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# Serum leptin and its relationship with metabolic variables in Arabs with type 2 diabetes mellitus

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**BACKGROUND AND OBJECTIVES:** Most studies on serum leptin in type 2 diabetes mellitus have focused on white populations. We studied serum leptin concentrations and parameters related to glycemic control and the association between leptin levels and anthropometric and metabolic factors in Arab patients with type 2 diabetes and in Arab control subjects.

**SUBJECTS AND METHODS:** Ninety-two patients (65 females and 27 males) with type 2 diabetes and 69 matched normal control subjects (48 females and 21 males) were included. Anthropometric measures (including body mass index [BMI] and waist:hip ratio) were assessed in all subjects. After an overnight fast, blood was collected for serum leptin assay. Other metabolic parameters including glucose, insulin, C-peptide, intact proinsulin, insulin resistance index (HOMA-IR), insulin-like growth factor 1 (IGF-1), lipids and hemoglobin A1c ($HbA_{1c}$) were determined.

**RESULTS:** Fasting serum leptin levels, IGF-1 and high-density lipoprotein (HDL) cholesterol were similar in patients with type 2 diabetes and control subjects. When obese subjects (BMI ≥30 kg/m$^2$) were analyzed separately, serum levels of leptin were significantly lower in patients compared to controls. In contrast, patients had higher fasting glucose, insulin, C-peptide, intact proinsulin, insulin resistance, total cholesterol, triglycerides, $HbA_{1c}$, and a larger waist circumference and waist-to-hip ratio than controls. Serum leptin correlated positively with BMI, negatively with waist-to-hip ratio, and demonstrated no relationship to other parameters.

**CONCLUSION:** Patients with type 2 diabetes in an Arab ethnic population showed evidence of an unfavorable metabolic profile despite having leptin levels similar to controls. Obesity influences serum leptin levels more significantly in type 2 diabetes, in which leptin levels tends to be low.

Type 2 diabetes mellitus is characterized by defects in insulin action and insulin secretion, free fatty acid metabolism, and fat distribution.[1-3] More recently, the adipocyte-derived hormone leptin has been implicated in the regulation of adipose mass[4] and has been reported to alter both insulin sensitivity[5-7] and insulin secretion.[8] Although it is clear that circulating leptin is positively correlated with various measures of adiposity,[9] the relationship of diabetes to plasma leptin concentration, independent of adiposity, is less clear.

Earlier reports variably suggest that circulating leptin is unchanged,[10-14] reduced,[15,16] and raised[17] in type 2 diabetes. However, studies of humans with untreated type 1 diabetes and animals with insulin deficiency consistently demonstrate that plasma leptin concentration and leptin mRNA are reduced.[18-21] The variable results in type 2 diabetes are not surprising given that subjects differed with respect to extent of obesity, age, gender and ethnic group. In the literature, most of the studies on serum leptin in type 2 diabetes have focused on white populations. In this study we investigated leptin concentrations and parameters related to glycemic control in Arabs with type 2 diabetes and in matched normal control subjects. We also assessed the relationship between leptin and anthropometric and metabolic variables.

## SUBJECTS AND METHODS

Ninety-two Arab patients with type 2 diabetes mellitus and 69 normal control subjects were included in the study. The patients and controls were matched by ethnic group, age, gender and body mass index (BMI). Patients had been diabetic for a median of 6 years

(range, 0.5-20.5 years). They were recruited from the endocrine clinics of Mubarak Al-Kabeer Hospital and the Hawalli region in Kuwait. Twelve patients were treated with diet only, 54 patients were treated with oral anti-diabetic agents and 26 were on insulin therapy. No patient or control had other known illness and none of the patients or controls was taking medications other than their anti-diabetic therapy (except for 42 patients taking either ACE inhibitors or angiotensin receptor blockers, 36 taking either statins or fibrates and 39 taking low-dose aspirin). Controls were healthy subjects who in the last 12 months were not taking any medication and had no family history of diabetes mellitus. The study protocol was approved by the local ethics committee and patients gave written informed consent.

Anthropometric measurements (including waist and hip) and blood pressures were assessed in all subjects. The waist and hip circumferences were measured using a flexible measuring tape directly applied to the subject's skin. The narrowest waist and widest hip circumferences were taken (after taking the average of three measurements for each individual). After 12 hours of an overnight fast, blood was collected for the measurement of serum leptin, glucose, insulin, C-peptide, intact proinsulin, insulin-like growth factor 1 (IGF-1), lipids and hemoglobin A1c ($HbA_{1c}$). Glycemic control was defined as excellent if the measured $HbA_{1c}$ was <6.5%, very good if $HbA_{1c}$ was 6.5% to 7.0%, good if $HbA_{1c}$ was 7.1% to 7.5%, acceptable if $HbA_{1c}$ was 7.6% to 8.0%, and poor if $HbA_{1c}$ was >8%. Insulin resistance was calculated using the HOMA-IR formula: Fasting insulin (μU/mL)×fasting glucose (mmol/L)/22.5.

