ORIGINAL ARTICLE

The impact of metformin therapy on serum leptin levels in Iraqi obese individuals with type 2 diabetes mellitus

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Abstract

Background: One of the primary factors causing obese individuals to develop diabetes is the regulation of appetite and metabolic rate by leptin. In Type 2 Diabetes (T2D), a change in leptin level is involved in the obesity pathophysiology and may lead to the progression of insulin resistance. Metformin was the first therapeutic choice to improve metabolic functions and insulin sensitivity in T2D. Comprehension of the impact of metformin on leptin levels may provide insight into its mechanisms of action in obese T2D patients. *Aim and Objectives*: This study aimed to examine how obesity affects leptin and type 2 diabetes and how metformin, with or without insulin, affects leptin levels. *Material and Methods* :Out of the 90 participants in the research, 60 had type 2 diabetes and were obese, while the remaining 30 subjects appeared to be in good health. Based on the type of therapy, diabetics were divided into four subgroups: Cohort 1 included 20 patients receiving metformin therapy, Cohort 2 included 20 patients receiving insulin therapy, Cohort 3 included 20 patients receiving metformin plus insulin therapy, and Cohort 4 consisted of 30 healthy controls, of which 20 were obese, and 10 were non-obese. *Results:* Compared to obese controls and other treatment cohorts, patients receiving metformin alone had lower leptin levels. In contrast to obese controls, leptin levels were slightly higher in cohorts receiving insulin alone and in cohorts receiving insulin plus metformin. In addition, obese controls exhibit noticeably higher leptin levels than non-obese controls. *Conclusion*: The main defects in T2D that cause elevated serum leptin content were body mass index and obesity. Leptin may be a useful predictor of response to metformin therapy.

Keywords: Obese, type 2 diabetes, leptin, metformin, insulin.

Introduction

Obesity is a chronic condition characterised by dysfunction or abnormal fat distribution of adipose tissue, which is significantly linked to elevated risks for various comorbidities that can impact overall health. The rising incidence of obesity and Type 2 Diabetes (T2D) has emerged as a major worldwide public health issue and has a strong epidemiological correlation [1]. Also, complications of T2D, including dyslipidemia, retinopathy, and nephropathy, are linked to obesity through hyperinsulinemia and hyperleptinemia, which signify insulin and leptin resistance [2, 3]. Free Fatty Acid (FFA) secretion and adipocytokines (tumor necrosis factor, adiponectin, resistin, and leptin), which function as moderators between insulin resistance and obesity, dyslipidemia, and inflammatory response, are the ways in which adipose tissue contributes to the pathophysiology of patients with T2D [4, 5]. The most common and well-

studied adipokine in the bloodstream is leptin, and there is a clear link between its levels and the percentage of body fat [6]

Leptin regulates insulin secretion peripherally, and its deficiency may lead to obesity, which can then be treated with leptin therapy [7, 8]. Leptin is highly correlated with both body fat mass and the size of adipocytes (fat cells). The transition from insulin resistance associated with obesity to frank T2D may be influenced by leptin [9]. Leptin regulates energy balance by increasing energy expenditure and suppressing appetite. It is primarily secreted in white adipose depots and brown adipose depots, the stomach, and the placenta. The production and release of leptin from adipose tissue is regulated by several substances, including insulin, steroids, and noradrenaline [10]. The first-line treatment for hyperglycemic patients who are obese or overweight and whose blood glucose cannot be controlled by a strict diet is metformin, which is a member of the biguanide class of medications. It can be taken either on its own or in combination with insulin or other medicines that lower blood glucose [11-13]. In addition to improving insulin signalling and cellular glucose uptake, metformin also works by reducing gluconeogenesis, which reduces excessive hepatic glucose synthesis and increases skeletal muscle glucose uptake while decreasing intestinal glucose absorption; it also increases fatty acid β-oxidation and suppresses the production of triglycerides and fatty acids [14]. Metformin is used to treat obesity because, in addition to its antihyperglycemic effect, it has many beneficial side effects, including anti-obesity properties. The well-established fact that metformin can increase leptin sensitivity, decrease or inhibit leptin secretion in cases of morbid obesity, and reverse leptin resistance explains the strong correlation between leptin hormone and metformin therapy [15]. This study aimed to evaluate the effects of obesity on leptin levels and the development of T2D, and to investigate how metformin, with or without insulin, affects leptin levels in patients with T2D.

