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Influence of various intermittent fasting regimens on body weight and glycemic control in streptozotocin-induced diabetic rats

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Abstract. – OBJECTIVE: This study aims to compare the effects of various intermittent fasting (IF) regimens, i.e., time-restricted fasting (TRF), alternate day fasting (ADF), and periodic fasting (PF) on body weight, glycemic control and associated metabolic parameters in streptozotocin-induced diabetic rats.

MATERIALS AND METHODS: Sixty male Sprague-Dawley rats (aged 3 months) were randomly assigned to the normal control (NC), diabetic control (DC), TRF, ADF, and PF groups. Type 2 diabetes was induced in all groups, except for the NC group, by intramuscular administration of streptozotocin (55 mg/kg). The IF interventions were administered for 6 weeks.

RESULTS: The rats in all the groups, except for the NC group, exhibited significant weight loss (31.4%, 46.4%, 31.0%, and 33.9% in the DC, TRF, ADF, and PF groups, respectively). The fasting blood glucose levels decreased to varying degrees, with the PF group showing the most significant decrease (77.0%), followed by the ADF (55.0%) and TRF (32.2%) groups. The plasma insulin levels were significantly lower in the experimental groups than in the NC group, but no significant effects were observed on the lipid profile.

CONCLUSIONS: The study findings indicate that while the IF protocols led to body weight loss, they exhibited varying effects on glycemic control and other metabolic parameters.

Key Words:
Intermittent fasting, Calorie restriction, Restricted eating, Alternate-day fasting, Periodic fasting, Glycemic control, Body weight, Weight loss, Diet, Diabetes.

Introduction

Type 2 diabetes mellitus (T2DM) is a common metabolic syndrome characterized by hyperglycemia and is associated with individual lifestyle and genetic variables. It results in reduced glucose utilization due to impaired insulin secretion and action, as well as systemic low-grade inflammation[1,2]. Globally, T2DM affects over 100 million people and is considered one of the leading causes of mortality[3,4]. T2DM is typically managed with glucose-lowering medications and with the use of insulin therapy in some cases[5,6]. However, intensive lifestyle intervention, including dietary energy restriction, is considered first-line treatment in the management of T2DM[7]. Literature has shown that intensive lifestyle intervention, when accompanied by a substantial weight loss, can improve glycemic control and thus reduce the metabolic complications of diabetes[8,9].

Food restriction, which leads to calorie restriction, is defined as a reduction in food consumption while maintaining basic nutritional levels[10]. It has long been established that calorie restriction helps control body weight and improve metabolic health[11]. Importantly, in human and animal studies[2,12-15] conducted on T2DM, food restriction has been reported to enhance pancreatic beta-cell function and blood glucose homeostasis. Furthermore, calorie restriction is the only scientifically validated strategy that has been shown to reduce

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insulin resistance and islet dysfunction, as well as slow aging[16]. In one such study, Larson-Meyer et al[17] found that a 25% calorie reduction, either through diet alone or a combination of diet and exercise, improved insulin sensitivity but decreased β-cell sensitivity in glucose-tolerant individuals who were overweight.

Several obesity experiments have shown that humans face challenges in maintaining daily calorie restriction over long periods of time[18]. However, intermittent fasting (IF) has been reported to improve compliance and has shown potential for improving metabolic risk factors, body composition, and weight loss in individuals with obesity[18-21]. It has been demonstrated that these favorable benefits are partly attributed to the shift in the body's preferred fuel source during fasting from glucose to fatty acids and ketones[18]. This transition in fuel source, which is known as metabolic reconditioning, has been identified as a potential basis for many of the therapeutic effects of IF. Ultimately, IF has been reported to reduce adiposity, particularly visceral and truncal fat, by creating modest energy deficits[22,23]. This weight loss may lead to increased levels of and sensitivity to leptin and adiponectin in patients, thus improving appetite control and reducing chronic inflammation and, thereby, lowering multiple risk factors for T2DM[24,25].

However, IF is associated with complications, such as hypoglycemia, ketoacidosis, dehydration, hypotension, and thrombosis, in individuals with diabetes[26,27]. Further, short-term fasting can lead to insulin resistance in humans[28], and the glucose utilization and blood pressure-lowering effects of time-restricted eating may worsen during eating periods, potentially due to changes in circadian rhythm[29]. Despite the acute complications and insulin resistance observed with short-term starvation in humans[28], exercise and IF have been recognized as important non-pharmacological tools for diabetes management and are accepted as adjunctive therapy for managing T2DM.

