Study on the Therapeutic Efficacy of Alogliptin on Obesity Induced Insulin Resistance

Ibrahim A. Ibrahim[1], Sherif Y. Saleh[2], Massy M. Al-Banna[3], and Yasmina K. Mahmoud[1]

[1]*Department of Biochemistry, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt.*
[2]*Department of Biochemistry, Faculty of Physical Therapy, Port Said University, Port Said, Egypt.*
[3]*Free Veterinarian* ***Corresponding author**: Tel. 01113712949_ 01026716102*
E-mail. massymorsy881@gmail.com

Abstract

Obesity is a pathological condition in which excess body fat has accumulated to the extent that it may have an adverse effect on health. Insulin resistance (IR) is defined where a normal or elevated insulin level produces an attenuated biological response due to impaired sensitivity of tissues to insulin. IR has been recently reported in various conditions including obesity, metabolic syndrome, diabetes mellitus. The current study investigated the influence of alogliptin on obesity induced insulin resistance in Wistar male rats. Eighteen rats were classified into three groups; (control, obese, and obese + alogliptin). The control group was offered a standard diet, while the other two groups consumed a high-fat diet for 12 weeks. The control and obese groups were administered water by gavage during the study's final month, whereas the obese + alogliptin group received daily therapy of alogliptin (20 mg/kg per day P.O.) Following the therapy, weight gain was determined and rats were sacrificed. Epididymal fat was weighed and blood samples were obtained from each rat to assess the three glycemic parameters: fasting blood glucose, fasting insulin, and the corresponding HOMA-IR. The development of IR was indicated by a significant rise in both insulin and HOMA-IR in the obese group. Rats did not progress to the diabetic stage, as indicated by non-significant variation in FBG between groups. On the other hand, treatment with alogliptin demonstrated significant improvement recognized by correction of hyperinsulinemia and decrease in HOMA-IR.

Keywords: Insulin resistance; High Fat Diet; Alogliptin; Obesity; HOMA-IR

Introduction

The pancreatic islets of Langerhans secrete the hormone insulin. It is crucial for controlling blood sugar levels. It increases the movement of glucose into the liver, fatty tissue, and muscle ***(Davids et al., 2020)***. On the other hand, defective regulation of glucose leads to diabetes mellitus ***(Lauterbach and Wunderlich, 2017)***. Reduced tissue sensitivity to insulin is a defining feature of insulin resistance (IR), a condition that is considered the linchpin in the progression of diabetes mellitus ***(Park et al., 2021)***.

Several studies demonstrated that obesity is a predisposing factor for IR. Since obesity has been increasingly spreading globally, a dramatic increase occurred in its closely-associated disease, type 2 diabetes mellitus (T2DM) ***(Kim and Lee, 2021)***.

Ota (2015) described the association between lipotoxicity, hyperinsulinemia, inflammation, and mitochondrial dysfunction with insulin resistance. Adipose tissue hypertrophy, immune cell proliferation, and an increase in inflammatory cytokines are all associated with obesity's pro-inflammatory state. These variables eventually result in persistent low-grade inflammation, which is crucial for the emergence of IR and, subsequently, type 2 diabetes ***(Asghar and Sheikh, 2017)***.

A metric that takes fasting insulin and fasting blood glucose (FBG) into account is the homeostatic model assessment of insulin resistance (HOMA-IR). Since it has been verified to be strongly correlated with the hyperinsulinemia-euglycemic clamp, it is regarded as a reliable indicator of IR ***(Song et al., 2007)***.

Lifestyle modifications, drug medication together with weight loss are recommended to ameliorate T2DM. Numerous potential medications have been thoroughly investigated in the last decades, including metformin, pioglitazone, GLP-1 receptor agonists, and SGLT-2 inhibitors ***(Mantovani and Dalbeni, 2021)***. More recently, the Food and Drug Administration (FDA) approved a new category of diabetic drugs entitled dipeptidyl peptidase 4 inhibitors (DPP-4I), to treat T2DM in adults ***(Singhal et al., 2024)***.

In the current study, we examined the efficacy of alogliptin, a member of DPP4 inhibitors, in treatment of obesity induced insulin resistance.

Material and methods

Drugs and chemicals

Alogliptin was purchased from a local pharmacy under the trade name Oliptina (Tenth of Ramadan for Pharmaceutical Industries, Diagnostic Reagent (rameda)). Each film is a coated tablet containing alogliptin benzoate 34 mg (Equivalent to 25 mg alogliptin).

