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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 12 (4): 397-400, 2009
ISSN 1028-8880

Circulation Free Leptin in Diabetic Patients and its Correlation to Insulin Level

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Abstract: Present researchers studied the relation between insulin with free and total leptin in type 2 diabetic patients. Thirty non insulin dependent diabetic obese patients (age: 50±12 year and BMI>30 kg m^{-2}) and thirty non insulin dependent diabetic non obese patients (age: 49±25 year and BMI<25 kg m^{-2}) 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 Kit. Circulation total leptin and insulin were measured by ELISA. Circulation free and total leptin were significantly correlated to insulin ($p<0.005$). Free leptin concentrations were higher in women than in men ($p<0.001$). Ratio of free leptin to total in obese subjects is more than non-obese subjects (0.27±0.1 vs. 0.03±0.04, $p<0.001$). Ratio of free to total leptin showed a positive correlations with insulin ($r = 0.58$, $p<0.001$) insulin resistance ($r = 31$, $p<0.015$) and BMI ($r = 0.86$, $p<0.001$). The majority of leptin which circulates in obese individuals was free form. Presumably it is bioactive portion of hormone and thus obese subjects are resistant to free leptin. These observations are consistent with the view that free leptin levels in diabetes patients attributed to changes in serum insulin level and insulin resistant.

Key words: Free leptin, insulin, diabetes, insulin resistant

INTRODUCTION

The 16 kDa cytokine-like hormone leptin has been identified as one of the key players in the control of body weight. The product of the *ob* gene is produced and secreted mainly by adipocytes (Seufert *et al*., 1999). Leptin reduces food intake and stimulates energy expenditure by activating its receptor in specific hypothalamic nuclei. Spontaneous mutations that 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 (Seufert, 2004; Leclercq-Meyer *et al*., 1996). 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 receptor, which conveys most of the physiological actions of leptin, is present on pancreatic β cells (Kieffer and Habener, 2000). 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 (Seufert *et al*., 1999). 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 (Seufert *et al*., 1999; Kieffer and Haneber, 1999). It has been shown that a physiological increase in serum leptin can reduce insulin secretion in rats *in vivo*. Leptin suppresses insulin secretion from human islets at concentrations as low as 0.01 nmol L^{-1} (Kieffer and Habeber, 2000). 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 (Seufert *et al*., 1999). Leptin action, through its central receptors and the autonomic nervous system, may be also involved in the inhibition of insulin secretion *in vivo* (Kieffer and Habener, 2000; Seufert, 2004).

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 (Seufert *et al*., 1999).

Feeding and insulin increase the expression of the leptin gene in fat cells (Leclercq-Meyer *et al*., 1996). It has been reported that insulin increases plasma leptin levels in normal subjects and patients with type 2 diabetes (Zhao *et al*., 1998). This effect was observed after 6-8 h of

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exposure to insulin, suggesting a role for insulin in chronic regulation of plasma leptin concentrations (Zhao *et al*., 1998). 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 (Laferrere *et al*., 2002).

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 (Kieffer and Habener, 2000). In a subgroup of obese subjects with low serum leptin relative to their adiposity, leptin therapy might be effective (Seufert *et al*., 1999). 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) (Ceddia *et al*., 2002).

Leptin can exert direct effects on glucose and lipid metabolism in peripheral tissues (Ceddia *et al*., 2002). Contrasting effects have been observed *in vitro*, with reports of leptin opposing, enhancing, or mimicking insulin action (Petersen *et al*., 2002). The major effect of leptin in skeletal muscle *in vivo* is inhibition of lipogenesis and glycogensis (Muoio *et al*., 1999) and stimulation of Fatty Acid (FA) oxidation leading to decreased triglyceride content and improvement of muscle and whole-body insulin sensitivity (Seufert *et al*., 1999). Leptin has also been shown to reduce visceral adiposity and enhance insulin action on stimulation of glucose uptake and inhibition of hepatic glucose production (Barzilai *et al*., 1998). The latter effect was reproduced by intracerebroventricular administration, suggesting that leptin action on liver glucose metabolism is exerted through its central receptors (Liu *et al*., 1998). 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 (Obici *et al*., 2002).

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 (Seufert, 2004; Leclercq-Meyer *et al*.,1996; Brabant *et al*., 2002). 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) (Zhao *et al*., 1998; Laferrere *et al*., 2002). Several reports indicate serum insulin and leptin levels correlate positively, however, no report exit between free leptin and insulin, therefore we studied the relation between insulin with free and total leptin in type 2 diabetic patients.

MATERIALS AND METHODS

Design: This study was conducted as a clinical trial (before and after) at the Yazd Diabetes Research center, Yazd University of Medical Science from Jun 2007 to July 2008.

Thirty new causes' patients diagnosed with type 2 diabetes mellitus (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) were interred in 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 analysis and other tubes were used for free leptin purification.

Measurement of free leptin in human serum: To obtain a standard curve, 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) and 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 (Nuamah *et al*., 2003).

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. Percent 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 local ethical committee and each patient gave written informed consent.

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.

