**ISSN: 0067-2904**

# Studying the Glycogen Synthase Kinase 3β and Arrestin β-1 Levels in Patients with Metabolic Disorders

**Asmaa Mohammed*, Fayhaa M. Khaleel**
*Department of Chemistry, College of Sciences for Women, University of Baghdad, Baghdad, Iraq.*

Received: 19/10/2024 Accepted: 13/5/2025 Published: 30/4/2026

**Abstract**

Metabolic disorders, characterized by concurrent metabolic abnormalities such as dyslipidemia, hyperglycemia, and obesity, involve dysregulated signaling pathways critical to energy homeostasis. This study investigates the interplay between the Glycogen Synthase Kinase 3β (GSK3β) enzyme and the protein Arrestinβ-1in individuals with metabolic disorders. A total of 135 samples were collected and divided into 2 groups: The first group was the controls, n= 46, and the second group the patients, which was divided into two groups: 45 patients with metabolic disorders patients (dyslipidemia, hyperglycemia, and obesity), and 44(T2DM) patients with obesity, Samples were collected from AL-Yarmouk Teaching Hospital from August to September 2024. Biochemical variables were assessed in all sample groups, including the quantification of GSK3β, Arrestinβ-1, and insulin by the ELISA technique . Glycemic and lipid profiles were measured. The findings indicated elevated GSK3β and Arrestin β-1 levels in the obese T2DM when compared with the control group. The statistical analysis showed a favorable association between insulin resistance andGSK3β and Arrestinβ-1. This study demonstrates that both GSK3β enzyme and Arrestin β-1 serve as effective biomarkers for predicting metabolic disorders. Their critical roles in metabolic disorders lead them to the development of these metabolic pathways. Disruptions in this signalling pathway result in beta cell damage which is one of the causes of diabetes, which is considered one of the Risk factors of metabolic disorders in addition to obesity and dyslipidemia.

Keywords: Glycogen Synthase Kinase 3β, Arrestinβ-1, Metabolic disorders, Insulin resistance, Type 2 diabetes.

## Glycogen Synthase Kinase 3β (GSK3β), Arrestin β- 1 دراسة لدى المرضى الذين يعانون من اضطرابات التمثيل الغذائي

**أسماء محمد عبدالجليل *, فيحاء مقدا خليل**

قسم الكيمياء ,كلية العلوم للبنات , جامعة بغداد , بغداد , العراق

الخلاصة

الاضطرابات الأيضية، التي تتميز باضطرابات الأيضية المتزامنة مثل خلل شحميات الدم وفرط سكر الدم والسمنة، تنطوي على مسارات إشارات غير منظمة ضرورية لاستقرار الطاقة. تبحث هذه الدراسة في التفاعل بين إنزيم جليكوجين سينثاز كيناز (GSK3β) وبروتين Arrestinβ−1 لدى الأفراد الذين يعانون من اضطرابات التمثيل الغذائي. تم جمع ما مجموعه 135 عينة وتقسيمها إلى مجموعتين: المجموعة الأولى كانت الضوابط، عددهم = 46، والمجموعة الثانية المرضى، والتي تم تقسيمها إلى مجموعتين: 45 مريضًا يعانون من اضطرابات التمثيل الغذائي

*Email: asmaa.Abd2305m@csw.uobaghdad.edu.iq

(خلل شحميات الدم وفرط سكر الدم والسمنة)، و44 مريضًا (داء السكري من النوع 2) يعانون من السمنة، تم جمع العينات من مستشفى اليرموك التعليمي من أغسطس إلى سبتمبر 2024. تم تقييم المتغيرات الكيميائية الحيوية في جميع مجموعات العينة، بما في ذلك تحديد كمية GSK3β و Arrestinβ1− والأنسولين بواسطة تقنية ELISA. تم قياس مستويات سكر الدم والدهون. وأشارت النتائج إلى ارتفاع مستويات GSK 3β وArrestinβ1− لدى مرضى السكري من النوع الثاني المصابين بالسمنة مقارنةً بالمجموعة الضابطة. وأظهر التحليل الإحصائي ارتباطًا إيجابيًا بين مقاومة الأنسولين وGSK3β و Arrestinβ−1. توضح هذه الدراسة أن كلاً من إنزيم GSK3β و Arrestinβ1− يعملان كمؤشرين حيويين فعالين للتنبؤ بالاضطرابات الأيضية. ويؤدي دورهما الحاسم في الاضطرابات الأيضية إلى تطور هذه المسارات الأيضية. ويؤدي أي خلل في هذا المسار الإشاري إلى تلف خلايا بيتا، وهو أحد أسباب مرض السكري، الذي يُعتبر أحد عوامل خطر الاضطرابات الأيضية، بالإضافة إلى السمنة وخلل شحميات الدم.

## 1. Introduction

Metabolic disorders are characterized by the presence of three to five metabolic defects [1], such as elevated blood sugar, dyslipidemia, cardiovascular disease (CVD), and diabetes. These conditions tend to occur together in a non-random manner. Furthermore, they are influenced by metabolic disorders associated with IR [2]. Recent studies have revealed a pathophysiological connection between T2DM and IR [3], which is a significant defect that precedes T2DM, CVD disease, and other metabolic diseases [4]. Impaired insulin-stimulated transport of the glucose transporter GLUT4 to the cell surface is one of the primary defects resulting from IR [5]. A key feature of IR is reduced insulin-stimulated sensitivity in skeletal muscle, which leads to compensatory hyperinsulinemia and many metabolic diseases [6]. Prolonged hyperinsulinemia, in turn, further aggravates IR.

