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# Comparison of Changes in Postprandial Serum Leptin Between Healthy and Type 2 Diabetic Individuals

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## ABSTRACT

**Introduction**: Leptin resistance may be intensified by insulin resistance. This vicious cycle between insulin resistance and leptin resistance may increase feelings of hunger and reduce energy expenditure and ultimately increase obesity. In this study, postprandial changes in leptin, insulin and glucose were compared between healthy subjects and patients with Type 2 diabetes mellitus (DM). **Methods**: Six men with Type 2 DM and seven healthy men (matched for age and body mass index), after 12 hours of fasting, ate the same Iranian meal (*chelo kebab kobibeh*) consisting of 46% carbohydrate, 21% protein, 33% fat and 743 kcal energy. Blood samples were obtained before and 1, 2 and 4 hours after the meal, and serum leptin, insulin and glucose levels were measured. The area under incremental curve (AUIC) computed using the Trapezoidal method with fasting values was deducted from each time point, yielding net postprandial changes. **Results**: Leptin levels at the first hour were significantly reduced in both groups and then increased at the second and fourth hours after the meal, although not reaching fasting values in the diabetic group at the 4th hour. AUIC for leptin was significantly lower in the diabetic group (p=0.023). Despite high levels before the meal, the early increase in insulin in the diabetic group was lower and declined more slowly than in the healthy group. The incremental area under the insulin curve was significantly lower in the control group than in the diabetic group (p=0.006). **Conclusion**: It appears that an increased leptin level two hours after the meal is due to increased serum insulin and glucose levels. Leptin AUIC after meals in people with Type 2 DM is lower than in healthy people and may be due to decreased responsiveness to insulin in adipocytes because of insulin resistance.

**Keywords**: Leptin, insulin, glucose, postprandial, type 2 diabetes mellitus

## INTRODUCTION

The classical perception of adipose tissue as a storage depot for fatty acids has been replaced recently by the notion that adipose tissue has a central role in lipid and glucose metabolism and produces a large number of hormones and cytokines, e.g. tumor necrosis factor-α, interleukin-6, adiponectin, leptin, and plasminogen activator inhibitor-1 (Engeli *et al.*,1999; Gideon *et al.*, 2008).

During evolution, fat tissue presumably acquired an intermediary role between nutritional status and essential body

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functions such as feeding behaviour and metabolism (Farooqi *et al*., 1999). A key (co-) regulator of these functions is leptin, which is principally produced by adipocytes (Considine *et al*., 1996). Leptin is considered a hormone as it regulates body weight by maintaining the balance between food intake and energy expenditure through signaling to the brain the changes in stored energy levels (Zhang *et al*., 1994; La Cava & Matarese, 2004).

Type 2 Diabetes mellitus (DM) affects 100 million people throughout the world. Among the various factors implicated in the causation of DM, the role of leptin is increasingly being investigated (Bhatta-charya *et al*., 2008). Leptin secretion has a circadian rhythm. It shows a nocturnal peak and mid-day nadir; this variation may be linked to the pattern of meal eating (Sinha *et al*., 1996). Meal time and insulin apparently play a major role because delaying meals for 6.5 hours caused a 4-7 hour shift in nadir and peak leptin levels (Schoeller *et al*., 1996). Perhaps insulin is the major determinant of leptin secretory pattern; although several studies showed that insulin infusions for 2-10 hours had no effect on plasma leptin concentrations (Boden *et al*., 1997). It is plausible that repeated daytime postprandial insulin release induces an increase in leptinemia that becomes apparent in the afternoon and at night (Perfetto *et al*., 2004). Two studies suggest that insulin is a signal that mediates the effect of a meal on leptin levels (Panarotto & Maheux, 1999) and it has been demonstrated that leptin concentrations increase only 6 h after insulin infusion in normal subjects and after 8.5 h in patients with Type 2 DM (Malmstroem *et al*., 1996).