IF is a broad term encompassing various fasting interventions and eating patterns that involve consuming very few to no calories for periods ranging from 12 hours to several consecutive days[18]. One such IF regimen is alternate-day fasting, in which days of fasting are separated by days of *ad libitum* food consumption. Another approach is periodic fasting, in which individuals fast for two days a week and consume food *ad libitum* for the remaining five days. Time-restricted feeding is another IF regimen wherein food consumption is limited to a specific window of time each day, and it often involves daily fasting periods ranging from 16 to 20h[30]. While several studies[31] on humans and animals have demonstrated the impact of IF on weight loss, glucose control, cardiovascular health, and brain function, there is limited research regarding the comparative effects of different forms of IF on T2DM. In order to fill in this research gap, the present study aimed to evaluate the effects of different IF regimens on body weight, glycemic control markers, and lipid profiles in Sprague-Dawley rats with streptozotocin-induced diabetes.

Materials and Methods

Materials

Streptozotocin and the formaldehyde were obtained from Cayman Chemical (Ann Arbor, Michigan, USA) and Merck (Darmstadt, Germany), respectively. Analysis reagents for the measurement of plasma glucose, triglycerides (TGs), high-density lipoprotein (HDL-C), low-density lipoprotein (LDL-C), total cholesterol, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were obtained from HUMAN Diagnostics (Germany). C-reactive protein (CRP) was evaluated qualitatively using the Latex Kit from Atlas Medical GmbH (Germany). The plasma insulin and leptin concentrations were measured using the competitive inhibition and sandwich ELISA kits, respectively, which were purchased from Cloud-Clone Corp., USA. Colorimetric kits were obtained from Ben S.r.l. Biochemical Enterprise (Italy) to quantitatively evaluate β-hydroxybutyrate (β-HBA) levels in plasma samples. All other chemicals and reagents used in the study were acquired from Sigma-Aldrich (USA).

Animals and Experimental Regimens

Sixty male Sprague-Dawley rats, aged 3 months and weighing 100-140 g, were acquired from the animal house of the College of Pharmacy at King Saud University in Riyadh, Saudi Arabia. The rats were housed in pathogen-free polyacrylic cages, with three rats per cage. They were provided with commercially available high-fat laboratory chow and had access to drinking water *ad libitum* for a duration of 4 weeks. The cages were placed in a well-ventilated animal house maintained at a temperature of 25ºC ± 2ºC, a relative humidity of 45%-55%, and a 12-h

photoperiod cycle. Throughout this period, the animals were weighed weekly, and the energy requirement (ER) was determined based on the average energy intake monitored over the course of 4 weeks.

Induction of Diabetes and Experimental Design

The rats were divided into one control group and four experimental groups, each consisting of 12 animals. Diabetes was induced in the experimental group animals, who were fasted overnight by a single intramuscular injection of streptozotocin (55 mg/kg) dissolved in freshly prepared citrate buffer (0.1 M, pH 4.5). The control group rats were administered the same dose of citrate buffer. To prevent hypoglycemia, the rats were given free access to a 10% glucose solution for 24 hours after the streptozotocin injection[32]. Diabetes was confirmed 72 h later with a glucometer (Accu-Chek; Roche, Germany) that was used to measure glucose levels in blood samples obtained through tail vein puncture. Rats with blood glucose levels exceeding 450 mg/dL were randomly assigned to different experimental groups, each consisting of 8 animals.

The following treatment regimens were followed for the control and experimental groups over a period of 6 weeks. Apart from this regimen, the rats in all the groups were given free access to potable drinking water:

- Group 1: This was the normal control (NC) group, in which non-diabetic rats received 100% of their ER.
- Group 2: This was the diabetic control (DC) group that comprised diabetic rats receiving 100% ER.
- Group 3: In the time-restricted feeding (TRF) group, diabetic rats received 50% ER on a daily basis for 8 hours a day and fasted during the remaining 16 hours.
- Group 4: In the alternate day fasting (ADF) group, diabetic rats were fasted on alternate days, where they received 50% ER. They received 100% ER on the remaining days.
- Group 5: In the periodic fasting (PF) group, diabetic rats received 100% ER for five consecutive days and 50% ER for two consecutive days each week.

