# Homocysteine and Visfatin Upregulation for Type 2 Diabetes Etiology

Sabaa Ahmed[1], Abdul-Rahim A. Ali [2], Murooj G. Jameel [2*], MayadaTalal Hamoo [2], Mohammed Fawzi Majeed [2], Dina Suhail M Saleh [2], Sarah Hussain Khalaf [2]

## Abstract

Background: Diabetes is a metabolic disorder characterized by insulin dysfunction. It has seen a surge in prevalence in recent years. Understanding the factors influencing diabetes pathogenesis is crucial for developing effective treatments. Recent studies have identified visfatin as a hormone with insulin-like properties, highlighting its potential role in diabetes. Methods: This study aimed to investigate the levels of homocysteine and its association with visfatin in diabetic patients compared to healthy individuals. Thirty patients with type 2 diabetes were enrolled, and their visfatin and homocysteine levels were measured. Results: Our findings revealed elevated levels of homocysteine and visfatin in diabetic patients compared to the control group. Statistical analysis demonstrated a significant difference between the two groups ($P < 0.05$). Furthermore, we observed a correlation between Apo.A1, Apo.B, and Apo.B / ApoA1 with visfatin and homocysteine levels, indicating a direct and significant relationship between visfatin levels and these apolipoproteins. Conclusion: The study underscores the importance of visfatin and homocysteine in diabetes pathophysiology. Elevated levels of these biomarkers in diabetic patients suggest their potential role as indicators or contributors to the disease. Further research into the mechanisms underlying their interaction may offer insights into novel therapeutic approaches for diabetes management.

**Keywords:** Diabetes mellitus, Homocysteine, Visfatin, Apolipoproteins, Case-control study

***Significance*** | This study showed homocysteine and visfatin levels in diabetes offered insights into pathogenesis and potential therapeutic targets for managing complications.

*Correspondence. Murooj G. Jameel, Department of Pharmacy, Al-Yarmouk University College, Iraq.
E-mail: muroojgazi@al-yarmok.edu.iq

Editor Mohamed Khadeer Ahamed Basheer, And accepted by the Editorial Board Apr 07, 2024 (received for review Jan 29, 2024)

## Introduction

Diabetes mellitus (DM) is a chronic disease that affects approximately 6% of people worldwide, and its prevalence is increasing (Townsend, 2000). According to Amos, McCarty, and Zimmet (1997), the total number of people with DM worldwide is estimated at 221 million, compared to 124 million in 1997. More than 90% of these patients have type 2 DM. There is no consensus on the pathogenesis of DM, but it is widely accepted that the cause is multifactorial, involving both genetic and environmental factors (Adeghate, Schattner, & Dunn, 2006). Environmental causes of DM types 1 and 2 may include an immune response to various stimuli and some viral infections (Roivainen et al., 1998).

High prevalence of genetic factors associated with obesity are major contributors to the pathogenesis of type 2 DM (Jameel, Hashim, & Al-Osami, 2022). Obesity results from a combination of inadequate physical activity, hormonal and genetic factors, or poor diet. Type 2 DM is now considered an epidemic in developed countries and is strongly associated with obesity, which is part of the metabolic syndrome (Yoon et al., 2006). Studies by several researchers have shown that DM is associated with obesity and metabolic syndrome, including hyperlipidemia and hypertension, with visceral intra-abdominal adipose tissue (Curat et al., 2006). Adipose tissue hosts

**Author Affiliation.**
[1] Biochemistry, Department of Biology, Mashad Branch College of Sciences, Islamic Azad University, 0000424, Iran
[2] Department of Pharmacy, Al-Yarmouk University College, Iraq.

**Please cite this article.**
Sabaa Ahmed, Abdul-Rahim A. Ali et al. (2024). Homocysteine and Visfatin Upregulation for Type 2 Diabetes Etiology, Journal of Angiotherapy, 8(4), 1-6, 9539

many hormones, including resistin, leptin, adipsin, adiponectin, angiotensin, and estradiol (Adeghate, 2004).

