Orginal Article Open Access
Iranian Journal of Diabetes and Obesity (IJDO)

Simultaneous Metformin and Sitagliptin Effect on Proteins Content Involved in Insulin Resistance in Human Adipose Tissue of Type 2 Diabetes: A Clinical Trial

Reza Didehdar[1,2*], Yousof Naghiaee[3], Javad Mohiti-Ardekani[3], Naeimeh Heiranizadeh[4], Masaoud Rahmanian[5]

[1]Department of Biochemistry and Molecular Biology, Faculty of Medicine, International Campus of Shahid Saduoghi University of Medical Sciences, Yazd, Iran.
[2]Department of Biochemistry, Faculty of Medicine, Zabol University of Medical Sciences, Zabol, Iran.
[3]Department of Biochemistry and Molecular Biology, Faculty of Medicine, Shahid Saduoghi University of Medical Sciences, Yazd, Iran.
[4]Department of General Surgery, Faculty of Medicine, Shahid Saduoghi University of Medical Sciences, Yazd, Iran.
[5]Department of Internal Medicine, Diabetes Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

Abstract

Objective: Obesity ultimately results in a variety of metabolic changes, the most significant of which is insulin resistance, which is a contributing factor to both type 2 diabetes and permanent insulin resistance? The purpose of this study was to determine whether these two medications break down insulin resistance concurrently and, if so, what modifications are made to the levels of the targeted proteins in the insulin signaling pathway in type 2 diabetic adipose tissue in humans.

Materials and Methods: A clinical trial is described in this article. We used (sodium dodecyl sulfate poly-acrylamide gel electrophoresis (SDSPAGE) and western blot analyses to identify the presence of insulin resistance-related proteins Insulin Receptor Substrate-1 (IRS-1), phosphatidylinositol 3 kinase (PI3K), and Mammalian Target of Rapamycin (mTOR) in the adipose tissue of type 2 diabetic patients. We looked at six individuals who were receiving simultaneous treatment with metformin and sitagliptin for three months, four of whom returned after treatment.

Results: Increases in glucose disposal, decreases in serum glucose levels ($P< 0.05$), decreased insulin resistance ($P< 0.05$), and changes in serum insulin levels were seen after concurrent therapy with metformin and sitagliptin. Conversely, an increase in the proteins implicated in insulin resistance, including PI3K, mTOR, and IRS-1, was noted in the adipose tissue of diabetic individuals ($P< 0.05$).

Conclusion: The insulin resistance-related proteins IRS-1, PI3K, and mTOR in type 2 diabetic adipose tissue were markedly improved by concurrent metformin and sitagliptin treatment.

Keywords: Insulin Resistance, Metformin, Sitagliptin, Diabetes, Glucose transporter 4

QR Code:

Citation: Didehdar R, Naghiaee Y, Mohiti-Ardekani J, Heiranizadeh N, Rahmanian M. Simultaneous Metformin and Sitagliptin Effect on Proteins Content Involved in Insulin Resistance in Human Adipose Tissue of Type 2 Diabetes: A Clinical Trial. IJDO 2025; 17 (3) :175-181

URL: http://ijdo.ssu.ac.ir/article-1-966-en.html

10.18502/ijdo.v17i3.19266

Article info:

Received: 13 February 2025

Accepted: 29 May 2025

Published in July 2025

Corresponding Author:

Reza Didehdar, Department of Biochemistry and Molecular Biology, Faculty of Medicine, International Campus of Shahid Saduoghi University of Medical Sciences, Yazd, Iran.

