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Hs-CRP and TNF- alpha in response to a stepwise incremental bicycle test in adult obese men

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Abstract

To compare of circulating levels of as tumor necrosis factor-α (TNF-α) and C reactive protein (CRP) as pro- inflammation cytokines between obese and none obese men and also to evaluate its response to a stepwise incremental bicycle test in obese men, eighty sedentary adult obese men (30 ≤ BMI ≤ 36, 40 ± 5 aged) and twelve none-obese men matched for age participated in this study. Fasting blood samples were obtained of two groups in order to measuring serum CRP, TNF-α, insulin and glucose. Depending of the values of insulin and glucose insulin resistance index (HOMA2) was calculated. Blood samples were also repeated in obese group after exercise test in order to determine all variables responses to exercise. Statistical analysis was performed with the SPSS software version 15.0 using an independent paired t-test. At baseline, Serum TNF-α, CRP, insulin and glucose concentrations and insulin resistance were significantly higher in obese group than in normal weight subjects ($p < 0.05$). Compared to pre-training, the CRP and TNF-α levels increased significantly ($P<0.01$) after acute exercise while glucose concentration decreased significantly in studied obese participants ($p<0.05$). No significant differences were found in insulin resistance by cycling exercise with compared to baseline ($p = 0.154$). This study suggests obesity is associated with systemic inflammation and a incremental cycle test have inflammatory properties regardless that is not related with glucose concentration.

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Introduction

Obesity is a chronic inflammatory disease and it is reported that various cytokines/adipocytokines play a key role in its prevalence. These Pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and C reactive protein (CRP) are produced by human adipose tissue dependent on the degree of obesity (Moschen *et al.,* 2010). Adipose tissue secretes a variety of bioactive mediators including adipocytokines such as adiponectin, leptin, resistin or classical cytokines such as the pro-inflammatory mediators TNF-α and interleukin 6 (IL-6) (Hotamisligil, 2006; Tilg *et al.,* 2006). High sensitivity C-reactive protein (hs-CRP) is an inflammatory mediator known to be related to inflammation, and cardiovascular diseases. Review of research evidence shows that Obesity is associated with a chronic inflammatory response characterized by abnormal production of these cytokine (Moschen *et al*., 2010).

TNF-α expression is enhanced in adipose tissue of obese subjects and reduced TNF-α serum levels are observed following weight loss (Sherman, 1991). A first, link between this pro-inflammatory cytokine and obesity a came from a study by Hotamisligil et al, who established the concept of a role for TNF-α/inflammation in obesity (Zureik *et al.,* 2011). Recent evidence has shown that obese subjects have higher levels of these pro-inflammatory mediators than none-obese healthy indivituals (Bal *et al*., 2010; Kutasy *et al.,* 2010; Kern *et al.,* 1995). It is reported they are associated with insulin resistance in obese people (Bal *et al.,* 2010).

The question is whether exercise as a none-pharmological intervention can affect circulation levels of these cytokines in obese men. Accumulating evidence indicates Conflicting data in this area. As some studies support the beneficial role of exercise training on these cytokines in obese or its related diseases (de Salles *et al.,* 2010; Sheu *et al.,* 2008) and the other finding suggested that exercise training does not affect them in these population (Kim *et al.,* 2008). Despite these findings, little information on the possible influence of acute exercise on these cytokines in obese subjects is available. Therefore, this study aimed to compare serum CRP and TNF-α between obese and none obese men and also to determine their responses to a cute exercise. We also investigated glucose and insulin resistance in studied obese subjects in this study.

Material and methods

Subjects

The main purpose of this study was to compare serum TNF-α, high-sensitivity C-reactive protein (hs-CRP) between obese and none-obese men and also to evaluate its response to a stepwise incremental bicycle test in obese men. For this purpose, eighty sedentary adult obese men ($30 \leq BMI \leq 36$, 40 ± 5 aged) and twelve none-obese men ($25 \leq BMI \leq 29$) matched for age participated in this study by accidentally. The Study Protocol was approved by the Ethics Committee of Islamic Azad University. Each participant received written and verbal explanations about the nature of the study before signing an informed consent form.