**Materials and Methods**

This case study focused on two distinct groups: patients diagnosed with type 2 diabetes and healthy individuals without diabetes, serving as the control group. Inclusion criteria for the case group involved individuals aged over 20 years with a minimum duration of one year since diabetes diagnosis and provided written consent to participate. Exclusion criteria for this group included known liver disease, renal failure, fever persisting for over a week without a known cause, active infection including viral infections, and pregnancy in female patients. The control group comprised non-diabetic individuals selected through age and gender matching from the same non-diabetic patient pool.

This study was conducted in accordance with ethical principles outlined in the Declaration of Helsinki. All participants provided informed consent before inclusion in the study. Confidentiality of participants' personal and medical information was strictly maintained throughout the research process. The study protocol was approved by the relevant institutional review board, ensuring adherence to ethical standards in human research. Any potential conflicts of interest were disclosed and managed appropriately. Data integrity and accuracy were maintained, and the results were reported transparently without manipulation or bias. The welfare and rights of the participants were paramount throughout the study, and steps were taken to minimize any potential risks or discomfort associated with participation.

**Procedure:**

This case-control study was conducted on known patients with type 2 diabetes admitted to Mashhad University Hospitals in 2020, approved by their endocrinologist. The control group was selected from non-diabetic patients admitted to the hospital, confirmed by a blood glucose test and approved by an internal medicine specialist or endocrinologist.

**Homocysteine:**

Homocysteine (Hcy) is a dietary amino acid produced by the breakdown of methionine. Hcy is also called L-homocysteine and the amino acid sulfanyl butanic acid (Townsend, 2000). Plasma Hcy levels depend on age, sex, and body weight (Amos, McCarty, & Zimmet, 1997). The presence of abnormally high levels of homocysteine in plasma or serum is called blood hyperhomocysteinemia. Although standard values are highly variable, in general, total homocysteine levels are considered to be mild at 12 to 30 mmol/L, and much higher homocysteine concentrations reflect levels above 100 mmol/L (Adeghate, Schattner, & Dunn, 2006).

The reference range for plasma homocysteine levels can vary depending on the methodology employed. Typically, reference values are stratified by age groups. For individuals aged 0 to 30 years, the reference range is approximately 4.6 to 8.1 µmol/l. Among those aged 30 to 59 years, men exhibit a range of 6.3 to 11.2 µmol/l, while women have a slightly lower range of 5.5 to 7.9 µmol/l. For individuals older than 59 years, the reference range is approximately 5.8 to 11.9 µmol/l (Townsend, T. J. 2000)

The study conducted by Ansari et al. (2020) in Pakistan aimed to assess the frequency of plasma homocysteine levels in type 2 diabetic patients. This cross-sectional study evaluated 90 patients with an equal gender distribution and a mean age of 60 years, focusing on fasting serum homocysteine and HbA1c levels. The findings revealed a significant increase in homocysteine levels among men ($p < 0.03$), individuals over 60 years of age ($p < 0.001$), and those with long-standing diabetes ($p < 0.0004$). However, the significance of these results was observed primarily in men, while women under 60 years did not show significant findings, attributed to variations in disease organ involvement and variations in sugar control among participants, with some undergoing initial treatment (Amos et al., 1997).

The procedure involved conducting a case-control study on known patients with type 2 diabetes admitted to Mashhad University Hospitals in 2020, approved by their endocrinologist. Control participants were selected from non-diabetic patients admitted to the hospital, confirmed through blood glucose tests and approved by internal medicine specialists or endocrinologists. Inclusion criteria for the case group included individuals aged over 20 years with at least 1 year of diabetes duration and written consent to participate. Exclusion criteria encompassed liver disease, renal failure, prolonged unexplained fever, infections including viral infections, and pregnancy in female patients. After obtaining informed consent, demographic data, including age, gender, education level, BMI, diabetes duration, and smoking status, were recorded, and relevant checklists were completed. Blood samples were collected in the fasting state and centrifuged to isolate serum, which was then examined for homocysteine and visfatin levels. Data analysis was conducted using SPSS software and appropriate statistical tests.

