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Full Length Research Article

INTERMITTENT FASTING MODULATES GLYCATED HEMOGLOBIN, SERUM INSULIN, AND HEXOKINASE IN STZ-INDUCED DIABETIC MALE WISTAR RATS

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ABSTRACT
Intermittent fasting (IF) is a recent non-pharmacological treatment used to manage type 2 diabetes mellitus (T2DM). Nevertheless, its relative effectiveness compared with conventional pharmacotherapy and its effects on major glycolytic enzymes are not well documented. This study was therefore designed to investigate the effects of various IF regimes on selected glycemic indicators and hepatic hexokinase activity in streptozotocin (STZ)-induced diabetic Wistar rats. The Wistar rats were induced with Type 2 diabetes by administering fructose followed by one low-dose injection of STZ (35 mg/kg). The animals were randomly assigned to six groups (n=3), namely: non-diabetic control, untreated diabetic control, metformin-treated (30 mg/kg), and three IF regimen groups (8, 12, and 15 hours daily). Serum glycated hemoglobin (HbA1c), insulin, and hepatic hexokinase activity were tested after the intervention period. Group E (12-hour IF regimen) had the greatest metabolic changes, with a significant decrease in HbA1c ($p<0.05$), an increase in serum insulin levels near metformin, and the highest significant increase ($p<0.05$) in hepatic hexokinase activity compared to other IF regimens and the untreated diabetic control. The 15-hour IF was less effective. The general glycemic control of 12-hour IF was similar to that of metformin. A middle-range 12-hour intermittent fasting diet is effective in glycemic control, restoration of insulin levels, and hepatic hexokinase activity in STZ-induced diabetic rats. The findings indicate an ideal fasting period with maximum metabolic advantage, thereby confirming IF as a powerful non-pharmacological approach, possibly through mechanisms such as improved glycolysis and increased insulin sensitivity.

Keywords: Diabetes mellitus, Intermittent fasting, Hexokinase, Streptozotocin.

INTRODUCTION

Over 100 million individuals globally have been affected by diabetes mellitus, which is also reported as one of the leading causes of death across the globe (Harding *et al.*, 2024). Diabetes mellitus is a metabolic disorder characterized by high fasting blood sugar, low insulin secretion, and insulin receptor insensitivity (Hudish et al., 2019). Hexokinase is an enzyme responsible for the phosphorylation of glucose, which is important for glucose uptake (Wasserman, 2022). It is known as an important enzyme that aids the capture and clamping of glucose from outside cells. Therefore, regulating the activity of hexokinase is vital for controlling blood glucose in diabetes mellitus (Shamansurova et al., 2019). A sustained increase in blood glucose levels can cause glucose to attach to the free amino group at the N-terminus of the beta chain of hemoglobin, independent of enzymatic action, resulting in the formation of glycosylated hemoglobin (Ajayi & Timothy, 2024). This explains the elevated levels of glycosylated hemoglobin in diabetes. Glycated hemoglobin (HbA1c) formation occurs in a non-enzymatic manner involving the binding of glucose to the N-terminal valine of the β-chain of hemoglobin. This results in the formation of a stable ketoamine through Amadori rearrangement (Ajayi & Timothy, 2024). Glycated hemoglobin reflects average blood glucose over the lifespan of erythrocytes (~120 days), making it a suitable marker of long-term glycemic control. When consistently high, HbA1c is closely linked with chronic diabetic complications, including retinopathy, nephropathy, neuropathy, and cardiovascular diseases (Sartore *et al*., 2023). These complications result from advanced glycation end products (AGEs), oxidative stress, and inflammation, which contribute to endothelial dysfunction and tissue damage. Glycosylated hemoglobin reflects the concentration of fasting and postprandial glucose for a period of 3 months, with the onset of glycosylation occurring from the 6th week of continuous high blood sugar (Ajayi & Timothy, 2024). Plant extracts and other pharmacological interventions that can lower glycosylated hemoglobin levels could be useful in the management of diabetes. Diabetes mellitus gradually results in damage to vital organs such as the nerves, kidneys, eyes, and even the heart. The majority of diabetic patients have type 2 diabetes mellitus, which is characterized by low insulin production by pancreatic beta cells. This ultimately progresses to insulin tolerance (Rachdaoui, 2020).