Experimental animals

Eighteen male albino Wistar rats, four weeks old and weighing between 120 and 130 grams, were

acquired from the lab animal house at the Faculty of Veterinary Medicine, Suez Canal University. Experimental procedure was permitted by the Institution of Animal Care and Use Committee (IACUC) at the Faculty Veterinary Medicine, Suez Canal University (Approval number is 201909).

Experimental design

Three equal groups of six rats each were created by random selection. After being put in separate cages, they were categorized as follows:

Group I (control)

This group served as control negative group. They were fed normal control diet all experimental period (12 weeks) and given tape water by oral gavage for the last 4 weeks.

Group II (obese)

This group served as control positive group. Rats were fed high-fat diet (HFD) till the end of the trial 12 weeks and given tape water by oral gavage for the last 4 weeks.

Group III (obese + alogliptin)

They were fed HFD till the end of the trial 12 weeks. Alogliptin was provided for 4 weeks (20 mg/kg per day P.O.). Alogliptin was suspended in 0.5 % CMC sodium to reach a final concentration of 2 mg/ml ***(Kabel, 2018)***.

Weight indices

A. Body weight gain

Experimental rats were weighed at the start and at 90 days (end of the experiment). weight gain was determined by subtracting the initial body weight from the final body weight.

B. Weight of the epididymal fat.

Collection of samples

Under light tetrahydrofuran inhalation anesthesia, blood was collected from retro-orbital venous plexus from overnight fasting rats on plain tubes using micro-hematocrit tubes. Then, serum was separated and stored at -20 ºC until biochemical analysis was performed, and collect epididymal fat.

Biochemical parameters

Serum fasting blood glucose was determined using commercially available diagnostic kits (Cat. No. BSIS 17-E, Spinreact, Spain) according to ***Nagel et al. (2006)***.

Serum fasting insulin level was determined by enzyme linked immunosorbent assay (ELISA) kit (Cat. No. BQ 130D, BQ Kit, Insulin ELISA, San Diego, California 92130, USA) according to manufacture instructions ***(Morgan and Lazarow, 1962)***.

HOMA-IR value as a measure of IR was calculated using the formula described by ***Kekow et al. (1988)*** as follows:

Fasting insulin (µU/L) × fasting glucose (mmol/L) / 22.5

Statistical analysis

Descriptive statistics were calculated and presented mean ± standard error mean (SEM). One-way ANOVA was used to compare between the three groups. Tukey`s post hoc tests were used for pairwise comparisons.

P value < 0.05 is considered statistically significant. All analysis was done using SPSS software for windows version 26 at significant levels 0.05.

Results

3.1. Weight gain and epididymal fat:

The obese rat group revealed significant (*P* <0.05) increase in weight gain as compared to control and alogliptin groups. However, non-significant variation was observed in the control rats and alogliptin group regarding weight gain. Epididymal fat was significantly higher in both obese and alogliptin group than control (Figure 1).

3.2. Serum fasting blood glucose levels:

No significant variations were observed in FBG levels between the control, obese group and that treated with alogliptin (Figure 2).

3.3. Serum fasting insulin levels and HOMA-IR:

Insulin levels in the obese group increased significantly (*P* <0.05) when compared to the control group, as seen in Table (1). Also, there was substantial decline in insulin level in obese rats treated with alogliptin upon comparing with obese group.

The obese group's HOMA-IR value was considerably (*P* <0.05) elevated when compared to that of the control group. Furthermore, obese rats treated with alogliptin showed a significantly (*P* <0.05) lower HOMA-IR value than the obese group.

Table 1: *Effect of treatment of obese rats with alogliptin for 4 weeks on serum fasting insulin levels and HOMA-IR.*

Group	Control	Obese	Obese + alogliptin
Insulin hormone (µIU/mL)	$7.98^{c} \pm 0.08$	$31.20^{a} \pm 0.53$	$24.47^{b} \pm 0.24$
HOMA-IR	$1.91^{c} \pm 0.10$	$7.49^{a} \pm 0.28$	$6.20^{b} \pm 0.23$

Values are demonstrated as means ± SEM

Means labelled with different superscripts are considered significant (*P* < 0.05)

