Turkish Journal of Endocrinology and Metabolism, (2005) 2 : 55 - 58 **ORIGINAL ARTICLE**

Relation Between Leptin and Insulin in Patients with Type 2 Diabetes Mellitus

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Leptin and insulin are reported to control glucose metabolism therefore, a relationship between these two hormones should reveal the metabolic effect of these hormones on glucose metabolism. In this study, the relationship between leptin and insulin was evaluated in obese diabetes type 2, BMI>30 kg/m2 (group A), and non-obese diabetes type 2 patients, BM<25kg/ m2 (Group B).

Study design: 49 subjected were studied. Of these, 32 subjects (4 male and 28 female) were (group A) and 17 subjects (8 male and 9 female) were (group B) and analyzed for Leptin, Insulin, and HbA1c.

Results: The results obtained showed leptin, insulin and HbA1c levels of 5.16± 1.2 µg/ L and 21.8±11.2 µg/L, 6.75±1.2 µlu/ml and 10.4 µlu/ml and %9.38 ± 0.56 and %8.76 ± 0.36 in groups B and groups A, respectively.

Discussion: The results of this study show that plasma concentrations increased with the percentage of body fat and to body mass index. Plasma leptin in obese diabetes, in comparison to non-obese diabetes individuals, was four times higher (p=0.001).

Statistical analysis indicates a direct correlation between fasting blood leptin and insulin (r=0.290 p=0.005) in group B, while this correlation is reverse in group A.

Key words: leptin,insulin,diabetes,obesity, Resistance, HbA1c

Introduction

Leptin, a 16 kDa circulating hormone produced and released primarily by adipose tissue. Leptin exerts a regulatory control mechanism on food intake via inhibition of neuropeptide Y and increases the basal metabolism rate with selectively promoting fat metabolism. (1) Leptin has two types of receptors; the long form and humorous short form. At the beginning direct leptin actions were thought to be exclusively confined to the central nervous system (CNS). It is now clear that there are multiplicities of peripheral target organs such as the pancreas, skeletal muscle, liver and gastrointestinal system (2,3).

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Leptin appears to play a range of roles as a growth factor in a number of different cell types, such as a mediator of energy expenditure and most importantly to interact with other hormonal mediators and regulators of energy and metabolism such as insulin, glucagon, growth hormone and glucocorticoids (5,6,7).

A large body of evidence indicates that leptin along with insulin exert an inhibitory effect on food intake, and an activation effect on the regulation of thermogenesis within the central nervous system (4,14). Leptin and insulin function as a critical signal to the brain in the long-term regulation of energy homeostasis (14,16). The exact relationship between leptin and insulin is not clear and is sometimes controversial (8,9). Some researchers failed to show a direct effect of leptin on the energy homeostasis (10), while other studies focused on the relationship between leptin and insulin, which share many properties as adiposity signals. Although insulin is secreted from pancreatic beta cells rather than from adipocytes, the secretion of both

hormones is influenced by the overall amount of fat stores as well as by short-term changes in energy balance (14). Moreover, insulin receptors are located in the same key hypothalamic areas as leptin receptors. Whereas insulin secretion is stimulated acutely in response to meals, leptin secretion is not.

Although the mechanisms governing leptin secretion have yet to be fully elucidated, insulin appears to play a key role. Most obese mammals have elevated plasma concentrations of leptin and insulin, and they appear to be resistant to leptin-induced anorexia (12). Therefore, the relationship between these two hormones should be revealed through the metabolic effects of these hormones on energy balance. In this study, the relationship between leptin and insulin was evaluated in obese diabetes type 2 and normal weight diabetes type 2 patients.

Material and Method

This cross-sectional study was carried out on patients with type 2 diabetes mellitus who had been referred to the Diabetes Research Center of Yazd. The diagnosis of diabetes mellitus was performed according to the world health organization's (WHO) criteria which had been reported by a WHO study group (1985).

Study design: 49 subjects were studies of which 32 (4 males and 28 females, mean age=54) were type 2 diabetic with a BMI of more than 30 kg/m^2 (group A) and 17 (8 males and 9 females, mean age=47) were type 2 diabetic with a BMI of less than 25 kg/m^2 (group B).

After measuring the weight and height and taking other necessary information, a blood sample was taken from each individual at fast (10-12 hours) for one time and serum was obtained. Samples were immediately frozen at-70°C until needed for analysis.

Leptin analysis: Samples were thawed at room temperature and serum leptin concentrations were determined using a sensitive ELISA kit. This assay has a limit of detection of 0.05 µg/L. Day to day CVs were typically 13% at 0.32 µg/L and 5.8% at 2.14 µg/L. Leptin ELISA Sandwich kit was obtained from DRG.Com.

Insulin analysis: Insulin concentrations were detected in serum using a human ELISA test kit after the serum samples were thawed at room temperature. This assay has a sensitivity margin of 0.5 µIu/ml. Insulin ELISA kit was obtained from Q-1-DIAPLUS.

