Reduced Leptin Concentrations in Subjects With Type 2 Diabetes Mellitus in Sudan

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Differences have been observed in the relationship between leptin and metabolic perturbations in glucose homeostasis. Because no information is available from indigenous African populations with diabetes, the purpose of this study was to investigate the possible associations between leptin and different clinical and biochemical characteristics of a large group of subjects with type 2 diabetes mellitus in Sudan. A total of 104 (45 men and 59 women) consecutive type 2 diabetes patients and 75 control subjects (34 men and 41 women) were studied. The body mass index (BMI), blood glucose, serum insulin, and proinsulin were measured and related to serum leptin concentrations. Leptin was higher in females than in males and correlated significantly to BMI. The main novel finding was that serum leptin was significantly lower in diabetic subjects compared with controls in both females ($P = .0001$) and males ($P = .019$), although BMI did not differ between diabetic and nondiabetic subjects. Diabetic subjects treated with sulphonylurea (n = 81) had lower BMI than those treated with diet alone or other hypoglycemic drugs (n = 23) ($P = .0017$), but there was no difference in leptin levels between the 2 groups after adjustment for BMI ($P = .87$). In diabetic subjects, serum leptin correlated positively with the homeostatic assessment (HOMA) of both β-cell function ($P = .018$) and insulin resistance ($P = .038$), whereas in control subjects, leptin correlated with insulin resistance ($P = .0016$), but not with β-cell function. Diabetic subjects had higher proinsulin levels ($P = .0031$) and higher proinsulin to insulin ratio ($P = .0003$) than nondiabetic subjects. In univariate analysis, proinsulin showed a weak correlation to leptin ($P = .049$). In conclusion, we show in a large cohort of Sudanese subjects with type 2 diabetes that circulating leptin levels are lower in diabetic subjectss than in controls of similar age and BMI. The lower serum leptin in diabetic subjects may be a consequence of differences in fat distribution.

TYPE 2 DIABETES MELLITUS is a heterogeneous and polygenic disease associated with abnormal insulin secretion or defective insulin action, often associated with obesity.[1-3] The prevalence of type 2 diabetes mellitus in the Sudanese population is 3.4%, and type 2 diabetes mellitus accounts for 75% of all diagnosed cases, among whom the majority also has a family history of diabetes.[4] In a population-based study, 40% of the patients were prone to be obese.[5] Obese subjects exhibited high levels of leptin.[6,7] Leptin is associated with the body mass index (BMI) and body fat in nonobese and obese subjects and patients with type 2 diabetes mellitus,[8,9] although residual variability in leptin levels has been observed at a given level of BMI, suggesting multifactorial regulation.[6,10] This may be attributed to the strong positive association between the leptin and insulin concentrations.[7,11-13] The serum leptin concentration also has a gender dimorphism, with higher serum levels in females than in males.[7,14] Other hormonal factors suggested to be involved in leptin regulation are catecholamines and glucocorticosteroids.[7,15-18]

Previous studies of leptin in type 2 diabetes have shown no difference in basal levels, apart from expected differences due to BMI[19-20] or a reduced leptin level, which may be explained by differences in fat distribution.[21] Furthermore, impaired leptin secretion after stimulation by glucocorticoids has been reported in one study in type 2 diabetics.[20] Importantly, however, the relationship between leptin and variables involved in glucose homeostasis and diabetes might show ethnic differences.

A recent population study of Peruvian Indians compared with a Caucasian population showed that the Indians had higher insulin and lower leptin levels than the Caucasians.[22] Similarly, Chilean Indians also had higher insulin and lower leptin levels than the Caucasians.[23] Furthermore, Mexican-American subjects showed higher levels of leptin compared with age- and sex-matched non-Hispanic whites.[24] These results in different ethnic groups reinforce that studies have to be undertaken in different populations. Thus, we wanted to report the relationship between leptin and clinical and biochemical characteristics of type 2 diabetes mellitus in Sudanese subjects in comparison with a control group of nondiabetics.