Tel: (98) 912 451 2859

Email: rezadid123@gmail.com

Orcid ID: 0000-0002-6283-1657

[DOI: 10.18502/ijdo.v17i3.19266]

Introduction

Obesity eventually results in a variety of metabolic changes, the most significant of which is insulin resistance, which is an obstacle to both Non-Insulin Dependent Diabetes Mellitus (NIDDM) and permanent insulin resistance. By identifying the proteins that regulate this system, pharmaceutical options may be able to address the altered mechanism of the insulin signaling pathway to intra-cells, particularly adipose tissue cells, which occurs in insulin resistance (1). Insulin activates its intracellular domain by linking itself to its receptor (IR) in the adipocyte membrane and modifying the spatial arrangement within this receptor. IR then triggers Insulin Receptor Substrate-1 (IRS-1) in the cytoplasm of these cells, which in turn triggers Phosphatidylinositol 3 Kinase (PI3K), which in turn triggers protein kinase B (Akt), which in turn triggers Mammalian Target of Rapamycin (mTOR). Glucose Transporter 4 (GLUT4) is brought to the membrane surface of muscle cells and adipocytes by the mTOR, which also increases its activity (2). In this instance, these cells take in more glucose and use it up. Patients with NIDDM have disruptions in this route and the way glucose enters these cells (3). The most prevalent kind of NIDDM which accounts for 90-95% of cases typically starts with insulin insensitivity, a disorder in which the body's muscles, liver, and adipose tissue do not react effectively to insulin (4). More than 40 years ago, Glycated Hemoglobin (HbA1c) was first discovered to be an "unusual" hemoglobin in those with diabetes. The hemoglobin in your red blood cells binds to glucose as it accumulates in your blood. The average plasma glucose during the preceding eight to twelve weeks is reflected in the HbA1c. Because of these characteristics, it is the recommended test for determining glycemic control in diabetics (5).

More than 40 years ago, HbA1c was first discovered to be an "unusual" hemoglobin in diabetic individuals. The hemoglobin in your red blood cells binds to glucose as it accumulates in your blood. The average plasma glucose during the preceding eight to twelve weeks is reflected in the HbA1c. Because of these characteristics, it is the test of choice for determining glycemic control in diabetics (5). The treatment of type 2 diabetic patients can be aided by a single medication, metformin, plus lifestyle modifications, weight control, physical activity and exercise, and training, according to the American Diabetes Association's therapeutic approaches (6). Patients with NIDDM are frequently treated with two medications: sitagliptin and metformin. The primary way that the biguanide metformin affects liver cells is by lowering gluconeogenesis, or the manufacture of glucose. Metformin: A) makes cells more sensitive to insulin; B) makes insulin receptors appear on the cell membrane surface; C) makes these receptors more expressed; D) makes insulin bind to its receptors, which lowers insulin resistance in people with NIDDM; and E) causes cells to uptake glucose via GLUT4. A novel medication called sitagliptin inhibits the Dipeptidyl Peptidase 4 (DPP4) enzyme. This enzyme, which is located in the pancreatic Langerhans islets, breaks down two substances known as Glucagon-like peptide-1 (GLP-1) and Glucose-dependent Insulin-tropic Peptides (GIP). GLP-1 and GIP boost insulin synthesis and secretion. When sitagliptin inhibits the DPP4 enzyme, more insulin is produced and secreted, and the breakdown of GLP-1 and GIP molecules takes longer (6).

There are few and infrequent human research on the insulin signaling system and the mechanism underlying these medications' effects in adipose tissue. Thus, the current study aims to demonstrate how two medications, sitagliptin and metformin, affect insulin resistance in human adipose tissue. It also uses laboratory techniques to identify issues with the insulin signaling pathway in adipose tissue in order to enhance it. In order

[DOI: 10.18502/ijdo.v17i3.19266]

to gain a better understanding of the mechanism underlying insulin resistance, this study uses human adipose tissue sampling to investigate the simultaneous effects of sitagliptin and metformin on elements of the insulin signaling pathway.

Materials and methods

6 individuals with type 2 diabetes, 3 men and 3 women, up to the age of forty, were treated for three months with sitagliptin and metformin concurrently; 4 of them-2 men and 2 women-returned following therapy. These patients, known as "New Cases," have not taken any medicine and were just identified based on a blood glucose test. Patients with diabetes who are on insulin, tablets, or other treatments have been removed. Since this research is analytical in nature, the sample size calculation procedure was conducted in accordance with earlier (11).

