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Determination of the Percentage of Blood Free Leptin Hormone in Diabetic Patients after Treatment with Metformin

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Abstract

Objective(s)
Plasma leptin exists in protein-bound and free forms, which may affect its hormonal bioactivity. The free and bound forms are changed by the body weight. The purpose of this study was to evaluate the effect of metformin on bound and free plasma leptin concentration.

Materials and Methods
Subjects recruited from Yazd Diabetes Research Centre, Yazd University of Medical Sciences. The participants were new cases of obese type 2 diabetes (BMI>30 kg/ m^2). Fasting bloods samples, before and after one month treatment with metformin collected. These samples used for the purification of free leptin by HPLC method and analysis of other biochemical parameters.

Results
The results indicated that, there was no significant difference observed between the free leptin forms before and after the one month treatment (71%±4.2 vs 72±5).

Conclusion
The one month treatment, reduced blood sugar in patients, but at the same time it did not have a reduction effect on the body weight. Therefore, in the free form of hormone after treatment comparing to before treatment it did not make a significant difference. This confirmed other reports which showed that the variation of free leptin depended on the body weight.

Keywords: Diabetes, Free leptin, Metformin, Obesity

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Introduction

Leptin, a 16 kDa circulation hormone produced and released primarily by adiposities, exerts a regulatory control on food intake and energy expenditure (1, 2). Plasma leptin concentration is correlated with the total fat mass, percentage of body fat and body mass index acting as sensing hormone in a negative feedback control from adipose tissue to the hypothalamus, the brain center responsible for satiety (3, 4).

A considerable portion of circulating leptin is bound to proteins; the amount bound to proteins is affected by the degree of adiposity and nutritional state (5, 6). 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 the cerebrospinal fluid (CSF) (7, 8).

Studies indicate that leptin improves glucose homeostasis because leptin is involved in the regulation of glucose transport. The importance of glucose utilization becomes more important in metabolic disease states such as obesity and type II (non-insulin-dependent) diabetes mellitus (5-7).

Relation between leptin and insulin seems to be complex. Some researchers failed to show a direct effect of leptin on the release of either insulin or glucagons in the isolated perfused rat pancrease (8). Other studies provide however, evidence that leptin can directly inhibit both basal and glucose-stimulated insulin secretion (9, 10).

Metformin (dimethylbiganide) is an insulin-sensitizing and antihyperglycemic agent used in the treatment of non-insulin-dependent diabetes mellitus (NIDDM). The exact mechanism of metformin is unknown, but one of its suggested actions is to increase the peripheral glucose disposal at lower insulin concentrations (11, 12). It has been shown that metformin treatment of obese adults with type 2 diabetes results in weight loss and improved glucose tolerance and lipid profiles. Furthermore, the use of metformin in nondiabetic obese adults has been demonstrated to cause reduced food intake and weight loss with reduction in fasting plasma glucose, cholesterol, and insulin concentration (13). Metformin reduces also, leptin levels in plasma and adipose deposit. It has been suggested that metformin primarily exerts its antihyperglycemic effect by decreasing hepatic glucose output through inhibition of gluconeogenesis (14).

Since metformin was reported to improve glucose tolerance and weight (15), therefore, this study aimed to evaluate the metformin effect on free and total leptin blood concentration during the period of one month.

Materials and Methods

Study subjects and design

In this study, twenty five new cases of type 2 diabetes patients were selected by clinical trial study, from Yazd Research Centre, Yazd University of Medical Sciences. All the participants were obese (BMI>30 kg/m^2). Patients having HbA1c greater than 9% were accepted as poorly controlled diabetes. A fasting blood sample was taken before and after treatment with 1000 mg metformin daily for one month. Glucose was measured by Biochemical auto analyzer via enzymatic-calorimetric method (Echoch- Italy). HbA1c percentage was measured by ion exchange chromatography and with Drew-DS5, England apparatus. 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). Sera, were separated immediately after centrifugation with 3000 g for 10 mins and stored at -20 °C until assaying for leptin and free leptin purification.

Free leptin purification

To obtain a standard pure, one vial containing 1 mg lyophilized leptin (sigma) was dissolved in 0.5 ml HCl (1.5 mM) and neutralized with 0.3 ml NaOH (7.5 mM) and applied to HPLC column, and then it was eluted with a linear gradient of solvent B (70% acetonitrile in 0.1% three fluroacetic acid (TFA) from 30 to 70% over 40 mins using chromatography pumps.

