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Tumor necrosis factor alpha as inflammatory cytokine is not associated with cardiovascular risk factors in presence obesity

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Abstract

It is generally accepted that obesity is associated with systemic inflammation and increased cardiovascular risk factor, although the physiopathological mechanisms underlying these associations are largely unknown. To analyze whether lipid profile of cardiovascular risk factors are associated with serum level of tumor necrosis factor alpha (TNF-α) in presence to obesity, thirty abdominally obese men (BMI ≥30 kg/m2) aged 34 to 41 years were participated in this study by accessible sampling. All participants reported being weight stable (±1kg) for 6 months prior to the study and engaged in physical activity less than once per week. Blood was collected from was drawn from the antecubital vein of all subjects after an overnight fast between 8:00 a.m. and 9:00 a.m. blood samples were used for evaluation serum TNF-α and lipid profile markers such as triglyceride (TG), total cholesterol (TC), low density lipoprotein (LDL) and high density lipoprotein (HDL). Pearson's correlation coefficients were used to evaluate the correlations between serum TNF-α and lipid profile markers. Findings from statistical analysis showed no significant correlation in serum TNF-α with TG, TC and LDL. Serum TNF-α was negatively correlated with HDL cholesterol in studied subjects (p = 0.002, r = 0.78). Based on these data, apart from HDL, serum TNF-α as inflammatory cytokine can not predict other cardiorespiratory risk factor in obese subjects directly.

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Introduction

The increasing prevalence of obesity and its dangerous consequences, along with the industrialization of societies and changes in lifestyle, highlights the prevention and treatment of obesity as a major challenge for healthcare systems. Obesity is associated with several chronic diseases including cardiovascular disease, type 2 diabetes, and metabolic syndrome (Hart *et al.*, 2007).

Mechanisms involved in positive energy balance, such as inflammation and release of some adipocytokines, are the determinant of the pathophysiology of the metabolic disorders in the diseases associated with obesity. Scientific evidence revealed that some peptide components regulate the energy balance. They also regulate the body weight through intervening with metabolism mechanisms. Evidence supports a close relationship between inflammatory cytokines and the size of visceral adipose tissue in obese subjects as well as the subjects with other chronic diseases (Alexandraki *et al.*, 2006; Boulet, 2008).

Adipose tissue secretes some biochemical mediator with different characteristics, such as adiponectin, leptin, resistin, visfatin, or classic cytokines such as peptide inflammatory mediators including IL-6 and TNF-α (Hotamisligil, 2006; Tilg *et al.*, 2006). Hotamisligil *et al.* were first to investigate the relationship between obesity and TNF-α proinflammatory cytokines, as well as its role in the relationship between obesity and inflammation (Hotamisligil *et al.*, 1993). Although TNF-α is secreted primarily by the adipose tissue, it can also be produced by macrophages and other cells. This cytokine has a major role in the inflammation process, and increase in its secretion can enhance the synthesis of some interleukins such as IL-8, which have an especially important role in the atherosclerosis development process (Gerszten *et al.*, 1999).

TNF-α has biological effects on different tissues, which leads to changes in the rate of metabolism. In addition, the inflammatory cytokine has a direct effect on glucose homeostasis and lipid metabolism (Sethi *et al.*, 1999). In obese patients, TNF-α is involved in the increased basal lipolysis in adipocytes (Yang *et al.*, 2011). External application of TNF-α increases lipolysis as well as the circulating free fatty acid levels (Kawakami *et al.*, 1987). However, the relationship between lipid profile markers or cardiovascular risk factors with lower levels of this inflammatory cytokine is not well-studied. This study aimed to determine the relationship between these variables in inactive obese men.

Methods

Human Subjects

In this study, the relationship between serums TNF-α and cardiorespiratory risk factors was determined in obese males. Thirty adult obese men were included in the present study through local advertising. Subjects were 34–41 years old with a body mass index (BMI) of 30–36 kg/m2. The study protocol was approved by the Ethics Committee of Islamic Azad University, Parand Branch of Iran. Written consent was obtained from each subject after the experimental procedures and possible risks and benefits were clearly explained.