Patients were given sitagliptin and metformin pills at the same time. 500 mg of metformin hydrochloride, HEXAL Brand, is manufactured in Germany (HEXAL AG) and is taken twice a day, in the morning after breakfast and in the evening after supper. Sitagliptin: JANUVIA brand, 50 mg, twice daily, after breakfast in the morning and after supper in the evening, produced in England under license to Merck.

Blood sample collection and processing

Prior to therapy, 10 ml of blood was drawn in a tube devoid of anticoagulant. Samples of blood were drawn and allowed to coagulate for 30 minutes. The clotted blood was centrifuged at 2000 × g for 10 minutes at 4°C to extract the serum.

Serum Biochemical Assessments

An auto-analyzer (Biosystems Model BA 400, Biosystems glucose kit) was used to measure the subjects' serum glucose levels. The Biosystems Model BA 400 auto-analyzer was used to assess the subjects' serum TG, TC, c-LDL, and c-HDL.

The ELISA technique and an insulin kit (MONOBIND, Inc.) were used to measure the participants' serum insulin levels. With a TOSOH G8 HPLC Analyzer (Tosoh Bioscience, Inc.), participants' blood HbA1c was determined. The HOMA-IR (resistance formula, insulin resistance) (7) was then used to calculate the participants' HbA1c. Patients were deemed insulin resistant and kept in this trial if their IR was more than 1.8-2. The serum that was separated was kept at -80°C.

Adipose tissue biopsy procedure

In short, participants (8) had a piece of subcutaneous adipose tissue removed by a surgeon colleague of this work. The participants were first given local anesthesia in order to remove adipose tissue samples from the subcutaneous region of the abdomen. Then, using a knife and surgical scissors, the surgeon sliced into the anesthetized abdomen region of each subject to remove the subcutaneous adipose tissue. Participants' adipose tissue samples were put into a 50 mm Falcon tube with a transfer media (9% saline, PBS phosphate buffered saline).

Isolation of human adipocytes from adipose tissue

Each participant's adipose tissue samples were treated in order to isolate fatty adipocytes. All of the following procedures were carried out in a sterile environment under the laminar hood. In order to accomplish this, we used extremely sharp sterile scissors to cut the adipose tissue sample in the plate into pieces that were 1-2 cubic centimeters in size until a papescent texture was achieved. Next, we placed a filter inside a sterile laboratory buffer and set the funnel on a Falcon tube; a tissue sample to get rid of the fat and red blood cell counts, we put the sliced fat in the filtered funnel and then put it in the saline or PBS buffer at room temperature.

After that, this specimen was weighed. A 50-milliliter Falcon tube was filled with the chopped tissue sample from the previous stage, and three milliliters of collagenase

[DOI: 10.18502/ijdo.v17i3.19266]

solution (1 gram of collagenase powder in one milliliter of solution, Gibco, Lot No. 1870848) were added for every gram of tissue in order to separate the adipocytes. To discover a mixture with a watery consistency, we put the tube in a shaker set to 37°C and 100 rpm for 90 minutes.

We monitored the level of digestion by slowly rotating the tubes every fifteen minutes. Cell culture medium was used to filter the resultant mixture under a laminar hood in order to separate the adipocyte cells from the cell leftovers.

After treatment processes

Patients with diabetes returned to the Center of Research-Therapy Diabetes of Yazd after three months. Subcutaneous adipose tissue and blood samples were obtained from patients both before and after treatment. As in the pre-treatment phase, all procedures were carried out on the patients' adipose tissue samples at this point, and the intended tests were conducted on their serum. Likewise, we conducted all procedures and testing for the control group.