IR states, such as T2DM, are associated with higher glycogen synthase kinase-3β (GSK3β) , which phosphorylates and inactivates glycogen synthase [7]. Glycogen synthase kinase-3β (EC 2.7.11.26) is a serine-threonine kinase involved in several physiological processes, such as glycogen metabolism and microtubule integrity. It phosphorylates more than one protein substrate. It can link various signals outside and inside the cell to maintain the proper balance. In addition, studies established that GSK3β plays a significant role in the development of IR, a key factor in diabetes. GSK3β impairs insulin action within cells and disrupts glucose utilization by phosphorylating and promoting the degradation of insulin receptor substrate-1 (IRS-1), thereby weakening insulin signaling. It has been proven that a defect in the regulation of this enzyme may lead to many disorders, including heart disease and diabetes [8].

Arrestins β-1are a family of cytoplasmic proteins that are quite tiny, with an average size of about 45 kDa and four different subtypes. Since these proteins can stop (or "arrest") signaling via G protein-coupled receptors (GPCRs), the term "arrestin" was coined [9]. As the primary role of these proteins is to stop GPCR cellular pathways, it is yet unknown if these non-canonical arrestin beta1 actions require prior recruitment by GPCRs. Comprehending arrestins β-1 independent function in metabolic homeostasis is crucial since they can function as signaling molecules on their own [10]. The GPCRs control almost every metabolic process, including the regulation of glucose and energy. An increase in Arrestin β-1 activity may hinder normal interactions between insulin and its receptors, contributing to IR, a hallmark of obesity [11].

The study aims to investigate a relationship between the GSK3β enzyme and the Arrestinβ-1 protein in patients with metabolic disorders and to explore the possibility of using them as biomarkers for these metabolic disorders

## 2. Methods

### 2.1 Patients and Control

This study was conducted at the College of Science for Women, University of Baghdad. Samples were collected from Al-Yarmouk Teaching Hospital; with a total of 135 samples selected for the study. The groups were divided according to body mass index(BMI) and metabolic diseases into three groups: G1 includes 46 healthy subjects that represent the control group, patients group with metabolic disorders (G2, n=45), and patients with T2DM who have obesity (G3, n= 44). The age range of the participants was (30 -55) years. The BMI and waist-to-hip ratio (WHR) was calculated using the following equations: BMI=: weight in kg / height $m^2$ [12], and WHR= waist cm/hip cm [13].

The study population consisted of healthy individuals as well as patients diagnosed with metabolic disorders including dyslipidemia, hyperglycemia, and obesity, and those with T2DM of 1 to 10 years duration who were receiving treatment with metformin and Diamicron. Exclusion Criteria in this study included individuals who use insulin injections and suffer from T1DM, thyroid diseases, heart disease, kidney disease, pregnant women, liver diseases, and hypertension.

Six mL of venous blood was drawn from each individual using a 10 mL syringe. The blood was subsequently transferred into a gel tube and allowed to clot. After coagulation, the samples were centrifuged at 3000 rpm for 10 min to separate and collect the serum. Afterwards, the serum was frozen at -20 ℃. The GSK3β, Arrestinβ-1, and insulin were evaluated using ELISA, Al-Kit and technology from Huma Reader HS made in (USA). The lipid profile including (serum cholesterol, triglycerides (TG), low-density lipoprotein (LDL-C), very low-density lipoprotein (VLDL) and fasting blood sugar (FBS) were also measured with a UV-visible spectrophotometer from Huma Reader HS (USA). The (AIP) was derived through mathematical calculations utilizing a logarithm of (TG/HDL-C) [14]. Homeostasis Model Assessment (HOMA) for IRusing HOMA IR =(FBG x Insulin / 405) [15].

### 2.2 Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics version 26 (SPSS Inc., Chicago, U.S.A.). Data are presented as mean ± standard error (SE). This study involved several tests, including the ANOVA test to assess the correlation coefficient (r) between parameters, the Tukey test, the ROC curve, and the analysis of differences among three independent variables. The statistical test employed linear regression analysis as the estimation method. The expected value, of P as significant was at $p \leq 0.05$ and non-significant at $p > 0.05$, was utilized to determine the statistical significance.

## 3. Results and Discussion

The results indicate that there was no significant difference in the ages of the three groups, However, a significant difference in WHR and BMI ($kg/m^2$) ,($p<0.001$) as indicated in Table 1. Additionally, the lipid profile showed significant differences, with mean values ± SE for cholesterol, TG , LDL-C and VLDL as indicated in Table 1, while, HDL-C demonstrated a significant decrease HDL demonstrated significant differences among the groups Atherogenic index plasma AIP showed a significant difference between the three groups. in insulin, insulin resistance (IR), and fasting blood sugar (FBS) levels in both G2 and G3 groups compared to G1. Insulin resistance was assessed using the HOMA-IR equation, and the results showed a statistically significant increase ($P<0.05$), as presented in Table 1.