Inclusion and exclusion criteria

All subjects were non-smoker and non-athletes. Participants reported being weight stable (±1kg) for 6 months prior to the study and engaged in physical activity less than once per week. Subjects with a history or clinical evidence of impaired fasting glucose or diabetes, recent heart failure, active liver or kidney disease and other chronic diseases, or who were on medications were excluded. In addition, exclusion criteria included supplementations that alter carbohydrate metabolism.

Anthropometry

All anthropometric measurements were made by the same trained general physician and under the supervision of the same pediatrician following standard protocols. Anthropometric measurements of participants were performed while they stood in light clothing without shoes. Height was measured to the

nearest 0.1 cm using a free-standing stadiometer. Body composition monitor (BF508-Omron made in Finland) with a precision error of less than 100 g was used to measure weight and body fat percentage of the subjects. The Body Mass index (BMI) was calculated using the formula body weight/height2 in terms of kg/m². Abdominal and hip circumferences were measured and their ratio (AHR) was calculated. Abdominal circumference and hip circumference were measured in the most condensed part using a non-elastic cloth meter.

Laboratory and clinical measurements

Subjects attended human lab on one morning at 08.00 h for documentation of their full history and a physical examination. Blood samples were collected, via the cannulated antecubital vein, between 8:00–9:00 a.m. after an overnight fasting for all subjects. All participants refrained from any severe physical activity 48 h before measurements. After sampling in ETDA- or serum-tubes, blood was immediately chilled on ice, centrifuged and aliquots were frozen at – 20C until assayed. Blood samples were analyzed for serum TNF-α, cholesterol, LDL cholesterol, HDL cholesterol, triglycerides. Serum TNF-α was determined by ELIZA method (Enzyme-linked Immunosorbent Assay for quantitative detection of human TNF-α). The sensitivity of the TNF-α assay was 5.0 Pg/mL. Intra and inter-assay coefficients of variation were 6.0 and 7.4%, respectively. Total cholesterol, HDL and LDL cholesterol and tryglicerides were measured using the colorimetric enzymatic method with COBAS MIRA from Roche (Lörrach, Germany).

Data analysis

The statistical software program SPSS (SPSS Co, Chicago IL, version 17) for windows were used for data analysis. All data were tested for normal distribution by the Kolmogorov-Smirnov test Pearson's correlation coefficients were used to evaluate the correlations between serum TNF-α and lipid profile markers. All statistical tests were performed and considered significant at a $P \leq 0.05$.

Results

Anthropometric and clinical characteristics of the study participants are described in Table 1 and 2. All values are represented as mean ± SD. All subjects was obese and non-athletes.

Table 1. Mean and standard deviation of anthropometrical markers in studied subjects.

Variables	Age (years)	Weight (kg)	Height (cm)	BMI (kg/m²)	BF (%)	AC (cm)	HC (cm)	AHO
M ± SD	38.1 ± 1.98	94 ± 7.7	173.2 ± 4.5	31.3 ± 1.89	31.9 ± 1.34	104 ± 4.6	106 ± 4.8	0.98 ± 0.02

BMI, body mass index; BF, Body fat percentage; AC, Abdominal circumference; HC, Hip circumference; AHO, abdominal circumference to Hip circumference ratio;

Table 2. Mean and standard deviation of anthropometrical markers in studied subjects.

Variables	Serum TNF-α (pg/ml)	Triglyceride (mg/dl)	Total Cholesterol (mg/dl)	LDL cholesterol (mg/dl)	HDL cholesterol (mg/dl)
M ± SD	34.76 ± 13.33	208 ± 33.	189 ± 53	132 ± 24	47.3 ± 4.4

Based on Pearson's correlation coefficients, there was no significant correlation in serum TNF-α with TG, TC and LDL cholesterol. But we observed that serum level of TNF-α was negatively correlated with HDL cholesterol (see Table 3).