**Results**

Data analysis involved the utilization of appropriate statistical methods, including the Shapiro-Wilk test to assess normality and subsequent use of parametric or non-parametric tests accordingly. For abnormal data, the Mann-Whitney test was employed, while parametric methods such as the Student's t-test were applied after confirming normality. Chi-square tests were utilized for nominal scale data analysis. Furthermore, Pearson correlation coefficient and regression were employed to explore variable relationships. SPSS v.26 served as the software for data analysis, with significance levels set at $p < 0.05$, where values less than 5% were denoted by

**Table 1.** Distribution of blood indicators in patients with type 2 diabetes and control group

| Variable | Group | | Normal | | Abnormal | Min | Max | Mean | SD | Statistic |
|---|---|---|---|---|---|---|---|---|---|---|
| | | N | % | N | % | | | | | p.value |
| | Control | 30 | 100.0% | 0 | 0.0% | 69.00 | 114.00 | 95.70 | 13.55 | t=.10.43 |
| FBS | Diabetes | 3 | 10.0% | 27 | 90.0% | 99.00 | 161.00 | 135.13 | 15.67 | P=0.0001* |
| | Total | 33 | 55.0% | 27 | 45.0% | 69.00 | 161.00 | 115.42 | 24.62 | |
| | Control | 30 | 100.0% | 0 | 0.0% | 4.10 | 5.90 | 5.00 | .53 | t=.17.85 |
| HBAIC | Diabetes | 0 | 0.0% | 30 | 100.0% | 6.50 | 8.80 | 7.65 | .61 | P=0.0001** |
| | Total | 30 | 50.0% | 30 | 50.0% | 4.10 | 8.80 | 6.33 | 1.45 | |
| | Control | 23 | 76.7% | 7 | 23.3% | 114.00 | 309.00 | 183.53 | 47.13 | t=0.35 |
| Chol | Diabetes | 20 | 66.7% | 10 | 33.3% | 100.00 | 309.00 | 179.00 | 53.06 | P=0.728 |
| | Total | 43 | 71.7% | 17 | 28.3% | 100.00 | 309.00 | 181.27 | 49.80 | |
| | Control | 22 | 73.3% | 8 | 26.7% | 80.00 | 287.00 | 155.60 | 60.17 | |
| TG | Diabetes | 25 | 83.3% | 5 | 16.7% | 57.00 | 489.00 | 155.53 | 105.39 | t=0.003 |
| | Total | 47 | 78.3% | 13 | 21.7% | 57.00 | 489.00 | 155.57 | 85.08 | P=0.998 |
| | Control | 18 | 60.0% | 12 | 40.0% | 26.00 | 93.00 | 55.30 | 21.63 | |
| HDL | Diabetes | 11 | 36.7% | 19 | 63.3% | 13.00 | 120.00 | 47.27 | 19.99 | t=1.49 |
| | Total | 29 | 48.3% | 31 | 51.7% | 13.00 | 120.00 | 51.28 | 21.04 | P=0.141 |
| | Control | 19 | 63.3% | 11 | 36.7% | 45.00 | 180.00 | 99.33 | 36.03 | |
| LDL | Diabetes | 17 | 56.7% | 13 | 43.3% | 43.00 | 180.00 | 97.47 | 38.56 | t=0.188 |
| | Total | 36 | 60.0% | 24 | 40.0% | 43.00 | 180.00 | 98.35 | 37.06 | P=0.851 |
| | Control | 22 | 73.3% | 8 | 26.7% | 60 | 1.30 | 93 | 17 | |
| Cr | Diabetes | 8 | 26.7% | 22 | 73.3% | 70 | 3.10 | 1.45 | 64 | t=.4.28 |
| | Total | 30 | 50.0% | 30 | 50.0% | 60 | 3.10 | 1.19 | 53 | P=0.0001* |

**Table 2.** Distribution of ApoB/ApoA1 in patients with type 2 diabetes and control group

| Group | Min | Max | Mean | SD | Statistic(p.value) |
|---|---|---|---|---|---|
| Control | .42 | .73 | .59 | .08 | t=3.61 |
| Diabetes | .46 | 1.06 | .73 | .19 | P.Value=0.002** |
| Total | .42 | 1.06 | .66 | .16 | |

**Table 3.** The mean homocysteine in the patient group and in the control group

| Group | Min | Max | Mean | SD | Statistic(p.value) |
|---|---|---|---|---|---|
| Control | 3.7 | 17.0 | 8.55 | 2.91 | t=2.85 |
| Diabetes | 4.8 | 28.1 | 11.45 | 4.74 | P.Value=0.006** |
| Total | 3.7 | 28.1 | 1.00 | 4.17 | |

"*", and values less than 1% by "**" (Henrieta Škovierová et al., 2016; M A Pajares & D Pérez-Sala, 2006).