Inclusion criteria/anthropometrical measurements

After obtaining written informed consent, the individuals were asked to complete questionnaires on anthropometric characteristics, general health, smoking, alcohol consumption, and present medications. Participants were included if they had not been involved in regular physical activity/diet in the previous 6 months. None of the participants had ongoing cardiovascular disease, infections, renal diseases, hepatic disorders, use of alcohol, and use of nonselective β blockers and presence of malignancy. Participants were non-athletes, non-smokers and non-alcoholics. In addition, exclusion criteria included inability to exercise and supplementations that alter carbohydrate-fat metabolism. Additional variables for this report included age, height and

weight, body mass index (BMI). Weight was measured by an electronic balance and height by a stadiometer. Abdominal obesity was determined as waist circumference measured in a standing position. BMI was calculated as weight in kilograms divided by the square of height in meters (kg/m2). The arterial systolic and diastolic blood pressures (BP) were calculated after they rested for 10 minutes with a mercury manometer with appropriate sleeves (Alpikado, japens) from the right arm, in sitting position on the condition that they had not eaten anything, had not taken any caffeine, had not smoked or exercised thirty minutes before the measurement, and then the averages were calculated. Subjects were advised to be well hydrated and to limit their physical activity the day before the evaluation.

Blood sampling and protocol

After anthropometric measurements, the individuals in the and none-obese groups were asked to attend Hematology Lab following 12 hours of overnight fasting, between the hours of 8 to 9 am for blood sampling. The subjects were advised to avoid any physical activity or exercise 48 hours before the blood sampling. Then, a 5 ml fasting blood samples were collected from brachial vein in sitting position. Serums were immediately separated and stored at -80° until the assays were performed. Blood sampling was performed in order to measuring serum CRO, TNF-α, insulin and glucose. Then, all participants of obese group were completed a stepwise incremental bicycle test (Mullis *et al.,* 1999) and blood samples were repeated immediately after exercise test. Insulin and glucose levels were used for the homeostasis model assessment of insulin resistance ($HOMA_{IR}$ = (fasting insulin (μIU/ml) × fasting glycemia (mmol/l))/22.5).

Serum CRP was determined by ELISA method (Diagnostics Biochem Canada Inc. High sensitivity C - reactive protein (Hs-CRP)). The Intra- assay coefficient of variation and sensitivity of the method were 5% and 10 ng/mL, respectively. Serum TNF-α was determined by ELISA method (Enzyme-linked Immunosorbent Assay for quantitative detection of human TNF-α total). The Intra- assay coefficient of variation and sensitivity of the method were 7.7% and 5.0 pg/mL, respectively.

Statistical analysis

Statistical analyses of data were performed using the SPSS software version 15.0. For the descriptive statistics after having checked the normality of the variables using the Kolmogorov-Smirnov test. Independent sample T-test was used to compare the serum levels of all variables between obese and none-obese subjects. Pre- to post training changes in obese group were determined by two-tailed T tests. P value of less than 0.05 was regarded as indicative of a signigcant difference.

Results

Anthropometric and metabolic characteristics of the study participants in the obese and none-obese groups are shown in Table 1. Data were expressed as individual values or the mean ± SD. At baseline, the data indicate higher CRP and TNF-α in obese group when compared to none-obese subjects ($p<0.05$). Fasting glucose, insulin and insulin resistance level in obese subjects were also significantly higher than those of none-obese subjects ($p<0.05$).

The statistical analysis in obese group showed that high-sensitivity C-reactive protein levels were significantly increased in response to exercise test when compared with baseline levels (from 1868 – 456 to 2914 – 636 ng/ml, p = 0.000, Fig 1). Compared to pre-training, the TNF-α levels increased significantly after exercise in the obese group (from 52 – 14 to 63 – 13 pg/ml, p = 0.21, Fig 2). Blood glucose concentration decreased after exercise test when compared to pre-training (from 104 – 13 to 98 – 11 mg/dl, p=0.032), but insulin resistance remained without significantly change in obese subjects did not improve significantly in the type 2 diabetic subjects (from 2.21 – 0.33 to 2.42 – 0.45, p = 0.116).

Table 1. Mean and standard deviation of Baseline level of anthropometric and metabolic characteristics of studied subjects.

Variables	obese group	None-obese group
Age (year)	40 ± 5	41 ± 4
Weight (kg)	75 ± 7	98 ± 11
Height (cm)	174 ± 7	175 ± 8
Body Fat (%)	23.11 ± 2.33	30 ± 3.33
Body mass index (kg/m^2)	24.8 ± 3.11	32.03 ± 3.45
Abdominal circumference (cm)	91 ± 6	105 ± 9
Glucose (mg/dL)	94 ± 7	104 ± 13
Systolic blood pressure (mmHg)	116 ± 7	128 ± 12
Diastolic blood pressure (mmHg)	78 ± 7	82 ± 10
Insulin (μIU/ml)	7.28 ± 2.32	8.75 ± 2.11
Insulin resistance	1.63 ± 0.36	2.21 ± 0.33
hs-CRP (ng/ml)	971 ± 211	1868 ± 456
TNF-α (pg/ml)	40 ± 5.6	52 ± 14