Discussion

Although controversial findings have been reported regarding the relationship between inflammatory or anti-inflammatory cytokines and markers of lipid profile or cardiovascular risk factors, they often suggested a very close relationship between them (Fain, 2006). However, the findings of this study did not show a significant relationship between serum levels of TNF-α with cardiovascular risk factors in obese adult men. In other words, in the present study,

serum levels of TNF-α were independent of the levels of cardiovascular risk factors such as TG, TC, and LDL in the studied population. These findings were observed in the obese population, while the majority of studies suggest that obesity and obesity-related diseases, such as type 2 diabetes, are on the one hand associated with systemic inflammation and increased inflammatory cytokine (Hultman, 1996), and on the other hand, they are associated with dyslipidemia and cardiovascular risk factors imbalance (Poirier, 2006). Higher levels of TNF-α, as a cytokine, in obese subjects compared to normal weight subjects have been reported by some recent studies (Hotamisligil *et al.*, 1995). The weight loss in obese patients with type 2 diabetes is associated with decreased TNF-α production and improved insulin resistance (Dandona *et al.*, 1998). TNF-α acts as a gene expression regulator in adipocytes. Since adipose tissue is an important source of TNF-α production, expression of this cytokine in human muscle and adipose tissue is increased in obesity (Hotamisligil *et al.*, 1995; Saghizadeh *et al.*, 1996). The literature suggests that TNF-α expression considerably increases in adipocytes of obese and insulin-resistant human subjects (Rotter *et al.*, 2003). Serum levels and TNF-α expression increases in adipose tissue of obese humans, and a reduction in its serum levels have been observed following weight loss (Dandona *et al.*, 1998). A recent review describes the characteristics of TNF-α as follows: TNF-α is an adipocytokine secreted from macrophages in adipose tissue and other tissues. In the adipose tissue, TNF-α accelerates lipolysis, and its increase damages both adipogenesis and lipogenesis. In the hypothalamus, TNF-α reduces appetite and stimulates the release of catabolic corticotropin hormones. In the liver, TNF-α increases the expression of the genes involved in the synthesis of free fatty acids and decreases the expression of the genes involved in the fatty acid oxidation (Bouassida *et al.*, 2010). The findings of a recent study show that plasma levels of TNF-α in obese diabetic subjects are about 30 percent higher than in the non-diabetic obese subjects. However, this response is not merely due to hyperglycemia, and the phenomenon of obesity along with hyperglycemia is responsible for increase in TNF-α in such patients (Merghani *et al.*, 2014).

Table 3. The relationship between Serum TNF-a and lipid profile markers in studied subjects.

	TNF-a (pg/ml)	Total Cholesterol (mg/dl)	Triglyceride (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
TNF-a (pg/ml) Pearson Correlatio Sig. (2-tailed) N	1 13	-.207 .497 13	-.244 .423 13	-.247 .416 13	-.777** .002 13
Total Cholesterol (mg/d) Pearson Correlatio Sig. (2-tailed) N	-.207 .497 13	1 13	.435 .138 13	.490 .089 13	.237 .436 13
Triglyceride (mg/di) Pearson Correlatio Sig. (2-tailed) N	-.244 .423 13	.435 .138 13	1 13	-.144 .639 13	.060 .846 13
LDL (mg/dl) Pearson Correlatio Sig. (2-tailed) N	-.247 .416 13	.490 .089 13	-.144 .639 13	1 13	.386 .193 13
HDL (mg/dl) Pearson Correlatio Sig. (2-tailed) N	-.777** .002 13	.237 .436 13	.060 .846 13	.386 .193 13	1 13

**.Correlation is significant at the 0.01 level (2-tailed).

It is known that obesity not only by disturbing some metabolic factors such as lipid profile markers including TG, TC, LDL, and HDL, but also in most cases, as an endocrine dysfunction of adipose tissue intensifies the outbreak of these diseases that are all associated with the metabolic syndrome (Bays *et al.*, 2005). Some observations have shown that TNF-α leads to increased production of vLDL which describe

the relationship between this cytokine and the plasma TG (Mari *et al.*, 2002). A direct and significant correlation between TNF-α with TG and cardiovascular disease have also been reported by some other studies (Ludvik *et al.*, 1995).

The findings of this research do not suggest a relationship between TNF-α, as an inflammatory cytokine, and cardiovascular indicators. However, the lack of relationship can be attributed to the low number of samples in the present study. Despite these observations, the considerable point of this study, even with a small number of samples, is the inverse significant relationship between this inflammatory cytokine and HDL, as good cholesterol, in obese men. Because, the findings of the present study suggest that the increase in systemic TNF-α levels in obese men is associated with decreased levels of HDL, which somehow indicates the relationship between cytokine profile and cardiovascular disease or metabolic syndrome in obese populations.

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