SERUM LEPTIN IN OBESITY AND ITS RELATION WITH FASTING GLUCOSE AND INSULIN RESISTANCE

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ABSTRACT
Studies in obesity have implicated adipose tissue secreted leptin in the development of insulin resistance, which in turn may lead to type II diabetes or metabolic syndrome. In this study, serum leptin was compared between non-active obese and normal weight men. We also determined its relationship with fasting glucose and insulin resistance in obese subjects. For this purpose, fasting blood samples were collected after overnight fast for measure serum leptin, insulin, glucose and insulin resistance of two groups. Data were analyzed by independent t test and Pearson's correlation coefficients. Serum leptin levels were higher in obese than in normal weight (p=0.000). Glucose, insulin and insulin resistance were higher in obese than normal weight men (p < 0.05). A significant positive correlation was found between serum leptin and fasting glucose in obese subjects (p = 0.000, r = 0.78). Obesity is associated with insulin resistance and leptin resistance. Despite relationship between leptin and insulin resistance in obese subjects,, fasting glucose concentration may be influenced by serum leptin directly.
KEYWORDS: Cytokine, Insulin, Leptin, Obesity,

INTRODUCTION

Over the past two decades, it has been found that certain peptide mediators secreted by adipose tissue, such as adiponectin, resistin, and leptin, play a major role in glucose and lipid homeostasis and the energy cost. They also found to be the effective components in the incidence of chronic diseases such as insulin resistance or dyslipidemia (Fasshauer *et al*., 2003). Adipocytes, as an active and complex endocrine organ, play an important role in the metabolism of the body by producing secretory materials such as hormones, prohormones, cytokines, and enzymes (Aldhahi *et al*., 2003). However, most studies have somehow noted their role in the dyslipidemia, insulin resistance, and obesity-related abnormalities (Ursula *et al*., 2004; Pradhan *et al*., 2001). Scientific studies have repeatedly reported the increased serum or plasma levels of leptin, resistin, and CRP and decreased levels of adiponectin in obese and insulin-resistant patients compared to normal weigh healthy individuals (Robert *et al*., 2007; Nayak *et al*., 2010). Both leptin and adiponectin play a role in regulating energy balance and insulin performance (Bouassida *et al*., 2010; Meier *et al*., 2004). Obesity is positively correlated with plasma leptin concentrations, so the amount of leptin secretion depends on the size and number of adipocytes. Weight loss, fasting, and starvation decrease leptin concentrations, and weight loss caused by some external interventions, such as diet and exercise, reduce leptin levels in obese populations (Eizadi *et al*., 2011). In contrast, leptin levels increase in response to weight gain and systemic inflammation (Aviva *et al*., 1991). Resting level of leptin in obesity-related diseases such as type 2 diabetes is significantly higher compared to the normal subjects due to the increased percentage of body fat (Schwartz *et al*., 1997) and impaired leptin secretion from adipose tissue (Garvey *et al*., 1991). In obese patients, increased adipose tissue is associated with hyperleptinemia and increased leptin resistance (Zhang *et al*., 2006). A study showed that leptin levels were not associated with acute response of insulin to glucose (Koebnick *et al*., 2008). In mice, increased plasma leptin temporarily inhibits insulin secretion (Cases *et al*., 2001). But this can only be limited to obese individuals and is a result of their increased leptin resistance. It has been shown that leptin resistance also increases in the pancreatic cells of the obese individuals and leads to the reduced control of insulin secretion by leptin (Kieffer *et al*., 2000). On the other hand, it is known that plasma levels of leptin are inversely associated with insulin sensitivity even after homogenization of body fat mass (Steinberger *et al*., 2003). However, there are limited studies on the relationship and effect of leptin on insulin resistance. The present study aimed at comparing the baseline levels of serum leptin between normal weight and obese men and determining the relationship between its serum levels with insulin resistance and blood glucose in obese men.

MATERIALS AND METHODS
Subjects

This study involved seventeen inactive healthy obese (BMI, 31.4 ± 1.5 kg/m2 and the same number non-obese men (BMI, 22.9 ± 38 kg/m2) that participated by accessible samples. Participants were non-athletes, non-smokers. Participants were included if they had not been involved in regular physical activity/diet in the previous 6 months. A

detailed history and physical examination of each subject was carried out. Those with chronic disease or metabolic disorders were excluded. In addition, exclusion criteria included supplementations that alter carbohydrate metabolism. After the nature of the study was explained in detail, informed consent was obtained from all participants.

Anthropometry and biochemistry

Anthropometrical and biochemical variables were measured. Body weight and height were measured on the same day to the nearest 0.1 kg and the nearest 0.1 cm, respectively. Body mass index (BMI) was calculated by dividing body mass (kg) by height in metres squared (m2). The abdominal circumference was measured to the nearest 0.1 cm, using a non-extendable flexible tape applied above the iliac crest and parallel to the ground; with the subject standing erect with abdomen relaxed. Hip circumference was measured at the maximum circumference between the iliac crest and the crotch while the participant was standing and was recorded to the nearest 0.1 cm.