After grouping, blood glucose levels were monitored on a weekly basis in all the animals in each group with a glucometer (Accu-Chek) through samples obtained by tail vein puncture. Similarly, body weight measurements were conducted weekly and recorded for all the animals in each group over the 6-week intervention period. After the 6-week study period, rats that were fasted overnight were humanly anesthetized *via* inhalation of carbon dioxide (CO_2). The rats were sacrificed, and blood was drawn from the orbital sinus using glass capillaries into EDTA-K2 microtubes and then centrifuged (2,500 rpm for 20 min) to separate the plasma. The plasma was then used for determining the levels of glucose, insulin, TGs, HDL-C, LDL-C, total cholesterol, ALT, AST, ALP, leptin, CRP, and β-HBA according to the protocols provided with the respective assay kits. The rats were then dissected, and the internal organs, including the liver, spleen, and kidneys, were immediately excised and weighed using an electronic laboratory weighing scale. The pancreas was also removed and placed in a 10% formaldehyde solution for histopathological investigation.

Histopathological Analysis

Portions of formaldehyde-fixed pancreatic tissues were longitudinally trimmed and subsequently dehydrated with increasing alcohol concentrations (50-100%), and this was followed by rinsing with xylene. Next, the dehydrated tissue pieces were embedded in paraffin to create blocks. These paraffin blocks were then sliced into sections with a thickness of 4 mm using an automated microtome. Finally, the sections were stained with hematoxylin and eosin, and evaluated under a microscope (Olympus Engineering Co., Ltd.) at a power of 200x to assess histopathological changes.

Statistical Analysis

All statistical analyses were conducted using GraphPad Prism (GraphPad Prism LLC.). Biochemical data are presented by their mean ± SEM values, while mean ± SD values present body weight. One-way analysis of variance was used to determine significant differences between various groups at a 95% confidence level ($p \leq 0.05$), and it was followed by Tukey's post-hoc test.

Results

Body Weight and Food Intake

The current study was conducted in two phases. In the first phase, 60 rats were randomly assigned to 5 groups, each consisting of 12 rats. All the groups received a commercial high-fat

