Percentage of Free to Total Leptin in Diabetic Patients and its Correlation to Blood Insulin, Glucose and Lipids

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ABSTRACT

INTRODUCTION: Leptin is a hormone resulting from the obesity gene which could be important in the pathogenesis of the insulin. Only limited data concerning the interaction of insulin, glucose tolerance and free leptin are available and no data exist on the potential influence of free vs. bound circulating leptin. We, therefore, studied percentage of free to total leptin in diabetic patients.

MATERIAL AND METHODS: Thirty non-insulin dependent diabetic obese patients (age: 50±20 years, BMI >30 kg/m²) and 30 non-insulin dependent diabetic non-obese patients (age: 50±20 years, BMI <25 kg/m²) were studied. Free leptin was purified by Gel filtration chromatography and the fractions were collected and then their free leptin was measured by a high sensitive ELISA method. Circulation total leptin and insulin was measured by ELISA.

RESULTS: Circulation total leptin was significantly correlated to insulin ($P < 0.005$). Percentage of free leptin to total in obese subjects was more than non-obese subjects (27% ± %1 vs. %3 ± %4, [$P < 0.001$]). Percentage of free to total leptin showed a positive correlation with insulin (r = 0.58 [$P < 0.001$]), insulin resistance (r = 31 [P < 0.015]) and BMI (r = 0.86 [$P < 0.001$]).

CONCLUSION: The majority of leptin which circulates in obese individuals was free form. Presumably it is bioactive protein of hormone and thus obese subjects are resistant to free leptin.

KEYWORLDS: Percent of free to total leptin, Insulin, Diabetes.

INTRODUCTION

Leptin is a 16-kD protein that is involved in the regulation of body weight. The primary amino acid sequence indicated that leptin may adopt a helical cytokine structure similar to interleukin-2 (IL-2) and growth hormone, and this led to the early indication that its receptor might be a member of the hemopoietin receptor family (1). Leptin reduces food intake and stimulates energy expenditure by activating its receptor in specific hypothalamic nuclei. Spontaneous mutations lead to a functional defect in either leptin or its receptor result in a complex syndrome that includes morbid obesity, hypothermia, infertility, hyperglycemia, decreased insulin sensitivity, and hyperlipidemia. Besides serving a weight-regulating function, leptin also plays a role in other processes including metabolism, reproduction, hematopoiesis, and immunity (2-4). Evidence has accumulated to suggest that insulin and leptin are closely related partners in physiological and pathophysiological conditions (e.g. coexistence of insulin resistance and leptin resistance in common obesity).

The long isoform (ObRb) of the leptin re-

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ceptor, which conveys most of the physiological actions of leptin, is present on pancreatic β cells (2). Although initial reports have produced conflicting results, it has now been demonstrated in a number of studies that leptin reduces insulin release from rodent and human β cells (1). In islets of leptin-deficient ob/ob mice, leptin inhibition of insulin secretion was smaller at high glucose concentrations and absent in the presence of glucagons-like peptide-1 (GLP-1), suggesting that it could be overcome by nutrient and incretion signals (1-3). It has been shown that a physiological increase in serum leptin can reduce insulin secretion in rats in vivo (4). Leptin suppresses insulin secretion from human islets at concentrations as low as 0.01 nmol/L (2). Leptin inhibition of insulin secretion appears to result from multiple effects: opening of KATP channels; activation of phosphodiesterase 3 B and consecutive reduction of intracellular cAMP; inhibition of protein phosphatase 1 (PP-1), and subsequent reduction in intracellular calcium (1). Leptin action, through its central receptors and the autonomic nervous system, may be also involved in the inhibition of insulin secretion in vivo (3,4).

Leptin also inhibits insulin biosynthesis. It has been shown that leptin decreases preproinsulin mRNA expression in a variety of β cell and islet preparations, including human islets (5).

