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Impact of Metformin on FBXW7 Protein and Total Fatty Acids in Diabetes Individuals

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Abstract: Patients with type 2 diabetes who are receiving metformin treatment should be closely monitored, particularly for progression, changes in metabolic parameters, and prediabetes. Our study aimed to evaluate FBXW7 protein levels and total fatty acids in type 2 diabetes patients receiving and not receiving metformin. One hundred volunteers participated in this study, divided into three groups: group 1: 30 untreated patients; group 2: 42 patients receiving metformin; and group 3: 28 controls. The protein FBXW7 and Total fatty acids were measured using an enzyme-linked immunosorbent assay. Lipid profiles and fasting serum glucose levels were also determined using fully automated methods for all participants. A fully automated method was also used to calculate glycated hemoglobin. After examining the effect of metformin on FBXW7 protein and total fatty acids in diabetic patients, the current study indicate that metformin significantly reduced FBXW7 levels in this group. Serum TFA levels also decreased after metformin treatment. The results demonstrate that metformin has a specific regulatory effect on FBXW7 and total fatty acids, which promotes diabetes management and fatty acid oxidation in diabetic patients. The effect of metformin on the expression of metabolic markers and FBXW7 protein in diabetic patients was significant. In conclusion, these findings highlight the importance of metformin and its effects on FBXW7 and total fatty acids in the diagnosis and progression of diabetes.

Keywords: FBXW7 protein, total fatty acids, type 2 diabetes, metformin

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Introduction

Type 2 diabetes (T2D) is a progressive metabolic disorder caused by insulin resistance in various cells in the body and peripheral tissues(1). It usually affects adults (2). Type 2 diabetes is multifactorial and may develop through lifestyle habits, adverse environmental factors, or genetic susceptibility. (3) Prolonged administration of Metformin can enhance the body's metabolism and facilitate effective fat utilization. The F-box protein is implicated in the onset and progression of diabetes-associated inflammation (4,5). Diabetes is often associated with lipid metabolic abnormalities that diminish insulin sensitivity and impair its efficacy. Inadequate insulin results in hyperglycemia and numerous disorders in glucose and lipid metabolic pathways. The concept of the "lipid triad" has been introduced. This denotes an elevation in circulation free fatty acids alongside hypertriglyceridemia and diminished high-density lipoprotein cholesterol, which is indicative of predominantly T2DM Therapies for

concurrent diabetes and dyslipidemia can be employed that possess both glucose metabolism and lipid-regulating properties. Metformin, an oral antidiabetic medication, can significantly decrease body weight and provide beneficial metabolic effects in the management of T2D (6,7). Metformin primarily lowers glucose levels by blocking hepatic production. Metformin can also diminish hyperglycemia by enhancing glucose use in insulin-sensitive tissues, including muscle cells and adipocytes. Besides its direct regulation of glucose metabolism, new clinical and experimental studies have revealed that metformin also positively influences body weight, lipid metabolism, and cardiovascular disease. Metformin can be regarded as an optimal oral hypoglycemic agent for managing type 2 diabetes mellitus, as it does not induce hypoinsulinemia, presents a minimal risk of hyperglycemia, and often exhibits mild side effects. metformin is extensively utilized in clinical settings; nevertheless, the molecular mechanisms governing body weight and lipid metabolism remain unclear(8). The FBXW7 gene encodes the F-box W7 protein, alternatively referred to as F-box protein WD1001.Also,FBXW7 is part of the F-box protein family, which participates in proteasome-mediated protein degradation regulated by ubiquitination. The FBXW7 comprises a diverse array of regulatory proteins that play direct roles in proliferation, metabolism, inflammation, differentiation, and the maintenance of stem cell status. So, the FBXW7 gene clearly functions as a tumor-suppressor gene in the development of follicular carcinomas. Mutations identified in the initial two exons of the gene are correlated with the onset of poorly differentiated thyroid carcinoma accompanied by lymph node metastases. In low colorectal carcinomas, cancer arose concurrently due to the activation of cell proliferation via the mTOR pathway, the development of active AKT, a crucial component among BAD cell death inhibitors, and cyclin E steroid receptors, which are also activated by the Notch ligand. The substrate is neogenin, which promotes the elongation of axons and dendrites. The FBXW7 molecule can bind the F-box protein to cyclin E, specifically to the phosphorylation-free secondary loop of its T-stage. It can modulate protein levels independently of phosphorylation. This information is particularly significant about the progression of pre-malignant hormone-dependent breast lesions, because cyclin E levels were elevated in regions absent of T-stage induction. It constitutes an anomaly to the regulations when nearly all eukaryotic cells undergo significant transformations during switching from the pseudo-G1 phase to mitosis, likely influenced by CDK2 activity. (9, 10). Metformin is an important hypoglycemic medication. (11,12). Metformin may additionally produce a lipid-lowering impact. The mechanisms underlying the lipid-lowering effect encompass enhancing insulin sensitivity, suppressing de novo lipogenesis in the liver, facilitating the utilization of glucose and fatty acids for energy production, and modulating the expression of various enzymes, including the upregulation of aminoacyl-tRNA synthetase complex-interacting multifunctional protein type 2 and the inhibition of sterol regulatory element-binding protein-1 gene expression. Additional research indicates that metformin can enhance the expression of certain microRNAs via AMP-activated protein kinase