**Table 1:** Clinical and biochemical parameters among studied groups .

| Groups / Parameters | Control G1 No. (46) | Metabolic disorders G2 No. (45) | T2DM with Obesity G3 No. (44) | P-value |
|---|---|---|---|---|
| **Age (year)** | 40.82±1.28[a] (40) | 40.84±1.10[a] (41) | 44.45±1.04[a] (47.5) | 0.071 |
| **BMI (kg/m$^2$)** | 22.85±0.17 [a] (23) | 36.51±0.74 [c] (36.3) | 34.01±0.65[b] (33) | 0.001** |
| **WHR** | 0.87±0.01[a] (0.9) | 1.12±0.02[c] (1.09) | 1.04±.0.02[b] (1.04) | 0.001** |
| **FBS (mg/dL)** | 97.79±1.96 [a] (93.1) | 131.58±2.34 [b] (130) | 212.67±6.71 [c] (217.35) | 0.001** |
| **Insulin ( IU/ mL )** | 3.36±0.11 [a] (3.5) | 6.05±0.12 [b] (6) | 7.66±0.17 [c] (7.9) | 0.001** |
| **IR** | 0.79±0.02 [a] (0.82) | 1.91±0.06[c] (1.9) | 4.05±0.19 [b] (4.2) | 0.001** |
| **Cholesterol (mg/dL)** | 173.91±1.11 [a] (174.95) | 209.17±2.30 [b] (207.5) | 213.56±2.45[b] (207) | 0.001** |
| **TG (mg/dL)** | 156.49±1.16[a] (154.3) | 204.38±2.97 [b] (201.8) | 217.87±1.91 [c] (220.95) | 0.001** |
| **HDL-C (mg/dL)** | 38.67±0.69 [b] (40) | 18.05± 0.48[a] (18.7) | 21.46±1.71[a] (16.85) | 0.001** |
| **LDL-C (mg/dL)** | 103.39±1.58 [a] (104) | 150.06±2.58 [b] (148) | 148.59±1.80[b] (145.75) | 0.001** |
| **VLDL-C (mg/dL)** | 31.00±0.22[a] (30) | 40.80±0.59[b] (40) | 43.46±0.38[c] (44) | 0.001** |
| **AIP** | 0.609 ±0.008 [a] (0.60) | 1.059± 0.013 [b] (1.03) | 1.051± 0.028 [b] (1.11) | 0.001** |

Body mass index: BMI, Waist to hip ratio : W/H ratio, Fasting Blood sugar: FBS, Homeostasis Model Assessment: HOMA, Insulin hormone: IR, Total cholesterol:TC, Triglycerides: TG, Low-Density Lipoprotein: LDL-C, Very Low-Density Lipoprotein: VLDL, High-Density Lipoprotein cholesterol: HDL-C, and Atherosclerosis index plasma: AIP Different letters (a, b, c) indicate values which reliably differed one from another within one line of the table according to the results of comparison using the Tukey test with Bonferroni correction,(a) represents the difference between G1 and G2 as shown in the FBS results, insulin,(b) represents the difference between G2 and G3 as shown in Triglyceride,(c )represents the difference between G3 and G2 as shown in VLDL.

The results indicated that the mean age level ± SE for G1, G2, and G3 showed a significant non-difference, which indicates an age matching between groups. A significant difference in BMI is a predictive measure of potential health risks associated with weight gain [16]. An increase in the index exceeding 30 kg/m² was observed in both groups. Obesity does not necessarily indicate the presence of DM or sclerosis. However, atherosclerosis contributes to the development of metabolic diseases. This result indicates that the individual does not experience excess weight and maintains good health. This finding aligns with other studies, which demonstrate that individuals with a BMI greater than 35 kg/m² have an elevated risk of developing T2DM compared to those with a BMI lower than 23 kg/m², as reported by Chan et al [17]. Similarly, Satman et al. established that BMI is a significant indicator of diabetes risk [18].

The WHR is a significant predictor of body fat percentage and its distribution. Findings from groups G2 and G3 indicate that individuals with obesity, T2DM, and metabolic disorders exhibit the highest fat accumulation around the waist and hips. This observation aligns with existing literature, as the WHR reflects total body fat content and distribution, particularly highlighting abdominal obesity, which is associated with an increased risk of metabolic diseases [19]. This is consistent with another study that measured the WHR as a diagnostic tool for obesity. This also agrees with Shungin D, Who confirmed that the WHR is a good predictor of diabetes and identified different aspects of the risk associated with obesity [20]. The result in G1, indicates that the fat distribution percentage in the hips and waist is uniform, and non-harmful. The current results indicate that BMI and WHR significantly contribute to the prevalence of metabolic disorders. Additionally, fasting blood sugar (FBS) serves as another parameter for predicting metabolic diseases, alongside BMI and WHR. The G2 result, indicates the onset of diabetes in the absence of anti-diabetic treatment. A high BMI is a contributing factor to elevated blood sugar levels. Exceeding 30 kg/m², as indicated above, along with age and. These factors significantly contribute to the development of DM, aligning with the findings of Tchernof et al. The factors contributing to fat storage and an elevated BMI include age, sex, and genetic predisposition. These factors also influence individuals with DM [21], In G3, this result is higher than in G2, and this is because they suffer from T2DM resulting from several factors secreted by adipose tissue impair glucose tolerance and cause damage to pancreatic cells, which leads to the development of diabetes [22] In patients with T2DM and obesity, the result is greater than in patients with metabolic disorders, due to the duration of diabetes, which ranges from 1 to 10 years, along with their adherence to oral anti-diabetic therapy.T2DM arises from a dysfunction in insulin production, specifically in the β-cells, resulting in heightened IR, as our findings indicate the high IR value in G3 group. This outcome signifies impairment of the pancreatic cells, which are responsible for controlling insulin output. The results indicated IR levels of G2was higher than that of the control group and G1, revealing a notable difference. This signifies the degree of damage to pancreatic cells in G2 patients with metabolic problems. This marginal increase in IR reflects the degree of damage to pancreatic cells in G2 patients with metabolic disorders. A marginal increase was observed in comparison to the control group, suggesting the initial stages of diabetes development. This marginal increase of IR in T2DM is partly attributed to the decreased insulin response in adipose and muscle tissues, resulting in glucose intolerance, which significantly contributes to the development of the condition [23].