Feeding and insulin increase the expression of the leptin gene in fat cells (6). It has been reported that insulin increases plasma leptin levels in normal subjects and patients with type 2 diabetes (7). This effect was observed after 6 to 8 hours of exposure to insulin, suggesting a role for insulin in chronic regulation of plasma leptin concentrations (7). It has also been shown that even a transient rise in insulin and glucose, similar to the metabolic response observed after a single meal, can prevent the decline in leptin observed with fasting, suggesting a physiological role of insulin and glucose in the short-term regulation of leptin secretion (8). In leptin-deficient, severely insulin-resistant lipodystrophic patients, leptin replacement therapy have been reported to improve glycemic control, decrease triglyceride levels and reverse insulin resistance (2). In a subgroup of obese subjects with low serum leptin relative to their adiposity, leptin therapy might be effective (1). However, in the vast majority of obese subjects, there is defective leptin action despite hyperleptinemia, suggesting leptin resistance, part of which may result from reduced entry of leptin into the central nervous system (CNS) (9). Leptin can exert direct effects on glucose and lipid metabolism in peripheral tissues (9). Contrasting effects have been observed in vitro, with reports of leptin opposing, enhancing, or mimicking insulin action (10). The major effect of leptin in skeletal muscle in vivo is inhibition of lipogenesis and glycogenesis and stimulation of fatty acid (FA) oxidation leading to decreased triglyceride content and improvement of muscle and whole-body insulin sensitivity (11). Leptin has also been shown to reduce visceral adiposity and enhance insulin action on both stimulation of glucose uptake and inhibition of hepatic glucose production (12). The latter effect was reproduced by intracerebroventricular administration, suggesting that leptin action on liver glucose metabolism is exerted through its central receptors (13). Interestingly, leptin and insulin inhibit the excitability of selective hypothalamic neurons by activating ATP-sensitive K+ channels, an effect mimicked by long-chain FA and possibly involved in the regulation of food intake and hepatic glucose fluxes by insulin, leptin, and FA (14). A considerable portion of circulating leptin is bound to proteins; the amount bound to protein is affected by the degree of adiposity and nutritional state (15,16). Although the physiological function of bound and free leptin are not well understood, it has been hypothesized that leptin is more active in its free form because this form is present in cerebrospinal fluid (CSF) (17,18). Several reports indicate that serum insulin and leptin levels correlate positively, however, no report exit between Ratio free to total leptin and insulin, therefore, we studied the relation between insulin, glucose and lipids with Ratio free total leptin in type 2 diabetic patients.

METHODS AND DESIGN

This study was conducted as a clinical trial at Yazd Diabetes Research Center, Shahid Sadoughi University of Medical Sciences.

Thirty obese diabetic patients (mean BMI = 37.1 Kg/m^2, age = 54±12) and thirty non-obese diabetes patients (mean BMI = 20.5 Kg/m^2, age = 49±25) entered the study. A fasting blood sample was taken and divided into two tubes. One was used for analysis of total leptin, insulin and other routine analyses and other tubes were used for free leptin purification.

Determination of free leptin in human serum: To obtain a standard cure, one vial containing 1 mg lyophilized leptin (sigma) was dissolved in 0.5 ml HCL (1.5 mM) and neutralized with 0.25 ml NaOH (7.5 mM) and applied with Marker Gel filtration Bludextran (Product Brand: Sigma Product Number: D4772) to Sephadex G-100 column chromatography (Amersham Biosource 1908-7101 [9/30 column]). Then it was eluted with 0.25 mM phosphate buffer (pH = 7.4). Fraction eluting were collected and assayed by a Sensitive ELISA method. (Catalogue Number: kap 2281: 96 determinations. Manufactured by Biosource Europe S-A.). It showed a single bound indicating free leptin fraction (19).

Serum sample (0.5 ml) was fractionated by same above Sephadex G-100 gel filtration. Fractions eluting between void and bed volumes were assayed by the ELISA method. Percentage of free leptin was then calculated by dividing free to total leptin and multiplying 100.

Insulin concentrations were measured by sandwich ELISA (Webster, Texas 77598-4217 USA, DSL). Total leptin concentrations were measured by sandwich ELISA (Biosource-EASIA Kit, KAP2281). This study was approved by the Ethics Committee and each patient gave written informed consent.

Calculation: percentage of leptin in the free –state (% free leptin) was calculated as the free plasma laptin concentration divided by the sum of free and bound plasma leptin concentrations, multiplied by 100.

Statistical analysis: Results of measured parameters have been represented mean ± SD. Paired T-test was used for statistical analysis results of free and total leptin, percentage of free to total leptin, glucose, LDL and HDL.

