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Metformin Affects Plasma Levels of Soluble Leptin Receptors in Type 2 Diabetes Mellitus and Metabolic Syndrome Patients

Najwa Hussein Ali* and Suzan Omer Rasool
Department of Pharmacology and Clinical Pharmacy, College of Pharmacy, University of Duhok, Duhok, Iraq.
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ABSTRACT

Background: Obesity and insulin resistance elevate plasma leptin, while it lowers plasma levels of soluble leptin receptors (sOB-R).
Objectives: To measure the levels of sOB-R in patients with type 2 diabetes mellitus (T2DM) and metabolic syndrome (MetS) and compare it to healthy controls. The study also aimed to investigate the influence of metformin on the levels of sOB-R in patients with T2DM and MetS.
Materials and methods: A cross-sectional study was conducted on 66 participants (33 patients and 33 controls). Patients were divided into two groups; Group I included 17 newly diagnosed T2DM patients, and Group II included 16 patients with MetS. Patients were studied before and 3 months after treatment with 850 mg metformin treatment. Group III included 33 non-obese healthy individuals as a control group. Measurements such as height, weight, waist circumference, hip circumference, blood pressure examination, and biochemical tests including fasting blood sugar, HbA1c, total cholesterol (Ch), triglyceride, high-density lipoprotein (HDL), insulin level, plasma sOB-R levels were performed. Ch/HDL ratio, non-HDL cholesterol, Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), Waist Hip Ratio (WHR), and Body Mass Index (BMI) were calculated.
Results: No statistically significant difference was found among the groups regarding sOB-R levels initially, but after metformin treatment, T2DM patients showed a significantly higher level (10.19 ng/ml). There was a significant increase in the levels of sOB-R after metformin treatment, as it increased from 7.82 ng/ml to 10.19 ng/ml in T2DM, and from 6.92 ng/ml to 7.82 ng/ml in MetS patients.
Conclusion: Metformin significantly increases the plasma levels of sOB-R in T2DM patients but only slightly increases these levels in MetS patients.
Keywords: Type 2 diabetes mellitus; Metabolic syndrome; Plasma levels of soluble leptin receptors; Metformin.

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INTRODUCTION

Obesity is defined as an increase in adipose tissue. It has an important role in the pathophysiology of diabetes and metabolic syndrome in industrialized, as well as in developing countries [1] since most T2DM patients are obese and have central visceral obesity [2].

The role of the adipose tissue in the pathophysiology of T2DM is through free fatty acid (FFA) secretion and behaving as an endocrine organ by secreting various adipocytokines (i.e. leptin, resistin, tumor necrosis factor, and adiponectin), which act as mediators between obesity and insulin resistance, dyslipidemia, and inflammation [3]. Leptin is the most frequent and extensively researched adipokine in the bloodstream, and the proportion of body fat is directly correlated with its levels [4].

The extracellular cleaved portion of the leptin receptor, also known as the soluble leptin receptor, has been determined to be the main leptin-binding protein in plasma (sOB-R) [5],

* Corresponding author: E-mail: najwahussein585@gmail.com
Phone number: +9647517850950

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which circulates at greater concentrations (and, as a result, produces a higher proportion of bound leptin) in lean individuals than in obese individuals [6]. Leptin's interactions with its cell-bound receptor or its half-life could be affected by this protein binding, allowing it to function as a regulator of leptin's physiological effects [7].

There is much evidence, that suggests sOB-R as a new biomarker for leptin sensitivity. A huge prospective study found that lower T2DM risk is linked to elevated plasma sOB-R levels [8]. Serum sOB-R may protect against future T2DM or function as a sign of protective characteristics, but its regulation is not yet known [9].

The first-line hypoglycemic treatment for patients with T2DM is metformin, it is a biguanide, a class of medication with herbal origins. It can be used alone or in conjunction with insulin or other glucose-lowering treatments [10]. Metformin works by reducing gluconeogenesis to decrease excessive hepatic glucose synthesis, it may also improve insulin signaling, cellular glucose uptake, boost fatty acid β-oxidation, and suppress fatty acid and triglyceride production [11]. In addition to its hypoglycemic property, metformin also exhibits several positive "side effects" such as anti-obesity and is currently used for the treatment of obesity [12].