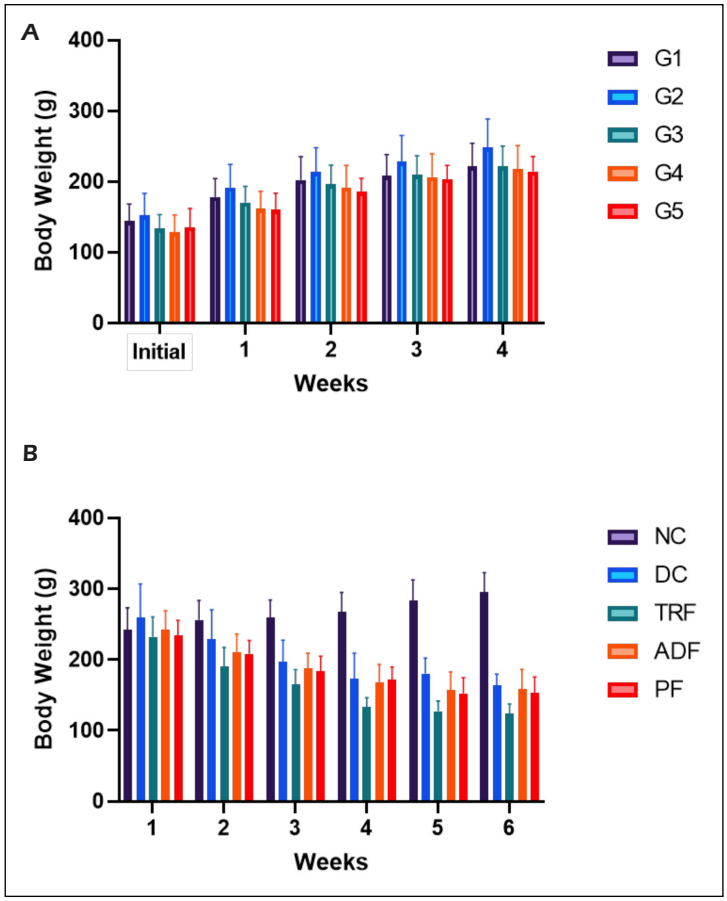

Figure 1. Changes in body weight in all the groups during the pre-intervention and six-week intervention periods. **A**, Body weight changes during the pre-intervention phase, that is, the 4-week high-fat diet feeding period. **B**, Changes in body weight during the 6-week experimental intervention in the control and experimental groups. The values plotted are mean ± SD. NC, normal control; DC, diabetic control; TRF, time-restricted feeding; ADF, alternate day fasting; PF, periodic fasting (n = 8 for each group).

diet (comprising 35% fat and 14% protein) and had *ad libitum* access to drinking water for four weeks. During this period, their daily food intake and weekly body weights were monitored. The rats consistently showed an increase in body weight, at a rate of 25% per week: their body weight doubled within 4 weeks till it reached 230-280 g (Figure 1a). The food intake during this period was calculated per kilogram of body weight, and the average daily intake ranged between 75 g and 85 g/kg of body weight, with a mean daily intake of 80 g/kg.

In the second phase, diabetes was induced in all the groups except for the NC group. Each experimental group consisted of 8 rats with blood glucose levels above 450 mg/dL. During this phase, all the groups, except for the NC group, showed significant weight loss over the 6-week study period (Figure 1b).

When data were expressed as the percentage difference in body weight at the end of the six-week intervention compared to the measurement taken at the first week, weight loss was found to be 31.4%, 46.4%, 31.0%, and 33.9% in the DC, TRF, ADF, and PF groups, respectively, and the changes were significantly greater than that in the NC group ($p < 0.0001$) (Figure 2). The percentage of body weight loss in the TRF group was significantly greater than that in the DC group ($p < 0.0001$), but no significant differences ($p > 0.05$) were observed between the other experimental groups (Figure 2).

Body Organ Weights

Table I displays the relative weights of different internal organs, namely, the liver, kidney, and spleen, expressed in relation to 100 g of body weight. It can be observed that there was no significant difference in liver and kidney weights (expressed as a percentage of the total body weight) between the groups. However, spleen weight was significantly ($p < 0.01$) lower in the DC, TRF, and PF groups than in the NC group.

Fasting Blood Glucose Levels

As depicted in Figure 3, in the NC group, the blood glucose levels consistently ranged between 85 and 107 mg/dL, while in the DC group, the mean fasting blood glucose was significantly

higher than that in the other groups ($p < 0.001$) and ranged between 555 and 581 mg/dL. However, in the three IF groups, blood glucose levels exhibited a decline to varying degrees over the course of 6 weeks. The greatest decrease in mean blood glucose was observed in the PF group (77.0%), and it was followed by the ADF (55.0%) and TRF (32.2%) groups (Figure 3).

Plasma Insulin Concentrations

The plasma insulin levels in the DC group were significantly lower than those in the control group, and a similar trend was observed in all the experimental groups ($p < 0.05$) (Figure 4). However, the insulin levels in the experimental groups were found to be significantly ($p < 0.05$) higher than those in the DC group. Interestingly, the PF group exhibited restoration of plasma insulin levels to near-normal levels, and this coincided with the restoration of fasting blood glucose levels to near-normal levels (Figure 4).

Plasma Lipid Profile

As shown in Figure 5, there was a significant increase in TG levels ($p < 0.001$) in the DC group compared to the NC group. In the fasting groups TRF and PF, TG levels were restored to normal