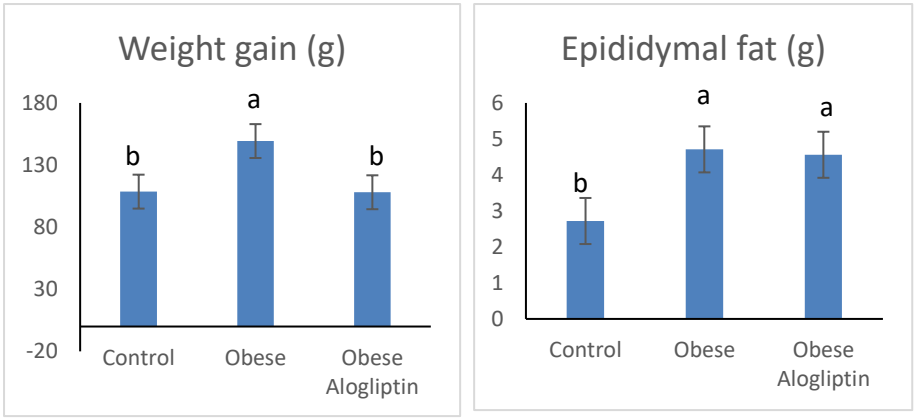

Figure 1: The effect of treatment of obese rats with alogliptin for 4 weeks on weight gain and epididymal fat.
Values are demonstrated as means ± SEM
Means labelled with different superscripts are considered significant ($P < 0.05$)

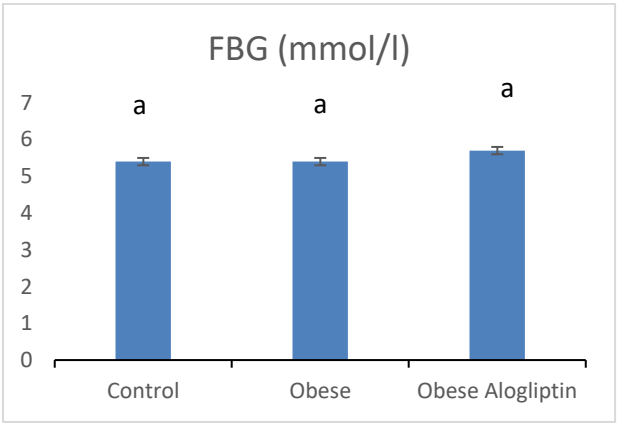

Figure 2: Effect of treatment of obese rats with alogliptin for 4 weeks on serum fasting blood glucose level.
Values are demonstrated as means ± SEM
Means labelled with different superscripts are considered significant ($P < 0.05$)

Discussion

Due to widespread of insulin resistance and its implication in obesity, exploring drugs for controlling insulin resistance could have great effect in management of obesity. The results obtained showed that feeding rats on HFD significantly increased body weight gain. These findings were consistent with other studies ***(Li et al., 2020; Wang et al., 2020)***. Treatment with alogliptin had a remarkable effect in reducing weight gain. Similarly

Ahmad et al. (2018) illustrated that alogliptin can decrease body weight and subsequently reduce weight gain .

Epididymal fat has also shown to be significantly increased due to HFD intake. ***Campos-Silva et al. (2015)*** demonstrated that high fat in diet increased genital fat deposition. Previous studies reported the ability of DPP4 inhibitors to decrease body fat ***(Khushwaha et al., 2019)***. However, our findings did not reveal significant reduction in epididymal fat in alogliptin treated group compared to obese group.

Our findings demonstrated that feeding rats HFD had no effect on their FBG level. The absence of high glucose made it challenging to assess how well alogliptin medication might affect the patient's blood sugar levels. However, other research showed that alogliptin lowered blood sugar and HbA1c ***(Kusunoki et al., 2015)***.

Similar to our results ***Kim et al. (2000)*** illustrated that there was no difference in blood glucose between groups fed normal diet and others fed HFD either with or without exercise. On the contrary, other studies indicated that HFD intake resulted in elevated blood glucose levels ***(Caroline et al., 2021)***. Variation of blood glucose level among different studies could be attributed to period of exposure to HFD.

In our study, the obese group's fasting insulin level increased dramatically, reaching a level four times greater than the control. Numerous studies have documented the impact of high-fat diet on the establishment of insulin resistance. ***Caroline et al. (2021)*** found that high-fat diet significantly elevated insulin, leptin, HOMA-IR and reduced adiponectin in obese rats.

Our findings also revealed a considerable reduction in serum insulin levels of alogliptin group compared with obese rats. This could be attributed to decrease fat in hepatic tissue which improves sensitivity of liver cells to insulin. Similar findings were reported by ***Vitola et al. (2009)*** who stated that reduction of liver content of fat improves insulin sensitivity.

The HOMA-IR model is predicated on the idea that the pancreas and liver interact to regulate blood glucose and insulin levels during fasting ***(Cacho et al., 2008)***. In the current study, FBG showed little difference between groups, consequently, HOMA-IR values nearly followed the same pattern as insulin levels. It was significantly increased in obese group. Similar results were obtained by ***Antunes et al. (2016)***.

