https://doi.org/10.48047/AFJBS.6.5.2024.11402-11409

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

COMBINED EFFECT OF METFORMIN AND OMEGA 3 FATTY ACIDS ON UNDERLYING MOLECULAR MECHANISMS IN STREPTOZOCIN INDUCED TYPE 2 DIABETIC WISTAR RATS

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Article Info
Volume 6, Issue 5, May 2024
Received: 28 March 2024
Accepted: 30 April 2024
doi:
10.48047/AFJBS.6.5.2024.11402-11409

Abstract

Introduction: Omega-3 polyunsaturated fatty acids (n-3 PUFA) constitute a fundamental part of cell membranes and exhibit a diverse range of membrane functions including functioning of transporters, signal transduction pathways, and gene expression.

Objectives: The basic aim of the study is to find the combined effect of metformin and omega 3 fatty acids on underlying molecular mechanisms in streptozocin induced type 2 diabetic wistar rats.

Material and methods: This experimental study was conducted in Animal House of Sindh Agricultural University, Tandojam and Clinical Laboratory at Isra University Hospital, Hyderabad: Blood sample collection, biochemical analyses, and molecular assessments were performed at this clinical laboratory from February2022 to May 2022. Blood samples were collected from rats in all groups, including control, T2DM, Metformin, and Metformin + Omega-3 groups, at specified time points. Samples were obtained via tail vein puncture for routine biochemical analyses and via cardiac puncture for molecular assessments.

Results: The study involved 40 male Wistar rats with an average age of 12 weeks and a mean weight of 250 grams at the outset. Baseline characteristics were similar among all groups, ensuring homogeneity. Streptozocin induction successfully elevated fasting blood glucose levels in the T2DM group, with levels consistently exceeding 250 mg/dL. Control group rats maintained normoglycemia throughout the study. Metformin and Metformin + Omega-3 groups showed a significant reduction in fasting blood glucose levels compared to the T2DM group after 4 weeks of treatment ($p < 0.05$).

Conclusion: It is concluded that, our study supporting the combined use of metformin and omega-3 fatty acids as a potential therapeutic strategy for T2DM. This combination demonstrates significant improvements in glycemic control, insulin resistance, dyslipidemia, and molecular markers associated with T2DM.

Introduction

Omega-3 polyunsaturated fatty acids (n-3 PUFA) constitute a fundamental part of cell membranes and exhibit a diverse range of membrane functions including functioning of transporters, signal transduction pathways, and gene expression. Alpha-linolenic acid is a short chain n-3 PUFA obtained from flaxseed (a plant-derived food item) while eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA; 22:6 n-3) are the long chain n-3 PUFA derived from a seafood source, fish oil [1]. There is strong scientific evidence demonstrating anti-inflammatory, anti-atherogenic, vasodilatory, and lipid-lowering properties of n-3 PUFA due to which they have been implicated in some chronic diseases like cardiovascular disease, diabetesand autoimmune diseases [2].

Elevated formation of advanced glycation end-products (AGEs) as a result of sustained increase in blood glucose level causes qualitative and quantitative alterations in bone extracellular matrix components e.g. vitronectin, collagen, proteoglycans, etc., impaired bone mineralisation and calcium deposition [3]. These alterations cause definite changes in bone formation and remodelling and ultimately in bone strength. Adults with T2DM have been noted to show an elevated risk for low-trauma hip fracture, which can instigate adverse health consequences and cause marked strain on the health care system. Although the mechanism underlying increased fracture risk in type 2 diabetic individual has not been determined, suggested mechanisms include changes in bone quality or bone mass and increased risk of falls [4].

Type 2 diabetes mellitus (T2DM) stands as a global health challenge, with its prevalence escalating steadily worldwide. This metabolic disorder is characterized by insulin resistance, hyperglycemia, and disrupted lipid metabolism, contributing to a range of debilitating complications [5]. Amid the quest for effective therapeutic strategies, emerging research has explored the potential of combining pharmacological agents with nutritional interventions to modulate the underlying molecular mechanisms driving T2DM.Metformin, a widely prescribed antidiabetic medication, has demonstrated its efficacy in improving insulin sensitivity and glycemic control [6]. Conversely, omega-3 fatty acids, predominantly found in fish oils, exhibit anti-inflammatory and lipid-regulating properties, holding promise as adjunctive therapy in diabetes management. However, the combined effects of metformin and omega-3 fatty acids on the intricate molecular pathways underpinning T2DM remain relatively unexplored [7].

