



## RESEARCH PAPER

## OPEN ACCESS

## Pro-inflammatory TNF- $\alpha$ in response to single bout cycling in type II diabetic patients

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### Abstract

Accumulating evidence suggest that type II diabetes is associated with systemic inflammation. This study aimed to determine relationship between serum TNF- $\alpha$  with glucose and insulin resistance and to evaluate the effect of a single bout moderate exercise on TNF- $\alpha$  in type II diabetes patients. For this purpose, blood samples of pre and immediately post cycling test were collected in order to measuring serum TNF- $\alpha$ , glucose and insulin in twelve adult males with type II diabetes. Insulin resistance was assessed using the homeostasis model assessment for insulin resistance formula derived from fasting insulin and glucose levels. Student's paired 't' test was applied to compare the pre and post exercise values. Pearson correlation coefficients were used to determine the associations between TNF- $\alpha$  with the blood chemistry parameters. There were no correlations between serum TNF- $\alpha$  concentrations with glucose and insulin and insulin resistance at baseline. Exercise test led to significant increase in TNF- $\alpha$  ( $p = 0.002$ ) and decrease in glucose concentration ( $p = 0.000$ ). No significant change were found in insulin ( $p = 0.154$ ) and insulin resistance at posttest when compared to pretest. Our findings indicate that acute exercise is associated with increased inflammation immediately after test and the change of serum TNF- $\alpha$  by exercise test is independent of glucose or insulin resistance in type II diabetes patients.

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## Introduction

It is generally accepted that chronic diseases are strongly correlated with inflammation resulting from the body's release of inflammatory cytokines as a result of injury or infection. Pro and inflammatory or antiinflammatory cytokines are secreted by different cell types and are released into circulation where they regulate different tissues through their local, central, or peripheral actions (Bruun *et al.*, 2003). Marked evidence indicates that chronic diseases such as heart disease, the metabolic syndrome, and type 2 diabetes have in common the increased concentration of circulatory cytokines as a result of inflammation (Ross, 1999).

Among cytokines, although tumor necrosis factor-alpha (TNF- $\alpha$ ) is also secreted by the adipose tissue, it can be secreted by macrophages and other cells (Puglisi *et al.*, 2008). TNF- $\alpha$  is primarily secreted by macrophages, and also by a broad variety of other cells including adipocytes (Beutler *et al.*, 1989; Giemeno *et al.*, 2005). This cytokine has an important role in inflammatory process and plays major role in the occurrence of metabolic disorders, including obesity and insulin resistance (Ye, 2008).

It has been previously reported that its elevated concentrations may promote the synthesis of some interleukins such as IL-8, whose function is to induce monocyte adherence contributing to the atherosclerotic process (Gerszten *et al.*, 1999). Abnormalities in serum or plasma TNF- $\alpha$  concentration have been implicated in metabolic disorders, such as obesity and insulin resistance, suggesting that perturbations of TNF- $\alpha$  metabolism may affect the onset of type 2 diabetes mellitus and the progression of the disease (Swaroop *et al.*, 2012), although the physiopathological mechanisms underlying these associations are largely unknown. These authors noted that the activation of proinflammatory pathways after exposure to TNF induces a state of insulin resistance in terms of glucose uptake in myocytes and adipocytes that impairs insulin signaling at the level of the Insulin

Receptor Substrate proteins (Nieto-Vazquez *et al.*, 2005).

During recent years, specific interventions promoting weight loss, exercise, or intake of antioxidants have been used by several investigators in an effort to decrease inflammatory cytokines. In a study by Lambert, 12-wk exercise program did not change plasma levels of TNF- $\alpha$  in elderly obese subjects (Lambert *et al.*, 2008). On the other hand, in another study, serum TNF- $\alpha$  decreased significantly by weight loss (Bastard *et al.*, 2000). Despite the inconsistent findings regarding the TNF- $\alpha$  in response to long-term exercise training, there are limited studies about the effect of short-time exercise test on this cytokine. Therefore, in this study we investigated serum TNF- $\alpha$  in response to single bout exercise in type II diabetes men.

## Material and methods

### Subject

This study involved seventeen non-trained adult obese men with type II diabetes aged 33-41 years that participated by accessible sampling ( $30 \leq \text{BMI} \leq 36$ ). The study aimed to evaluate serum TNF- $\alpha$  response to a stepwise incremental bicycle test in mentioned diabetes subjects. Each participant received written and verbal explanations about the nature of the study before signing an informed consent form. The ethics approval was taken from Islamic Azad University of Iran ethical committee.