Since postprandial leptin and insulin changes are closely linked, impairment in insulin metabolism may affect postprandial regulation of leptin levels. We hypothesised that postprandial leptin response is altered in diabetic subjects. So we examined the effect of meals on circulating leptin levels in healthy men and age / BMI matched patients with Type 2 DM who were not on insulin therapy.

## METHODS

Based on a previous study by Stella *et al.* (2001) for detecting the minimum difference in leptin levels between groups with statistical power > 80 %, we calculated that 6 persons was sufficient for each group. In this clinical trial, subjects were 6 patients with type 2 diabetes mellitus and 7 healthy volunteers matched for age and body mass index. The diabetic subjects were not on insulin therapy and had no chronic or acute kidney and hepatic diseases. Diabetes mellitus was diagnosed based on two fasting blood sugar (FBS) >126 mg / dl and their medical history. The protocol was approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (AJUMS), Ahvaz, Iran. All subjects signed an informed consent prior to the study.

After 12 hours of overnight fasting, all subjects consumed the same Iranian meal (Chelo kebab kobibeh) consisting of 46% carbohydrate, 21% protein, 33% fat and 743 kcal energy. Venous blood samples were taken four times including after overnight fasting status and three times postprandial, that is, one, two and four hours after the meal. These samples were analysed to evaluate changes in serum levels of leptin, insulin and glucose. The analyses of leptin and insulin were carried out using RIA kits (Karen *et al*., 2002). Glucose was measured enzymatically using an auto analyser. All analyses were conducted at Tawhid Laboratory, Ahvaz, Iran.

The area under the incremental curve (AUIC) was calculated by using the trapezoidal method according to Matthews *et al.* (1990). The initial values were deducted from each time value for each person, thus yielding the net postprandial changes. Descriptive statistics were run on all measurements and results were listed as

means ± standard deviation. After doing the Kolmogorov–Smirnov test for assessing normality, repeated measures were used to determine changes from baseline across time. Independent sample *t*-tests were used to compare between groups and also Pearson correlations were used to determine if variables were correlated. All statistical analyses were conducted using SPSS software (version 17, Chicago, IL). Significance was set at $p < 0.05$.

## RESULTS

Descriptive characteristics are shown in Table 1 as means±SD. There were no significant differences between fasting leptin in healthy and diabetic groups. However significant differences were observed between fasting levels of insulin (Table 1).

There was a direct significant correlation between BMI and fasting leptin in two groups (Healthy: $p$=0.005, $r$=0.906; Diabetic: $p$=0.005, $r$=0.944). But correlation between age and fasting leptin was observed only in the healthy group ($p$=0.025, $r$=0.815). In the healthy group, there was no significant correlation between fasting leptin with leptin AUIC, fasting insulin and fasting glucose; but in the diabetic group there were significant correlations between fasting leptin with fasting insulin ($p$=0.021, $r$=0.879) and fasting glucose ($p$=0.018, $r$= -0.890).

At the first hour after meal, leptin levels in two groups declined significantly ($p$=0.001), and increased significantly after

**Table 1.** Descriptive characteristics of healthy subjects and patients with Type 2 diabetes mellitus (mean±SD)

| | *Healthy (n=7)* | *Diabetic (n=6)* | *P-value* |
|---|---|---|---|
| Age (yr) | 43.1 ± 4.9 | 44.4 ± 3.6 | 0.58 |
| Height (cm) | 172.1 ± 3.7 | 175.2 ± 3.9 | 0.2 |
| Weight (kg) | 76.6 ± 5.1 | 80 ± 4.7 | 0.23 |
| BMI (kg / m$^2$) | 25.8 ± 1.2 | 26.6 ± 1.1 | 0.66 |
| disease duration (yr) | - | 2.8 ± 1.2 | - |
| Fasting Leptin (ng / ml) | 9.06 ± 2.66 | 10.42 ± 2.99 | 0.405 |
| Fasting Insulin (µIu / ml) | 5.99 ± 1.54 | 13.73 ± 4.61 | 0.006 |
| Fasting Glucose (mg / dl) | 81.71 ± 4.30 | 152.83 ± 12.19 | 0.001 |