signaling pathways, which expedite the degradation of specific mRNAs and suppress the expression of critical genes involved in fatty acid synthesis, including sterol regulatory element-binding protein-1, fatty acid binding protein 3, and 3-hydroxy-3-methylglutaryl-CoA reductase, thereby further reducing the levels of total fatty acids and cholesterol in the liver (13,14,15). This study aims to elucidate the role of Metformin in the onset and progression of diabetes by assessing the alterations in FBXW7 and total fatty acids in diabetic patients following Metformin medication.

Methods

In this study, 100 individuals aged 30-60 years were selected to provide a serum sample. With a BMI ranging from 25.1-30.4 to all participants.

They were divided into three groups:

G1: 30 patients T2D without treatment and G2: 42 patients T2D with metformin treatment and G3: 28 control in the first group; and

The patients were divided with the help of the Diabetes and Endocrinology Department at Baghdad Hospital - Medical City into three groups based mainly on the results of the HbA1c test and after examining the symptoms theoretically by the specialist: the first group was diabetic patients without treatment, while the second group was diabetic patients with metformin use. In addition to the control group with normal levels of HbA1c.

Individuals with hypertension, kidney disease, liver disease, heart disease and smoking were not included. Participants provided informed consent, and the study received approval from both the hospital and the university.

Criteria for Inclusion

Result and Discussion

The statistical ANOVA test was employed to compare the three groups:

Individuals aged 30 to 60 years with type 2 diabetes, patients without treatment, and a control group.

Criteria for exclusion

Patients with chronic diseases, including hypertension, heart, liver, kidney, bone, tumors, stomach and intestines, patients taking medications that affect the results other than metformin, metabolic disorders, type 1 diabetes, smokers will be excluded.

Collection of blood samples and laboratory analysis

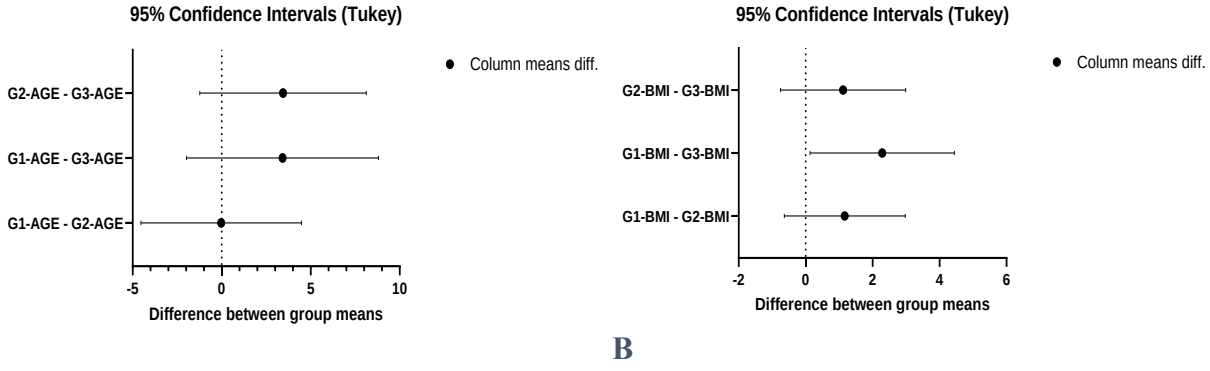
Ten milliliters of venous blood were drawn from the participants. Aliquots of the blood samples were separated. Whole blood was used to measure HbA1c. After five milliliters of blood were allowed to clot, serum was separated using centrifugation at 5000 rpm for fifteen minutes at room temperature. They separated the serum into vials. Lipid profile and fasting blood glucose (the sample obtained without a tourniquet) were measured using spectrophotometry on the same day using a portion of the extracted serum. In order to distinguish the remaining blood, it was divided into aliquots in labeled storage vials and then frozen at −20°C for further analysis of FBXW7 protein and total fatty acids, which were measured using enzyme-linked immunosorbent assay (ELISA).