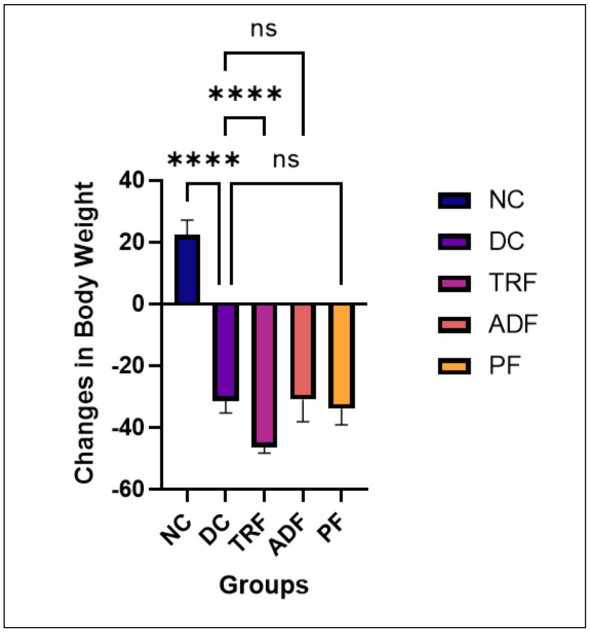

Figure 2. Percentage changes in body weight after the six-week experimental intervention. Body weight changes are expressed as percentage (%) difference after the intervention period compared to the weight in the first week of the intervention. The values plotted are mean ± SD. ****$p < 0.0001$; ns = not significant ($p > 0.05$). NC, normal control; DC, diabetic control; TRF, time-restricted feeding; ADF, alternate day fasting; PF, periodic fasting (n = 8 for each group).

Table I. Organ weights of different groups expressed as a percentage of the total body weight.

Groups	Liver	Kidney	Spleen
NC	3.7 ± 0.62	0.68 ± 0.11	0.24 ± 0.02
DC	3.4 ± 0.19	0.80 ± 0.13	0.14[a] ± 0.03
TRF	3.5 ± 0.28	0.85 ± 0.15	0.16[a] ± 0.04
ADF	3.2 ± 0.70	0.65 ± 0.06	0.19 ± 0.05
PF	3.4 ± 0.34	0.80 ± 0.14	0.11[a] ±0.03

Values are expressed as mean ± SD. a significantly different compared to the NC group at $p < 0.01$. NC, normal control; DC, diabetic control; TRF, time-restricted feeding; ADF, alternate day fasting; PF, periodic fasting (n = 6 for each group).

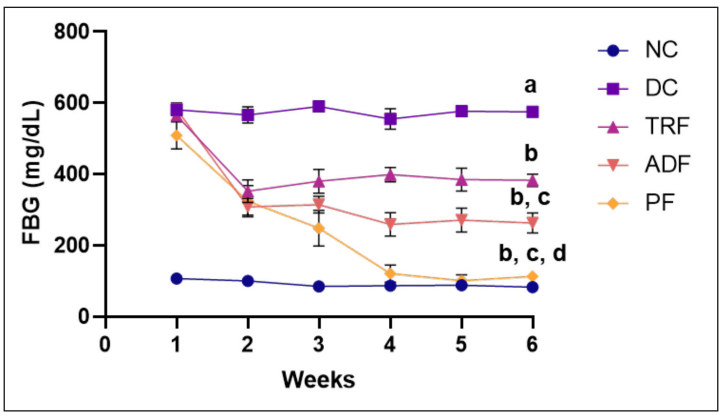

Figure 3. Fasting blood glucose levels over the six-week intervention period. The values plotted are mean ± SEM. a significantly different from the NC group at $p < 0.0001$; b significantly different from DC group at $p < 0.0001$; c significantly different from TRF group at $p < 0.0001$; d significantly different from the ADF group at $p < 0.0001$. NC, normal control; DC, diabetic control; TRF, time-restricted feeding; ADF, alternate day fasting; PF, periodic fasting (n = 8 for each group).

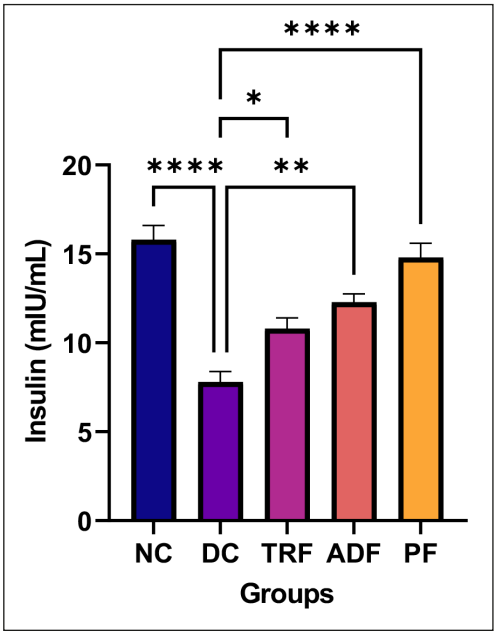

Figure 4. Plasma insulin concentrations after the six-week experimental intervention. The values plotted are mean ± SEM. *$p < 0.05$; **$p < 0.01$; ****$p < 0.0001$. NC, normal control; DC, diabetic control; TRF, time-restricted feeding; ADF, alternate day fasting; PF, periodic fasting (n = 6 for each group).