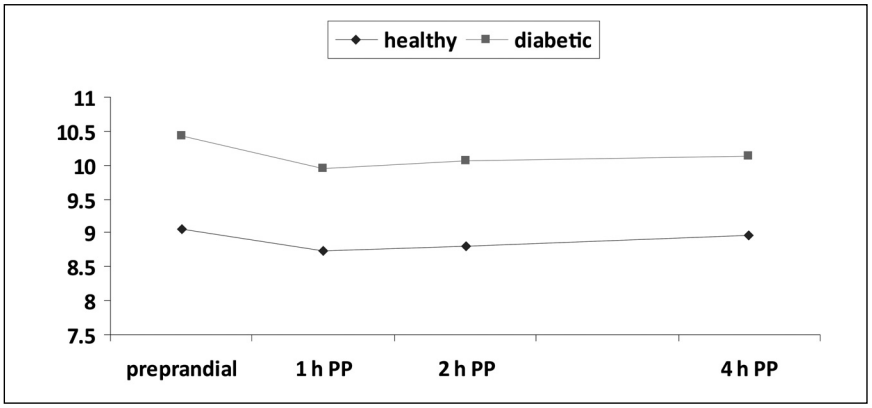


**Figure 1.** Leptin pre (Time 0) and postprandial (Times 1, 2 and 4) changes in healthy and diabetic groups

two hours after the meal (p<0.005) but did not reach pre-prandial levels (Figure 1). In the healthy group, there was a significant increase in serum leptin from second hour to fourth hour after the meal ($p$=0.017) but this increase was not statistically significant in the diabetic group. At the fourth hour after the meal, leptin concentration reached fasting level in healthy ($p$=0.002) but not in the diabetic group. $p$-values for comparisons of leptin levels in each group before and at three intervals after the meal are listed in Table 2 while all values are listed in Table 3.

Leptin AUIC was significantly higher in the healthy group ($p$= 0.023) but insulin AUIC ($p$=0.006) and glucose AUIC (p=0.001) was significantly higher in the diabetic group.

## DISCUSSION

In this study, leptin levels declined at the first hour after the meal in two groups but increased towards the end of the study. The mean AUIC of leptin concentration was lower in the diabetic group than in the control. The initial decrease in leptin concentration observed at the first measurement after the meal (1st hour) occurred in both groups. The early decline seems to be due to the diurnal cycle (Sinha *et al*., 1996; Stella *et al*., 2001). However, in previous studies (Silva *et al*., 2008; Szalecki & Janas, 2009), this decline lasted up to the second hour in the healthy group and fourth hour after a meal in the diabetic group. It is probably because consuming a high fat, low carbohydrate meal causes lower insulin secretion and consequently more delays in leptin secretion; the greater delay in the diabetic group may be due to insulin resistance

The later increase may be due to initial increase in glucose and insulin; but as insulin sensitivity is not normal in DM patients, it can be concluded that lower leptin response in the DM group, despite higher levels of insulin and glucose, is due to impairment in adipose tissue response to insulin. In addition, leptin AUIC, as an indicator of net postprandial secreted leptin, is lower in the diabetic group, whereas

**Table 2.** *P* values for leptin levels comparisons before and 3 times after meal in healthy subjects and patients with Type 2 diabetes mellitus

| | *1 hour postprandial* | | *2 hours postprandial* | | *4 hours postprandial* | |
|---|---|---|---|---|---|---|
| | *healthy* | *diabetic* | *healthy* | *diabetic* | *healthy* | *diabetic* |
| Fasting | 0.001 | 0.001 | 0.002 | 0.005 | 0.225 | 0.002 |
| 1 hour postprandial | - | - | 0.394 | 0.058 | 0.038 | 0.028 |
| 2 hours postprandial | - | - | - | - | 0.017 | 0. 421 |