Clinical studies have found that metformin has particular influences at the level of the hypothalamic centers which are responsible for satiety and hunger, leading to a reduction in food intake in both diabetic and non-diabetic patients [13]. According to existing evidence, the weight loss property of this medication may be influenced by increased leptin sensitivity [12]. Hence, the current study was performed to assess the levels of sOB-R in patients with T2DM and MetS and investigate the effect of metformin treatment on the levels of sOB-R.

MATERIALS AND METHODS

This was a cross-sectional study carried out at the Endocrine Department of Azadi Teaching Hospital and private endocrine clinics in the Duhok/Kurdistan region/Iraq. The study covered a period of 7 months (December 2021 to June 2022). Patients were enrolled if their levels of HbA1c were $\geq$ 6.5% and they had been prescribed metformin in a dose of 850 mg twice daily. They were contacted and informed about the study procedure and biochemical tests before being asked to participate in the study. Patients were excluded if they were previously diagnosed with T2DM, T1DM patients, pregnant and lactating women, or chronic diseases like thyroid disorders, chronic inflammatory diseases, chronic renal failure, hepatic failure, and malignancy. Besides, those who declined to participate and lost to follow-up were excluded.

Study flow

The study was carried out on sixty-six participants, thirty-three patients, and thirty-three controls. Based on eligibility criteria, two groups of patients were formed; the first group (Group I) included seventeen newly diagnosed T2DM patients diagnosed according to American Diabetes Association (ADA) [14] (10 males and 7 females), and the second group (Group II) included sixteen patients with MetS (4 males and 12 females), diagnosed according to the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) diagnostic criteria [15]. Patients in both groups were treated with metformin at a dose of 850 mg, twice daily for 3 months. Group III included thirty-three (19 males and 14 females) non-obese, healthy individuals, of comparable age and gender (Figure 1).

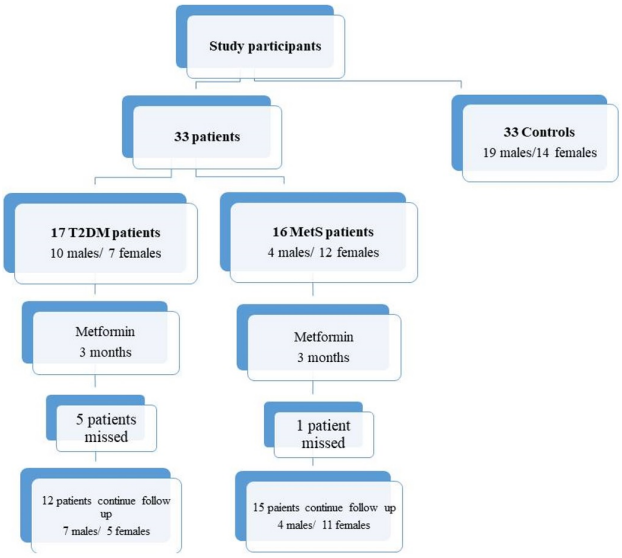

Figure 1. Study flow diagram.

Data collection

The data were collected through direct interviews with the subjects and using a specific data collection form designed to collect general information about subjects before blood sampling. A clinical examination including weight, height, waist circumference, hip circumference, and blood pressure was performed, and biochemical measurements (fasting blood sugar, HbA1c, total cholesterol, triglyceride, HDL, insulin level, and plasma sOB-R levels) were tested for all patients at the beginning and after 3 months. For the control group, the measurements were performed only once, without treatment with metformin. Ch/HDL ratio, non-HDL cholesterol, Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), Waist-Hip Ratio (WHR), and Body Mass Index (BMI) were calculated.