Results from Table 1 (Student's t-test) indicated a statistically significant difference in FBS, HBA1C, and Cr ($p < 0.05$). Additionally, the distribution of ApoB/ApoAl exhibited a significant difference between the two groups ($p = 0.002$**) with mean values of 0.73 ± 0.19 in the patient group and 0.59 ± 0.08 in the control group (Table 2). Similarly, the distribution of homocysteine revealed a significant difference between the patient and control groups ($p = 0.006$**), with mean homocysteine levels of 11.45 ± 4.74 µmol/L and 8.55 ± 2.91 µmol/L, respectively (Table 3). Homocysteine metabolism involves complex pathways, as illustrated in Figure 1.I, where homocysteine serves as an intersection point for regeneration and transulfurization pathways. This non-protein sulfur amino acid is synthesized from methionine and regulated by enzymatic processes requiring vitamins B12, B6, and 5,10-methyltetrahydrofolate (Henrieta Škovierová et al., 2016; M A Pajares & D Pérez-Sala, 2006).

**Discussion**

Diabetes is a significant global metabolic disorder affecting approximately 280 million individuals worldwide (Tsutomu Hirano, 2018). Despite its apparent simplicity, diabetes leads to numerous complications over time, significantly impacting individuals' quality of life and performance (Yanling Wu et al., 2014). Complications such as retinopathy, nephropathy, neuropathy, diabetic ulcers, dietary restrictions, daily medication requirements, and psychological effects contribute to mortality rates in diabetic patients, which are 2 to 2.5 times higher than the general population (de Marco et al., 1999). One of the primary reasons for diabetes progression and its complications is the increase in insulin resistance, particularly notable in type 2 diabetes. Adipose tissue, more abundant in diabetic individuals, produces various markers in the body, including visfatin (Mona Mohamed Ibrahim Abdalla Abdalla, 2022). Although visfatin is secreted from other organs, adipose tissue remains its primary source, promoting glucose utilization by the liver and adipose tissue. Visfatin exhibits an inverse association with insulin, and its increased levels correlate with reduced insulin secretion. However, the precise nature of this relationship remains ambiguous. Therefore, to elucidate the intricate relationship between visfatin and type 2 diabetes, this study aimed to investigate homocysteine levels and their association with visfatin levels in both diabetic and healthy individuals.

In this study, 30 patients with type 2 diabetes and 30 healthy individuals were included. The mean age of the patients was 54.34 ± 7.82 years, while the mean age of the control group was 53.27 ± 8.89 years, with no significant difference between the two groups ($P > 0.05$). The mean body mass index (BMI) in the diabetes group was 30.19 ± 4.55 kg/m², slightly higher than the control group's mean BMI of 28.11 ± 3.73 kg/m², but the difference was not significant ($P > 0.05$). The mean diastolic blood pressure in the patient group was 82.30 ± 2.77 mm Hg, significantly lower than the control group's mean diastolic blood pressure of 89 ± 14.70 mm Hg ($P < 0.05$). The mean level of visfatin in the diabetic group was 22.75 ± 5.26 ng/ml, significantly higher than the control group's mean visfatin level of 13.77 ± 1.84 ng/ml ($P < 0.05$), consistent with findings reported by Laudes et al. (2010). Additionally, the study found a significant relationship between apolipoprotein A, apolipoprotein B levels, and the ApoB/ApoA1 ratio with visfatin levels ($P < 0.05$), while no association was observed between these parameters and homocysteine levels ($P < 0.05$), aligning with the findings of Chen et al. 2006.