Additionally, there is a significant difference in total cholesterol levels among the three groups, with higher cholesterol percentages observed in the two groups affected by DM. This is due to exhibit dyslipidemia resulting from elevated TC levels. Dyslipidemia results in an imbalance of blood sugar, causing substantial harm to β-cells. This aligns with Wong, who indicated that metabolic diseases stem from elevated TC, reduced beneficial HDL-C, and an increase in detrimental LDL-C. These factors contribute to dyslipidemia and arteriosclerosis, ultimately impairing blood sugar regulation and damaging β-cells [24]. Also, there was a significant difference in the results for TG between the three groups, where we noticed a significant increase in G3 and G2 as compared with G1. This is due to insufficient insulin secretion, which causes an increase in the liver's secretion of VLDL with a delay in removing lipoproteins rich in TG. This is mainly due to increasing the levels of substrates for the synthesis of TG [25].

Furthermore, it was observed that diabetic patients exhibit high IR, a primary contributor to dyslipidemia. This is consistent with the findings of Goldberg et al [26]. On the other hand, our results for HDL mg/dL showed a significant difference between the three groups: G1, G2, and G3, as it was proven that low HDL is an independent risk factor for chronic heart disease

due to its important role in reducing heart disease. The present findings showed a significant reduction in HDL for people with T2DM who have excessive obesity, as well as those who have a metabolic disorder, and this indicates a high risk of heart disease. Numerous studies have demonstrated that higher HDL levels can lower the risk of heart chronic disease thus contributing to prolonging life expectancy [27]. This study revealed a significant increase in LDL and VLDL in the G2 metabolic disorders group and G3 obesity group with T2DM compared to G1 control group. The findings indicate that high levels of LDL and VLDL are associated with heart diseases, particularly atherosclerosis, corroborating numerous studies. This is consistent with Murtadha et al., that people who are obese and have T2DM face a greater risk of developing CVD. Impaired clearance of plasma lipoprotein particles, LDL, has been proven crucial in arteriosclerosis and CVD [28]. The results AIP showed a significant increase in G2 metabolic disorders and G3 obesity and T2DM patients compared to the control group G1.

These values are regarded as a reliable biomarker for predicting DM, a contributing factor to metabolic disorders. To explain these results, there are biological mechanisms, such as an increase in the AIP leading to dyslipidemia, which is one of them. On the other hand, a study has confirmed that it is a calculated indicator that accurately predicts atherosclerosis and future atherosclerosis risk. Studies have confirmed that the AIP value may deteriorate further, especially in patients with T2DM and obesity, consistent with current results [29].

Table 2 presents the mean ± SE values of GSK3β and Arrestin β-1 With now a significant difference between G2 and G1, but there is a significant increase compared to G3.

**Table 2**:The GSK3β and Arrestin β-1 levels in patients and control groups.

| Groups / Parameters | Control G1 No. (46) | Metabolic disorders G2 No. (45) | T2DM with Obesity G3 No. (44) | P-value |
|---|---|---|---|---|
| **GSK3β (ng\mL)** | 3.53±0.06 [a] (3.47) | 3.20±0.05 [a] (3.1) | 16.16±0.62 [b] (16.5) | 0.001** |
| **Arrestin β-1 (pg\mL)** | 273.98±21.30 [a] (218.6) | 311.16± 20.23 [a] (244) | 520.75±27.25 [b] (543.75) | 0.001** |

Glycogen Synthase Kinase 3β (GSK3β), Arrestin β-1
a, b, c, Different letters indicate values which reliably differed one from another within one line of the table according to the results of comparison using the Tukey test with Bonferroni correction. (b) represents the difference between G3 and G2.(a) represents no difference between G1 and G2.

The results of GSK3β indicated that there was no significant difference between G1 and G2; however, G3 exhibited a significant increase. This is because it significantly regulates blood glucose levels and is involved in insulin deficiency and resistance [30]. These results are consistent with other studies which indicated that GSK 3β enzyme is elevated in patients with T2DM. The primary cause of T2DM is a defect in the function of β-cells in the pancreas, characterized by high IR and inadequate insulin secretion. This dysfunction may stem from a weakness in mitochondrial function, adversely impacting the β-cells. Deficiencies in either of its two mitochondrial functions impact the enzyme glycogen synthase [31], which plays a role in mitochondrial energy metabolism. Numerous studies indicate that the AKT/IRS-1 pathway is critical in pancreatic β-cell death. In T2DM patients. This leads to a defect and disruption of the GSK3β and AKT pathways and the inhibition of

its host mitochondria. Another study has indicated that high GSK3β is the leading cause of impaired glucose metabolism in patients with T2DM, fat accumulation, and inhibition of protein synthesis [32].

While Arrestin-β1 showed results a significant difference between the three groups, as we noticed a significant increase in G3 patients with T2DM and obesity compared to G2 patients with metabolic disorders.
Arrestinβ-1 is a crucial intracellular protein involved in regulating fundamental metabolic processes, including glucose homeostasis and energy balance. Increased serum levels of arrestinβ-1 indicate heightened IR and diminished β-cell function. This occurs due to enhanced insulin signalling, which promotes the degradation IRS-1, thereby underscoring IR. Arrestin-β-1 also works to desensitize the glucagon-like peptide 1 GLP-1 receptor in pancreatic cells, which enhances insulin secretion [33].