Blood sampling

Seven milliliters of venous blood were drawn from the patients after overnight fasting. Two milliliters of blood were placed in EDTA tubes and used for measuring HbA1c, which was measured by immunofluorescence quantitative analyzer (Biozek DCR1000). The residual 5 ml of blood was placed in a plain gel tube and centrifuged for 10 minutes at 3000 rpm after being left to clot for 30 minutes at room temperature. The obtained serum was used for measuring fasting blood sugar (FBS) and lipid profile, which were calculated using an automatic biochemistry analyzer (KENZA 240 TX) and insulin level, which was measured using an immunoassay analyzer Cobas E411 a fully automated device that uses the Sandwich principle. The rest of the serum was kept in Eppendorf tubes at -20°C to -28°C so that the Sandwich enzyme immunoassay method could be used to measure sOB-R levels.

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Ethical consideration

Ethical clearance was obtained from the Committee of Local Research Ethics, planning, and Scientific Research Division Department of the General Directorate of Health of Duhok on October 24, 2021, reference number 24102021-10-28.

Statistical analysis

The data were converted into a computerized database, which utilize range and logical data cleansing techniques to check for inaccuracies and correct errors. A statistician's expert opinion was requested. The statistical calculations were performed by JMP pro 14.3.0. The general characteristics of patients and healthy controls were presented in mean (SD) or number (%). The homogeneity of age and gender in the study was examined by ANOVA one-way and Pearson Chi-squared test, respectively. The comparisons of plasma levels of sOB-R among MetS, T2DM, and healthy individuals were examined by ANOVA one-way. The pairwise comparisons were examined by Tukey test. The correlations of plasma levels of sOB-R with HbA1c and HOMA-IR in T2DM and MetS patients were examined by bivariate correlation model. The effects of metformin on plasma sOB-R levels in T2DM and MetS patients were examined by paired t-test. The significant level of difference was performed with a P-value < 0.05.

RESULTS

There were no statistically significant differences regarding age and gender among the study groups, whereas in BMI and WHR, MetS patients possessed the highest values. The mean BMI of MetS patients (33.08) was higher than that of healthy controls and T2DM patients (26.55 and 28.67) (P-value $<$ 0.0001). Considering WHR, the MetS group possessed the highest value, followed by T2DM and healthy controls (1.05, 1.04, 0.98) respectively (P-value = 0.006) as shown in Table 1.

The study showed no statistically significant difference in the levels of soluble leptin receptors among study groups before treatment with metformin (P-value = 0.7386). After treatment with metformin, the results demonstrated significant differences among the study groups, with T2DM patients recording the highest value (10.19 ng/ml), followed by MetS patients and healthy controls (7.82 ng/ml, 7.09 ng/ml) respectively (P-value = 0.036) (Table 2).

There was a non-significant positive correlation between HbA1c and plasma levels of sOB-R in T2DM patients (r = 0.476, P-value = 0.117 and a non-significant negative correlation between HbA1c and plasma levels of sOB-R in MetS patients (r = 0.050, P-value = 0.864) (Table 3).

There was a non-significant positive correlation found between HOMA-IR and plasma levels of sOB-R in T2DM patients (r = 0.470, P-value = 0.169) and a non-significant negative correlation was found between HOMA-IR and plasma levels of sOB-R in MetS patients (r = 0.189, P-value = 0.517) (Table 4).

When administered metformin to MetS patients resulted in a significant increase in the levels of sOB-R, which increased from 6.92 ng/ml to 7.82 ng/ml (P-value = 0.035). In T2DM patients, treatment with metformin showed a significant increase in the level of sOB-R from 7.82 ng/ml to 10.19 ng/ml (P-value = 0.0001) (Table 5).

DISCUSSION

The bioactivity of leptin in circulation is regulated by a sOB-R, which has an inverse relationship with both adiposity and circulating leptin levels [7]. The theoretical framework of the study showed that the age and gender of all participants were semi-identical among the three groups. This is because choosing the research individuals was intentionally done to have homologous individuals in all groups to eliminate the influence of age and gender differences among the groups on other measurements.