Intermittent fasting involves reducing caloric intake intermittently (Duregon *et al*., 2021). This includes several hours daily or a full 24-hour period, as seen during religious seasons such as Lent or Ramadan, or for health reasons such as weight loss. When used for health reasons, intermittent fasting involves withholding of food for several hours during the day, a full day, once a week, or twice a week (Grajower & Horne, 2019). Yuan *et al*. (2022) noted insulin resistance in individuals who practiced short-term starvation. Several studies have suggested that IF may serve as an alternative to drugs in the management of diabetes, owing to its ability to improve insulin sensitivity and muscle glucose uptake, thereby enhancing glucose utilization (Corley et al., 2018; Kumar et al.,

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2019). The literature is replete with medicinal plants and their anti-diabetic potential. However, there is a paucity of information on the role of IF in ameliorating type 2 diabetes mellitus by improving glycated hemoglobin, hexokinase, and serum insulin levels.

MATERIALS AND METHODS

Eighteen healthy Wistar rats weighing 121.20 ± 2.25 g were purchased from the animal holding unit of the Department of Biochemistry, Kaduna State University. The rats were kept in a well-ventilated cage with a temperature of 25℃ – 27℃, a photoperiod of 12 hours light and dark, and a relative humidity of 45% – 50%. Animal handling was conducted in accordance with the National Institutes of Health guidelines for the handling and use of laboratory animals. The rats were kept for 1 week to acclimate with access to a standard rat chow diet and unlimited water. The 18 Wistar rats involved in this study were randomly selected into six groups (A, B, C, D, E, F), each having three animals as described:

Group A- Negative Control (Distilled water and rat chaw)
Group B- Positive control (Diabetic, non-treated)
Group C- Standard drug (Diabetic +30mg/kg body weight of Metformin)
Group D- Diabetic + 8 hours IF
Group E- Diabetic + 12 hours IF
Group F- Diabetic + 15 hours IF

Diabetes Induction

Type 2 diabetes mellitus was induced in the animals in groups B, C, D, E, and F by administering fructose: 10% w/v in drinking water for 7 days (ad libitum) followed by a single 35 mg/kg intraperitoneal administration of STZ (fresh in 0.1 M citrate buffer, pH 4.5), given after the 7-day fructose period. The rats were thereafter allowed unlimited access to glucose water to avoid hypoglycemia. After 72 hours of STZ administration, diabetes was diagnosed, and the rats with a fasting blood glucose level of more than 200 mg/dl were confirmed as diabetic. This was done using the One Touch UltraEasy Blood Glucose Monitoring System and a glucometer after blood was drawn from the tail vein. The non-diabetic groups received an intraperitoneal injection of freshly prepared 0.1 M citrate buffer (pH 4.5) without STZ.

Drugs/Chemicals

Streptozotocin (STZ) was obtained from Peace Standard Pharmaceutical Ind. Ltd, Ilorin, Kwara State, while Metformin was obtained from Dialogue Pharmaceuticals, Kaduna, Kaduna State. The kits used for the assay of glycated hemoglobin, serum insulin, and Hexokinase were products of Elabscience. Other reagents used in this study were of the highest grade and prepared following strict specifications using distilled water and calibrated glassware.

Preparation of Serum and tissue homogenate

The procedure described by Yakubu and Salimon (2016) was adopted to prepare serum and tissue supernatants. At the end of the intermittent fasting intervention, the animals were sacrificed. Diethyl ether fumes were used to anesthetize the animals; the jugular veins were cut, and 6 mL of blood was collected into plain tubes. The collected blood was centrifuged at 1252 × g for 12 minutes, after which the serum was collected and stored at 4 °C until use for analysis. The liver tissues were excised, properly washed with normal saline, and allowed to dry on filter paper. Teflon homogenizer (Heidolph Silent Crusher M) was used to homogenize the tissues, which were then centrifuged at 10,000g for 15 min at 4°C.