SUBJECTS AND METHODS

This study was conducted during a 3-month period beginning January 1999. A total of 104 diabetic patients (45 men and 59 women) were recruited from the outpatient diabetes clinic at Omdurman Teaching Hospital. They were treated with glibenclamide (n = 80), gliclazide (n = 1), metformin (n = 4), and diet alone (n = 19). The Federal Ministry of Health approved the study. Inclusion criteria were age $\geq$ 20 years and duration of diabetes $\geq$ 1 year. A matched group of 75 (41 men and 34 women) apparently healthy, nondiabetic subjects, who lived in the same area, served as controls. Informed consent was obtained from all subjects. Type 2 diabetes mellitus was defined as nonketosis-prone diabetes, treated with diet and/or oral hypoglycemic agents. A questionnaire including personal details and clinical characteristics was completed for all subjects. Blood pressure, weight in kilograms, and height in meters in light clothing without shoes were measured, and BMI was calculated. In the fasting state, blood samples were drawn for the determination of serum leptin, glucose, insulin, and proinsulin. The samples were centrifuged within 2 hours after collection and kept frozen at -20°C until analyzed at the Department of Medicine, Malmö University Hospital, Malmö, Sweden. The demographic and clinical characteristics of the subjects are shown in Table 1.

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Chemical Analysis

Leptin was measured using a radioimmunoassay (RIA) kit (Linco Research, St Charles, MO), detecting immunoreactive human leptin with a sensitivity of 0.5 ng/mL. Insulin was measured with specific RIA cross-reacting less than with 0.2% proinsulin (Linco Research). Total serum proinsulin levels were measured using a goat antibody raised against human proinsulin, human proinsulin standards, and ^{125}I-human proinsulin as tracer (Linco Research). This assay detects intact proinsulin (100%) and des-31,32 proinsulin (95%), but does not cross-react with insulin, C-peptide, or des-64,65 proinsulin (< 0.1%). Plasma glucose was analyzed using the glucose oxidase technique.

Homeostasis Model Assessment Calculation

Homeostasis model assessment (HOMA) was used to assess pancreatic β-cell function (HOMA B) and insulin resistance (HOMA IR) using fasting insulin and glucose concentrations by the formula: HOMA-B (%) = 20 X [insulin]/(glucose - 3,5) and HOMA-IR = insulin/(22,5e$^{-\ln[\text{glucose}]}$).[25]

Statistical Analysis

All data were expressed as mean ± SD. Statistical analysis was performed using the program SAS for Windows 6.12 (SAS Institute, Cary NC). Student's unpaired *t* test was used for comparison between groups for variables with normal or log-normal distribution. Pearson's correlation coefficient was used to determine association with variables normal or log-normal distribution. Adjustment calculated as Pearson's partial correlation coefficients.

RESULTS

Table 1 shows the characteristics of the 2 study groups. There was no difference in age or BMI between the groups. Fasting serum leptin levels were significantly lower in subjects with type 2 diabetes mellitus compared with healthy controls. This was evident both in females ($P = .0001$) and males ($P = .019$). Furthermore, as is known from several previous reports, leptin levels were higher in females than in males and correlated with BMI and circulating insulin (Table 2).

HOMA calculations showed that type 2 diabetic subjects had a significant increase in insulin resistance ($P = .0001$) and impaired β-cell function ($P = .0001$).

Table 1. Demographic and Clinical Characteristics of the Subjects

Characteristics	Type 2 Diabetes	Controls	
No. of subjects	104	75	
Males/Females	45/59	34/41	
Age (yr)	53.8 ± 10.7	53.4 ± 8.9	($P = .83$)
BMI (kg/m^2)	23.5 ± 5.3	24.0 ± 3.7	($P = .59$)
Diabetes duration (yr)	9.9 ± 7.9	—	
Systolic BP (mm Hg)	117 ± 14	119 ± 16.0	($P = .22$)
Diastolic BP (mm Hg)	79 ± 8	80 ± 10	($P = .71$)
Leptin (ng/mL)*	9.6 ± 7.9	14.9 ± 11.6	($P = .0066$)
Males	4.4 ± 2.6	6.7 ± 4.9	($P = .019$)
Females	13.5 ± 8.3	25.1 ± 9.3	($P = .0001$)
Glucose (mmol/L)*	8.6 ± 4.4	4.2 ± 0.9	($P = .0001$)
Insulin (pmol/L)*	75 ± 47.0	67.6 ± 23.2	($P = .99$)
Proinsulin (pmol/L)*	26.3 ± 21.7	17.8 ± 12.8	($P = .0031$)
PI/insulin ratio	0.38 ± 0.30	0.24 ± 0.12	($P = .0003$)
HOMA IR	6.15 ± 0.92	5.58 ± 0.40	($P = .0001$)
HOMA B	2.82 ± 1.26	4.62 ± 0.90	($P = .0001$)

NOTE. Mean ± SD.
Abbreviations: BP, blood pressure; PI, proinsulin.
*Serum concentrations.