The frozen samples were first applied to C_{18} reverse-phase cartridge columns, (16) (Waters Sep-Pak Cartridge Column, Milford, Massachusetts, USA) washed with 2 ml aqueous 0.1% TFA (A). Analytical HPLC was performed on a Nucleosil C_{18} gel column (10 nm pore size, 5 μm particle size, Shimadzu, Tokyo, Japan) with solvents A and B (70% acetonitrile in 0.1% TFA). The leptin was eluted with a linear gradient of solvent B from 30 to 70% over 40 mins, using chromatography pumps. Elution of the peptide was monitored by measuring UV absorption at 220 nm (SPD-6AV model, Shimadzu, Tokyo, Japan) (17).

Statistical analysis
Results of measured parameters have been presented with mean±SD. Collected Data analyzed by SPSS for Windows package. Leptin, HbA1c, glucose, levels compared via an independent t- Test. For all statistical assessments, a value of *P*<0.05 was accepted to be statistically significant.

Results
The characteristic of patients, before and after treatment are presented in Table 1. The results indicate no significant decrease in weight after one month treatment.

Table 1. Weight and BMI of diabetic patients, before and after treatment with metformin.

	Before treatment			**After treatment**			*P*
	No.	Mean	SD	No.	Mean	SD	
Weight(kg)	25	72.4	11.2	25	72.6	10.5	0.157
BMI (kg/m²)	25	39.3	2.9	25	39.5	2.7	0.457
Age (Y)	25	54	12	54	12		

For achieving the percentage of free leptin, the fraction of HPLC column samples (free leptin form) was compared with the standard diagram. The percentage of each sample was calculated by the amount of free hormone divided by the total leptin, multiplied by 100. The averages of results before and after treatment are reported in Table 2. No significant difference was seen between the averages. The fasting insulin levels of patients, before and after the treatment were measured which are presented in Table 2. The average insulin, before and after treatment were 12.5 μIU/l, 14.8 μIU/l respectively, which also, had no significant difference.

The average of blood glucose level in patients before treatment was 214 mg/dl which reduced to 164 mg/dl after treatment with metformin which showed a significant difference (*P*<0.05) (Table 2).

Table 2. The percentage of free leptin, leptin, insulin, glucose and HbA1c in diabetic patients before and after the treatment with metformin.

	Before treatment			**After treatment**			*P*
	No.	Mean	SD	No.	Mean	SD	
%Free leptin	25	71	4.2	25	72	5	NS *
Leptin (ng/ml)	25	2.2	2.96	25	2.24	2.54	NS
Insulin (μIU/ml)	25	12.5	15.8	25	14.8	13.2	NS
Glucose (mg/dl)	25	214.4	55.3	25	164	62.9	0.002
HbA1c %	25	9.8	2	25	9.1	2.7	NS

＊Not significant

The average of HbA1c before treatment was 9.8%, which reduced to 9.1% after the treatment but, it was not statistically significant (Table 2).

Discussion

Metformin is an Anti-diabetes agent, which is used widely for the reduction of blood glucose level in diabetic patients type 2 (18, 19, 21).

The use of a dosage of 1000 mg each day for one month caused a significant reduction in blood glucose level (Table 2). This report indicates that one month treatment of metformin reduced the blood level glucose significantly, without any change in weight (Table 1).

In a study by Freemak and Kay, it was indicated that the effect of metformin for 2 and 6 months periods, caused the reduction of blood glucose level and the body fat mass (20, 23). In a study carried out by Fruehwald-Schultes (22), fifteen normal – weight men, were treated with metformin (850 mg twice daily) or placebo for a 15-day period in a cross-over study. The results indicated that metformin did not affect body weight (P=0.839) and body fat mass (P=0.916), however, after the treatment serum leptin concentration was reduced (P<0.01). In our study metformin reduced the blood glucoe but did not affect leptin level in type 2 diabetic patients (Table 1, 2).

The results of this study also, show that the percentage of free leptin hormone is correlated with the patient's weight. Although after one month of medication the blood glucose level reduced significantly but, it did not have a reduction effect on the weight. Therefore, the percentage of free leptin hormone before and after treatment was 71%±4.2 and 72%±5 respectively and confirmed other studies which reported that the free hormone level is dependent on the amount of body fat (13, 14, 25, 26).

In a study by Magni *et al* it was shown that the free leptin increased by the amount of body fat (13). The study by Michelladt (24), on 6 lean and 6 abdominally obese women indicated that although, the total plasma leptin concentration was more than 6 fold greater in obese compared with lean women, but the percentage of leptin in the bound form was greater in lean than obese subjects. Also, the results of Yannakouli showed that free leptin had a positive relation with the amount of body fat (14).

Conclusions

The results of this study show that the changes of blood free hormone level is not correlated with the blood glucose level, however it may be affected by weight and BMI in the diabetic patients.

Acknowledgment

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