All participants refrained from any severe physical activity 48 h before measurements. Basal concentrations of leptin, insulin and glucose were measured at 08:00 h after an overnight fast. Glucose was determined by the oxidase method (Pars Azmoon kit, Tehran). Serum separated to determine insulin and leptin by ELIZA. Intra- assay and inter-assay coefficient of variation of the method for serum leptin were 4.2and 6.7 %respectively.Insulin resistance index was assessed by homoeostasis model assessment: [fasting insulin (μ/ml) $\times$ fasting glucose (mmol/l)] / 22.5 (Afreen *et al*., 2013).

Statistical Analysis

Pearson's correlation coefficients were used to evaluate the associations between serum leptin with HOMA-IR and fasting glucose in obese subjects. After normal distribution of variables with a Kolmogorov–Smirnov test, An Independent sample T-test was used to compare all variables between obese and normal weight subjects. A P-value of < 0.05 was considered to be statistically significant.

RESULTS

In this study, serum leptin was compared between obese and normal weight men. Table 1 shows anthropometrical and metabolic markers between two groups. Base on statistical data, we observed that serum leptin was significantly higher in obese subjects than normal weight men ($p = 0.000$, Fig 1). Fasting glucose was significantly higher in obese than normal weight subjects ($p = 0.008$).

Insulin ($p = 0.001$) and insulin resistance ($p = 0.000$) were also higher in obese subjects (see table 1). Based on data by Pearson's partial correlation coefficients, non-significant correlation (borderline) was found between serum leptin and insulin resistance in obese subjects ($p = 0.052$, $r = 0.41$, Fig 2). In contrast, there was a strong, positive, linear relation between serum leptin and fasting glucose in obese subjects ($p = 0.000$, $r = 0.78$, Fig 3).

| Table 1: Mean and standard deviation of anthropometric and metabolic characteristics of studied group.		
Variables	Obese group	Normal group
Age (year)	38.5 (2.2)	38.6 (1.6)
Height (cm)	172 (4.6)	173 (1.90
Weight (kg)	93 (7.6)	68 (2.2)
Body mass index (kg/m^2)	31.4 (1.5)	23 (0.38)
Body Fat (%)	32.5 (1.9)	21.9 (0.97)
Visceral fat	13.2 (2.0)	7.4 (1.0)
Abdominal circumference (cm)	104 (5)	87 (3)
Hip circumference (cm)	105 (5)	93 (5)
WHO	0.99 (0.02)	0.94 (0.05)
Insulin (IU/ml)	16.7 (2.6)	12.9 (2.9)
Glucose (mg/dl)	95 (13)	83 (11)
HOMA-IR	3.89 (0.79)	2.65 (0.71)
Leptin (ng/ml)	12.3 (4.8)	3.5 (0.81)
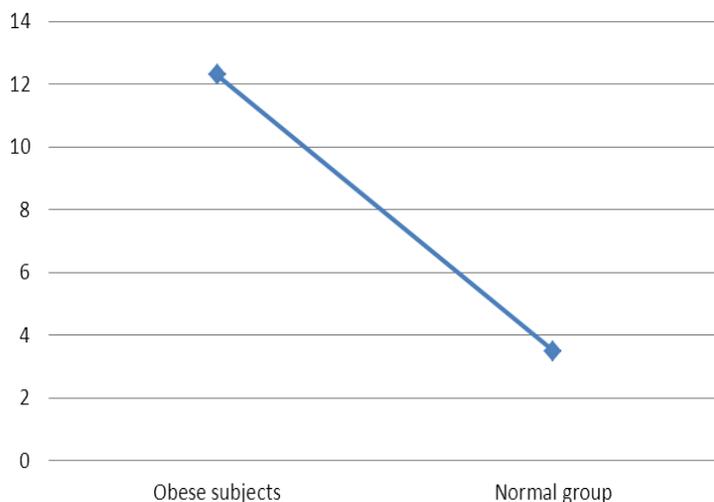
Fig 1: Serum leptin between obese and normal weight. Serum leptin levels were significantly higher in obese subjects than normal weight individuals.
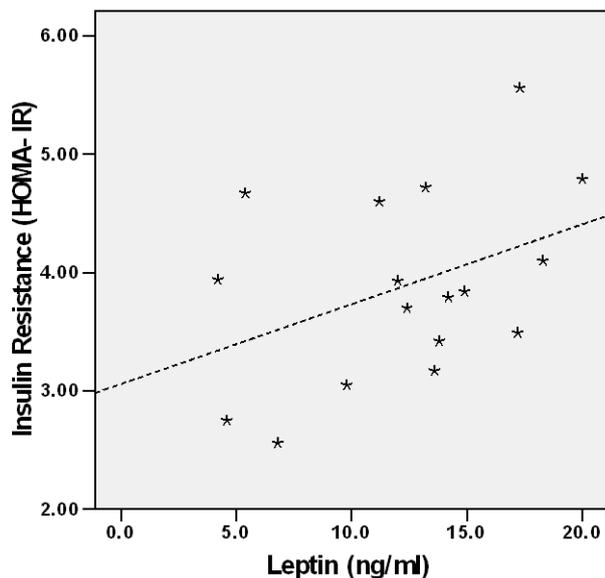