### Inclusion and exclusion criteria

Inclusion criteria for study group were determined as existing type 2 diabetic for at least two years. None of diabetic subjects had participated in regular exercise for the preceding 6 months, nor did all subjects have stable body weight. Subjects were reported to be non-smokers, not currently taking supplements of any kind. Presence of previous coronary cardiac disease, chronic airway disease, and impaired hepatic dysfunction, and presence of any acute disease were determined as exclusion criteria. All subjects had a body mass index (BMI) of more than 30 kg/m<sup>2</sup>. Information about medication use, including

hypertension medications, statins, oral hypoglycemic agents, and insulin, was obtained from a questionnaire completed at the time of medical examination.

#### *Exercise protocol and measurements*

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. BMI was calculated as kilograms per square meter. Abdominal obesity was determined as waist circumference measured in a standing position. The subjects were advised to avoid any physical activity or exercise 48 hours before the exercise test. Cycling exercise test was a YMCA standard test on leg ergometry cycle (Tunturi, made in Finland). This protocol was performed in 5 continuous stage without rest between stages. Each stage lasted 3 minute (Mullis et al., 1999). Pre and post exercise blood samples were collected of all participants. All blood samples were taken following an overnight 12-hour fast. Blood samples were analyzed for glucose, insulin and serum TNF- $\alpha$ .

#### *Assay*

After sampling in EDTA- or serum-tubes, blood was immediately chilled on ice, centrifuged and aliquots were frozen at  $-80^{\circ}\text{C}$  until assayed. Serum TNF- $\alpha$  was determined by ELISA method (Enzyme-linked Immunosorbent Assay for quantitative detection of human TNF- $\alpha$  total). The Intra-assay coefficient of variation and sensitivity of the method were 6% and 5.0 pg/mL for TNF- $\alpha$ . Glucose was determined by the oxidase method (Pars Azmoon kit, Tehran). Insulin was determined by ELISA method (Demeditec, Germany) and the intra- assay and inter-assay coefficient of variation of the method were 2.6% and 2.88 respectively. To estimate insulin resistance, the homeostasis model assessment (HOMA) index was calculated as fasting insulin concentration ( $\mu\text{U/ml}$ )  $\times$  fasting glucose concentration ( $\text{mmol/l}$ )/22.5. (Matthews et al., 1985)

#### *Statistical analyses*

Data were expressed as individual values or the mean  $\pm$  SD. Data were analyzed by computer using SPSS software version 15.0. Normal distribution of data was analyzed by the Kolmogorov-Smirnov normality test. Student's Pre- and post exercise TNF- $\alpha$  contents were compared between conditions using a paired-samples t-test. The association between serum TNF- $\alpha$  concentration and blood biochemistry parameters were assessed using Pearson's correlation coefficient. An alpha-error below 5% was considered as statistically significant.

#### **Results**

Anthropometric characteristics of the study participants are shown in Table 1. All values are given as mean and standard deviation.

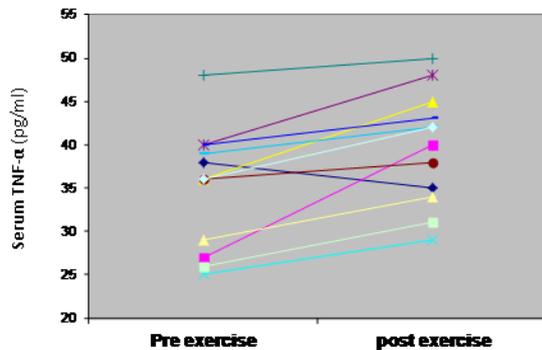
**Table 1.** The descriptive anthropometric features of studied patients.

Variable	Mean	Standard deviation
Age (years)	37	3.96
Weight (kg)	101	13.2
Height (cm)	177	13.2
Body mass index (kg/m <sup>2</sup> )	32	3.19
Abdominal circumference (cm)	107	9.6
Hip circumference (cm)	108	8.96

Data of Pearson's correlation coefficient method showed no significant correlation between serum TNF- $\alpha$  with serum insulin ( $p \geq 0.045$ ). There were no correlations between serum TNF- $\alpha$  concentrations and fasting glucose concentration ( $p \geq 0.045$ ) and insulin resistance ( $p \geq 0.045$ ).