Material and Methods

In this study, participants were randomly assigned to treatment groups from September 2022 to January 2023 using a computerized random number generator to ensure concealment and minimize selection bias, thereby creating comparable cohorts for a randomized controlled study. Prior to participation, all subjects provided written informed consent. Participants were fully informed about the study's objectives and the potential benefits of their involvement. Out of the ninety participants in the survey, sixty had type 2 diabetes and were obese, while the remaining thirty were seemingly in good health. Patients with type 2 diabetes, cancer, thyroid disorders, chronic inflammatory diseases, chronic renal failure, chronic hepatic failure, and pregnant or nursing women were excluded from the study. In addition, few were lost to follow-up because they refused to participate

Patients with diabetes mellitus were grouped into four cohorts based on the type of therapy used. Cohort 1 consisted of twenty patients (8 males and 12 females), ranging in age from 30 to 62 years, who were treated with metformin. Cohort 2 consisted of twenty patients (6 males and 14 females) aged 32-65 years who were treated with insulin. Cohort 3 consisted of twenty patients (4 males and 16 females) aged 32-65 years, treated with metformin plus insulin. Cohort 4 consisted of

thirty healthy controls, aged 30-65 years, grouped into twenty obese individuals with body mass index (BMI) ≥30 kg/m² and ten non-obese with BMI≤30 kg/m². Waist-to-Hip (W/H) ratio and BMI were computed as obesity indicators.

In this study, participants' blood samples were assembled after a fasting period of 8–10 hours. Eight millilitres of venous blood were placed into two types of labelled tubes: one for serum (gel tube) and one containing EDTA to prevent clotting. The resulting serum after centrifugation was carefully stored at −20°C until it was time for testing.

The observed glycosylated haemoglobin (HbA1C) and Fasting Plasma Glucose (FPG) values were recorded using Gluc2 and A1C-3 kits with the cobas C111 analyser. The DRG leptin ELISA kit was used to estimate the serum leptin level.

Our study sample size was determined depending on the expected differences in serum leptin levels among the study groups. A total of 90 participants, including 60 patients with T2D and 30 healthy controls, was considered sufficient to achieve a significance level of 0.05 and obtain 80% statistical power. It was chosen to ensure the detection of meaningful differences between groups while also accounting for potential variability in the data and possible dropouts during the study.

Statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS) version 26.0. One-way Analysis of Variance (ANOVA) was employed to assess differences among the study groups. A value of $p < 0.05$ was considered indicative of statistical significance. The study was carried out in accordance with the Declaration of Helsinki (1964) and received ethical approval from the Ethics Committee of Mustansiriyah University/National Diabetes Center.

Results

Our results revealed that the mean serum leptin level, when comparing the cohort of obese participants with non-obese controls, was considerably elevated in the obese cohort, as shown in Table 1. At the same time, in comparison with the non-obese cohort, the obese cohort exhibited a considerably elevated BMI mean and median W/H ratio. In contrast, the mean FPG and HbA1C did not indicate any considerable difference between the obese cohort and the non-obese controls. Contrastingly, although there was no considerable variation in mean age between the three diabetes treatment cohorts, the mean age was considerably elevated in the treatment cohort compared to the control cohort. There was a decrease in serum leptin in the metformin-treated cohort when compared to the other treatment cohorts and controls. Still, no meaningful change was observed in mean serum leptin between the three treatment cohorts and controls. Serum leptin content was increased in the cohort receiving combination metformin and insulin treatment than in the other treatment cohorts and control subjects. The mean "HbA1C" showed a "significant" elevation in the insulin and combination cohort compared to the "metformin" cohort, and it was significantly higher in the three treatment cohorts when compared to controls. Mean "FPG" showed "significant" elevation in the insulin cohort when compared with the "metformin" cohort, and increased significantly in three treated cohorts when compared with controls. Between the insulin and "metformin" cohorts and the combination cohort, there was no notable differences were observed.

Table 1: Differences between obese and non-obese healthy controls

Cohorts Characteristics	Non-obese (N=10)	Obese (N=20)	*p*
Age (years)	41.5 ± 3.65	44.8 ± 1.78	0.37
BMI (kg/m^2)	25.1 ± 0.58	34.9 ± 0.97	< 0.001*
W/H ratio (cm)	0.89	1.01	< 0.001*
FBG (mg/dl)	96.4 ± 2.5	98.4 ± 3.37	0.7
HbA1C (mg/dl)	5.7 ± 0.23	5.5 ± 0.15	0.48
Leptin (ng/ml)	25.7 ± 6.13	123.4 ± 12.37	< 0.001*

Note: A p-value < 0.05 is considered significant. fasting plasma glucose (FPG), glycated haemoglobin (HbA1c), Body Mass Index (BMI), and Waist-to-Hip (W/H) Ratios.

Table 2: Differences between the diabetes treatment cohorts and obese, healthy controls

Cohort Characteristics	Control obese (n=20)	Metformin (n=20)	Insulin (n=20)	Metformin + Insulin (n=20)	*p*
Age (years)	44.8 ± 1.78	48.9 ± 2.02	52.5 ± 2.38	52.5 ± 1.74	0.02*
BMI (kg/m^2)	34.9 ± 0.97	35 ± 0.98	33.7 ± 0.59	34.6 ± 0.085	0.74
W/H ratio (cm)	1.01	1	0.98	1.01	0.67
FPG (mg/dl)	98.4 ± 3.37	169.7 ± 11.32	220.1 ± 23.95	193.6 ± 15.51	< 0.001*
HbA1C %	5.5 ± 0.15	8 ± 0.38	9.3 ± 0.5	9.1 ± 0.35	< 0.001*
Leptin (ng/ml)	123.4 ± 12.37	106.7 ± 9.02	126 ± 12.65	141.8 ± 14.02	0.25