**Table 3:** The correlation coefficient between difference parameters and IR.

| The studied groups / Parameters | | Insulin resistance | | |
|---|---|---|---|---|
| | | **Control Group G1 No. (46)** | **Metabolic disorders Group G2 No. (45)** | **T2DM with Obesity Group G3 No. (44)** |
| **Age (years)** | r | -.042 | .021 | .035 |
| | P | .784 | .892 | .823 |
| **BMI (kg/m$^2$)** | r | -.205 | -.106 | -.057 |
| | P | .171 | .487 | .715 |
| **WHR** | r | -.155 | -.034 | -.260 |
| | P | .304 | .824 | .088 |
| **FBS (mg/dL)** | r | .082 | .805** | .965** |
| | P | .590 | .000 | .000 |
| **Cholesterol (mg/dL)** | r | -.373* | .136 | .621** |
| | P | .011 | .374 | .000 |
| **Triglyceride (mg/dL)** | r | .038 | .262 | .711** |
| | P | .801 | .082 | .000 |
| **HDL-C (mg/dL)** | r | .278 | -.056 | .559** |
| | P | .062 | .717 | .000 |
| **LDL-C (mg/dL)** | r | -.370* | .072 | .158 |
| | P | .011 | .637 | .306 |
| **VLDL-C (mg/dL)** | r | .060 | .266 | .711** |
| | P | .692 | .077 | .000 |
| **Insulin (uIU/mL)** | r | .859** | .795** | .901** |
| | P | .000 | .000 | .000 |
| **GSK3β (ng\mL)** | r | .008 | .262 | .312* |
| | P | .960 | .082 | .039 |
| **Arrestin β-1 (pg\mL)** | r | -.078 | -.243 | .714** |
| | P | .606 | .108 | .000 |

***Correlation is significant at the 0.05 level.**
****Correlation is significant at the 0.01 level.**

A weak positive correlation was identified in Group G3 between IR and GSK3β, along with IR and Arrestinβ-1, as shown in Table 3. IR plays a significant pathophysiologic role in T2DM and is also a risk factor for the development of CVD [34]. These results show that an increase in IR indicates and predicts an increase in the vital indicator GSK3β. Insulin activates GS due to its phosphorylation of GSK3β, which is involved in the activity of glycogen synthase, as this enzyme works to convert glucose into glycogen, which agrees with

a previous study. Insulin plays a vital role in facilitating the transport of glucose across the membrane to specific tissues, and this agrees with both previous studies, which showed that insulin stimulation has a strong effect on increasing protein expression in many pathways, most notably GSK3β, IR, and IRS-1 in middle-aged and elderly adults who suffer from obesity [35]. There is also a positive correlation between IR and Arrestinβ-1 in G3, which is due to the physiological role of Arrestinβ-1 within β-cells. This is consistent with previous data, which confirmed the role of Arrestinβ-1 in regulating insulin secretion and the proliferation of β-cells in the pancreas [36].

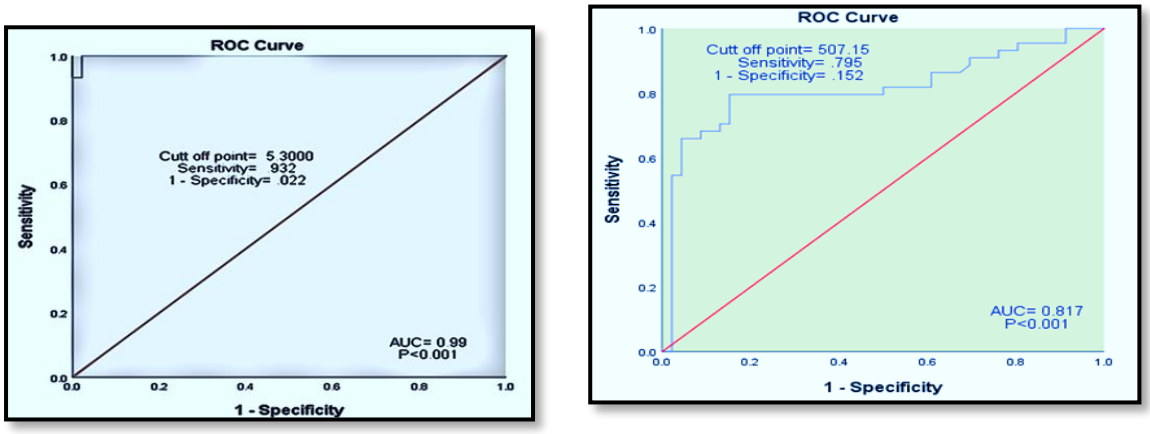
In contrast, a positive correlation has been noticed between IR and TG, FBG, TC, HDL-C, and VLDL in G3. The results elucidate mechanisms by which competition with glucose for cellular entry impairs glucose oxidation, leading to hypertriglyceridemia and potential IR. Moreover, elevated TG levels diminish both the quantity and quality of adipocyte insulin receptor activity. Additionally, one study suggests that elevated triglycerides may precede the onset of diabetes rather than solely result from it, as high TG levels were observed in individuals without diabetes, a finding that contrasts with previous research [37,38] .

Table 4 indicates that the area under the curve for the enzyme GSK3β is 0.999, which signifies an excellent result. Also, Arrestin β-1 gave a very good result =0.817. This suggests that the indicator is highly effective for both groups, G1 and G2.