Determination of Hexokinase, glycated hemoglobin, and serum Insulin

Hexokinase activity was assayed using a spectrophotometer to measure the absorbance of the sample as described by Scheer *et* al (1978). Glycated hemoglobin was assayed spectrophotometrically as described by Aba and Azuzu (2018). Serum insulin was measured using the ELISA method (ALPCO insulin rat) as described by Al-Quraishy et al. (2015).

Statistical analysis

The results obtained were expressed as means ± standard error of the means (SEM). Data were analyzed using one-way analysis of variance. Duncan's post hoc test was used to compare significance between groups, using the Statistical Package for the Social Sciences (SPSS) version 26 (SPSS Inc., Chicago, IL, USA).

RESULTS

The results are presented in figures. Figure 1 shows the effect of intermittent fasting on glycated hemoglobin of diabetic Wistar rats. From the results, serum glycated hemoglobin was significantly reduced ($P<0.05$) in group E and F treated with 12 and 15 hours of intermittent fasting respectively when compared to groups A, B, C and D. Similarly, there was a significant increase ($P<0.05$) in serum insulin level in the animals in groups C and E when compared to group B and no significant difference in the serum insulin level of groups D and F compared to group B (Figure 2). The effect of intermittent fasting on liver hexokinase was depicted in Figure 3. The results showed a significant increase ($P<0.05$) in liver hexokinase activity in group E compared with groups B, C, D, and F. However, the increase in liver hexokinase activity in group E was comparable to that in group A.

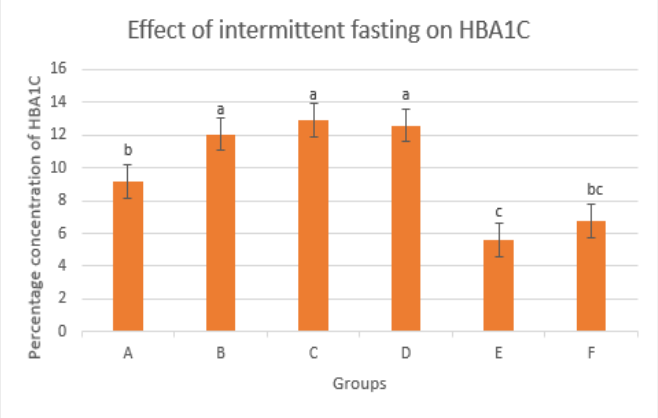

Figure 1: Effect of Intermittent Fasting on serum glycated hemoglobin level of STZ-induced diabetic Wistar rats

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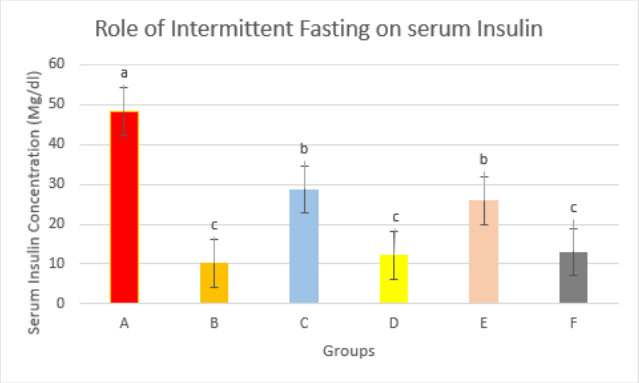

Figure 2: Effect of Intermittent Fasting on serum insulin level of STZ-induced diabetic Wistar rats

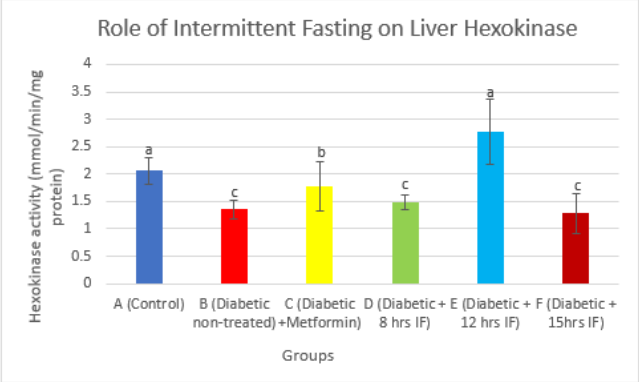

Figure 3: Effect of Intermittent Fasting on liver Hexokinase activity of STZ-induced diabetic Wistar rats