Fig 1: Relationship between serum leptin and insulin resistance in obese individuals. A borderline correlation
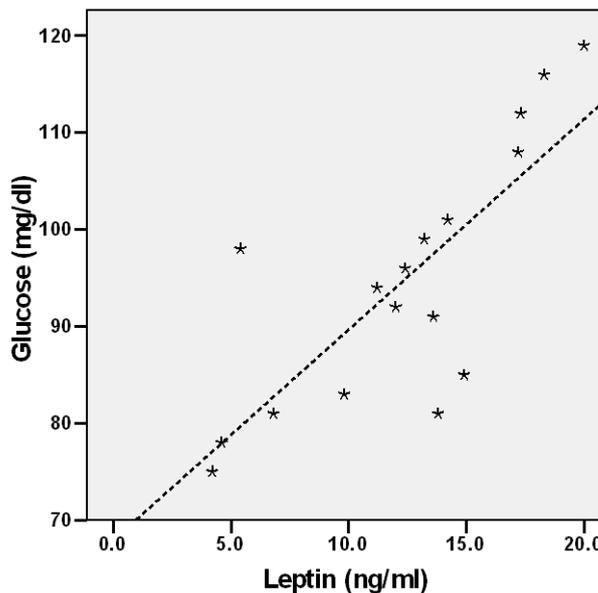
Fig 1: Relationship between serum leptin and fasting glucose in obese individuals. A significant positive correlation.

Regulation of body weight is a complex behavior that is a function of the physiological and genetic behaviors and other environmental interventions. In this regard, energy balance is one the most important factors (Jequier *et al*., 1999). Leptin is one the most effective peptide mediators in the obesity phenomenon so that the increased fat tissue, especially abdominal obesity, is associated with its higher levels (Jequier *et al*., 1999; Ozcelik *et al*., 2004). The findings of this study showed that serum levels of leptin in obese men were significantly higher compared to the

normal weight men. In other words, the findings of this study showed that the baseline levels of leptin in the obese men were 4 times higher compared to normal weight men. Leptin is known as an anti-obesity or anti-starvation hormone which functions as an afferent signal in the feedback regulation of body weight by controlling food intake and energy cost through affecting the path of the hypothalamus, pituitary, and gonads (Sinha *et al*., 1997). It can be concluded that the increased levels of leptin is associated with reduced food absorption and delayed starvation, but the question is why the obese individuals have l higher eptin levels compared to the ordinary individuals. To answer this question, researchers have pointed to the leptin resistance in the obese individuals (Martin *et al*., 2008). The evidence suggests that the internal leptin resistance is caused by obesity, and obesity-induced leptin resistance damages some peripheral tissues such as liver, pancreas, platelets, blood vessels, and myocardium (Martin *et al*., 2008). On the other hand, it has been suggested that higher secretion of peptide hormone depends on the adipose tissue metabolic activity rather than the actual mass of the adipose tissue (Woods *et al*., 2000).

It is worth mentioning that leptin levels are also affected by nutritional statuses as it decreases by starvation and increases by feeding (Rajala *et al*., 2004; Kim *et al*., 2001). These effects are somehow intervened by insulin and glucose (Rajala *et al*., 2004; Kim *et al*., 2001). Daily injections of leptin lead to the reduced food absorption, body weight, and fat mass as well as a balanced glycemic through increasing insulin performance (Park *et al*., 2008). Some studies attributed the higher leptin levels in type 2 diabetes to higher levels of body fat and its impaired secretion by the adipose tissue compared to healthy or normal weight individuals (Steinberger *et al*., 2003). It was also found that changes in the level of free fatty acids during starvation somehow affect the leptin secretion trough sending a message to adipose tissue (Considine *et al*., 1997). The hypothalamic function of leptin is relatively well known. However, the expression of its receptors in other tissues such as the endocrine part of pancreas indicates that leptin not only affects the hypothalamus, but also directly affects the other peripheral tissues (Kieffer *et al*., 2000). For example, some studies reported the inhibitory effect of leptin on insulin gene expression and its secretion from beta cells in humans and animal models (Kieffer *et al*., 2000; Kulkarni *et al*., 1997; Poitout *et al*., 1998). Despite these statements, in this study, although a direct linear relationship was observed between leptin serum levels and insulin and insulin resistance in obese male population, the relationship was not statistically significant which can be due to the low number of samples. Despite the lack of significant relationship between insulin and insulin resistance, this peptide hormone had a positive and significant correlation with fasting glucose levels. In other words, increased serum leptin levels in the studied obese men were associated with increased fasting glucose concentration. The significant relationship between leptin and glucose and the lack of relationship between leptin and insulin or insulin resistance implies the indirect role of leptin in the regulation of blood glucose levels.

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