The mean age of patients with type 2 diabetes was 65.3 years, while in the control group it was 64.5 years. There was no significant difference between the two groups regarding age, gender, and body mass index, which is consistent with findings similar to our study. However, similar to our study, the body mass index in diabetic patients in the study conducted by Chen et al. was higher than in the control group. Chen et al. found that fasting blood sugar, HbA1c, serum insulin, and insulin resistance were significantly elevated in diabetic patients compared to controls. Although only the HbA1c level was assessed in our study, similar results might have been observed if other markers were examined, as these variables typically change in tandem. Additionally, systolic and diastolic blood pressure were found to be significantly higher in diabetic patients compared to controls, which aligns with our study's findings. While Chen et al. observed higher creatinine levels in diabetic patients compared to the control group, no significant difference was found between the two groups in our study, possibly due to the limited sample size in both studies. In terms of visfatin levels, diabetic patients had a mean visfatin level of 31.9 ng/ml, significantly higher than the 15.8 ng/ml observed in healthy individuals, consistent with our findings. Chen et al. demonstrated that even after adjusting for age, sex, body mass index, and lipid profile, visfatin levels remained significantly higher in patients with type 2 diabetes compared to normal individuals, consistent with our study's results, indicating an association between diabetes and elevated visfatin levels (Chen et al. 2006). These findings emphasize the link between diabetes incidence and increased visfatin levels.

In a study examining the association of adipocytokines with heart risk factors in patients with type 2 diabetes, 85 individuals with diabetes and 30 non-diabetics were included. The mean age of patients with type 2 diabetes was 61.76 years, whereas the mean age of the control group was 58 years. No significant difference was observed between the two groups regarding age and sex distribution. While the body mass index (BMI) in the diabetic patient group was higher compared to the control group, similar to our study, there was no significant disparity in BMI between the two

groups. Key indicators of diabetes, such as blood glucose, HbA1c, serum insulin, and insulin resistance, were notably elevated in the diabetic patient group compared to the control group, aligning with our study's findings. These findings underscore the significant increase in these variables among patients with diabetes ($P < 0.05$). Moreover, the mean systolic blood pressure in diabetic patients was significantly higher than that in the control group, consistent with our study's results. However, no significant difference in diastolic blood pressure was observed between the two groups.

In the diabetic group, the level of apolipoprotein A was measured at 131.20 mg/dL, significantly higher than the control group's measurement of 10.75 mg/dL ($P < 0.05$). In a study by Barmjoo et al., aimed at investigating the correlation between serum visfatin and progressive B cell destruction in diabetic patients, 118 non-diabetic individuals were studied alongside 64 type 2 diabetes patients and 58 type 1 diabetes patients. The mean age of diabetic patients was 56 years, comparable to the control group's mean age of 51 years, with no significant difference noted. While the majority of patients were male, the study revealed higher systolic and diastolic blood pressure levels in the patient group compared to the control group, along with elevated fasting blood sugar levels, body mass index, and HbA1c levels in diabetic patients. These findings align with our study and major research on diabetic patients compared to healthy individuals, indicating that markers such as BMI, fasting blood sugar, and HbA1c levels are influenced by diabetes. Barmjoo et al.'s study further demonstrated a correlation between visfatin levels and circulating insulin levels across all patient groups, suggesting that higher visfatin levels coincide with lower insulin levels. Additionally, visfatin levels were significantly elevated in patients with type 2 diabetes compared to non-diabetics, corroborating the association between diabetes incidence and increased visfatin levels, as indirectly confirmed by our study's findings linking diabetes and its complications to visfatin levels (Abel et al. 2006).

## Conclusion

In conclusion, diabetes presents a complex interplay of factors impacting individuals globally. Our study elucidated the significant association between elevated visfatin levels and type 2 diabetes, reaffirming previous research. Understanding these connections is crucial for advancing diabetes management and reducing associated complications, ultimately improving patient outcomes and quality of life.

## Author contributions

A.A., A.R.A.A., M.G.J., M.T.H., M.F.M., D.S.M.S., S.H.K. developed the Study design, and wrote, reviewed, and edited the paper.

## Acknowledgment

The authors thanked the Department.

## Competing financial interests

The authors have no conflict of interest.