levels, but this was not observed in rats of the ADF group. In terms of total cholesterol levels, a significant ($p < 0.05$) increase was observed in all the experimental groups compared to the NC group. When compared to the DC group, both the TRF and PF groups exhibited comparable total cholesterol levels. The LDL-C levels did not significantly differ between any of the study groups. The HDL-C levels were the highest in the ADF group, and it was followed by the PF group. The HDL-C levels in the TRF and DC groups were comparable but higher than those in the control group. The HDL-C levels were significantly ($p < 0.05$) higher in the DC and all the fasting groups compared to the NC group, with the ADF group showing significantly ($p < 0.05$) higher levels than the DC group (Figure 5).

Plasma Leptin Concentrations

The leptin levels were significantly ($p < 0.05$) lower in the DC and TRF groups than in the NC group (Figure 6). Surprisingly, the plasma leptin levels were the highest in the ADF group and were significantly ($p < 0.001$) higher than that in all the groups. However, the leptin levels in the PF group did not differ significantly compared to the NC group (Figure 6).

Liver Function Profile

The levels of various indicators of liver function and the inflammatory marker CRP are presented in Table II. It was observed that the induction of diabetes resulted in an increase in the levels of AST, ALT, and ALP to varying degrees in all the experimental animals compared to the control animals, but the levels in the fasting groups were lower than those in the DC group. Within the experimental groups, the levels of AST, ALT, and ALP were the lowest in the ADF group. However, CRP was not detected in any of the groups.

Plasma β-HBA Concentrations

The levels of β-HBA were measured as an indicator of fatty acid oxidation, and the results are presented in Figure 7. The β-HBA levels were significantly ($p < 0.05$) lower in the NC group than in all the other groups, but they were not significantly different ($p > 0.05$) between the DC, TRF, ADF, and PF groups.

Table II. Effect of various intermittent fasting regimens on liver function markers.

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)	CRP
NC	29.2 ± 4.6	32.5 ± 1.3	7.0 ± 0.83	Negative
DC	65.1[a] ± 8.9	54.0[a] ± 6.2	21.0[a] ± 0.74	Negative
TRF	35.7[b] ± 4.7	47.2[a] ± 2.8	17.0[a,b] ± 1.06	Negative
ADF	24.0[b] ± 2.8	42.4 ± 2.4	15.0[a,b] ± 0.94	Negative
PF	26.4[b] ± 0.7	47.5[a] ± 1.3	18.0[a] ± 0.75	Negative

Values are expressed as mean ± SEM. [a]significantly different from the NC group at $p < 0.01$; [b]significantly different from the DC group at $p < 0.05$. NC, normal control; DC, diabetic control; TRF, time-restricted feeding; ADF, alternate day fasting; PF, periodic fasting (n = 6 for each group). ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; CRP, C-reactive protein (qualitative assay).

Histopathology Findings

When micrographs of the histological sections of the pancreas from different groups were observed, the sections from the NC group revealed dark pancreatic acini and pale islets of Langerhans (Figure 8). Conversely, the sections taken from the DC group exhibited pale islets of Langerhans with vacuolation and congested blood vessels (Figure 8; DC). Similar observations were made in the TRF group, where the pancreatic acini and islets of Langerhans displayed some vacuolation, congested blood vessels with inflammatory cells (mainly lymphocytes), and collagenous fibers around the blood vessels (Figure 8; TRF). In the photomicrographs from the ADF group, dark acini and dilated blood vessels accompanied by some inflammatory cells were observed (Figure 8; ADF). In the PF group, the pancreas exhibited dark acini, and the pancreatic islets were found to be rich in blood capillaries (Figure 8; PF).