**Table 4:** The ROC Curve analysis of Arrestin β-1and GSK3β of patients and control groups.

| Parameters | Area under the curve | Std.Error [a] | Asymptotic Sig. [b] | Asymptotic 95% Confidence Interval Lower Bound-Upper Bound | Sensitivity | Specificity |
|---|---|---|---|---|---|---|
| | | | | 0.995-1.0 | 0.932 | 0.022 |
| **GSK3β** | 0.999 | 0.002 | < 0.001 | 0.722-0.912 | 0.795 | 0.152 |
| **Arrestin β-1** | 0.817 | 0.049 | < 0.001 | | | |

**a. Under the nonparametric assumption**
**b. Null hypothesis: true area = 0.5**




a. GSK3β b. Arrestin β-1

**Figure1**: The ROC curve analysis of Arrestin β-1and GSK3β metabolic disorders.

Table 4 and Figure 1 represent a curve for GSK3β and Arrestin β-1 biomarkers to differentiate between metabolic disorders from controls. The result for GSK3β yielded an area under the curve of (0.999). This result is significant, demonstrating that GSK3β is an effective indicator for metabolic disorders. It directly contributes to glucose formation and is involved in glycogen synthase activity. Kinases serve as critical targets for diagnosing

metabolic diseases such as obesity, T2DM, and prediabetes. The findings of McManus et al. support these results, which indicate that hyperactivity of GSK3β results in defects in pancreatic islets associated with diabetes and in the subgenual area. The hypothalamus impairs glucose homeostasis, and thus targeting GSK3β provides a way to combat multiorgan dysfunction that leads to the development of diabetes and thus increases metabolic diseases [39]. Also, the ROC analysis of Arrestin β-1 gave a very good result =0.817, proving that Arrestin β-1 is a clear indicator to differentiate metabolic disorders from control. Arrestin β-1 is a plasma membrane that has a role in regulating the cell cycle and also joins many subsequent signals that play a role in ageing and also has a role in functions related to the G protein, which modifies metabolic responses such as insulin secretion and glucose balance. Functional studies of β-cells have shown that activation of G protein receptors can modulate B- cell signaling and protein phosphorylation, suggesting that G protein receptors in beta cells promise targets for developing antidiabetic treatments. In patients with T2DM, Arrestin-β1 plays a vital role in decreased homeostatic stability, which leads to less efficient metabolic processes, as indicated by van Gastel et al [40], who affirmed that it contributes to the regulation of different types of cells, including GSK3β, which is consistent with other studies, including Peterson et al [41].

## Conclusion

This study concludes that the GSK3β enzyme and Arrestin β-1 are reliable biomarkers for predicting metabolic disorders, owing to their fundamental roles in metabolic disorders pathways. Cellular functions, particularly in pancreatic β- cells, are regulated by inhibiting signals through G protein-associated receptors. Defects in this signaling pathway result in β-cell damage, contributing to IR and impairing the production of GSK3β, an enzyme crucial for glucose organization and synthesis. These results in a defect in glucose production, causing an increase in blood sugar levels and contributing to metabolic disorders syndrome.

## References

**[1]** F. Ahsan, "Metabolic Syndrome: Mini Review," *JSM Human Nutrition & Food Science*, vol. 5, no. 6, pp. 1–5, 2022. DOI: 10.21123/bsj.2022.19.2.0340.

**[2]** H. A. Riyam and O. F. Layla, "Studying Myostatin as a Diagnostic Indicator in Sera of Iraqi Women with Metabolic Syndrome," *Baghdad Science Journal*, vol. 21, no. 5, pp. 1609–1616, 2024. DOI: 10.21123/bsj.2023.8180.

**[3]** C. Hooper, R. Killick, and S. Lovestone, "The GSK3 Hypothesis of Alzheimer's Disease," *Journal of Neurochemistry*, vol. 104, pp. 1433–1439, 2020. DOI: 10.3390/nu12041032.

**[4]** D. E. James, J. Stöckli, and M. J. Birnbaum, "The Aetiology and Molecular Landscape of Insulin Resistance," *Nature Reviews Molecular Cell Biology*, vol. 22, pp. 751–771, 2021. DOI: 10.1038/s41467-023-36549-2.

**[5]** S. J. Humphrey, D. E. James, and M. Mann, "Protein Phosphorylation: A Major Switch Mechanism for Metabolic Regulation," *Trends in Endocrinology & Metabolism*, vol. 26, pp. 676–687, 2015. DOI: 10.1038/s41467-023-36549-2.

**[6]** D. Einhorn, G. M. Reaven, R. H. Cobin, E. Ford, O. P. Ganda, and Y. Handelsman, "American College of Endocrinology Position Statement on the Insulin Resistance Syndrome," *Endocrine Practice*, vol. 9, no. 3, pp. 237–252, 2024. DOI: 10.1101/2024.09.06.24313199.

**[7]** S. E. Nikoulina, T. P. Ciaraldi, S. Mudaliar, P. Mohideen, L. Carter, and R. R. Henry, "Potential Role of Glycogen Synthase Kinase-3 in Skeletal Muscle Insulin Resistance of Type 2 Diabetes," *Diabetes*, vol. 49, pp. 263–271, 2021. DOI: 10.3390/cells10123490.

**[8]** E. Beurel, S. F. Grieco, and R. S. Jope, "Glycogen Synthase Kinase-3 (GSK3): Regulation, Actions, and Diseases," *Pharmacology & Therapeutics*, vol. 148, pp. 114–131, 2024. DOI: 10.1126/sciadv.abm8786.

**[9]** V. V. Gurevich and E. V. Gurevich, "Biased GPCR Signaling: Possible Mechanisms and Inherent Limitations," *Pharmacology & Therapeutics*, vol. 211, p. 107540, 2020. DOI: 10.1124/pharmrev.121.000302.

**[10]** L. N. Zhuang, W. X. Hu, M. L. Zhang, S. M. Xin, W. P. Jia, and J. Zhao, "Beta-Arrestin-1 Protein Represses Diet-Induced Obesity," *Journal of Biological Chemistry*, vol. 286, no. 32, pp. 28396–28402, 2023. DOI: 10.1124/pharmrev.121.000302.

