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Increased IL-6 and is associated with decreased glucose concentration during cycling exercise

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Abstract
Review of research findings show higher circulating interleukin 6 in diabetic patients than healthy people. The aim of our work was to study evaluate effects of exercise on serum interleukin 6 and insulin sensitivity in type II diabetic patients. Blood samples were derived from brachial vein before and immediately after YMCA cycling protocol of 16 healthy non-trained adult men with type II diabetic that participated in this study by voluntarily. Blood samples were used for measuring serum interleukin 6 and insulin sensitivity of all patients. We observed an increase in serum interleukin 6 by exercise test when compared to baseline. Glucose concentration was decreased in response to exercise test, while insulin sensitivity did not change. Based on these data, we can conclude that acute exercise test increase serum interleukin 6 and decrease glucose concentration in the absence of changes in insulin sensitivity in diabetic patients.

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Introduction

Accumulating evidence indicates a high prevalence of diabetes and impaired glucose tolerance (IGT) in the older population (Ohang *et al.,* 2003). It is also important to note that multiple risk factors for type 2 diabetes associated with aging including increased adiposity and decreased physical activity predispose older people to develop glucose intolerance and increased insulin resistance (Bloem *et al.,* 2008). Although there is considerable evidence that insulin resistance and beta cell dysfunction are two major factors in diabetic prevalence but it seems that other factors such as Hormonal disorders also contribute to diabetes.

On the other hand, Epidemiologic studies clearly suggests type II diabetic as an inflammation diseases and Systemic inflammation has been long known to be a key factor in insulin resistance and glucose homeostasis (Rotter *et al.,* 2003). Among inflammation cytokine, interleukin 6 have the important role in type II diabetes (Carey *et al.,* 2006).It has been established that diabetic patients have higher circulating IL-6 than non-diabetic subjects (Fontana *et al.,* 2007; Kopp *et al.,* 2003). Several studies have reported that expression of IL-6 and TNF-α, two 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). According to recent epidemiologic studies, IL-6 did not increase whole-body glucose disposal in either healthy subjects or patients with type 2 diabetes, whereas it reduced insulin concentrations in the patients to values comparable with those of the healthy subjects, suggesting that IL-6 might have favorable effects on insulin action (Petersen *et al.,* 2005). However there have been only a few studies on the fact that an increase in IL-6 leads to a decrease in blood glucose levels.

Regular exercise particularly aerobic exercise training offers protection against all cause mortality and A number of studies have demonstrated intervention studies that physical training is effective non-pharmacological treatment in patients with chronic heart diseases, type 2 diabetes and symptoms related to the metabolic syndrome. In this area, some previous study have been demonstrated that exercise training for long time is associated with decreased systemic inflammation in type II diabetes or other chronic disease (King *et al.,* 2003). Of course conflicting information are observed in this area yet. On the other hand, the role of short time or acute exercise on inflammation or non-inflammatory cytokines such as Il-6 and insulin sensitivity has received limited attention. In this study, we investigated serum Il-6 and insulin sensitivity as well as glucose concentration responses to one session cycling exercise in a group of type II diabetic men.

Material and methods

Subjects

In this study, we investigated the effect of a single bout cycling exercise on serum IL-6, glucose concentration and insulin sensitivity in sixteen sedentary adult men with type II diabetic (aged, 43 ± 5 years; body weight, 92 ± 7). All patients were selected for participation in study by accessible sampling. The study was conducted with the approval of the Ethics Committee of Islamic Azad University, Iran. Those patients were included if they had not been involved in regular physical activity or diet in the previous 6 months. Each participant received written and verbal explanations about the nature of the study before signing an informed consent form.

Inclusion and exclusion criteria

Participants were non-athletes, non-smokers and non-alcoholics. Inclusion criteria for study group were determined as existing type 2 diabetic for at least two years. Exclusion criteria included medications that alter carbohydrate metabolism and inability to exercise. We also excluded people who had any self reported physician diagnosed chronic disease (arthritis, stroke, cancer, heart attack, chronic cough, or bronchitis), injury and abnormal exercise electrocardiogram.

Anthropometrical and biochemical measurements

The weight and height of the participants were measured by the same person when the participant had thin clothes on and was wearing no shoes by using the standard hospital scales. Abdominal circumference and hip circumference were measured in the most condensed part using a non-elastic cloth meter. Body mass index (BMI) was calculated as weight (kg) divided by squared height (m). The arterial systolic and diastolic blood pressures (BP) were calculated after they rested for 10 minutes with a mercury manometer with appropriate sleeves from the right and left 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.

