# STUDY ON PLASMA LEPTIN LEVELS IN TYPE 2 DIABETES MELLITUS PATIENTS WITH OVERWEIGHT OR OBESITY

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**Summary**

***Objectives:*** To investigate plasma leptin level and its association with some risk factors in type 2 diabetes mellitus (T2DM) patients with overweight or obesity. ***Subjects and methods:*** A cross-sectional descriptive and comparable study was conducted on 266 patients assigned into three groups, including T2DM patients with overweight or obesity (study group, n = 104), non-overweight/non-obese atients with T2DM (disease-control, n = 109) and healthy control subjects (n = 53). Plasma leptin levels were quantified with ELISA Kit at the Department of Pathophysiology, Vietnam Military Medical University. ***Results:*** For type 2 diabetic patients with obesity/overweight, we found no significant difference in plasma leptin levels between the study group and the disease-control and healthy subjects (0.43; 0.35-0.53 ng/mL vs 0.42; 0.34-0.52 ng/mL and 0.46; 0.36-0.60 ng/mL, respectively) with $p > 0.05$. In the overweight or obese diabetic patients, there was a positive correlation of leptin levels with age ($r = 0.228$; $p = 0.02$), waist circumference (WC) ($r = 0.638$; $p < 0.001$), hip circumference (HC) ($r = 0.473$; $p < 0.001$), W/H ($r = 0.471$; $p < 0.001$) and serum creatinine levels ($r = 0.508$; $p < 0.001$). Additionally, plasma leptin levels were higher in overweight/obese patients who were diabetic for more than 5 years (0.47 ± 0.16 ng/mL) than in those with diabetes less than 5 years (0.38 ± 0.15 ng/mL) ($p < 0.05$). Significantly higher blood leptin levels were noted in the group with hypertension than in normotensive individuals ($p < 0.05$).

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**Date received: 03/3/2023**

**Date accepted: 18/4/2023**

**http://doi.org/10.56535/jmpm.v48i4.317**

***Conclusion:*** There were no significant differences between the levels of leptin in the study group compared with disease-control and healthy control groups. Moreover, leptin levels in the study group correlate positively with age, WC, HC, W/H, blood creatinine level, and diabetic duration. The leptin levels were significantly higher in obese diabetic patients with hypertension than in normotensive subjects.

** Keywords: Type 2 diabetes mellitus; Plasma leptin level; Overweight; Obesity.*

## INTRODUCTION

Obesity prevalence has risen dramatically in recent years. According to the World Health Organization, the global projected prevalence by 2030 is estimated to be roughly 1.9 billion people with obesity/overweight [1]. Obesity is reported to be linked with an increased risk of developing insulin resistance, whose progression over time leads to type 2 diabetes. Obese subjects with T2DM are at higher risk of cardiovascular disease. Thus, obesity management is one of the primary treatment goals of T2DM [2]. Leptin was first discovered to be a product of the obese gene in 1994. It is an adipokine produced and secreted primarily from adipose tissue with many functions. Its main function is to help regulate the body's energy balance by supporting energy expenditure and constraining fat mass by limiting energy intake. Leptin acts as a cytokine promoting inflammatory responses and has detrimental effects on organs [3]. Diabetic patients with obesity/overweight are more likely to get higher chances of cardiovascular diseases and are gradually vulnerable to developing obesity-related complications. However, leptin concentration and its association with T2DM have not been completely understood. Thus, the current study was conducted: *To investigate plasma leptin levels and their association with clinical and subclinical characteristics of diabetic patients with obesity/overweight.*

## SUBJECTS AND METHODS

### 1. Subjects

A total of 266 patients were classified into 3 groups: 104 subjects were diabetic patients with overweight/ obesity as the study group, 109 non-overweight/non-obese patients with T2DM as the disease-control, and 53 were healthy control subjects. These participants visited or were being treated at the Department of Endocrinology

and Diabetes and National Heart Institute - Bach Mai Hospital during the period 2018-2022.

* *Exclusion criteria:* Patients had comorbid chronic conditions including severe sepsis, elevated plasma osmotic pressure, etc. or cancer; those who refused to enroll in the study.

**2. Methods**

- This was a cross-sectional descriptive design in which differences among three groups were compared.

- Patients were performed a clinical examination, past medical history, disease detection time, smoking status (yes/no), and exercise status (yes/no).