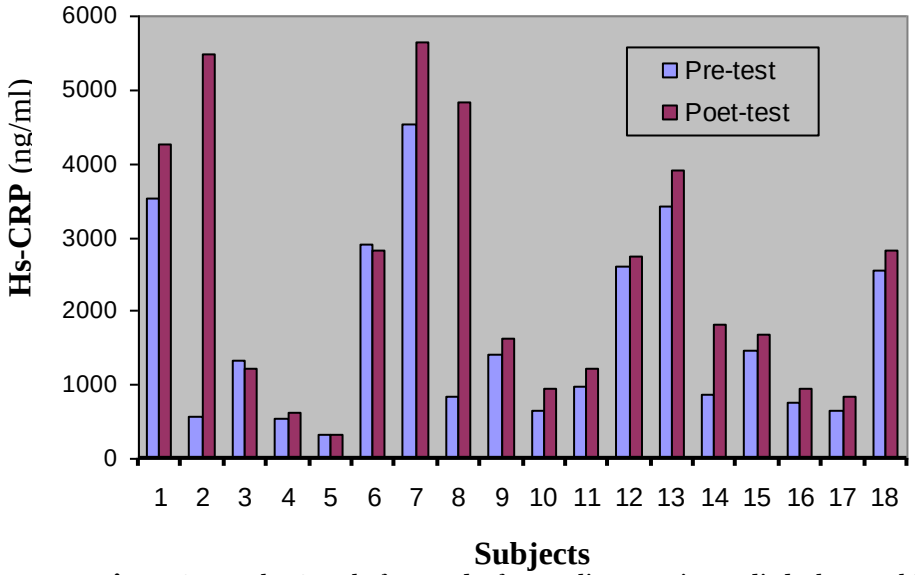
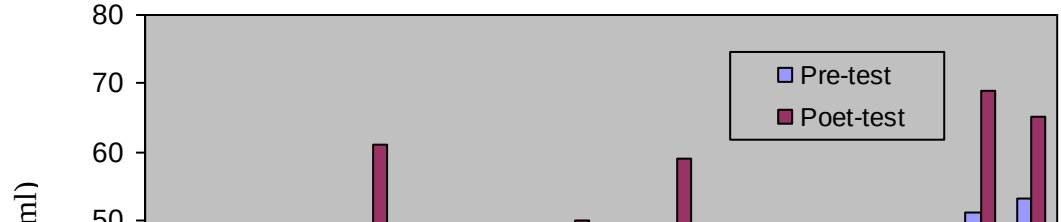


Fig 1. Serum hs-CRP before and after cycling test in studied obese subjects. Serum hs-CRP levels exhibited a statistically significant increase at the end of cycling exercise when compared to pre-test values. Each number on the horizontal axis represents one subject.



Discussion

In present study, at first, we also observed higher serum CRP and TNF-α, glucose and insulin resistance in obese subjects than none obese participants. Our study finding showed that an incremental cycling exercise increases serum CRP and TNF-α in adult obese men.

To support these findings, the serum levels of both TNF-alpha and CRP were found elevated in the obese group when compared to normal weight subjects (Bal *et al.,* 2010). These authors suggested that Insulin resistance is one of the major causes of obesity-related complications due to increased secretion of TNF-alpha and CRP together with android obesity (Bal *et al.,* 2010). It has been established that TNF-α is produced 7.5 times more by the adipose tissue in obese subjects compared with lean counterparts (Sharman *et al*., 2004).

It was reported that expression of TNF-α as a major pro-inflammatory cytokines, is markedly regulated at the transcriptional level and increased in human fat cells from obese subjects and patients with insulin resistance (Rotter *et al.,* 2003). C-reactive protein (CRP), a marker of systemic inflammation, is a powerful predictor of adverse cardiovascular events (Amina *et al.,* 2010). The CRP belongs to a family of molecules called the pentraxins. Structurally CRP is composed of five identical non-glycosylated polypeptide subunits (pentraxin; penta=five, ragos=berries), each 23 kDA in mass, held together by non-covalent bonds to form a disc-like pentagonal ring (Kony *et al.,* 2004; Mendall *et al.,* 2000). The CRP is predominantly synthesized in the liver and is regulated by pro-inflammatory cytokines, primarily the tumour necrosis factor-alpha and interleukin-6 (IL-6). During an acute-phase response, there is a rapid increase in the production of CRP (≥10,000-fold), resulting in the release of elevated quantities

into the circulation (Sahoo *et al.,* 2009). TNF-α have both been demonstrated to be involved in the pathophysiology of insulin resistance (Rotter *et al*., 2003). That was among the first inflammatory mediators implicated as predictors or pathogenetic markers of insulin resistance (Hotamisligil *et al.,* 1993; Hirosumi *et al.,* 2002; Lang *et al.,* 1992). It has been established that adipose tissue including subcutaneous adipose tissue is a major source of TNF-α compared to hepatic tissue in human obesity (Hotamisligil, 2006). TNF-α expression is also enhanced in adipose tissue of obese subjects and reduced TNF-α serum levels are observed following weight loss after long term exercise/diet program (Dandona *et al*., 1998). Also, Kern et al first described a signigcant increase in adipose TNF-α mRNA levels with increasing adiposity and showed a signigcant decrease with weight loss (Kern *et al.,* 1995).