Western blot analysis

By using SDSPAGE and Western blot techniques, the proteins IRS-1, PI3K, and mTOR in the cellular extract of the participants' adipose tissue were separated. After hours at room temperature, a 5% skimmed milk solution was used to block the nitrocellulose membranes. Then, using Santa Cruz Biotechnologies mice monoclonal antibodies for each target protein, membranes were treated with primary antibodies for an entire night at 4°C. After cleaning the membranes, we added chemiluminescence substrate to them in Gel Doc (G: BOX, Syngene), connected them to a computer, and used Gene Snap Image Acquisition software (Syngene) to image the protein bands.

Statistical analysis

Using non-parametric (Wilcoxon) tests in SPSS version 17.0 and Graph Pad Prism 7.04 software, respectively, we evaluated statistically significant rates among the targeted categories. IMAGE J 1.50e software was used to examine protein bands.

Ethical considerations

ID Code for Ethics: This paper, IR.SSU. MEDICINE.REC.1396.54, was published on July 18, 2017, by the Shahid Sadoughi University of Medical Sciences, Yazd, Ethics Committee. As of 2018-08-26, 1397/06/04, the IRCT registration number is IRCT201710 18036870N2, and it was last updated on 2019-02-06, 1397/11/17. Each participant completed a questionnaire and gave their informed consent.

Results

Clinical specifications of the participants

The participants mean age was 44 (±10.1) years old. In Table 1, we provided clinical criteria.

Table 1.Clinical characteristics of diabetic patients before and after combined sitagliptin–metformin treatment

Characteristic	Diabetic patients		*P*-value
	Before Treatment (n=6)	After Treatment (N=4)	
BMI (Kg/m^2)	33 (±3)	33(±4)	0.249
Serum glucose (mg/dl)	219 (±39)	150 (±29)	0.028
Serum insulin (µU/ml)	10.9 (±2.2)	6.3 (±1.5)	0.463
HOMA-IR	5.2 (±0.7)	2.4 (±0.7)	0.046
Serum triglyceride (mg/dl)	264 (±27)	205 (±17)	0.173
Serum cholesterol (mg/dl)	228 (±23)	216 (±05)	0.046
LDL-cholesterol (mg/dl)	134 (±04)	102 (±17)	0.075
HDL-cholesterol (mg/dl)	42 (±4)	45 (±4)	0.345
HbA1c (%)	7.9 (±0.7)	5.4 (±0.5)	0.043

Values are mean ± SEM, *P< 0.05, after treatment vs before treatment in diabetic participant

[DOI: 10.18502/ijdo.v17i3.19266]

When metformin and sitagliptin were taken together, type 2 diabetic patients' fasting serum glucose levels dropped after treatment compared to before ($P< 0.028$), as did their insulin levels ($P< 0.043$) and HOMA-IR levels ($P< 0.046$). Additionally, their HbA1c levels ($P< 0.043$) and total cholesterol levels ($P< 0.046$) decreased after treatment compared to before treatment.

Effect of simultaneous metformin and sitagliptin treatment on IRS-1, PI3K, m-TOR contents (protein levels)

Following simultaneous therapy with sitagliptin and metformin, the contents of IRS-1 increased statistically significantly ($P< 0.035$) in comparison to pretreatment, the levels of PI3K increased statistically significantly ($P< 0.043$) in comparison to prior therapy and the contents of mTOR increased statistically significantly ($P< 0.042$) in comparison to prior therapy.

Discussion

Due to the rise in adipose tissue signaling insulin pathway proteins, IRS-1, PI3K, and mTOR, the concurrent use of metformin and sitagliptin treatment was found to be significantly helpful in lowering insulin resistance. This result is consistent with prior research (9). The use of metformin or thiazolidinedione alone has not been shown to significantly improve the activation of these signaling components in the literature, and the highest level of insulin stimulation has been used in (9,10). A prior study found that in NIDDM, thiazolidinedione and metformin treatment together might dramatically boost maximum and submaximal IR, atypical protein kinase C (aPKC), IRS-1/PI3K, and PKBβ insulin signaling (11). Compared to reports of earlier trials assessing the single use of either medicine, the observed improvement was more significant. Another trial found that both monotherapy with either sitagliptin or metformin and beginning treatment with a combination of both medications resulted in significant sustained glucose improvements; type 2 diabetes individuals tolerated the therapies well over a 104-week period (12). Telmisartan and sitagliptin monotherapy or dual treatment produced the best results, activating PPAR-α in the liver with extended incretin (13).