## References

Abel Lo´ pez-Bermejo, Berta Chico-Julia`, et al. (2006). Serum Visfatin Increases With Progressive beta-Cell Deterioration. Diabetes, 55(10), 2871–2875.

Adeghate, E. (2004). An update on the biology and physiology of resistin. Cell Mol Life Sci, 61(19-20), 2485-96.

Adeghate, E., Schattner, P., & Dunn, E.J.A.o.t.N.Y.a.o.s. (2006). An update on the etiology and epidemiology of diabetes mellitus. 1084(1), 1-29.

Amos, A., McCarty, D., & Zimmet, P. (1997). Diabet Med 14 Suppl 5, S1.

Ansari, R., et al. (2014). Hyperhomocysteinemia and neurologic disorders: a review, 10(4), 281-288.

Ansari, T., et al. (2020). Frequency of elevated plasma homocysteine (Hcy) levels among type 2 Diabetes mellitus (T2DM) patients, 27(03), 635-640.

Chen Miao-Pei et al. (2006). Elevated Plasma Level of Visfatin/Pre-B Cell Colony Enhancing Factor in Patients with Type 2 Diabetes Mellitus. J Clin Endocrinol Metab, 91(1), 295–299.

Curat, C., et al. (2006). Macrophages in human visceral adipose tissue: increased accumulation in obesity and a source of resistin and visfatin. 49(4), 744-747.

de Marco R., Locatelli F., Zoppini G., Verlato G., Bonora E., Muggeo M. (1999). Cause-specific mortality in type 2 diabetes. The Verona Diabetes Study. Diabetes Care, 22, 756–761.

Henrieta Škovierová et al. (2016). The Molecular and Cellular Effect of Homocysteine Metabolism Imbalance on Human Health. Int. J. Mol. Sci., 17, 1733.

Kumar, J., et al. (2015). No evidence of a causal relationship between plasma homocysteine and type 2 diabetes: a Mendelian randomization study. 2, 11.

Laudes M, Oberhauser F, Schulte DM, Freude S, Bilkovski R, Mauer J, Rappl G, Abken H, Hahn M, Schulz O, Krone W. (2010). Visfatin/PBEF/Nampt and resistin expressions in circulating blood monocytes are differentially related to obesity and type 2 diabetes in humans. Horm Metab Res, 42, 268–273.

M A Pajares and D Pérez-Sala. (2006). Betaine homocysteine S-methyltransferase: just a regulator of homocysteine metabolism?. Cell Mol Life Sci, 63(23), 2792-803.

Marti-Carvajal, A.J., et al. (2017). Homocysteine-lowering interventions for preventing cardiovascular events, 8.

Mona Mohamed Ibrahim Abdalla Abdalla MMI. (2022). Role of visfatin in obesity-induced insulin resistance. World J Clin Cases, 10(30), 10840-10851.

Murooj G Jameel, Zainab M. Hashim, & Mohammad H. Al-Osami. (2022). Prevalence of AGER Gene Polymorphism in Post Menopause Iraqi Sample with Osteoporosis and Osteopenia in type 2Diabetes Mellitus. raqi J Pharm Sci, 31(2).

Mursleen, M.T., et al. (2017). Implication of homocysteine in diabetes and impact of folate and vitamin B12 in diabetic population. 11, S141-S146.

Roivainen, M., et al. (1998). Several different enterovirus serotypes can be associated with prediabetic autoimmune episodes and onset of overt IDDM. 56(1), 74-78.

Townsend, T. J. (2000). A decade of diabetes research and development. International Journal of Diabetes and Metabolism, 8, 88-92.

Tsutomu Hirano. (2018). Pathophysiology of Diabetic Dyslipidemia. J Atheroscler Thromb, 25(9), 771-782.

Yanling Wu1, Yanping Ding, Yoshimasa Tanaka and Wen Zhang. (2014). Risk Factors Contributing to Type 2 Diabetes and Recent Advances in the Treatment and Prevention. Int. J. Med. Sci., 11.

Yoon, K.-H., et al. (2006). Epidemic obesity and type 2 diabetes in Asia. 368(9548), 1681-1688.