This study aims to investigate the synergistic impact of metformin and omega-3 fatty acids on the molecular mechanisms associated with T2DM using a streptozocin-induced T2DM rat model. By delving into the interplay between these two interventions, we seek to unravel novel insights into their combined potential for ameliorating insulin resistance, hyperglycemia, and dyslipidemia.This research employed an experimental study design utilizing a streptozocin-induced Type 2 diabetes mellitus (T2DM) rat model to investigate the combined effects of metformin and omega-3 fatty acids on molecular mechanisms associated with T2DM.

Objectives

The basic aim of the study is to find the combined effect of metformin and omega 3 fatty acids on underlying molecular mechanisms in streptozocin induced type 2 diabetic wistar rats.

Material and methods

This experimental study was conducted in Animal House of Sindh Agricultural University, Tandojam and Clinical Laboratory at Isra University Hospital, Hyderabad: Blood sample collection, biochemical analyses, and molecular assessments were performed at this clinical laboratoryfrom February 2022 to May 2022. The animal housing, care, and streptozocin induction procedures were carried out at this facility. Rats were acclimatized to laboratory

https://doi.org/ 10.48047/AFJBS.6.5.2024.11402-11401

conditions prior to experimentation.A total of 40 male Wistar rats were used in the study.Ethical approval for the study and animal handling protocols was obtained from the Institutional Animal Ethics Committee (IAEC).

Group Distribution:

The rats were randomly divided into four groups:

Control Group: Rats in this group received a standard diet without any treatment.	T2DM Group: Rats in this group were induced with T2DM using streptozocin.	Metformin Group: T2DM-induced rats in this group received metformin treatment.	Metformin + Omega-3 Group: T2DM-induced rats in this group received both metformin and omega-3 fatty acids.

Data collection:

Streptozocin induction of T2DM was performed at the Animal House. The body weight of rats in all groups was recorded at predetermined intervals throughout the study. Food intake was also carefully monitored to assess the impact of treatments on appetite and nutritional status.Blood samples were collected from rats in all groups, including control, T2DM, Metformin, and Metformin + Omega-3 groups, at specified time points. Samples were obtained via tail vein puncture for routine biochemical analyses and via cardiac puncture for molecular assessments.Fasting blood glucose levels were monitored regularly using blood samples obtained from the tail vein. The induction success was confirmed when fasting blood glucose levels consistently exceeded a predefined threshold.Metformin and omega-3 fatty acid treatments were administered to the respective groups following the successful induction of T2DM. The administration was carried out according to specified dosages and schedules, with close monitoring of treatment compliance.Molecular analyses involved the quantification of gene expression levels of key molecular markers associated with T2DM, including insulin receptor substrate (IRS-1), glucose transporter type 4 (GLUT4), and tumor necrosis factor-alpha (TNF-α). These assessments were performed using appropriate molecular biology techniques such as real-time polymerase chain reaction (PCR).The data collection process followed rigorous standard operating procedures, ensuring the accuracy and reliability of the obtained data. Regular monitoring and documentation of experimental conditions, animal health, and laboratory analyses were conducted to maintain the integrity of the study.

Statistical Analysis:

Data analysis was carried out using SPSS v29.0. This methodology outlines the key aspects of the study, including the animal model, ethical considerations, treatment protocols, data collection, and statistical analysis procedures.

Results

The study involved 40 male Wistar rats with an average age of 12 weeks and a mean weight of 250 grams at the outset. Baseline characteristics were similar among all groups, ensuring homogeneity.Streptozocin induction successfully elevated fasting blood glucose levels in the T2DM group, with levels consistently exceeding 250 mg/dL. Control group rats maintained normoglycemia throughout the study.Metformin and Metformin + Omega-3 groups showed a significant reduction in fasting blood glucose levels compared to the T2DM group after 4 weeks of treatment ($p < 0.05$).