After anthropometrical measurements, pre and post exercise blood samples were collected for measuring serum IL-6, glucose and insulin of all patients. The subjects were advised to avoid any physical activity or exercise 48 hours before the exercise test. Cycling exercise test was performed according to a YMCA standard test on leg ergometery cycle (Tunturi, made in Finland). This protocol was performed in 5 continues stage without rest between stages. Each stage lasted 3 minute (Mullis *et al.,* 1999).

Glucose was determined by the oxidase method (Pars Azmoun, Tehran, Iran). Insulin was determined by ELISA method (Demeditec, Germany) and the intra- assay and inter-assay coefficient of variation of the method were 1.79 and 5.99 (%) respectively. Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) was calculated by the formula fasting blood glucose (mg/dl) x insulin (uIU/ml)/405 (Duncan *et al.,* 1995). Serum IL-6 was determined by ELISA method, ((Enzyme-linked Immunosorbent Assay for quantitative detection of human IL-6). The Intra-assay coefficient of variation and sensitivity of the method were 3.4% and 0.92 pg/mL, respectively.

Statistical analysis

Normal distribution of data was analyzed by the Kolmogorov-Smirnov normality test. Student's t-tests for paired samples were performed to determine whether there were significant within-group changes in the outcomes. All statistical tests were performed and considered significant at a $P \leq 0.05$.

Results

Higher level of serum IL-6 and lower insulin sensitivity in type II diabetic patients than healthy people was reported by many previous studies. Therefore, citing to previous finding, we only investigated the effect of above mentioned exercise test on diabetic patients. Table 1 presents anthropometrical and biochemical features of studied patients at baseline. All values are given as mean and standard deviation. Following statistical analysis, the data showed that cycling exercise test led to increase in serum IL-6 when compared to baseline in studied patients (2.01 ± 0.71 to 4.38 ± 2.26, pg/ml, p = 0.002, Fig 1). These data indicates acute response of this inflammatory cytokine to a single bout exercise. Insulin sensitivity is an important marker in diabetes diagnosis. Compared to pre-exercise, insulin sensitivity did not change after exercise test (0.50 ± 0.03 to 0.51 ± 0.06, p = 0.449). No significant differences was found in serum insulin by cycling exercise with compared to baseline (8.26 ± 1.69 to 9.11 ± 3.42, μIU/ml, P = 0.222). Despite the lack of change of insulin and insulin sensitivity, cycling exercise test led to significant decrease in glucose concentration in all participants (237 ± 73 to 221 ± 74, mg/dl, p = 0.000, Fig 2).

Table 1. The descriptive anthropometric and biochemical features of studied patients.

Descriptive Statistics

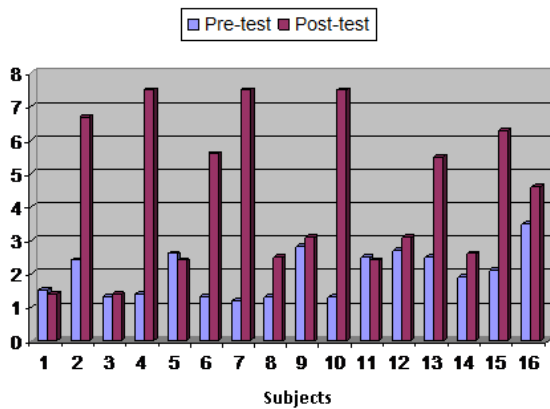|	N	Minimum	Maximum	Mean	Std. Deviation
Age (yesr)	16	32	49	42.69	5.263
Height (cm)	16	165	182	173.06	5.221
Weight (kg)	16	80	100	92.00	6.501
Systole (mmHg)	16	10	18	13.13	1.708
Diastole (mmHg)	16	7	10	8.44	.892
BMI (kg/m2)	16	27	34	30.65	2.030
Glucose (mg/dL)	16	136	385	237.13	72.779
Insulin (µIU/ml)	16	6.20	11.50	8.2563	1.68759
Insulin sensitivity	16	.45	.58	.4994	.03376
IL-6 (pg/mL)	16	1.2	3.5	2.019	.7129
Valid N (listwise)	16				

Fig. 1. The diagram shows serum IL-6 before and after cycling test in studied patients. Serum IL-6 levels exhibited a statistically significant increase at the end of cycling exercise when compared to pre-test values. Each pair of columns represents pre and post values of IL-6 in each subject.