Anthropometric assessments

Participants' height and weight were measured while standing and attired in loose clothing. Upon conducting the measurement twice, an average was computed. Body Mass Index, (BMI) is calculated by dividing weight (in kilograms) by height squared (in meters).

G1: 30 patients T2D without treatment and G2: 42 patients T2D with metformin treatment and G3: 28 control

. The analysis revealed that there was no statistically significant difference in age across the groups, indicating that age was well-matched among the participants as shown in Figure 1, A significant difference in body mass index (BMI) was noted between G1 and G3 as shown in figure 1b



A B

Figure.(1) The Confidence Intervals (95%) and the Variation in Group Means for A: Age, B: BMI

Kruskal-Wallis test have been used as a nonparametric test for analysis of results among the three groups. The results showed no significant deference in the mean ranks of the data as shown in Table 1. In many cases, the Kruskal - Wallis H test is the preferred method for comparing more than one independent sample. (16)

Table (1): Kruskal-Wallis test of FBXW7 protein, and TFA in all studied groups.

Parameters		GI	G2	G3
FBXW7 protein ng/ml	Mean ranks	51.8	41.7	42.9
	Median	2.96	2.94	2.93
	P value 0.29 Kruskal-Wallis statistic 2.43			
TFA umol/l	Mean ranks	55.7	61.4	72.5
	Median	15.2	16.3	18.9
	P Value 0.18 Kruskal-Wallis statistic 3.33			

***For groups and presenting them with the symbols below:**
G1: diabetics without treatment
G2: diabetics taking metformin
G3: control group

The present results in (Table 1) show that metformin significantly reduces the levels of FBXW7 protein and total fatty acids in diabetic patients after analyzing its effect on them.

- Effects of metformin on total fatty acids

In our study, we observed a decrease in the levels of total fatty acids in diabetic patients taking metformin in the second group. Metformin led to the removal of fatty acid desaturation enzymes by reducing insulin sensitivity, increasing fatty acid oxidation, changing their composition and profiles, and significantly reducing their activity in diabetic patients compared with diabetic patients who do not take any treatment and the control group (17). Metformin is a first-line pharmacological intervention to control blood sugar and reduce insulin resistance (18).

- Effects of metformin on FBXW7 protein

When compared to the earlier study on type 2 diabetes, Suhayla K. Mohammed's study revealed that FBXW7 protein was closely linked to G1 (newly diagnosed patients). They

also proposed that serum FBXW7 protein could be used as an early diagnostic test in diabetic patients, and that metformin

significantly lowers the level of FBXW7. This protein was selected as a marker for the current study. The mechanism by which FBW-7 inhibits EZH2 in type 1 diabetes was elucidated in the first investigation of FBXW-7 protein in type 1 diabetes in 2021, suggesting that FBW-7 is a promising target for type 1 diabetes treatment (19, 20) .Our study results also showed that metformin has a certain regulatory effect on FBXW7 protein, which helps regulate blood sugar. Metformin had a significant effect on FBXW7 protein expression and metabolic parameters, which were significantly decreased in type 2 diabetes patients taking metformin. While the first group of type 2 diabetes patients who did not take the treatment showed higher levels of both FBXW7 protein and total fatty acids than the other groups.

In nodular rats, overexpression of FBW-7 prevents beta cell death in islets and pro-inflammatory cytosine release in splenocytes. (21)

Table (2): Kruskal-Wallis test of TG, Cholesterol, HDL, LDL, VLDL, FBS, and HbA1c in all studied groups.