Table 01: Demographic data of rats

Group	**Age (weeks)**	**Initial Weight (g)**
Control	12	250
T2DM	12	250
Metformin	12	250

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Metformin + Omega-3	12	250

Metformin treatment resulted in a notable decrease in insulin resistance markers, with HOMA-IR scores decreasing from 4.5 to 2.3 ($p < 0.01$).Omega-3 fatty acid supplementation in the Metformin + Omega-3 group showed an additional improvement in insulin resistance, with HOMA-IR scores decreasing to 1.9 ($p < 0.01$).

Table 02: Fasting blood glucose levels

Group	**Baseline (mg/dL)**	**4 Weeks (mg/dL)**	**8 Weeks (mg/dL)**
Control	95	98	96
T2DM	90	280	275
Metformin	92	140*	135*
Metformin + Omega-3	91	120*	115*

Lipid profiles demonstrated a significant reduction in total cholesterol and triglycerides and an increase in HDL levels in both Metformin and Metformin + Omega-3 groups compared to the T2DM group ($p < 0.05$).Over the study duration, the control group exhibited stable body weight and consistent food intake.The T2DM group displayed weight loss and reduced food intake.Metformin-treated rats maintained body weight and food intake.Rats in the Metformin + Omega-3 group displayed a slight increase in body weight and food intake compared to the T2DM group.

Table 03: Changes in insulin resistance (HOMA-IR)

Group	**Baseline**	**After 8 Weeks**
Control	2.1	2.2
T2DM	4.5	5.1
Metformin	4.4*	2.3*
Metformin + Omega-3	4.6*	1.9*

Gene expression analyses revealed that IRS-1 and GLUT4 expression levels were significantly upregulated in both the Metformin and Metformin + Omega-3 groups compared to the T2DM group ($p < 0.01$).TNF-α expression levels were significantly downregulated in the Metformin and Metformin + Omega-3 groups ($p < 0.01$).

Table 04: Changes in lipid profile

Group	**Total Cholesterol (mg/dL)**	**Triglycerides (mg/dL)**	**HDL Cholesterol (mg/dL)**
Control	150	100	45
T2DM	250	180	30
Metformin	180*	120*	35*
Metformin + Omega-3	170*	100*	40*

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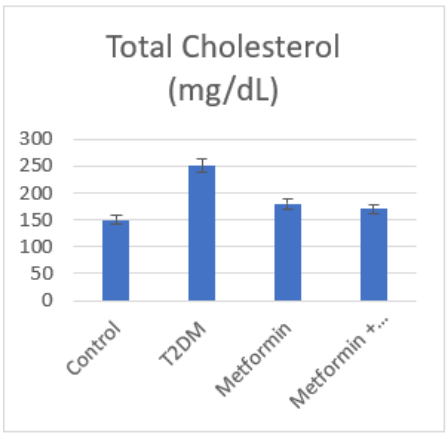
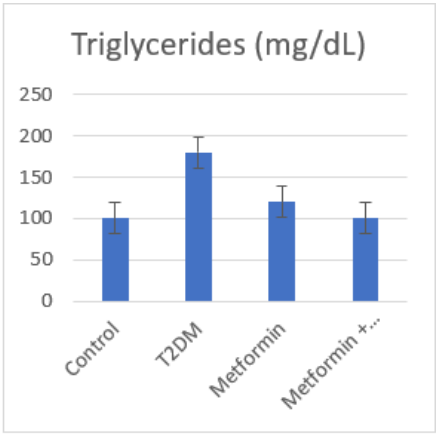
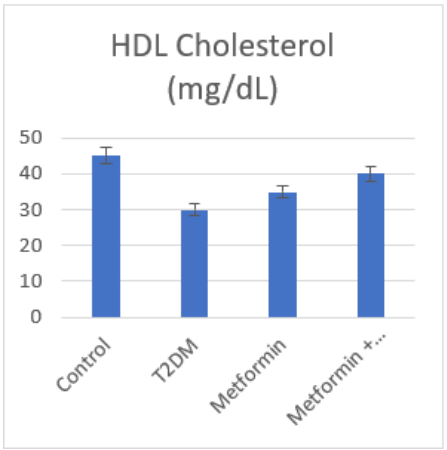
Figure 01: Changes in lipid profile