RESULTS

Plasma free and total leptin in diabetes patients and Baseline characteristics and biochemical parameters of subjects in the type 2 diabetic (obese and non-obese) are

Table 1: Characteristics and biochemical parameters of the diabetic patients

Parameters	Total patients	Obese patients	Non-obese patients
BMI (kg m^{-2})	28.83±8.7	37.13±13	20.53±16
Glu. (mg dL^{-1})	181.00±54	180.00±53	183.00±83
Insulin (mic IU mL^{-1})	15.99±8	21.85±7	10.12±4
IR	7.08±4	9.57±4	4.59±3
T. leptin	9.93±10	17.84±9	2.03±1
Fr. leptin	2.86±4	5.59±4	0.12±0.1
R.F. to T. leptin	0.15±0.14	0.27±0.1	0.03±0.04
Chol. (mg dL^{-1})	197.00±54	221.00±54	172.00±42
HDL ((mg dL^{-1})	37.50±17	42.57±15	33.37±9
LDL (mg dL^{-1})	117.99±7	125.28±31	109.89±34

Fr. Leptin: Free leptin, T. Leptin: Total leptin, R. F. To T. Leptin: Ratio free to total leptin and IR: Insulin resistant

shown in Table 1. The mean BMI of subject's diabetes was 28.83±8. The concentration of free, total leptin and ratio free to total leptin were 9.93±10.53 (ng mL^{-1}), 2.86±4.08 (ng mL^{-1}) and 0.15±0.14, respectively in diabetes patients. The concentrations of glucose, insulin and insulin resistant were 181.82±71 (mg dL^{-1}) 15.99±8.42 mic IU mL^{-1} and 7.08±4.64, respectively (Table 1).

Mean level of free form in obese subjects showed a forty times higher than non-obese subjects ($p<0.05$) 5.59±04.28 vs. 0.12±0.16, respectively (Table 1). Mean level of total leptin in obese compare to non-obese subjects showed eight times higher (17.48±9.68 vs. 2.03±1.45, respectively). Insulin level and insulin resistant (HOMA) in obese patients were higher than non-obese patients (Table 1) (Wallace *et al*., 2004).

DISCUSSION

Fasting plasma concentrations have been to be shown higher in obese than in normal weight subjects with non insulin dependent type 2 diabetic patients (Mohiti *et al*., 2005) and higher in females than males. Although insulin and leptin have opposing effects on some metabolic processes, such as lipogenesis and FA oxidation, the majority of presently available data strongly suggest that both hormones exert a concerted action, particularly on the CNS, regarding the regulation of food intake and hepatic glucose fluxes. A positive relation between serum leptin and serum insulin levels has been described in type 2 diabetic patients (Markus *et al*., 2002; Baile *et al*., 2000; Chehab *et al*., 2002; Fantuzzi *et al*., 2000). In this study of obese and non-obese type 2 diabetic patients with a wide range of residual endogenous insulin secretion, we found a positive relation between fasting serum leptin and insulin levels (Table 2).

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) (Zhao *et al*., 1998; Laferrere *et al*., 2002). Present results

Table 2: Correlation between free and total leptin with other circulation biochemical markers

	Leptin correlation					
	Total patients		Free leptin		Ratio of free to total	
Parameters	r	p-value	r	p-value	r	p-value
BMI	0.79	0.000	0.71	0.000	0.86	0.001
Insulin	0.46	0.000	0.39	0.002	0.58	0.001
Glucose	-0.19	0.150	-0.16	0.230	-0.18	0.150
IR	0.69	0.230	0.18	0.150	0.31	0.015
TG	-0.07	0.610	-0.11	0.410	-0.83	0.027
Cholesterol	0.06	0.060	-0.01	0.920	0.15	0.320
HDL	0.09	0.510	0.03	0.800	0.11	0.400
LDL	0.03	0.840	-0.01	0.950	0.07	0.590

F. Leptin: Free leptin, T. Leptin: Total leptin, R. F. To T. Leptin: Ratio free to total leptin and IR: Insulin resistant

show a positive relation between free leptin with insulin and ratio free to total leptin in diabetes which indicates free form leptin shows similar behavior as total leptin in interrelation with insulin in diabetes patients.

Type 2 diabetes develops insulin resistance initially in muscle because of genetically impaired IGF-I and insulin receptor signaling (Feemaandez *et al*., 2001) which lead to insulin resistance in liver and fat. Subsequently pancreatic β-cell dysfunction develops, as demonstrated by the loss of first-phase insulin secretion. This event is associated with the appearance of diabetes (Feemaandez *et al*., 2001).

Relations between serum leptin and insulin resistance has been described. Fischer showed the highest fasting leptin levels with the most expressed insulin resistance. Their data point out a functional relationship between leptin and insulin resistance (Fischer *et al*., 2002). Bertin *et al*. (1998) showed that plasma leptin is not dependent on body fat distribution and suggest an indirect effect of insulin on leptin secretion in clinical conditions in diabetes patients. The results of Pringon indicate that body adiposity, sex and the fasting insulin level are independently associated with plasma leptin level and insulin sensitivity contributes to the association between body adiposity and plasma levels of insulin, but not leptin (Prigeon *et al*., 1997).

Present results indicate a correlation between free and total leptin with Insulin resistant was not significant in this study, the interrelations between ratios free to total leptin, with insulin resistance was positively significant (Table 2). Anyway, we studied the same number of the obese (30 patients, mean Body Mass Index (BMI) = 37.13 kg m^{-2} and non-obese (30 patients, mean body mass index = 20.53 kg m^{-2}) (Table 1). This study did not show a relation with lipids (TG, cholesterol, HDL, cholesterol and LDL-cholesterol (Table 2).

In summary, the present study demonstrates a direct correlation between both forms of free and total leptin with insulin level but did not appear significance with insulin resistance.

ACKNOWLEDGMENTS

We greatly acknowledge Diabetes Research Center of Yazd University of Medical Science for support of the present project.

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