Blood samples were immediately centrifuged at 2500 rpm at 4°C for 15 minutes and the supernatants were stored at -70°C until analysis. Serum leptin was measured by radioimmunoassay (Human Leptin RIA kit, Linco Research, St. Charles, Missouri, USA).[22] Plasma glucose, triglycerides and total cholesterol were measured by an enzymatic colorimetric test using an automatic colorimeter (Hitachi 717, Boehringer Mannheim Gmbh Diagnostica, Mannheim, Germany). Insulin and C-peptide concentrations were measured by radioimmunoassay and the double antibody C-peptide kit was used for the measurement of C-peptide.[23] Intact proinsulin was measured by an ELISA method (DAKO Diagnostica, Cambridgeshire, UK). IGF-1 was measured by an ELISA (Diagnostic System Laboratories, Texas, USA). The inter-assay and intra-assay coefficients of variation (CV) were 3.4% and 2.5% for leptin (at 5.8 μg/L), 1.4% and 1.2% for triglycerides (at 1.2 mmol/L), 3.3% and 2.8% for total cholesterol (at 3.5 mmol/ L), 7.0% and 7.8% for insulin (at 30.7 mU/L), 1.9% and 3.0% for C-peptide (at 2929 pmol/L), 4.5% and 5.2% for intact proinsulin (at 4.2 pmol/L), and 3.1% and 6.0% for IGF-1 (at 16μg/ L), respectively. Hemoglobin, packed cell volume, white cell count, platelets, and liver and renal function were measured by routine laboratory techniques.

Data are expressed as mean and the standard error of the mean or median and range. Data in patients and controls were compared using the Mann-Whitney U test or the unpaired t test as appropriate. Correlation between variables was sought using the Spearman rank correlation coefficient (rho). Non-normally distributed variables (insulin, C-peptide and intact proinsulin) were normalized by log-transformation prior to analysis. The Statview program was used for statistical analysis. Differences were considered statistically significant when the *P* value was <.05.

**Table 1.** Clinical characteristics of patients with type 2 diabetes and controls.

| | Patients | Controls | *P* value |
|---|---|---|---|
| Number | 92 | 69 | |
| Males | 27 | 21 | |
| Females | 65 | 48 | |
| Age (years) | 41±1 | 40±1 | NS |
| Body mass index (kg/m²) | 32.1±0.6 | 31.6±1.4 | NS |
| Waist circumference (cm) | 100.6±1.5 | 95.7±2.1 | .08 |
| Waist:hip ratio | 0.90±0.06 | 0.80±0.02 | .0001 |
| Systolic blood pressure (mm Hg) | 134±2 | 133±3 | NS |
| Diastolic blood pressure (mm Hg) | 83±2 | 78±2 | .02 |

Values are mean ± SEM. NS: not statistically significant

## RESULTS

Hemoglobin, white cell count, packed cell volume, platelets, creatinine, and liver function tests were within the normal range and similar in patients and controls. Glycemic control in the patients was excellent in 9 patients (10%), very good in 12 (13%), good in 40 (44%), acceptable in 12 (13%), and poor in 19 patients (20%). Patients tended to have a higher waist circumference, waist-to-hip ratio and diastolic blood pressure than controls (Table 1).

Fasting serum leptin levels were similar in patients with type 2 diabetes and control subjects. When obese subjects (BMI ≥30 kg/m²) were analyzed separately, serum levels of leptin were significantly lower in obese patients compared to obese controls (Table 2), and the differences in leptin remained significant even after corrections for the differences in insulin. Women in each group had significantly higher leptin levels than men (patients, *P*=.03; controls, *P*=.002). Gender differences

remained significant even after correction for differences in BMI (patients, $P$=.038; controls, $P$=.0001).

Patients had significantly higher fasting glucose, insulin, C-peptide, intact proinsulin, total cholesterol, triglycerides, HOMA-IR and $HbA_{1c}$ than controls (Table 3). However, fasting serum levels of IGF-1 and HDL cholesterol were similar in patients and control subjects.

Serum leptin correlated positively with BMI (rho=0.49, $P$=.003) and negatively with waist-to-hip ratio (rho=−0.27, $P$=.049) and $HbA_{1c}$ (rho=-0.27, $P$=.04). Serum leptin demonstrated no relationship to other metabolic parameters or to duration of diabetes. Multivariable linear regression analysis between leptin and age, diabetes control ($HbA_{1c}$), weight, BMI, waist circumference and waist-to-hip ratio in patients showed only a strong association with BMI ($P$=.02) and a tendency of negative association with waist-to-hip ratio ($P$=.07), but not with other variables.