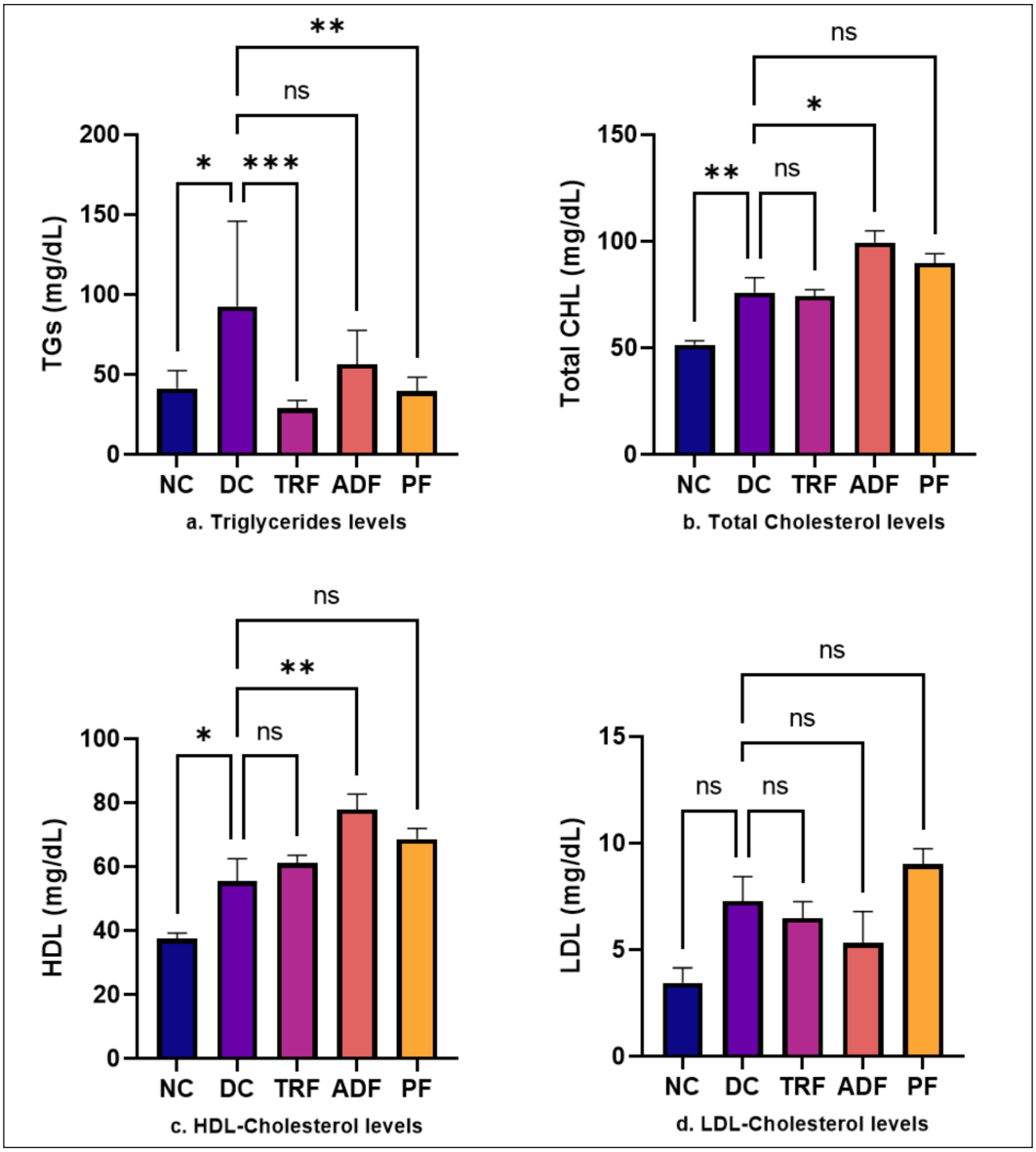

Figure 5. Plasma lipid profile levels after the six-week experimental intervention. The trends in TG, total cholesterol, HDL-C, and LDL-C after 6 weeks of intervention are depicted in panels **a**, **b**, **c**, and **d**, respectively. The values plotted are mean ± SEM. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns = not significant ($p > 0.05$). NC, normal control; DC, diabetic control; TRF, time-restricted feeding; ADF, alternate day fasting; PF, periodic fasting (n = 6 for each group). TGs, triglycerides; total CHL, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