Alogliptin significantly decreased HOMA-IR. This was also the findings reported by ***Kutoh et al. (2023)***. They suggested that regulation of insulin resistance and beta-cell function determines the effectiveness of alogliptin.

Conclusion

The findings of our study revealed

that consumption of diet containing high fat played a significant part in the development of insulin resistance demonstrated as high insulin level and HOMA-IR. It could be also concluded that, alogliptin produced remarkable amelioration of the adverse effect of obesity through improving tissue sensitivity to insulin leading to considerably lower insulin and HOMA-IR.

References

Ahmad, M.F., Babu, D.M. and Pradhan, A., (2018): Evaluation of anti diabetic drug alogliptin for the treatment of obesity in rats. Journal of Drug Delivery and Therapeutics 8, 209-216.

Antunes, L.C., Elkfury, J.L., Jornada, M.N., Foletto, K.C. and Bertoluci, M.C., (2016): Validation of HOMA-IR in a model of insulin-resistance induced by a high-fat diet in Wistar rats. Archives of endocrinology and metabolism 60, 138-142.

Asghar, A. and Sheikh, N., (2017): Role of immune cells in obesity induced low grade inflammation and insulin resistance. Cellular immunology 315, 18-26.

Cacho, J., Sevillano, J., de Castro, J., Herrera, E. and Ramos, M., (2008): Validation of simple indexes to assess insulin sensitivity during pregnancy in Wistar and Sprague-Dawley rats. American journal of physiology-endocrinology and metabolism 295, E1269-E1276.

Campos-Silva, P., Furriel, A., Costa, W.S., Sampaio, F.J. and Gregório, B.M., (2015): Metabolic and testicular effects of the long-term administration of different high-fat diets in adult rats. International braz j urol 41, 569-575.

Caroline, O.B., Ebuehi, O.A., Cecilia, O.A. and Kayode, O.A., (2021): Effect of Allium sativum extract in combination-with orlistat on insulin resistance and disrupted metabolic hormones in high fat diet induced obese rats. Scientific African 14, e00994.

Davids, S.F.G., Matsha, T.E., Peer, N., Erasmus, R.T. and Kengne, A.P., (2020): The 7-year change in the prevalence of insulin resistance, inflammatory biomarkers, and their determinants in an urban south african population. Journal of Diabetes Research 2020.

Kabel, A.M., (2018): Zinc/alogliptin combination attenuates testicular toxicity induced by doxorubicin in rats: Role of oxidative stress, apoptosis and TGF-β1/NF-κB signaling. Biomedicine and Pharmacotherapy 97.

Kekow, J., Ulrichs, K., Müller-Ruchholtz, W. and Gross, W.L., (1988): Measurement of Rat Insulin: Enzyme-Linked Immunosorbent Assay With Increased Sensitivity, High Accuracy, and Greater Practicability Than Established Radioimmunoassay. Diabetes 37, 321-326.

Khushwaha, S., Behl, T., Pandey, R.K., Kamboj, A., Babu, A. and Goyal, A., (2019): Beneficial effect of sitagliptin on high fat diet induced

obesity in wistar rats. Obesity Medicine 14, 100084.

Kim, C.-H., Youn, J.H., Park, J.-Y., Hong, S.K., Park, K.S., Park, S.W., Suh, K.I. and Lee, K.-U., (2000): Effects of high-fat diet and exercise training on intracellular glucose metabolism in rats. American Journal of Physiology-Endocrinology and Metabolism 278, E977-E984.

Kim, J. and Lee, J., (2021): Role of obesity-induced inflammation in the development of insulin resistance and type 2 diabetes: history of the research and remaining questions. Annals of pediatric endocrinology & metabolism 26, 1.

Kusunoki, M., Sato, D., Nakamura, T., Oshida, Y., Tsutsui, H., Natsume, Y., Tsutsumi, K. and Miyata, T., (2015): The beneficial effects of the DPP-4 inhibitor Alogliptin on hemoglobin A1c and Serum lipids in Japanese patients with type 2 diabetes. Drug research, 18-22.

Kutoh, E., Kuto, A.N., Ozawa, E., Akiyama, M. and Kurihara, R., (2023): Alogliptin: a unique DPP-4 inhibitor that regulates adipose tissue insulin resistance and atherogenic lipids.

Lauterbach, M.A. and Wunderlich, F.T., (2017): Macrophage function in obesity-induced inflammation and insulin resistance. Pflügers Archiv-European Journal of Physiology 469, 385-396.