Hemoglobin A1c measurement: HbA1c was measured by Ion-Exchange chromatography using the DS5 Pink-300 test kit (Drew Scientific Limited. UK) from whole blood immediately after taking the blood sample from the individual. HbA_{1c} was obtained from Drew Scientific Limited. UK. Other kits and substances were obtained from Sigma chemical company or other company.

Glucose, triglycerides and cholesterol were measured by a colorimetric method using Autoanalyser RA-1000.

Statistical Analysis

All results are expressed as the mean ± SD. The obese group was compared to the non-obese group using a two-tailed students't-test. Correlation between groups was tested by the Pearson and Spearman test. For comparison of mean levels of leptin between males and females, the Mann-Whitney test was carried.

Results

Plasma Leptin in type 2 diabetic patients

Leptin concentration of fasting blood subjects was measured by the above method. As shown in table 1, the mean level of leptin obtained was 21.8±11.2 µg/L in group A and 5.16± 1.2µg/L in group B.

The results of this study show that plasma leptin concentrations in group A increase with body mass index. Plasma leptin in obese diabetics (group A) when compared to non-obese diabetic patients (group B) shows more than a four-fold increase (p=0.001, table 1).

Table 1. The characteristic and results analysis of two groups

Variable	Group A (No:32)	Group B (No:17)	P Val
Leptin (µg/L)	21.8±11.2	5.16±1.20	0.001
Insulin (µIU/mL	11.44 ± 5.6	6.75±1.20	0.078
FBS (mg/dL)	174.62±9.97	177.58±13.25	0.86
$HbA_{1c(\%)}$	8.76±0.36	9.38±0.56	0.340
Cholesterol(mg/	222.15±11.82	194.05±9.38	0.053
Triglyceride(mg	219±11.18	254±41.10	0.299

Our statistical analysis results show a significantly positive correlation between leptin and insulin

(r=0.59, p=0.<05) in group B (table 3). while the correlation between leptin and insulin is negative and not significant (r=-0.089, p=0.62) in group A (table 2).

Table 2. Correlation between leptin and other analyses in group A

Leptin	*Correlation*®	P.V
Insulin	-0.033	0.873
FBS	-0.213	0.242
HbA_{1c}	-0.095	0.605
Cholesterol	0.050	0.786
Triglyreceride	-0.101	0.582
WHR	0.211	0.245
BMI	0.020	0.914

Insulin and hemoglobin A_{1c} in type 2 diabetic patients

Insulin of fasting blood subjects was measured in both groups. The mean level obtained was 11.4±5.8 µIu/ml in group A and 6.75±1.2 µIu/ml in group B (table 1), The mean value of HbA_{1c} obtained was 8.76 %± 0.36 in group A and 9.38% ± 0.56 in group B. The results of the measurements obtained for FBS, triglycerides and cholesterol of both groups are shown in Table 1.

Table 3. Correlation between leptin and other analyses in group B

Leptin	Correlation®	P.V
Insulin	0.598	0.005
FBS	-0.190	0.464
HbA1c	-0.273	0.289
Cholesterol	0.361	0.154
Triglyceride	-0.032	0.903
WHR	-0.093	0.611
BMI	0.179	0.327

Discussion

The paradoxical observation contributes to the question of whether alteration in leptin action contributes to diabetes caused by obesity or lipodystrophy merely as a correlate with these phenomena or if they occur as a consequence of insulin resistance (12,13). The statistic analysis show a significantly positive correlation between leptin and insulin in group B (table 3). These correlations may express a cooperative effect between these two hormones in the control of body weight in this group (14), while the correlation between leptin and insulin is negative and not significant in group A (table 2). This may express an uncooperative effect of these two hormones on the control of body weight (18, 19). Our results indicate that plasma insulin levels are higher in either obese diabetes patients or obese non-diabetes individuals (results not shown) when compared to non-obese diabetes (table 1). Since other factors measured are very similar in the two groups (see table 1), therefore the increase in insulin levels in group A may be caused by the impaired action of leptin signaling in cells (20, 21). Kellerer and co-workers (21) have assessed the effects of insulin concentration on leptin signaling pathway in rat-1 and HEK293 cells. Their results suggest that insulin concentration may contribute to the pathogenesis of leptin (21,22). It has also been reported which leptin effects on glucose metabolism differ between lean mice and hyperglycemia and hyper insulinemia obese animals (19,23).

The observation that leptin levels are elevated in proportion to body fat (r=0.211,p=0.245) is a generally accepted idea, and that most obese individuals are leptin-resistant (20). Resistance to the actions of leptin could be caused by decreased leptin transport through the blood-brain barrier (25, 26) or to reduced signaling distal to the leptin receptor (24, 25). Peripheral signals such as glucocorticoids may also interfere with leptin's interaction with its receptor and produce central leptin resistance (27,28).

Conclusion: fasting levels of plasma HbA_{1c}, glucose and triglycerides are all similar in obese and non-obese groups. Therefore, circulating leptin levels appear to be one of the best biological markers of obesity and that hyperleptinemia is closely associated with several metabolic risk factors related to insulin resistance in the obesity syndrome.

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