In the current study, healthy individuals had the lowest in BMI and WHR; this phenomenon may be related to dietary habits, awareness of hypertension, and diabetes. However, the BMI was significantly different in the T2DM and MetS patients in comparison with the control group. We noticed that patients with MetS had a much higher BMI than the control group, and then patients with T2DM. In this context, previous studies reported that MetS is a group of the following illnesses; obesity, high fasting glucose, hypertension, and dyslipidemia [16, 17]. For the WHR parameter, the result revealed that each group of patients (MetS and T2DM) was over-dominated by the control healthy group, in this particular measurement. According to this finding, the relationship between certain chronic diseases and body features is most strongly influenced by central obesity. Truncal adipocytes are thought to be endocrine organs with high metabolic activity that secrete inflammatory mediators into the bloodstream. These explanations very well agree with the conclusions reported by a previous study [16, 17].

It has been found in this study that in T2DM patients, the correlation value of plasma levels of sOB-R with glycemic control (HbA1c) was statistically non-significant, moderately positive (r = 0.476, P-value = 0.117), whereas, in MetS patients, there was a weak positive correlation (r = 0.050, P-value = 0.864). These findings agreed with the results of Sommer et al., who reported that HbA1c was not associated with sOB-R in simple regression models ($\beta = 0.04$, P-value = 0.70) [9].

However, the results of the current study disagreed with those of Morioka et al., who enrolled 289 T2DM patients from the Japanese population in the study. He found that plasma sOB-R levels were significantly and positively associated with HbA1c ($\beta = 0.158$, P-value = 0.001) in a multivariate analysis [18].

In the present study, the correlation between plasma sOB-R levels and insulin resistance differed between the two groups of patients, with a non-significant positive correlation (r = 0.470, P-value = 0.169) in diabetic patients and a non-significant negative correlation (r = 0.189, P-value = 0.517) in MetS patients.

The data from the present study were in high agreement with the findings of Morioka et al., in Japan, who enrolled 289 participants with T2DM in their study, and found that plasma sOB-R levels were not significantly associated with HOMA-IR ($\beta = 0.006$, P = 0.899) in the multivariate analyses [18]. However, our finding disagrees with the result of another study conducted in Japan by Ogawa et al., which showed that serum sOB-R level was significantly and negatively correlated with HOMA-IR (r = -0.483, P-value $<$ 0.0001) in 96 diabetic men, and (r = 0.592, P-value $<$ 0.0001) in 54 diabetic women. The correlations between serum sOB-R level and HOMA-IR were significant in men even after adjustment for age and BMI [19].

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Table 1. Comparison of general information among study groups before treatment.

Characteristics	Study groups			P-value	Pairwise comparisons
	Healthy controls (n=33)	MetS (n=16)	T2DM (n=17)		
	G1	G2	G3		
Age (years)	49.80 (9.82)	52.00 (9.76)	49.00 (8.35)	0.638	NA
Gender (no, %)					
Male	19 (57.58)	4 (25.00)	10 (58.82)	0.071	NA
Female	14 (42.42)	12 (75.00)	7 (41.18)		
Body Mass Index (kg/m^2)	26.55(3.56)	33.08(4.76)	28.67(3.99)	< 0.001	G2 vs. G1 (P-value = 0.000) G2 vs. G3 (P-value = 0.002)
Waist-Hip Ratio	0.98 (0.09)	1.05 (0.10)	1.04 (0.08)	0.006	G2 vs. G1 (P-value = 0.005) G3 vs. G1 (P-value = 0.013)

One-way ANOVA was performed for statistical analyses. Except for gender, the Chi-square test is used.
The post hoc analysis was performed using Tukey test.
The bold red numbers show significant differences.
The values are in the mean (SD) except for gender (number and%).
NA = not available.

Table 2. Comparison of plasma levels of soluble leptin receptors among study groups.