Discussion

In this study, cycling exercise for short time (acute exercise) increased serum interleukin 6 in studied patients. On the other hand, it seems that a single bout cycling with moderate intensity led to significant increase in serum Il-6 in type 2 diabetic patients. Despite the increased this cytokine, but exercise test didn't significant change in insulin sensitivity in these patients. Of course, glucose concentration was decreased in response to exercise test. Obesity is related with systemic inflammation caused by Abnormal levels some cytokines. It was

previously reported that adipose tissue secretes some peptides bioactive mediators including adipocytokines such as leptin, resistin, pre-B-cell enhancing factor/Nampt/visfatin or classical cytokines such as the pro-inflammatory mediators tumor necrosis factor a (TNFa) and interleukin 6 that play key role in obesity and its related diseases such as type 2 diabetic or metabolic syndrome (Hotamisligil, 2006; Tilg *et al.,* 2006).
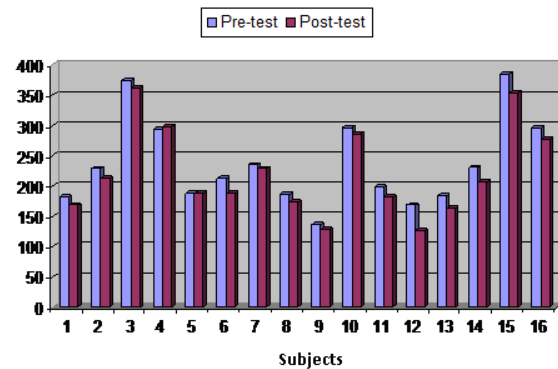

Fig. 2. The diagram shows glucose concentration before and after cycling test in studied patients. Glucose concentration levels exhibited a statistically significant decrease at the end of cycling exercise when compared to pre-test values. Each pair of columns represents pre and post values of glucose in each subject.

Pro-inflammatory cytokines such as interleukin 6 are produced by human adipose tissue dependent on the degree of obesity (Moschen *et al.,* 2010). It is also important to note that, the whole body adipose tissue

mass could contribute to 15e35% of the body's total circulating IL-6 making the adipose tissue a key IL-6 producing organ (Mohamed-Ali *et al.,* 1997). Despite our findings, recent epidemiologic studies indicates inverse relation between the level of daily physical activity and the incidence of type 2 diabetes is now well established by the results from several prospective epidemiological studies (Manson *et al.,* 1992; Manson *et al.,* 1991). In present study, we observed exercise test was associated with both increased IL-6 and decreased glucose concentration. Based on these data, it is likely that increased serum Il-6 follow up exercise affects glucose metabolism. In this regard, it was reported that IL-6 enhances insulin-stimulated glucose transport (Stouthard *et al.,* 1996) or glycogen synthesis (Weigert *et al.,* 2004; Weigert *et al.,* 2005) in myotubes and/or adipocytes. It has been established in a recent study that IL-6 can be enhance AMP-activated protein kinase (AMPK) in both skeletal muscle and adipose tissue (Kelly *et al.,* 2004). It is also important to note that AMPK plays a key role in the regulation of fuel metabolism in skeletal muscle because its activation stimulates fatty acid oxidation and may increase glucose uptake via mechanisms thought to involve enhanced insulin signaling transduction (Manson *et al.,* 1992; Manson *et al.,* 1991; Stouthard *et al.,* 1996). On the other hand, although in most cases IL-6 has been recognized as an inflammatory cytokine, but increase in its serum levels after one session exercise has anti-inflammatory characteristics. In accordance with these observations, the data of a recent study acute IL-6 enhances insulin-stimulated glucose disposal in humans in vivo by via an increase in translocation of GLUT4 from intracellular pools to the plasma membrane, while the effects of IL-6 on glucose and lipid metabolism in vitro appear to be mediated by AMPK (Carey *et al.,* 2006).

It should be noted that our previous study on obese men showed that one session of biking exercise bike leads to a concomitant increase in IL-6 and decrease in blood glucose concentration (Eizadi *et al.*, 2001). Overall, the findings of this study showed that a relatively low intensity and duration biking exercise would lead to increased serum levels of IL-6 in patients with type-II diabetes. The findings also showed that despite the absence of changes in insulin sensitivity, biking exercise in this study was associated with a significant reduction in blood glucose. Based on evidence from previous studies, the effect of exercise on IL-6 levels in the present study appears to be an anti-inflammatory one. Furthermore, increased IL-6 concurrent with decreased glucose in response to exercise somehow supports the anti-inflammatory role of IL-6. In support of the said discussion, findings of a recent study showed that high levels of IL-6 after exercise are followed by increased levels of IL-10 as an anti-inflammatory cytokine which is indicative of the anti-inflammatory properties of IL-6 (Steensberg *et al.*, 2003). Another study also stated that the increase in IL-6 after exercise would lead to inhibition of TNF-α secretion as another inflammatory cytokine (Matthys *et al.,* 1995; Mizuhara *et al.*, 1994; Fiers, 1991). Hence, according to the study's findings as well as the said discussions, it is possible that the acute increase in IL-6 subsequent to exercise through indirect routes such as reduced levels or expression of inflammatory cytokines such as TNF-α or increased levels or expression of non-inflammatory mediators such as IL-10 would lead to lowered blood glucose in patients with type II diabetes.

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