After exercise test, Serum TNF- $\alpha$  was significantly ( $p = 0.002$ ) increased (pre to post, from  $35 \pm 6.93$  to  $3.39.75 \pm 6.55$  pg/ml,  $p = 0.002$ ) when compared to pretest value. After exercise test was completed, glucose levels decreased significantly (pre to post,

from  $101 \pm 9.28$  to  $96 \pm 8.6$  mg/dl,  $p = 0.000$ ). There were no significant differences for serum insulin (pre to post, from  $8.46 \pm 2.73$  to  $9.76 \pm 3.66$   $\mu$ IU/ml,  $p = 0.154$ ) or insulin resistance (pre to post, from  $2.10 \pm 0.67$  to  $2.32 \pm 0.92$ , HOMR-IR,  $p = 0.362$ ) in studied patients.



**Fig. 1.** Mean ( $\pm$  standard deviation) of serum TNF- $\alpha$  concentration in pre and post test in diabetic subjects. Results indicated a significant increase in this cytokine immediately after exercise test when compared to pretest. Each line in chart represent pre and post values of one subject.

### Discussion

The major finding of this investigation was that one session exercise test involved a stepwise incremental bicycle test increase serum TNF- $\alpha$  in adult men with type 2 diabetes. We also observed significant decrease in glucose by exercise test. Despite a improving in glucose concentration, but insulin or insulin resistance remained without change after exercise test.

Clear evidence has established that exercise is a cornerstone in the treatment of obesity (Pasman *et al.*, 1999). On the other hand, exercise training is associated with several important health benefits such as improving in insulin sensitivity or blood lipid profile, blood pressure (Lesnika *et al.*, 2001). TNF- $\alpha$  as a proinflammatory cytokine is emerging to be a pivotal component in a number of metabolic diseases, such as obesity, diabetes, dislipidemia and atherosclerosis (Sethi *et al.*, 1999) and is produced in response to infection to confer immunity to the host (Apostolaki *et al.*, 2010).

This cytokine is a pleiotropic, proinflammatory mediator whose function is implicated in a wide range of inflammatory, infectious, autoimmune and malignant conditions (Apostolaki *et al.*, 2010). Increased TNF- $\alpha$  production has been shown in adipose from tissue derived obese rodents or human subjects and it has been established as a causative factor in obesity-associated insulin resistance and the pathogenesis of type 2 diabetes (Aguirre *et al.*, 2000).

Marked evidence indicates that Regular exercise training have a protection role against all cause mortality and Much evidence suggests that physical training is effective as a treatment in patients with chronic heart diseases, type 2 diabetes and symptoms related to the metabolic syndrome (Petersen *et al.*, 2006). A large body of evidence suggests to Conflicting findings about the effect of exercise on inflammation profile. Among them, Several studies have found no significant changes in some cytokines (Hammett *et al.*, 2006; Fischer *et al.*, 2004; Bautmans *et al.*, 2005; Marcell *et al.*, 2005), while significant reductions in inflammatory markers have been observed following training without changes in BMI or body fat in elderly participants (Kohut *et al.*, 2006; Stewart *et al.*, 2005).

Therefore exercise training particularly long-term exercise have beneficial role in obese subjects or patients with type II diabetes. Despite numerous long-term studies with aim to effect on cytokines, there are limited studies regarding the effects of short time or acute study in this area. In our study, we observed significantly increase in serum TNF- $\alpha$  in response to cycling test. This data supports inflammatory property of exercise immediately after exercise cessation. In this regards, although endurance exercise training induces an anti-inflammatory response (Lira *et al.*, 2009), but the authors noted that in stress-related situations such as exhaustive exercise, the adipose tissue shows increased expression of cytokines that act both locally and distally with autocrine, paracrine and endocrine

effects (Rosa Neto *et al.*, 2009; Lira *et al.*, 2009). Acute exhaustive exercise induces sapro-inflammatory response in the adipose tissue, enhancing IL-6 and TNF- $\alpha$  level (Rosa Neto *et al.*, 2009). Although exercise protocol in present study was not an exhaustive exercise, but it seems that short-time exercise test in present study was heavy and prolix for studied patients.

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