as mean ± standard deviation (SD). Independent t-test and paired t-test were used to evaluate changes in variables. General linear regression analysis and Pearson's correlation were performed to calculate a correlation between variables in response to training. P-values less than 0.05 were considered statistically significant.

## RESULTS

Anthropometric, physiological and metabolic characteristics of subjects are shown in Table 1. The results showed that body weight, body mass index (BMI), body fat percent and WHR were decreased ($P<0.05$) after aerobic training. Maximum oxygen consumption, on the other hand, increases significant ($P<0.05$) in the training group compared with the control group. Plasma lipocalin-2, LDL-c,TG, TC, and insulin resistance determined by HOMA-IR decreased ($P<0.05$) and HDL-c increased ($P<0.05$) after 8 weeks aerobic training (Table 1).For hs-CRP, there was no significant different between aerobic training group and control group after 8 weeks exercise. Pearson's correlation demonstrated a positive relationship between, adipokin (Lcn-2) levels at baseline ($P<0.05$) with body fat percent, WHR and BMI. No significant relationship between HOMA-IR with biochemical variables were found in the endurance group after 8 weeks intervention.

## DISCUSSION

Insulin resistance can be defined as a condition in which normal concentrations of insulin produce an inadequate metabolic response by insulin sensitive tissues (skeletal muscle, liver, and adipose tissue) (Kahn, 1978). The hallmarks of impaired insulin sensitivity in these three tissues are decreased insulin-stimulated glucose uptake into skeletal muscle, impaired insulin-mediated

**Table 1: Anthropometric and metabolic characteristics of study subjects (mean ± SD)**

| Variables | Control group | | Training (Endurance group) | |
|---|---|---|---|---|
| | Pre test | Post test | Pre test | Post test |
| Body weight (kg) | 81.30 ± 6.76 | 81.29 ± 6.40 | 83.65 ± 7.04 | 80.90 ± 6.77 * |
| BMI (kg/m$^2$) | 28.49 ± 10.45 | 28.50 ±10.43 | 28.67 ± 1.50 | 27.67 ± 1.41 * |
| %Body fat | 23.56 ± 1.53 | 23.36 ± 1.65 | 23.66 ± 2.22 | 22.48 ± 2.0 * |
| WHR | .90 ± .03 | .90 ± .03 | .93 ± .02 | .89 ± .03 * |
| $VO_{2max}$ (ml.kg$^{-1}$.min$^{-1}$) | 35.76 ± 3.37 | 35.96 ± 3.23 | 35.9 ± 2.77 | 43.68 ± 2.39 *† |
| Triglyceride (mg/dl) | 1.61± 2.03 | 1.58 ± 2.03 | 1.71± 4.56 | 1.48 ± 4.41 |
| Cholesterol (mg/dl) | 1.89 ± 2.7 | 1.88 ± 2.6 | 1.96 ± 4.22 | 1.85 ± 3.33 |
| LDL-c | 1.27 ± 2.57 | 1.27 ± 2.39 | 1.32 ± 3.1 | 1.24 ± 2.74 * |
| HDL-c | 38.01 ± 4.98 | 38.23 ± 5.27 | 35.4 ± 6.81 | 42.1 ± 6.51 * |
| Lipocalin2 (ng/ml) | 23.56 ± 2.26 | 23.02± 2.8 | 23.79 ± 2.82 | 19.33 ± 2.45 *† |
| HOMA-IR | 52.56± 12.54 | 51.41± 11.93 | 55.65 ± 10.99 | 54.12 ± 12.66 |
| Hs-CRP | .83 ± .88 | .86 ± .88 | .78 ± .73 | .72 ± .55 |

* P<0.05 for between-group differences.

† P<0.05, pretraining vs. posttraining values.

inhibition of hepatic glucose production in liver, and a reduced ability of insulin to inhibit lipolysis in adipose tissue. On the other hand, Lipocalin2 has been identified as a novel adipokine associated with obesity, type2 diabetes and the metabolic syndrome. The effects of aerobic training on Insulin resistance and plasma Lcn2 are still unclear, thus this study aimed to investigate the effects of aerobic training on these factors in obese young men. In this study, results showed that Plasma Lipocalin-2 decreased (P<0.05, 11.2%) in response to 8 weeks aerobic training compared to the control group. Choi (2009) indicated that there was no significant change in the Lcn2 in obese women after 12 weeks moderate exercise training. This discrepant result may be attributed to variation in the exercise protocols and differences in subject. On the other hand, there was the positive relationship between plasma lipocalin2 and body fat percent at baseline populations. The results showed that body weight; body mass index (BMI), body fat percent and WHR were decreased after aerobic training, thus exercise-induced changes in body fat, especially visceral adipose tissue, may attribute to plasma Lipocalin2 decrease and after the training. The results are in agreement with previous reports showing that there was a significant positive relationship between plasma lcn2 levels with body mass, body fat percentage and WHR, suggesting that the increased fat mass might account for the elevated blood levels of this adipokine in obese individuals. Wang (2007), showed a higher concentration of Lcn2 in obesity and this adipokine is positively related to the BMI, Waist circumference and body fat percentage. Choi et al (2008) demonstrated that a positive relationship between Lcn2 and body mass and Damirchi et al. (2011) showed a positive relationship between Lcn2 level with waist circumference, fat mass and BMI. Body fat percent decreased 8.8% after 8 weeks aerobic training, thus it seems that the aerobic training could offer a sufficient stimulus for plasma Lcn2 decreases. We found a significant related between Lcn2 and insulin resistance determined by HOMA-IR. A number of previous studies have investigated the effect of aerobic exercise training on insulin sensitivity in obese humans with impaired glucose tolerance. These studies have demonstrated that chronic aerobic exercise training resulted in improved glucose tolerance during glucose clamp conditions and ultimately improved insulin sensitivity. Arciero *et al* showed that effect of aerobic exercise training in humans with impaired glucose tolerance or mild T2DM resulted in increased whole-body glucose disposal during hyperglycemic clamp conditions (Arciero, 1999). Choi et al reported that HOMA-IR is not a very sophisticated measure of insulin resistance, although it has been used widely in clinical and epidemiological studies (Choi et al,

2009).Results showing no significant relationship between Lcn2 and hs-CRP after 8 weeks aerobic training. Suggesting that decrease of the other inflammatory markers might decrease Lcn2 and CRP concentration. Serum CRP levels correlated with serum IL-6 and TNF-$\alpha$ concentration in this study, which then affects the production of CRP by the liver. Additional research is needed to examine whether exercise induced change in IL-6 and TNF-$\alpha$ concentrations, decreases CRP. We did not measure IL-6 and TNF-$\alpha$ in the present study. If we could measure these inflammatory markers, we could carefully explain the decrease of plasma Lcn2 in response to 8 weeks exercise training in obese men.

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