- Blood pressure was measured using the Korotkoff method.

- In each group, measurement of weight and height and calculation of BMI (Body Mass Index) was undertaken. This was similarly done with WC, HC, and W/H.

* *Laboratory test:* All patients were done laboratory testing.

- Biochemistry blood tests (glucose, kidney function, blood lipid test, HbA1c, etc.).

- Early morning blood draws were collected (after a minimum fasting of 8 hours). Plasma leptin levels were quantified with ELISA Kit at the Department of Pathophysiology, Vietnam Military Medical University.

- Control group: defined as subjects who visit hospital for medical check-ups at the Department of Medical Examination according to the National Heart Institute's requirement had normal health based on the results of medical examination.

* *Diagnostic criteria used in the current study:*

- Diagnosis of diabetes according to the American Diabetes Association (ADA) criteria.

- The current WHO criteria to classify overweight and obesity for the Asians (2000).

- The criteria for obesity regarding the larger WC and W/H according to the Southeast Asia Diabetes Association (ADA) criteria.

- Diagnosis of hypertension according to Vietnamese National Heart Association guidelines (2016).

- The 2019 European Society of Cardiology Guidelines for diagnosis of dyslipidemia.

* *Data processing:* By using SPSS 20.0 software.

## RESULTS

Table 1: Study participants' characteristics.

| Variables | | Study group (n = 104)(1) | Disease control (n = 109)(2) | Healthy control (n = 53)(3) | p |
|---|---|---|---|---|---|
| Gender | Male (n, %) | 46 (44.23) | 58 (53.21) | 19 (35.85) | 0.1 * |
| | Female (n, %) | 58 (55.77) | 51 (46.79) | 34 (64.15) | |
| Age | Average (years) | 63.90 ± 10.25 | 63.21 ± 10.10 | 66.02 ± 8.71 | 0.187 ** |
| Duration of diabetes | Average (years) | 8.39 ± 7.29 | 7.90 ± 6.56 | - | 0.839 *** |
| | ≤ 5 years | 39 (37.50) | 47 (43.12) | - | 0.403 * |
| | > 5 years | 65 (62.50) | 62 (56.88) | | |

*(* $\chi^2$ test; ** Kruskal Wallis test, *** Mann-Whitney U-test)*

There was no difference in sex ratio and mean age across the three groups ($p > 0.05$).

Table 2: Serum leptin levels of the study participants.

| Study participants | Leptin levels (ng/mL) | | | p * |
|---|---|---|---|---|
| | Median | Percentile | | |
| | | *25* | *75* | |
| Study group | 0.43 | 0.35 | 0.53 | 0.385 |
| Disease control | 0.42 | 0.34 | 0.52 | |
| Healthy control | 0.46 | 0.36 | 0.60 | |

*(* Kruskal Wallis test)*

No differences in plasma leptin concentrations across the groups were recorded ($p > 0.05$).

Table 3: Association of leptin levels with age, gender and illness detection time in diabetic patients with obesity/overweight (the study group).

| Variables | | Leptin levels (ng/mL) | p* |
|---|---|---|---|
| Gender | Male (n = 46) | 0.43 ± 0.14 | 0.541 |
| | Female (n = 58) | 0.45 ± 0.18 | |
| Age | ≤ 60 (n = 34) | 0.39 ± 0.13 | 0.052 |
| | > 60 (n = 70) | 0.46 ± 0.17 | |
| Duration of diabetes | ≤ 5 years (n = 39) | 0.38 ± 0.15 | 0.008 |
| | > 5 years (n = 65) | 0.47 ± 0.16 | |

*(* Independent T-test)*

No differences in leptin concentrations by age and gender were observed in the study group. Differences in diabetic duration exist in patients who were diabetic for more than 5 years having higher leptin levels than those with less than 5 years ($p < 0.05$).