Li, L., Li, R., Zhu, R., Chen, B., Tian, Y., Zhang, H., Xia, B., Jia, Q., Wang, L. and Zhao, D., (2020): Salvianolic acid B prevents body weight gain and regulates gut microbiota and LPS/TLR4 signaling pathway in high-fat diet-induced obese mice. Food & function 11, 8743-8756.

Mantovani, A. and Dalbeni, A., (2021): Treatments for NAFLD: state of art. International journal of molecular sciences 22, 2350.

Morgan, C.R. and Lazarow, A., (1962): Immunoassay of insulin using a two-antibody system. Proceedings of the Society for Experimental Biology and Medicine 110, 29-32.

Nagel, J.M., Tietz, A.B., Göke, B. and Parhofer, K.G., (2006): The effect of telmisartan on glucose and lipid metabolism in nondiabetic, insulin-resistant subjects. Metabolism 55, 1149-1154.

Ota, T., (2015): Obesity-induced inflammation and insulin resistance. Frontiers Media SA.

Park, H.M., Lee, H.S., Lee, Y.-J. and Lee, J.-H., (2021): The triglyceride–glucose index is a more powerful surrogate marker for predicting the prevalence and incidence of type 2 diabetes mellitus than the homeostatic model assessment of insulin resistance. Diabetes research and clinical practice 180, 109042.

Singhal, S., Patil, V.M., Verma, S. and Masand, N., (2024): Recent advances and structure-activity relationship studies of DPP-4 inhibitors as anti-diabetic agents. Bioorganic Chemistry, 107277.

Song, Y., Manson, J.E., Tinker, L., Howard, B.V., Kuller, L.H., Nathan, L., Rifai, N. and Liu, S., (2007): Insulin sensitivity and insulin secretion determined by homeostasis model assessment and risk of diabetes in a multiethnic cohort of women: the Women's Health Initiative Observational Study. Diabetes care 30, 1747-1752.

Vitola, B.E., Deivanayagam, S., Stein, R.I., Mohammed, B.S., Magkos, F., Kirk, E.P. and Klein, S., (2009): Weight loss reduces liver fat and improves hepatic and skeletal muscle insulin sensitivity in obese adolescents. Obesity 17, 1744-1748.

Wang, Y., Yao, W., Li, B., Qian, S., Wei, B., Gong, S., Wang, J., Liu, M. and Wei, M., (2020): Nuciferine modulates the gut microbiota and prevents obesity in high-fat diet-fed rats. Experimental & molecular medicine 52, 1959-1975.

Arabic Summary

السمنة هي حالة مرضية تتراكم فيها الدهون الزائدة في الجسم إلى الحد الذي قد يكون له تأثير سلبي على الصحة. يتم تعريف مقاومة الأنسولين عندما يؤدي مستوى الأنسولين الطبيعي أو المرتفع إلى استجابة بيولوجية مخففة بسبب ضعف حساسية الأنسجة للأنسولين. تم رصد مقاومة الانسولين مؤخرًا في حالات مختلفة بما في ذلك السمنة ومتلازمة التمثيل الغذائي ومرض السكري. بحثت الدراسة الحالية في تأثير الألوجليبتين على مقاومة الأنسولين الناجمة عن السمنة في ذكور فئران ويستار. تم تقسيم ثمانية عشر فأرًا إلى ثلاث مجموعات؛ (الضابطة والسمنة والسمنة + الأوغليبتين). وقد تم تغذية المجموعة الضابطة على نظام غذائي قياسي، في حين تناولت المجموعتان الأخريان نظامًا غذائيًا غنيًا بالدهون لمدة 12 أسبوعًا. تم إعطاء المياه للمجموعات الضابطة والسمنة عن طريق التجريع خلال الشهر الأخير من الدراسة، في حين تلقت مجموعة السمنة + الألوجليبتين علاجًا يوميًا بالألوجليبتين (20 مجم / كجم يوميًا عن طريق الفم). بعد العلاج، تم تحديد الزيادة في الوزن ووزن الدهون البربخية و معايير نسبة السكر في الدم: جلوكوز الدم الصائم، والأنسولين الصائم، وHOMA-IR. تطور مقاومة الانسولين من خلال الارتفاع الكبير في كل من الأنسولين وHOMA-IR في المجموعة التي تعاني من السمنة المفرطة. لم تتقدم الفئران إلى مرحلة مرض السكري، كما يتضح من التباين غير الكبير في سكر الدم الصائم بين المجموعات. من ناحية أخرى، أظهر العلاج بالألوجليبتين تحسنًا كبيرًا من خلال تصحيح فرط أنسولين الدم وانخفاض HOMA-IR.