**[11]** D. Y. Oh and J. M. Olefsky, "G Protein-Coupled Receptors as Targets for Anti-Diabetic Therapeutics," *Nature Reviews Drug Discovery*, vol. 15, no. 3, pp. 161–172, 2023. DOI: 10.1124/pharmrev.121.000302.

**[12]** A. M. Nabaa and K. M. Fayhaa, "Studying the Role of Heme Oxygenase-1 in Obese Patients," *Baghdad Science Journal*, vol. 21, no. 7, pp. 2246–2254, 2024. DOI: 10.21123/bsj.2023.8406.

**[13]** A. H. Abedi, S. Khosravi, M. Hassabi, M. Poorsaid, B. Hassanmirzaei, and A. Asgari, "Comparing the Accuracy of Waist-Hip Ratio Calculation by the BIA Device versus the Manual Method," *Ann Appl Sport Sci*, vol. 4, no. 2, pp. 9–15, 2016. [Online]. Available: http://aassjournal.com/article-1-289-en.html

**[14]** M. Dobiásová and J. Frohlich, "The Plasma Parameter Log (TG/HDL-C) as an Atherogenic Index: Correlation with Lipoprotein Particle Size and Esterification Rate in ApoB-Lipoprotein-Depleted Plasma (FER(HDL))," *Clinical Biochemistry*, vol. 34, no. 7, pp. 583–588, 2023. DOI: 10.1186/s12933-023-01886-5.

**[15]** O. F. Layla, A. A. Baydaa, and S. D. Ashgan, "Comparison Study between Adipsin Levels in Sera of Iraqi Patients with Diabetes and Neuropathy," *B S J*, vol. 20, no. 3, pp. 726–733, 2023.

**[16]** P. Björntorp, "Abdominal Fat Distribution and the Metabolic Syndrome," *Journal of Cardiovascular Pharmacology*, vol. 20, suppl. 8, pp. S26–S28, 2024. DOI: 10.1002/oby.24070.

**[17]** J. M. Chan, E. B. Rimm, G. A. Colditz, M. J. Stampfer, and W. C. Willett, "Obesity, Fat Distribution, and Weight Gain as Risk Factors for Clinical Diabetes in Men," *Diabetes Care*, vol. 17, pp. 961–969, 2022. DOI: 10.2337/diacare.17.9.961.

**[18]** I. Satman, B. Omer, and Y. Tutuncu, "Twelve-Year Trends in the Prevalence and Risk Factors of Diabetes and Prediabetes in Turkish Adults," *European Journal of Epidemiology*, vol. 28, pp. 169–180, 2022. DOI: 10.1007/s10654-013-9771-5.

**[19]** A. Shatha, S. Refif, and H. Rusul, "Variants of MMP-9 and TIMP-1 Levels Could be a Predictor of an Early Development of Cardiovascular Diseases in Type 2 Diabetes among Iraqi Patients," *Iraqi Journal of Science*, vol. 56, no. 1C, pp. 622–632, 2015. [Online]. Available: https://orcid.org/0000-0003-1629-3488

**[20]** D. Shungin, T. W. Winkler, and D. C. Croteau-Chonka, "New Genetic Loci Link Adipose and Insulin Biology to Body Fat Distribution," *Nature*, vol. 518, no. 7538, pp. 187–196, 2022. DOI: 10.1210/clinem/dgac140.

**[21]** A. Tchernof and J. P. Després, "Pathophysiology of Human Visceral Obesity: An Update," *Physiological Reviews*, vol. 93, no. 1, pp. 359–404, 2021. DOI: 10.1155/2021/881243.

**[22]** P. A. Kern, S. Ranganathan, C. Li, L. Wood, and G. Ranganathan, "Adipose Tissue Tumor Necrosis Factor and Interleukin-6 Expression in Human Obesity and Insulin Resistance," *American Journal of Physiology. Endocrinology and Metabolism*, vol. 280, no. 5, pp. E745–E751, 2021. DOI: 10.1155/2021/8812431.

**[23]** B. Nanda, R. K. S. Mahapatra, N. Devi, S. Swain, R. Padhy, and R. Rattanet, "Study of Serum Omentin-1 in Relation to Insulin Resistance in Type II Diabetes Mellitus," *Journal of Dental and Medical Sciences*, vol. 14, no. 12, pp. 12–21, 2023. DOI: 10.21123/bsj.2022.6535.

**[24]** F. N. Wong, K. H. Chua, J. Tan, C. M. Wong, and U. R. Kuppusamy, "Glycaemic Control in Type 2 Diabetic Patients with Chronic Kidney Disease: The Impacts on Enzymatic Antioxidants and Soluble RAGE," *PeerJ*, vol. 6, p. e4421, 2020. DOI: 10.1186/s12944-020-01303-w.

**[25]** I. J. Goldberg, "Lipoprotein Lipase and Lipolysis: Central Roles in Lipoprotein Metabolism and Atherogenesis," *Journal of Lipid Research*, vol. 37, pp. 693–707, 2019. DOI: 10.2147/DMSO.S222271.

**[26]** M. Cao, Z. W. Zhou, B. J. Fang, C. G. Zhao, and D. Zhou, "Meta-Analysis of Cholesteryl Ester Transfer Protein TaqIB Polymorphism and Risk of Myocardial Infarction," *Medicine*, vol. 93, no. 26, p. e160, 2019. DOI: 10.21123/bsj.2019.16.3(Suppl.).0747.