RESULTS

Percentage of free to total leptin in diabetes patients: Table 1 demonstrates evaluation of the differences between mean parameters in the diabetics (obese and non-obese). The mean BMI of subject's diabetes was 28.83 ± 8. The concentration of total leptin and percentage of free to total leptin were 17.87 ± 9 (ng/ml), 2.03 ± 1 and %27 ± %1, %3 ± %4, respectively in obese and non- diabetes patients. The concentrations of glucose and insulin were 180 ± 53 (mg/dl), 183 ± 83 and 21.85 ± 8.42, 10.12 ± 4 Iu/ml, respectively in obese and non-obese-diabetes (Table 1).

The mean level of percentage of free to total leptin in obese subjects showed a nine times higher than non-obese subjects ($P < 0.05$) %27 ± 1% vs. %3 ± 4%, respectively. Mean level of total leptin in obese subjects compared with non-obese ones was eight times higher (17.48 ± 9.68 vs. 2.03 ± 1.45, respectively). Insulin level in obese patients was higher than non-obese patients (Table 1). There was a direct correlation between percentage of free to total leptin with insulin level and insulin resistance. Correlation of total leptin with insulin level was significant, but it was not significant with insulin resistant (Table 2).

Table 1- Characteristics and biochemical parameters of the diabetic patients

	Total patients	Obese patients	non-obese patients
BMI	28.83 ± 8.7	37.13 ± 13	20.53 ± 16
Glu. (mg/dl)	197 ± 54	180 ± 53	183 ± 83
Insulin (µIu/ml)	15.99 ± 8	21.85 ± 7	10.12 ± 4
T.leptin	9.93 ± 10	17.84 ± 9	2.03 ± 1
P.F.to T.leptin	15% ± 0.14	27% ± 1%	3% ± 4%
Chol. (mg/dl)	197 ± 54	221 ± 54	172 ± 42
HDL (mg/dl)	37.5 ± 17	42.57 ± 15	33.37 ± 9
LDL (mg/dl)	117.99 ± 7	125.28 ± 31	109.89 ± 34

DISCUSSION

There is controversial correlation between leptin with insulin secretion. Fishers showed a functional relationship between leptin and insulin resistance (19). An indirect effect of insulin on leptin secretion was investigated in clinical conditions by Bertin (20). We have reported the relationship between insulin with free and total leptin in type 2 diabetic patients (21). We have reported similar relation in PCOS patients. (22).

There are two free and bounded forms of leptin in Blood. Circulation free form changes by obesity, fasting and other factors. In this study the correlation of percentage of free to total leptin was evaluated in diabetes. A considerable of plasma leptin is in free form in circulation. Although the physiological function of bound and free leptin are not well understood, it has been hypothesized that leptin is more active in its free form because this form is present in cerebrospinal fluid (CSF) (23, 24). The results of the present study demonstrated that the decline in both percentage of free to total leptin were blunted in persons with non-obesity demonstrating the obesity affected the degree of binding leptin to soluble binding protein in blood.

CONCLUSION

The results of this study indicate a direct correlation between percentage of free to total leptin with insulin level and insulin resistance. Correlation of total leptin with insulin level was significant but it was not significant with insulin resistant. These results indicate that free leptin form may be more responsible to insulin secretion in these patients.

Table 2- Correlation of total leptin and percent of free to total leptin with other circulation biochemical markers

Leptin correlation	Total patients		Percentage of free to total	
	r	p	r	p
BMI	0.79	0.001*	0.86	0.001*
Insulin	0.46	0.001*	0.58	0.001*
Glucose	-0.19	0.15	-0.18	0.15
IR	0.69	0.23	0.31	0.015*
TG	-0.07	0.61	-.83	0.027*
Cholesterol	0.06	0.06	0.15	0.32
HDL	0.09	0.51	0.11	0.4
LDL	0.03	0.84	0.07	0.59

*Correlations are significant (P<0.05)

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Abbreviations:

F.Leptin: Free leption, T.Leptin: Total leptin
P. F. To T. Leptin: Percentage of free to total leptin,
IR: Insulin resistant, FFM: Free fatty Mass

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