Note: A p-value < 0.05 is considered significant. fasting plasma glucose (FPG), glycated haemoglobin (HbA1c), Body Mass Index (BMI), and Waist-to-Hip (W/H) Ratios

Discussion

Our results demonstrate a correlation between leptin levels, obesity, and T2DM, as well as the effect of various management regimens, including metformin, insulin, and their combination, on leptin levels. Leptin regulates obesity-induced inflammation, food intake, and BMI. It has been shown to be an indicator of insulin resistance, independent of the amount of body fat mass [16]. Therefore, higher baseline leptin levels may reflect higher insulin resistance and, thus, predict the more pronounced reduction in total daily insulin dose in response to metformin due to improved insulin sensitivity [17]. The study revealed that the average FPG in obese controls was marginally higher than in non-obese controls [17]. This finding aligns with the research by Nnamdi, Ubuo, *et al.,* which reported no notable alteration in FPG and HbA1C levels with the degree of obesity [18]. However, other authors found that FPG is much greater in obese

individuals than in non-obese individuals. This finding may be explained by the pancreatic beta cells' capacity to counteract insulin resistance [19]. Some studies involving both obese and non-obese human subjects have shown a significant positive relationship between body fat percentage and serum leptin levels [20, 21]. These results corroborate the findings of Considine et al., who found that obese individuals have higher serum leptin concentrations, which are correlated with both BMI and fat mass. The obese individuals exhibited significantly increased mean serum leptin than in non-obese controls [20]. However, this study demonstrated that the mean serum level of "leptin" did not significantly differ amongst the three treatment cohorts and the non-diabetic controls among the diabetic cohorts. These results were in line with the study conducted by Ambad, Jha, *et al.,* which states that it is normal to expect a complex interaction between insulin resistance and leptin and insulin [21]. Other research, such as Onyemelukwe, Ogoina, *et al.,* contested these results, which discovered that individuals with T2D exhibited significantly elevated serum leptin levels compared to non-diabetic controls [22]. Wu, Song *et al.* discovered that diabetic individuals had somewhat higher serum leptin levels than non-diabetic subjects. A significant positive relationship was found between leptin and insulin amount in non-obese diabetic subjects [23]. The fact that the current study allowed for a fair comparison between diabetic and non-diabetic cohorts by controlling for both cohorts' BMIs, which is actually the best predictor of serum leptin, could be one reason for the differences between it and the earlier studies [24]. This result could be attributed to the fact that the serum-associated leptin, which is primarily produced by adipocytes, is strongly correlated with "BMI", or fat mass, and that the reduction in BMI that is seen following treatment is what causes the decrease in leptin levels [25].

The results suggest that monitoring leptin levels may provide important perspectives into the impact of various treatment regimens in obese T2DM patients. Leptin is crucial to the pathophysiology of obesity-related insulin resistance; medications that improve leptin sensitivity may give better metabolic outcomes. The measurement of leptin in clinical practice may help tailor modern management plans and reduce complications associated with T2D and obesity. In contrast, compared to non-diabetic controls and other treated cohorts, the diabetes metformin cohort in the current study had lower serum leptin levels. Mueller made similar findings to Stanhope *et al.* and Mick, Wang *et al.,* who discovered that metformin enhances glucose absorption and decreases the secretion of leptin from cultured adipocytes [26, 27]. Serum leptin was found to decrease with metformin treatment, according to Sardu, D'Onofrio *et al.* [28]. Additionally, Kim *et al.* found that metformin improved leptin resistance in "obese" individuals. They examined the potential metabolic effects of this medication on adipose tissue function and hormone regulation in individuals with T2D [29]. Furthermore, compared to the diabetes metformin cohort and controls, the diabetes combination cohorts and the diabetes insulin cohort had higher leptin levels. This result was in line with research by Pereira, Cline *et al.,* who looked at how insulin affected the level of leptin. They concluded that insulin causes adipocytes to secrete more leptin [30].

According to reports from other researchers, there is a negative correlation between blood glucose and leptin measurement and a positive correlation between insulin amount and leptin amount. This

suggests that increased insulin secretion causes an increase in leptin secretion from adipose tissue [20, 21].

Nevertheless, these findings support the notion that serum leptin levels are linked with glycemic status, body composition, and the type of antidiabetic therapy administered. The high leptin levels, particularly in insulin-treated patients, may reflect underlying adipose tissue dysfunction and insulin resistance, underscoring leptin's influential role as a biomarker for disease progression and response to management in T2DM.

Conclusion

BMI and obesity are primary defects in type 2 diabetes that contribute to the elevated serum leptin level. For obese individuals with diabetes, metformin enhances leptin resistance, which provides benefits that extend beyond blood sugar control. Combination therapy, which includes metformin and insulin, may help treat patients with higher leptin levels. In contrast, insulin-treated groups exhibit persistent insulin resistance and adipose dysfunction.

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