**[27]** H. A. Murtadha and Z. H. Saba, "Evaluation of Oxidative Stress in Iraqi Male Patients with Myocardial Infarction and Type 2 Diabetes Mellitus," *Iraqi Journal of Science*, vol. 64, no. 10, pp. 4918–4929, 2023.

**[28]** E. Al Shawaf, E. Al-Ozairi, F. Al-Asfar, A. Mohammad, S. Beloushi, and S. Devarajan, "Atherogenic Index of Plasma (AIP): A Tool to Assess Changes in Cardiovascular Disease Risk Post Laparoscopic Sleeve Gastrectomy," *Journal of Diabetes Research*, vol. 2020, pp. 1–9, 2020. DOI: 10.21123/bsj.2022.6535.

**[29]** J. E. Gleason, E. A. Szyleyko, and D. M. Eisenmann, "Multiple Redundant Wnt Signaling Components Function in Two Processes During C. elegans Vulval Development," *Developmental Biology*, vol. 298, pp. 442–457, 2020. DOI: 10.3390/nu12041032.

**[30]** S. K. Marafie, R. M. Al-Shawaf, J. Abubaker, and H. Arefanian, "Palmitic Acid-Induced Lipotoxicity Promotes a Novel Interplay Between Akt-Mtor, IRS-1, and FFAR1 Signaling in Pancreatic Beta-Cells," *Biology Research*, vol. 52, p. 44, 2021. DOI: 10.1007/s10565-021-09644-7.

**[31]** I. Usui, T. Imamura, J. Huang, H. Satoh, S. K. Shenoy, R. J. Lefkowitz, C. J. Hupfeld, and J. M. Olefsky, "β-Arrestin-1 Competitively Inhibits Insulin-Induced Ubiquitination and Degradation of Insulin Receptor Substrate 1," *Molecular and Cellular Biology*, vol. 24, pp. 8929–8937, 2022. DOI: 10.3390/jpm12010023.

**[32]** M. M. Mariappan, M. Shetty, K. Sataranatarajan, G. G. Choudhury, and B. S. Kasinath, "Glycogen Synthase Kinase-3β is a Novel Regulator of High Glucose- and High Insulin-Induced Extracellular Matrix Protein Synthesis in Renal Proximal Tubular Epithelial Cells," *Journal of Biological Chemistry*, vol. 283, no. 45, pp. 30566–30575, 2024. DOI: 10.1111/1753-0407.13470.

**[33]** T. P. Ciaraldi, L. Carter, S. Mudaliar, and R. R. Henry, "GSK-3β and Control of Glucose Metabolism and Insulin Action in Human Skeletal Muscle," *Molecular and Cellular Endocrinology*, vol. 315, no. 1–2, pp. 153–158, 2023. DOI: 10.1016/j.bbadis.2023.166726.

**[34]** K. R. Short, J. L. Vittone, and M. L. Bigelow, "Effect of Supervised Progressive Resistance-Exercise Training Protocol on Insulin Sensitivity, Glycemia, Lipids, and Body Composition in Asian Indians with Type 2 Diabetes," *Diabetes*, vol. 67, pp. 179–183, 2019. DOI: 10.1016/j.rehab.2018.11.001.

**[35]** G. Iaccarino, D. Franco, S. Sorriento, T. Strisciuglio, E. Barbato, and C. Morisco, "Modulation of Insulin Sensitivity by Exercise Training: Implications for Cardiovascular Prevention," *Journal of Cardiovascular Translational Research*, vol. 14, pp. 256–270, 2021. DOI: 10.3390/cells10123490.

**[36]** M. Thomsen, A. Varbo, A. Tybjærg-Hansen, and B. G. Nordestgaard, "Low Nonfasting Triglycerides and Reduced All-Cause Mortality: A Mendelian Randomization Study," *Clinical Chemistry*, vol. 60, no. 5, pp. 737–746, 2021. DOI: 10.1373/clinchem.2013.219881.

**[37]** M. Arca, C. Veronesi, L. D'Erasmo, C. Borghi, F. Colivicchi, G. M. De Ferrari, "Association of Hypertriglyceridemia with All-Cause Mortality and Atherosclerotic Cardiovascular Events in a Low-Risk Italian Population: The TGREAL Retrospective Cohort Analysis," *Journal of the American Heart Association*, vol. 9, no. 19, p. e015801, 2020. DOI: 10.1161/JAHA.119.015801.

**[38]** S. Tesfaye, N. Chaturvedi, S. E. Eaton, J. D. Ward, C. Manes, C. Ionescu-Tirgoviste, "Vascular Risk Factors and Diabetic Neuropathy," *The New England Journal of Medicine*, vol. 352, no. 4, pp. 341–350, 2020. DOI: 10.21123/bsj.2023.7566.

**[39]** E. J. McManus, "Role that Phosphorylation of GSK3 Plays in Insulin and Wnt Signalling Defined by Knock-In Analysis," *EMBO Journal*, vol. 24, pp. 1571–1583, 2023. DOI: 10.1038/s41467-023-36549-2.

**[40]** J. van Gastel, J. O. Hendrickx, H. Leysen, P. Santos-Otte, L. M. Luttrell, B. Martin, and S. Maudsley, "Arrestin-Based Receptor Signaling Paradigms: Potential Therapeutic Targets for Complex Age-Related Disorders," *Frontiers in Pharmacology*, vol. 9, p. 1369, 2022. DOI: 10.3390/jpm12010023.

**[41]** Y. K. Peterson and L. M. Luttrell, "The Diverse Roles of Arrestin Scaffolds in G Protein-Coupled Receptor Signaling," *Pharmacological Reviews*, vol. 69, pp. 256–297, 2021. DOI: 10.1038/s41467-021-23656-1.