Parameters		GI	G2	G3
TG	Mean ranks	56.8	71.6	47.6
	Median	152	182*	117
	P Value 0.007 Kruskal-Wallis statistic 9.7			
Cholesterol	Mean ranks	176	195	178
	Median	60.4	64.0	61.0
	P Value 0.78 Kruskal-Wallis statistic 2.67			
HDL	Mean ranks	54.4	66.9	60.9
	Median	19.5	23	23.9
	P Value 0.26 Kruskal-Wallis statistic 2.6			
LDL	Mean ranks	65.35	58.94	67.61
	Median	129.6	122.5	131.9
	P Value 0.4967 Kruskal-Wallis statistic 1.3			
VLDL	Mean ranks	56.82	71.62	47.63
	Median	30.40	36.54	23.41
	P Value 0.0076 Kruskal-Wallis statistic 9.751			
FBS	Mean ranks	39.19	87.03	31.36
	Median	94.80	179.7	89.45
	P Value <0.0001 Kruskal-Wallis statistic 64.34			
HbA1c	Mean ranks	44.00	92.00	14.50
	Median	6.000	8.500	5.350
	P Value <0.0001 Kruskal-Wallis statistic 102.0			

***For groups and presenting them with the symbols below:**
G1: diabetics without treatment
G2: diabetics taking metformin
G3: control group

Some interesting findings were observed from the comparison of several metabolic parameters between diabetic individuals in the experimental and control groups. About Very low-density lipoprotein (VLDL), significant differences were observed (Table 2). VLDL levels differed significantly in

the diabetic and control groups. A typical feature of diabetes is dysregulation of lipid metabolism, which may be the reason for the elevated VLDL levels in diabetic groups. Another reason for this increase may be due to decreased clearance of VLDL particles by diabetic individuals and increased hepatic synthesis, these results are consistent with several recent studies (22,23). Hemoglobin A1c (HbA1c) and fasting blood glucose (FBG) levels differed significantly across the groups studied (Table 2). These markers are key indicators of glycemic control. Poor glucose control and chronic hyperglycemia, a hallmark of diabetes, are reflected in the elevated FBG and HbA1c levels in the diabetic group. Diabetes can be diagnosed in the laboratory by measuring HbA1c only, which is a validated method (24). There were slight differences between the diabetic and control groups in terms of total cholesterol, HDL, and LDL values.

This means that diabetes may not always significantly alter these lipid profiles, even if it has a significant impact on glucose metabolism. Since some people may not show the usual symptoms of dyslipidemia, this also underscores the complexity of lipid metabolism in diabetes.

Since it is formed in diabetic patients and is closely related to the exposure of red blood cells to blood glucose concentrations while in the blood, causing sugar to bind to the hemoglobin molecule, the HbA1c test was used as the baseline test in this study to stratify the research groups. Since the HbA1c value cannot be replaced in the treatment of people with Type 1 Diabetes and Type 2 Diabetes , it is the best test for predicting diabetes(25 ,26).

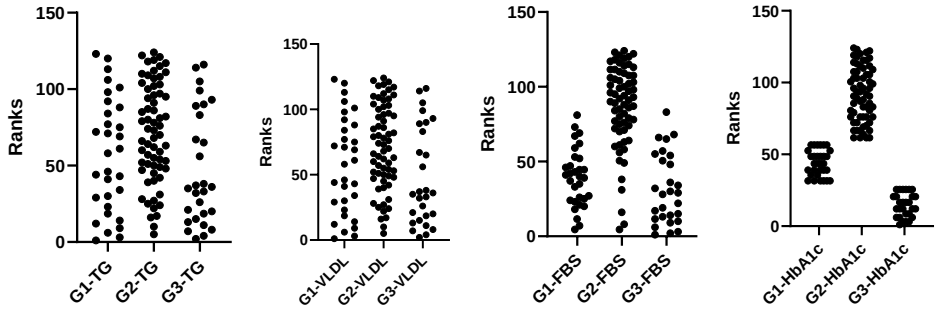


Figure (2): Kruskal-Wallis statistic test for variables TG, VLDL, FBS, and HbA1c.

Conclusion

compared to the non-medication diabetic group and the control group, the T2DM group on metformin had reduced TFA levels in addition to lower FBXW7 protein levels. The data' notable decline highlights the possible significance of TFA and FBXW7 protein as biomarkers for diabetes and its development. Furthermore, TFA has been found to be a crucial indicator of diabetes, offering important information for future studies or medical uses.

Ethical approval

The studies were carried out in accordance with the ethical standards outlined in the 1964 Helsinki Declaration and its subsequent revisions, as well as comparable ethical standards, as well as the ministry of Iraqi health protocols. The studies were approved by the Committee of the University of Baghdad College of Science for Women. Every individual participant participating in the study gave informed consent.

Adherence to ethics the writers declare they have no conflicting interests.

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