**Table 3.** Pre and postprandial and AUIC values for insulin, glucose and leptin in healthy subjects and patients with Type 2 diabetes mellitus (mean±SD)

| | *Insulin (μIu/ml)* | | *Glucose (mg/dl)* | | *Leptin (ng/ml)* | |
|---|---|---|---|---|---|---|
| | *Healthy* | *Diabetic* | *Healthy* | *Diabetic* | *Healthy* | *Diabetic* |
| Fasting | 5.99 ± 1.54 | 13.73 ± 4.61 | 81.71 ± 4.30 | 152.83 ± 12.19 | 9.06 ± 2.66 | 10.42 ± 2.99 |
| 1hpp | 40.03 ± 4.70 | 38.5 ± 3.78 | 163.14 ± 5.55 | 241.33 ± 6.62 | 8.74 ± 2.65 | 9.95 ± 3.02 |
| 2hpp | 17.14 ± 2.25 | 34.67 ± 2.34 | 114.14 ± 4.10 | 219.67 ± 11.15 | 8.81 ± 2.60 | 10.07 ± 2.97 |
| 4hpp | 6.07 ± 1.30 | 21.67 ± 3.93 | 82.43 ± 2.37 | 162 ± 10.92 | 8.97 ± 2.57 | 10.13 ± 2.98 |
| AUIC | 50.86 ± 3.99 | 64.10 ± 9.40 | 130.79 ± 10.38 | 197.92 ± 10.25 | -0.76 ± 0.31 | -1.28 ± 0.39 |

insulin AUIC and glucose AUIC are higher in the diabetic group, which supports the hypothesis that glucose insensivity causes impairment in leptin secretion (Malmstroem *et al*., 1996; Panarotto & Maheux, 1999; Silva *et al*., 2008; Szalecki & Janas, 2009).

Feeding studies clearly indicate that while insulin sharply increases after meals or glucose infusions, it takes several hours before leptin also increases (Karen *et al*., 2002). In this study, it was also observed that leptin levels started to increase one hour after the increase in insulin and glucose, although it was lower in DM patients. The relationship between leptin and insulin appears to be complex. *In vitro* insulin stimulates leptin secretion by adipocytes after two hours (Barr *et al*., 1997). In humans, insulin infusion appears to prevent a diurnal decline of circulating leptin and increases leptin levels; this effect of insulin appears to be impaired in individuals who are insulin resistant (Saad *et al*., 1998).

Studies in insulin-deficient diabetic rats showed that plasma leptin concentrations decreased proportionally to those of insulin and inversely to those of glucose, independent of weight loss, consistent with the decrease resulting from decreased glucose transport into cells (Havel *et al*., 1998). The decline in insulin with fasting or poorly controlled Type 2 DM leads to a change in adipocytes fuel uptake that could regulate leptin synthesis and release; however, no correlation was found between percentage changes in leptin and changes in insulin (Brent *et al*., 1999). In isolated adipocytes, insulin causes leptin release in the presence of glucose but not in the presence of the 2-deoxyglucose analogue, suggesting that cellular uptake and metabolism of glucose is somehow coupled to leptin secretion (Bornstein *et al*., 1998).

Insulin plays an important role in leptin secretion regulation. After release from adipocytes, leptin is transported through the blood-brain barrier to the central nervous system. It causes hunger inhibition and a sensation of satiety by inhibiting the secretion of neurotransmitters, including neuropeptide Y (NPY) and stimulation of melanocyte-stimulating hormone (MSH) and cocaine amphetamine-regulated transcript (CART) release (Tucholski & Otto-Buczkowska, 2011). In this study, insulin resistance was found to lead to disturbance in leptin secretion. It is therefore assumed that it causes an increase in fat tissue to retrieve serum leptin. This fatty weight gain causes a cycle that increases both body weight and insulin resistance.

Obesity and visceral fat accumulation increase the risk of Type 2 DM, dyslipidemia, and cardiovascular disease (Almanza-Pérez *et al*., 2008; Rasouli & Kern, 2008; Fasshauer & Bluher, 2009). Dissections of the molecular mechanisms such as the adipo-insular axis and leptin role that relate obesity to insulin resistance are needed for prevention and treatment of these disorders.

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