**Table 2.** Levels of serum leptin (ng/mL) in non-obese and obese subjects.

| | Non-obese | Obese |
|---|---|---|
| Patients | 22.2±1.7 | 19.4±1.2 |
| Controls | 21.3±2.0 | 21.1±2.1 |
| *P* value | NS | .002 |

Values are mean±SEM; Obese: BMI≥30.0 km/m²

**Table 3.** Fasting levels of metabolic variables in patients with type 2 diabetes and controls.

| | Patients | Controls | *P* value |
|---|---|---|---|
| Glucose (mmol/L) | 9.8±0.5 | 5.3±0.1 | .0001 |
| Insulin (pmol/L) | 29.4±6.2 | 10.3±2.2 | .003 |
| Intact proinsulin (pmol/L) | 37.5±3.6 | 8.6±1.2 | .0001 |
| C-peptide (pmol/L) | 738±88 | 582±35 | .05 |
| HOMA-IR | 16.4±5.1 | 2.8±0.5 | .006 |
| $HbA_{1c}$ (%) | 9.6±0.3 | 6.0±0.3 | .0002 |
| IGF-1 (µg/L) | 25.3±2.1 | 24.5±1.7 | NS |
| Triglycerides (mmol/L) | 1.59±0.10 | 1.35±0.16 | .03 |
| Total cholesterol (mmol/L) | 5.95±0.14 | 5.38±0.18 | .01 |
| HDL (mmol/L) | 1.19±0.07 | 1.36±0.14 | NS |
| Leptin (ng/mL) | 20.2±1.5 | 20.9±1.8 | NS |

Values are mean±SEM

## DISCUSSION

It has recently been shown that leptin levels display ethnic differences. For example, a large population study from the United States has showed that leptin levels were higher in non-Hispanic blacks than in whites with subjects of Mexican-American origin having leptin levels in between.[24] Inhabitants of the Pacific island of Kitava had lower leptin levels than whites,[25] whereas subjects of south Asian origin seem to have a higher leptin level than whites or Chinese.[26,27] In subjects of African origin, the information is not conclusive since studies showing higher leptin than in other ethnic groups[24] or no difference[28,29] have been published. To further explore whether differences exist in leptin levels in Arabs with and without diabetes, we assessed leptin levels in obese and non-obese Arabs with type 2 diabetes and in obese and non-obese normal control Arabs. We demonstrated that leptin levels are similar in non-obese type 2 diabetics compared with matched non-obese controls. However, leptin was lower in obese type 2 diabetics than in obese controls even after correction for the differences in insulin levels between the two groups.

Most cross-sectional studies have shown no difference in leptin levels between diabetic and non-diabetic individuals of similar body weight.[30-36] One study in Saudi Arabian male patients with metabolic syndrome (and type 2 diabetes) and coronary artery disease demonstrated increased levels of leptin in patients compared with controls.[37] However, there is a single report of reduced leptin levels in obese individuals with poorly controlled diabetes.[38] Consistent with our findings is a recent report that obese type 2 diabetics had lower leptin levels than obese control subjects.[38] In the same study, obese subjects with less beta-cell dysfunction and controlled diabetes had leptin levels only slightly lower than those of control subjects. These results were similar to our findings of a negative association between leptin and $HbA_{1c}$. Moreover, if all subjects (obese and non-obese) had been combined into a single group,[38] the difference in leptin levels compared with control subjects would have been attenuated as was the case in our study, and this was reflected clearly when a multivariable regression analysis was applied and $HbA_{1c}$ did not seem to be an independently associated variable in our study.

Similarly, the association between high leptin levels and subsequent weight gain deserves attention. Pima Indians who gained at least 3.0 kg/year had lower baseline leptin levels than those whose weight remained stable.[39] It remains to be seen how these findings compare with studies in other populations. Nevertheless, our study design does not allow definitive testing of these hypotheses since we do not know the time of diabetes onset in relation to changes in insulin secretion or changes in body fat or weight. Our finding of the significant positive correlations between serum leptin and measures of adiposity like BMI was in agreement with observations

in other populations.[38-40] However, the observed negative relationship between leptin and waist-to-hip ratio in this group of subjects seems difficult to explain, but less association was found by multiple regression analysis. An opposite relationship between leptin and waist-to-hip ratio was found for different age groups of women; there was a positive relationship in pre-menopausal and a negative relationship in postmenopausal women.[41] In our study, however, similar numbers of postmenopausal subjects (patients and controls) were studied (less than 10 subjects) and none of the subjects was taking hormone-replacement therapy.

In conclusion, non-obese Arabs with type 2 diabetes have leptin levels similar to control subjects. However, obesity in Arabs with type 2 diabetes is associated with low serum leptin levels. This probably suggests that leptin levels display an ethnic difference.

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