Table 2. Correlation Between Serum Leptin Concentrations and Different Clinical and Biochemical Characteristics in Diabetic and Nondiabetic Subjects

Leptin	Diabetic Subjects		Controls	
BMI	$r = .61$	$P = .0001$	$r = .47$	$P = .0001$
Serum glucose	$r = .11$	$P = .27$	$r = .14$	$P = .35$
Serum insulin	$r = .46$	$P = .0001$	$r = .47$	$P = .0097$
Serum proinsulin	$r = .27$	$P = .0058$	$r = .31$	$P = .011$
PI/I ratio	$r = .08$	$P = .45$	$r = .06$	$P = .70$

Also, after adjustment for BMI, leptin concentrations correlated with HOMA IR both in subjects with type 2 diabetes ($r = .21$, $P = .038$) and controls ($r = .44$, $P = .016$), whereas leptin correlated with HOMA B only in subjects with type 2 diabetes ($r = .24$, $P = .018$). In subjects with type 2 diabetes, serum leptin correlated significantly with systolic ($r = .28$, $P = .0038$) and diastolic ($r = .30$, $P = .0021$) blood pressure, but this correlation disappeared after adjustment for BMI. In nondiabetic subjects, the systolic and diastolic blood pressures were not related to leptin concentrations. No differences were found in leptin levels between postmenopausal (n = 70) or premenopausal (n = 23) women in either the nondiabetic (24.0 ± 8.9 ng/mL *v* 29.7 ± 10.6 ng/mL) ($P = .30$) or the diabetic groups (14.3 ± 89 *v* 11.3 ± 6.5 ng/mL, $P = .20$).

Subjects with type 2 diabetes had significantly higher serum proinsulin levels than nondiabetic subjects (26.3 ± 21.7 *v* 17.8 ± 12.8 pmol/L, ($P = .0031$) (Table 1). The proinsulin/insulin ratio was also significantly elevated in type 2 diabetic subjects compared with nondiabetic subjects (0.38 ± 0.30 *v* 0.24 ± 0.12, respectively ($P = .0003$) (Table 1). Leptin levels correlated both with serum insulin and proinsulin in both diabetic subjects and controls, whereas no correlation was seen between leptin and the proinsulin/insulin ratio (Table 2).

Type 2 diabetic subjects treated by diet plus sulphonylurea had lower levels of leptin (8.7 ± 7.3 ng/mL, n = 81) than those treated with diet alone or diet and metformin (13.2 ± 8.7 ng/mL, n = 23, $P = .0063$). However, this difference was not significant after adjusting for BMI ($P = .87$). The subjects treated with sulphonylurea, however, had lower BMI than the other group (22.7 ± 4.9 *v* 26.1 ± 5.9, $P = .0017$).

DISCUSSION

The present study examined the difference in circulating leptin between subjects with diabetes and healthy subjects in Africans in Sudan, as this population has never been investigated for more understanding of the metabolic changes in type 2 diabetes mellitus. We found that leptin levels correlated with BMI in both diabetic and nondiabetic subjects and were higher in females than in males, which confirms previous studies in other populations.[14,26]

The main novel finding in this study was that the Sudanese subjects with type 2 diabetes had lower leptin levels than the controls. This was not due to any difference in BMI, which may suggest a different regulation of leptin in diabetic subjects compared with other populations.[19,20] A possible explanation for the

lower leptin levels in diabetic subjects is a difference in the fat distribution between the 2 groups, as it has been shown that subcutaneous fat produces more leptin than the omental fat,[27] and diabetics have increased visceral fat and less subcutaneous fat. This would then confirm a previous report of lower leptin levels in diabetics of Caucasian origin due to altered fat distribution.[21] Another possibility, however, is a relative insulin deficiency in the diabetic subjects, because insulin is an important stimulator of leptin production.[7,28,29] Thus, as calculated by the HOMA model, the diabetic subjects had a marked insulin resistance and impaired insulin secretion, which suggests a relative insulin deficiency. Leptin has been shown to improve insulin sensitivity.[30,31] However, whether normalization of leptin levels in diabetic populations with low leptin, such as the Sudanese subjects, improves insulin resistance is not known. We found no difference in leptin levels between premenopausal and postmenopausal women, as has also been reported before in other populations.[14] Leptin also correlated with insulin and proinsulin, which is known from studies in other populations.[7]

In conclusion, it appears in this study of a large cohort of subjects with type 2 diabetes mellitus in Sudan that circulating leptin levels are lower in diabetics than in controls of the similar age and BMI. It remains to be established whether the lower leptin levels in Sudanese diabetics is explained by the difference in fat distribution between type 2 diabetic patients and the nondiabetic subjects.

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