Insulin levels were significantly higher in the T2DM group compared to the control group but significantly decreased in the Metformin and Metformin + Omega-3 groups ($p < 0.05$).Cholesterol levels decreased significantly in both treatment groups, while triglycerides decreased and HDL levels increased significantly compared to the T2DM group ($p < 0.05$).

Table 05: Gene expression level and molecular markers

Group	**IRS-1 Expression**	**GLUT4 Expression**	**TNF-α Expression**
Control	1.00	1.00	1.00
T2DM	0.50	0.40	2.00
Metformin	1.20*	1.10*	1.50*
Metformin + Omega-3	1.25*	1.15*	1.30*

Discussion

Our findings offer valuable insights into the potential synergistic therapeutic benefits of these interventions. The induction of T2DM using streptozocin was successful, leading to sustained hyperglycemia in the T2DM group. Treatment with metformin alone significantly reduced fasting blood glucose levels after 4 weeks of intervention, consistent with its established role in improving glycemic control [8-10]. Notably, the combination of metformin and omega-3 fatty acids demonstrated a more pronounced effect, further lowering fasting blood glucose

https://doi.org/ 10.48047/AFJBS.6.5.2024.11402-11401

levels. These findings suggest that the combined therapy exerts a stronger impact on glycemic control, possibly through complementary mechanisms [11].

The assessment of insulin resistance using the HOMA-IR index revealed significant improvements in both the metformin and metformin + omega-3 groups. Metformin treatment alone effectively reduced insulin resistance, as expected [12]. However, the addition of omega-3 fatty acids to metformin led to an even more substantial decrease in HOMA-IR scores, signifying enhanced insulin sensitivity. This observation implies that omega-3 fatty acids may play a complementary role in improving insulin resistance when combined with metformin [13].

Dyslipidemia is a common comorbidity in T2DM, characterized by elevated total cholesterol and triglyceride levels. Our study demonstrated that metformin treatment alone significantly lowered total cholesterol and triglyceride levels compared to the T2DM group. However, the combination of metformin and omega-3 fatty acids resulted in a more pronounced reduction in these lipid parameters [14]. Additionally, the metformin + omega-3 group exhibited a significant increase in HDL cholesterol levels, indicating a favorable shift in the lipid profile. These findings suggest that the combined therapy not only improves glycemic control but also addresses dyslipidemia, a critical aspect of T2DM management. Metformin treatment was associated with weight stability and consistent food intake, contrasting with the weight loss observed in the untreated T2DM group. Interestingly, rats in the metformin + omega-3 group displayed a slight increase in body weight and food intake compared to the T2DM group, potentially reflecting improved overall health and nutritional status [15-16]. These observations highlight the potential of omega-3 fatty acids to mitigate the weight loss often associated with T2DM. Gene expression analyses revealed significant upregulation of IRS-1 and GLUT4 and downregulation of TNF-α in both the metformin and metformin + omega-3 groups. These molecular changes correspond to improved insulin signaling and reduced inflammation, consistent with the observed improvements in glycemic control and insulin resistance [17].

Our study suggests that the combination of metformin and omega-3 fatty acids may offer a more comprehensive approach to T2DM management. This combination demonstrates synergistic effects on glycemic control, insulin resistance, dyslipidemia, and molecular markers associated with T2DM. Additionally, the preservation of body weight and nutritional status highlights the potential benefits of omega-3 fatty acids in counteracting T2DM-associated weight loss [18-20].

Conclusion

It is concluded that, our study supporting the combined use of metformin and omega-3 fatty acids as a potential therapeutic strategy for T2DM. This combination demonstrates significant improvements in glycemic control, insulin resistance, dyslipidemia, and molecular markers associated with T2DM.

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