Table 4: Correlation of leptin levels with risk factors of diabetic patients with obesity/overweight.

| Variables | | | Leptin levels (ng/mL) | p |
|---|---|---|---|---|
| Hypertension | Presence (n = 78) | Mean ± SD | 0.46 ± 0.17 | 0.039 * |
| | Absence (n = 26) | Mean ± SD | 0.38 ± 0.12 | |
| Lipid disorders | Presence (n = 80) | Mean ± SD | 0.44 ± 0.16 | 0.393 * |
| | Absence (n = 24) | Mean ± SD | 0.41 ± 0.17 | |
| WC | High (n = 48) | Median (IQR) | 0.54 (0.46-0.63) | < 0.001 ** |
| | Normal (n = 56) | Median (IQR) | 0.35 (0.28-0.39) | |
| W/H | High (n = 59) | Mean ± SD | 0.51 ± 0.16 | < 0.001 * |
| | Normal (n = 45) | Mean ± SD | 0.34 ± 0.09 | |
| Smoking | Yes (n = 12) | Mean ± SD | 0.44 ± 0.16 | 0.979 * |
| | No (n = 92) | Mean ± SD | 0.44 ± 0.16 | |
| Exercise | Yes (n = 7) | Mean ± SD | 0.44 ± 0.12 | 0.939 * |
| | No (n = 97) | Mean ± SD | 0.44 ± 0.16 | |

*(* Independent T-test; ** Mann-Whitney U-test)*

Significantly higher blood leptin levels were noted in the group with hypertension, large WC and large W/H than in normotensive individuals and those with no change of WC and W/H ($p < 0.05$).

Table 5: Correlation of leptin levels with anthropometric indices, age, hypertension, and period with diabetes in the study participants.

| Variables | Correlation | |
|---|---|---|
| | r | p |
| BMI (kg/m$^2$) | - 0.06 | 0.543 |
| WC (cm) | 0.638 | < 0.001 |
| HC (cm) | 0.473 | |
| W/H | 0.471 | |
| Age (year) | 0.228 | 0.02 |
| SBP (mmHg) | - 0.019 | 0.848 |
| Duration of diabetes (year) | 0.178 | 0.071 |

*(r: Pearson)*

Leptin concentrations were also found to be strongly positively correlated to WC, moderately with HC and W/H ($p < 0.05$).

Table 6: Correlation of leptin levels with a biochemistry blood test in the study participants.

| Variables | Correlation | |
|---|---|---|
| | r | p |
| Blood glucose (mmol/L) | 0.148 | 0.231 |
| HbA1c (%) | -0.044 | 0.658 |
| Creatinine (µmol/L) | 0.508 | < 0.001 |
| Triglyceride (mmol/L) | 0.053 | 0.594 |
| Cholesterol (mmol/L) | 0.048 | 0.627 |
| HDL-C (mmol/L) | - 0.019 | 0.851 |
| LDL-C (mmol/L) | - 0.026 | 0.796 |

*(r: Pearson)*

There was a moderately positive correlation between leptin and creatinine levels ($p < 0.05$).

## DISCUSSION

As can be seen from the findings, we recorded no difference in age, sex ratio, and diabetes duration across the study participants ($p > 0.05$).

Regarding the leptin levels, the study group showed higher values (0.43; 0.35 - 0.53 ng/mL) than the disease-control and healthy control (0.42; 0.34 - 0.52 ng/mL) and (0.46; 0.36 - 0.60 ng/mL), respectively. This difference was, however, not significant across the groups ($p > 0.05$) (*Table 2*). Leptin was first discovered in 1994 to be adipokine produced and secreted primarily from adipose tissue with many functions. Nevertheless, its main role is to help regulate the body's energy balance by supporting energy expenditure and constraining fat mass by limiting energy intake. There have been numerous studies supporting that serum leptin levels are higher in obese individuals. Despite this increase, there is no *fat mass* reduction or *appetite suppression. Thus, a question has been raised about the presence of selective leptin resistance. There are several causes of this mechanism. Firstly,* there is s*aturation* in the *transport* of *leptin into* the *central nervous system in obese individuals. Secondly, there is the activation of pro-inflammatory cytokine*s, *typically suppressors* of *cytokine signaling* 3 (SOCS-3), thereby exerting the *physiological* effects *following leptin binding* to *its receptor*. Nevertheless, due to selective leptin resistance, leptin acts to inhibit appetite and controls energy expenditure. Its effects on the sympathetic nervous system remain preserved via inflammatory markers (C-reactive protein) and metabolic mediators that adversely affect the cardiovascular system. This action is observed in mice [4]. In a Multi-Ethnic Study of Atherosclerosis (MESA) study on the relation of leptin to left ventricular hypertrophy by Matthew A et al. (2013) with 1.464 participants, leptin concentrations were reported to be 19.4 ± 21.6 pg/mL/median: 12.1 pg/mL. Their study also revealed a relationship between leptin levels and measures of LV structure and function, suggesting that leptin is a mediator *affecting the cardiovascular system* [5].