It was found that thiazolidinedione and metformin treatment together enhanced aPKC activation in diabetic muscles to the level of enzyme-specific activity in healthy individuals does not prove that aPKC's insulin actions are entirely typical. According to the results, thiazolidinedione-metformin treatment did not raise muscle aPKC, and the lower total activated aPKC may restrict glucose transport in NIDDM' intact muscles (2,14).

Thiazolidinedione-metformin treatment could significantly restore IRS-1/PI3K and PKBβ/Akt2 activation, and it did not result in lingering abnormalities in glucose disposal after treatment, since PKBβ/Akt2 and IRS-1 levels did not decrease in diabetics (9,10,15,16). Following sitagliptin-metformin treatment, alterations in diabetic adipose tissues most likely reflect primary alterations in the liver and muscle tissues. Insulin signaling in adipose tissues is positively impacted by both medications. The present investigation reports improvements in insulin resistance, which is generally indicated by prevalent repairs in all assessed signaling proteins.

Conclusion

Lastly, we must ascertain whether the blood insulin level is corrected during sitagliptin-metformin treatment since extremely high insulin levels can raise hepatic lipids (17), which may aid in the development of atherosclerosis in NIDDM. In our study, HDL cholesterol was not restored, but serum levels of total cholesterol, glucose, triacylglycerol, and LDL cholesterol dropped. Overall, long-term trials of sitagliptin-metformin treatment must assess changes in key metabolic and clinical factors. Additionally, after short-term combination sitagliptin-metformin treatment, diabetic adipose tissues showed notable

[DOI: 10.18502/ijdo.v17i3.19266]

improvements in metabolic variables and insulin signaling pathways, indicating the effectiveness of treatment, particularly in the early stages of NIDDM. Furthermore, the study's findings might have been impacted by variables like nutrition, exercise, and past drug use. To avoid having an influence on the findings of future study, it is advised that these issues be investigated in subsequent studies.

Acknowledgments

We strictly state that there are no conflicts of interest and financial interest in this article on the preparation of materials and tests and the writing of this article.

Funding

Because an article is a result of my dissertation, it funding sponsor is of Shahid Sadoughi University of Medical Sciences, Yazd.

Conflict of Interest

We strictly state that there are no conflicts of interest and financial interest in this article on the preparation of materials and tests and the writing of this article.

Author contributions

R**.** D**:** developed and conceived the analysis; collection of the data; provided information and instruments for analysis; carried out the analysis; writing the first draft of manuscript. J. MA: participated in the analysis of the data. Y. N: participated in the collection of the data. M. R: Referral of individuals with diabetes. N. H: collected human adipose tissue samples from the patients in the outpatient operating room. All the authors critically revised the manuscript, agree to be fully accountable for the integrity and accuracy of the study, and read and approved the final manuscript.