	Study groups' Mean (SD) of leptin receptor level (ng/ml)				
	Healthy controls (n=33)	MetS (n=16)	T2DM (n=17)	P-value	Pairwise comparisons
	G1	G2	G3		
Before metformin treatment	7.09 (3.77)	6.92 (2.51)	7.82 (3.14)	0.738	NA
	Healthy controls (n=33)	MetS (n=15)	T2DM (n=12)	P-value	Pairwise comparisons
After metformin treatment	7.09 (3.77)	7.82 (2.64)	10.19 (3.07)	0.036	G3 vs. G1 (P-value=0.010)

One-way ANOVA was performed for statistical analyses.
The Tukey test was performed for post hoc analyses.
The bold red numbers show significant differences.
NA = not available.

Table 3. Correlation of plasma levels of soluble leptin receptor with glycemic control in T2DM and MetS patients.

	Correlations	Confidence Interval CI		P-value
	r -Value	Lower 95%	Upper 95%	
T2DM	0.476	– 0.133	0.825	0.117
MetS	0.050	– 0.493	0.565	0.864

Table 4. Correlation of plasma levels of soluble leptin receptors with insulin resistance in T2DM and MetS patients.

	Correlations	Confidence Interval CI		P-value
	r -Value	Lower 95%	Upper 95%	
T2DM	0.470	– 0.225	0.849	0.169
MetS	– 0.189	– 0.654	0.379	0.517

Table 5. Effects of metformin on plasma soluble leptin receptors levels in T2DM and MetS patients.

	mean (SD) of sOB-R (ng/ml)			
	Before	After	Mean diff (95% CI)	P-value
Study groups	MetS			
	6.92(2.51)	7.82(2.64)	0.89(0.07 to 1.71)	0.035
	T2DM			
	7.82(2.66)	10.19(2.71)	1.85(1.04 to 2.65)	0.0001

Paired t-test was performed for statistical analyses.
The bold red numbers show significant differences.

In the current study, T2DM patients who are treated with metformin exhibited a statistically highly significant increase in the levels of sOB-R, (7.87 ng/ml to 9.71 ng/ml; P-value = 0.0001), a statistically significant increase in sOB-R levels was also showed in MetS patients after treatment with metformin (6.92 ng/ml to 7.82 ng/ml; P-value = 0.035). This was the most important and novel finding of this study. These results were in high agreement with those reported by Tang et al., who tested the effects of metformin on plasma sOB-R levels in newly diagnosed T2DM patients. Results showed that metformin up-regulates the expression of leptin receptors (OB-Ra, -Rb, -Rc, and Rd) in the liver. For the upregulation of the long-signaling isoform OB-Rb, a significantly higher dose of metformin is essential. In T2DM patients, this effect was followed by an increase in sOB-R levels [20].

This research had a number of limitations, the first of which was a very small sample size, and the second was that some of the cases weren't identified during the follow-up.

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CONCLUSION

Metformin has significant effect on increasing the plasma levels of sOB-R in T2DM, but a lesser effect in MetS. Further studies with a larger sample size, extended duration, and more frequent follow-up are recommended to examine the effect of other drugs on plasma levels of sOB-R and to estimate the plasma levels of sOB-R and its correlation to other conditions.

ETHICAL DECLARATIONS

Acknoweldgements

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Ethics Approval and Consent to Participate

Ethical clearance was obtained from the local Research Ethics Committee of Duhok General Directorate of Health, Department of Planning and Scientific Research Division, on October 24th, 2021, reference number 24102021-10-28. Patients were contacted and informed about the study procedure and biochemical tests being asked to participate in the study. Patients who agreed to participate signed form C provided by the Research Ethics Committee.

Consent for Publication

Not required (the study did not include individualized personal information).

Availability of Data and Material

The data supporting this study's findings is available from the corresponding author, Ali NH, upon reasonable request.

Competing Interests

The authors declare that there is no conflict of interest to disclose.

Funding

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Authors' Contributions

Conceptualization and methodology: Ali NH and Rasool SO. Data collection: Ali NH. Statistical analysis: Ali NH and Rasool SO. Writing: Ali NH. Review and editing: Ali NH and Rasool SO Supervision: Rasool SO, funding acquisition: Ali NH. Both authors have read and agree with the published final draft of the manuscript.

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