Vo Minh Phuong's study (2019) on leptin levels, plasma adiponectin, and leptin-to-adiponectin ratio among overweight/obese populations indicated that plasma leptin levels were the highest in obese individuals (10.74 ± 5.61 ng/mL), followed by overweight group (9.74 ± 5.76 ng/mL) and control

group (6.75 ± 5.17 ng/mL), ($p < 0.05$) [6]. The difference in our study is, however, not statistically significant across the groups. The different findings across the research are due to a *strong positive association* of body fat percentage with serum leptin production and *elevated blood leptin concentrations* [7]; *serum leptin levels are greater* in *females than* in *males; estrogens increase leptin levels* (19), whereas androgens decrease leptin levels [8]; In the meantime, reductions in *leptin levels* have been attributed to *diabetes medications, diet, and exercise programs etc.* There is growing evidence that some anti-diabetic drugs are shown to reduce serum leptin levels, including *statins, sitagliptin*, *metformin*, pioglitazone, liraglutide and empagliflozin (Katsiki Niki et al., 2018) [3]; atorvastatin (Werida Rehab et al., 2021) [9]. Additionally, high-fiber meals and regular exercise also lead to lower leptin levels [10]. This may explain the reasons why leptin concentrations in the current study were not high, particularly in the study group. This difference may be due to patients' characteristics *being primarily older adults (mean age of* 63 years) and longer diabetes duration (average 8 years); most patients were diabetic for more than 5 years (62.5% in the study group and 56.88% in the disease control). Besides, the use of many drugs during treatment including tablets (metformin, janomet, sitagliptin etc) or insulin injection combined with tablets or *atorvastatin,* a *drug* used to treat *dyslipidemia*. These factors contribute to low levels of leptin. Our findings are consistent with Alzamil Hana et al.'s report (2020), in which there was no difference in leptin concentrations in both T2DM and healthy controls and no relation between leptin levels and BMI in T2DM subjects [11].

Regarding the association between plasma leptin levels and some anthropometric features including WC, HC, and W/H in the study group (*Table 4, Table 5*), we recorded a moderate-to-strong positive correlation between them ($p < 0.05$). Our findings are in line with Onyemelukwe et al.'s report (2020) in which leptin concentrations were positively correlated with WC in T2DM ($r = 0.71$, $p < 0.05$). This might be attributable to the fact that leptin is one of the cytokines excreted from adipose tissue. Thus, the positive correlation of leptin levels and WC, HC, and W/C is reasonable. Additionally, diabetes duration and comorbidities are factors affecting the progression of diabetes.

Besides, the relationship between leptin levels and hypertension was also found in the literature review. Table 4 shows that the leptin levels were significantly higher in obese diabetic patients with hypertension than in normotensive subjects ($p < 0.05$). Our results were similar to the study by Vo Minh Phuong (2019): levels of leptin were positively related to hypertension, WC, and H/C in obesity and overweight in male patients (r ranging from 0.369 - 0.373), $p < 0.05$) [6]. Additionally, leptin concentrations were shown to be positively correlated with creatinine ($r = 0.508$, $p < 0.001$) (*Table 6*). Similar findings were found in Shihi et al.'s research (2022) [12] on the relationship between leptin levels and creatinine. As observed from table 3 and table 5, diabetic subjects over the 5-year period had greater leptin concentrations than those within 5 years, and levels of leptin were positively correlated to age. As such, older age and longer diabetes duration proportionally increased the risk of major renal events and hypertension. Moreover, hypertensive diabetic participants had higher leptin levels compared to normotensive individuals or those without diabetic kidney disease. Thus, the relationship of leptin concentrations with age and diabetes duration in the present study was found to be reasonable.

## CONCLUSION

There were no significant differences between the levels of leptin in the study group compared with disease-control and healthy control groups. Additionally, leptin levels are positively correlated with age, WC, HC, W/H, blood creatinine levels, and the duration of diabetes. The leptin levels are significantly higher in obese diabetic patients with hypertension than in normotensive subjects.

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