It was concluded that CRP in combination with age, hypertension, and diabetes were the most outstanding risk factors associated with CVD in this population (Panagiotakos *et al*., 2008). In a recent study, weigh loss was associated with decreased serum CRP (Valsamakis *et al.,* 2004). It is also speculated that exercise training decreases resting levels of TNF-alpha and C-reactive protein and increases adiponectin (AD) and insulin sensitivity (de Salles *et al.,* 2010). In another study, weight loss by 5% of initial weight in nondiabetic obese women led to significant improvement in inflammation cytokines such as CRP, TNF-α and glucose concentration (Sheu *et al.,* 2008). Whereas some authors have not found any changes in CRP after 12 mo of an exercise program (Kim *et al.,* 2008), others have reported reductions in this cytokine following an exercise regimen for 6 months (Campbell *et al.,* 2008).

Despite many studies on response of these inflammatory cytokines to chronic exercise training and Contradictory findings in this area, little information on the possible influence of acute exercise on these cytokines in obese subjects is available. In present study, compared to pre-training, the serum CRP and TNF-α level increased significantly after acute exercise in studied obese subjects.

In this context, although exercise is known as an anti-inflammatory intervening factor, our study showed that a moderate cycling exercise would lead to the significant increase in serum concentrations of CRP and TNF-α. This hypothesis is made that one session of exercise is associated with immediate increase in circulatory levels of these inflammatory cytokines, but changes in the muscle concentration of these cytokines differ from their serum levels in response to muscle activity. It is also important to note that not only does adipose tissue release cytokines, but also skeletal muscles express cytokines that have direct autocrine and paracrine effects (Saghizadeh *et al.,* 1996). In this area, a number of studies have demonstrated that exercise may induce local anti-inflammatory effects in skeletal muscle that may not be reflected in the systemic circulation (Gielen *et al.,* 2003). These investigators found that aerobic training reduced TNF-a gene expression in skeletal muscles but had no effect on levels of these cytokines in the systemic circulation (Gielen *et al.,* 2003).

On the other hand, although a single bout exercise is associated with acute increase of TNF-α and CRP cytokines, it is likely that the anti-inflammatory effect of exercise is associated with decreased gene expression and the likely and reduction of the receptors of these cytokines. In line with this hypothesis, in Huang study, although acute exercise did not significantly change plasma adiponectin concentration at 2 hours or 18 hours after the exercise, the expression levels of AdipoR1 significantly increased in both skeletal muscle and liver compared with control group (Huang *et al.,* 2007). In this area, it seems that the measuring of inflammation cytokines does not directly account for the beneficial effects of physical activity.

On the other hand, it is also possible that the beneficial effects of a single bout exercise on these inflammatory cytokines represent a delayed rather than acute response, Because, studies on some other inflammatory or anti-inflammatory cytokines show that, although exercise does not lead to acute changes of these hormonal variables, its beneficial effects appear in the recovery period after exercise. So that, it was reported a decline in leptin concentrations in trained rowers 30 min after maximal rowing exercise (30 min) (Jurimae *et al.,* 2005). In another study it was found that Adiponectin was unchanged immediately after a 6.5 km rowing at the individual anaerobic threshold and significantly increased above pre-exercise values after 30 min of recovery (Jurimae *et al.,* 2006).

Exercise in the present study, although associated with a significant increase in serum levels of CRP and TNF-α and no change in insulin resistance, led to a significant reduction in blood glucose concentration. It must be pointed out that lack measuring of serum IL-6 was one of the main limitations of this study. It is possible that despite an increase in CRP and TNF-α in this study, decreased glucose concentration is correlated with serum changes in IL-6. Several studies have reported that IL-6 increased glucose infusion rate (Julia *et al.,* 2010) and glucose oxidation without affecting the suppression of endogenous glucose production during a hyperinsulinemic euglycemic clamp in healthy humans (Petersen *et al.,* 2006). The potential role of IL-6 on the activity of AMPK, that is one effective factor in membrane transport of glucose, should not be ignored (Jakobsen *et al.,* 2001).

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