DISCUSSION

Our results revealed that intermittent fasting (IF) reduced serum glucose and HbA1c and enhanced hepatic hexokinase activity and serum insulin levels in STZ-induced diabetic Wistar rats. Predictably, the STZ administration induced hyperglycaemia with a similar pattern of type 2 diabetes mellitus through the destruction of the pancreatic beta-cells, consequently lowering the concentration of insulin. This was clear in the untreated diabetic (Group B) with high serum glucose and HbA1c levels, and low serum insulin levels and hexokinase activity.

After treatment with different intermittent fasting periods, the most favourable metabolic change was observed in Group E (12-hour IF). The animals treated with 12-hour IF demonstrated a significant decrease in HbA1c, a significant rise in hexokinase activity, and a recovery of insulin levels to almost metformin levels. These effects observed for the duration of the study indicate that moderate fasting periods could be better glucose regulators than sustained fasting periods. The STZ-mediated reduction in hexokinase activity in diabetic rats is consistent with other findings indicating that STZ inhibits glycolytic enzymes through a process involving insulin resistance (Yoopum et al., 2023). The concept of intermittent fasting has been investigated as a non-pharmacological treatment method that can revitalize the β-cell activity and improve pancreatic insulin secretion (Patel et al., 2024). This is a possibility, as indicated by the higher serum insulin levels after 12 hours of IF in the current study, and could be attributed to reduced hyperglycaemia.

The mechanistic explanation for the improved glycaemic profile could be the observed rise in hepatic hexokinase activity. The initial step in glycolysis is catalyzed by hexokinase, which facilitates hepatic glucose uptake and converts glucose into glucose-6-phosphate; thus, upregulation decreases the amount of glucose in the blood that can be glycated (Aedh *et al*., 2023). The coincidental decrease in serum insulin in some IF groups indicates increased peripheral insulin sensitivity, consistent with prior reports that time-restricted feeding supports insulin receptor signaling, leading to a decline in fasting glucose and improved metabolic control of enzymes (Al Sarayreh et al., 2025).

The effect of IF also seems to be complementary to that of metformin, which mainly lowers glycaemia by reducing hepatic gluconeogenesis and increasing peripheral insulin sensitivity (Barroso *et al*., 2024). Metformin suppresses endogenous glucose production, whereas IF promotes glycolytic flux by elevating hexokinase activity and possibly reinstating beta-cell responsiveness. The reduced effect of the 15-hour IF (Group F) over the 12-hour regimen indicates that there might be a U-shaped relationship: moderate fasting maximizes the metabolic benefits, but over time, the counter-regulatory hormones (e.g., glucagon and cortisol) are activated, which may neutralize the positive effects (Herman *et al*., 2025). The combination of a decreased HbA1c, improved insulin levels, and higher hexokinase activity indicates increased insulin sensitivity and greater hepatic glucose phosphorylation.

The small sample size (n = 3 per group) in the study could limit the statistical power and generalizability. However, the 12-hour intermittent fasting diet yielded the best reproducible results in glycaemic regulation and liver hexokinase activity in STZ-diabetic rats and was equivalent to those of metformin. Such findings are consistent with emerging evidence on time-restricted feeding and support further mechanistic research into IF as a supplemental treatment for diabetes management.

The study demonstrates that a 12-hour intermittent fasting regimen significantly improves glycaemic control and promotes liver hexokinase activity in STZ-induced diabetic rats, with effects comparable to metformin. These findings imply that intermittent fasting could be a promising alternative therapeutic strategy in the management of diabetes; however, a larger-scale study is needed to validate the mechanism of action

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