References

1. Zhang C, Klett EL, Coleman RA. Lipid signals and insulin resistance. Clinical lipidology. 2013;8(6):659-67.
2. Guo S. Insulin signaling, resistance, and the metabolic syndrome: insights from mouse models to disease mechanisms. The Journal of endocrinology. 2014;220(2):T1-T23.
3. Cline GW, Petersen KF, Krssak M, Shen J, Hundal RS, Trajanoski Z, et al. Impaired glucose transport as a cause of decreased insulin-stimulated muscle glycogen synthesis in type 2 diabetes. New England Journal of Medicine. 1999;341(4):240-6.
4. Hui H, Tang G, Go VL. Hypoglycemic herbs and their action mechanisms. Chinese Medicine. 2009;4:1-1.
5. World Health Organization. Use of glycated haemoglobin (HbA1c) in diagnosis of diabetes mellitus: abbreviated report of a WHO consultation. Geneva: World Health Organization [Internet]. 2011.
6. Inzucchi SE, Bergenstal RM, Buse JB, Diamant M, Ferrannini E, Nauck M, et al. Management of hyperglycemia in type 2 diabetes, 2015: a patient-centered approach: update to a position statement of the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes care. 2015;38(1):140-9.
7. Sera N, Hida A, Imaizumi M, Nakashima E, Akahoshi M. The association between chronic kidney disease and cardiovascular disease risk factors in atomic bomb survivors. Radiation research. 2013;179(1):46-52.
8. Carswell KA, Lee MJ, Fried SK. Culture of isolated human adipocytes and isolated adipose tissue. Methods in Molecular Biology (MIMB)2012:806:203-14.
9. Beeson M, Sajan MP, Dizon M, Grebenev D, Gomez-Daspet J, Miura A, et al. Activation of protein kinase C-ζ by insulin and phosphatidylinositol-3, 4, 5-(PO4) 3 is defective in muscle in type 2 diabetes and impaired glucose tolerance: amelioration by rosiglitazone and exercise. Diabetes. 2003;52(8):1926-34.
10. Luna V, Casauban L, Sajan MP, Gomez-Daspet J, Powe JL, Miura A, Rivas J, Standaert ML, Farese RV. Metformin improves atypical protein kinase C activation by insulin and phosphatidylinositol-3, 4, 5-(PO 4) 3 in muscle of diabetic subjects. Diabetologia. 2006;49:375-82.
11. Temofonte N, Sajan MP, Nimal S, Pastoor T, Fumero C, Casaubon L, et al. Combined thiazolidinedione–metformin treatment synergistically improves insulin signalling to insulin receptor substrate-1-dependent phosphatidylinositol 3-kinase, atypical protein

[DOI: 10.18502/ijdo.v17i3.19266]

kinase C and protein kinase B/Akt in human diabetic muscle. Diabetologia. 2009;52:60-4.
12. Williams-Herman D, Johnson J, Teng R, Golm G, Kaufman KD, Goldstein BJ, et al. Efficacy and safety of sitagliptin and metformin as initial combination therapy and as monotherapy over 2 years in patients with type 2 diabetes. Diabetes, Obesity and Metabolism. 2010;12(5):442-51.
13. Souza-Mello V, Gregorio BM, Cardoso-de-Lemos FS, de Carvalho L, Aguila MB, Mandarim-de-Lacerda CA. Comparative effects of telmisartan, sitagliptin and metformin alone or in combination on obesity, insulin resistance, and liver and pancreas remodelling in C57BL/6 mice fed on a very high-fat diet. Clinical science. 2010;119(6):239-50.
14. Karasik A, Aschner P, Katzeff H, Davies MJ, Stein PP. Sitagliptin, a DPP-4 inhibitor for the treatment of patients with type 2 diabetes: a review of recent clinical trials. Current medical research and opinion. 2008;24(2):489-96.
15. Casaubon L, Sajan MP, Rivas J, Powe JL, Standaert ML, Farese RV. Contrasting insulin dose-dependent defects in activation of atypical protein kinase C and protein kinase B/Akt in muscles of obese diabetic humans. Diabetologia. 2006;49(12):3000-8.
16. Kim YB, Ciaraldi TP, Kong A, Kim D, Chu N, Mohideen P, et al. Troglitazone but not metformin restores insulin-stimulated phosphoinositide 3-kinase activity and increases p110β protein levels in skeletal muscle of type 2 diabetic subjects. Diabetes. 2002;51(2):443-8.
17. Farese RV, Sajan MP, Yang H, Li P, Mastorides S, Gower WR, et al. Muscle-specific knockout of PKC-λ impairs glucose transport and induces metabolic and diabetic syndromes. The Journal of clinical investigation. 2007;117(8):2289-301.

[DOI: 10.18502/ijdo.v17i3.19266]