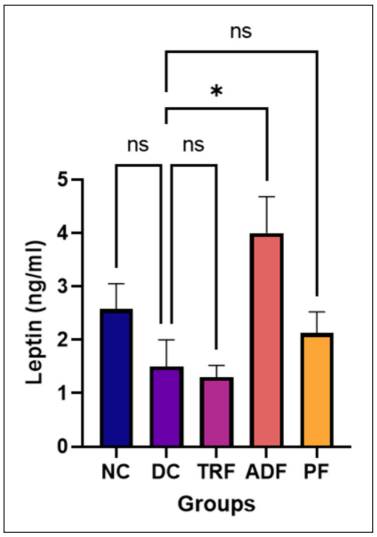

Figure 6. Plasma leptin concentrations after the six-week experimental intervention. The values plotted are mean ± SEM. *$p < 0.05$, ns = not significant ($p > 0.05$). NC, normal control; DC, diabetic control; TRF, time-restricted feeding; ADF, alternate day fasting; PF, periodic fasting (n = 6 for each group).

Discussion

The current study examines the pathophysiological and metabolic profiles, as well as histological features, of diabetic rats that were subjected to various forms of IF. To the best of our knowledge, this is the first investigation to compare the effects of various IF regimens, so the results would be useful as a basis for future animal and human studies seeking to find the optimal IF regimen. The results of our study demonstrated the effectiveness of various forms of IF, namely time-restricted feeding (TRF), alternate-day fasting (ADF), and periodic fasting (PF), in reducing plasma glucose levels to varying degrees compared to untreated diabetic rats. These findings are in alignment with previous studies[33-36] investigating the impact of IF on blood glucose levels in rats. For example, Hsu and associates[37] demonstrated that TRF with 18 h of daily fasting improved glycemic levels in diabetic rats. Additionally, a study by Antoni et al[38] reported that IF, a form of TRF, had beneficial short- and medium-term effects on glucose and lipid homeostasis. In contrast, Varady et al[19] found that 8 weeks of ADF had no effect on glucose homeostasis; thus, their results indicated that ADF might not improve blood glucose levels. Even though the present findings are promising, it should be noted that none of the IF regimens resulted in normalized blood glucose levels or a complete reversal of the negative effects of diabetes on body and organ weights. Therefore, further long-term studies (conducted over more than 12 months) are necessary to explore the safety and effectiveness of IF as a weight loss and glucose and lipid metabolism control strategy.

Even though the rats in the IF groups in the present study exhibited weight loss, the weights of the liver, kidney, and spleen of diabetic rats were not significantly affected by any of the regimens. However, the spleen weight was significantly lower in the diabetic control (DC) rats and the rats on the TRF and PF regimens than in the NC group. Similar to our findings, Shawky et al[39] reported that IF caused a significant decrease in body weight. Yet, they reported that it led to a significant decrease in liver and stomach weights. The differences between the two studies could be attributable to the restriction on water intake during fasting times in the Shawky et al[39] study, while water was available all the time to the rats in the current study, which maintained extracellular fluid volume, thus preventing dehydration[40]. The study of Shawky et al[37] also reported that IF led to a significant increase in packed cell volume,

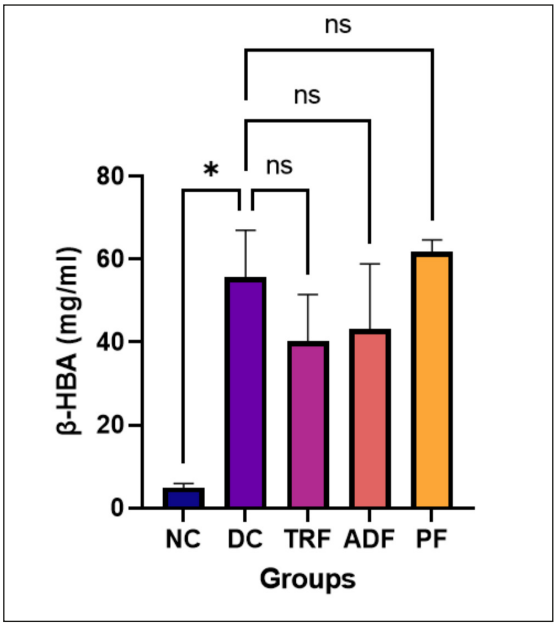

Figure 7. Plasma β-HBA concentrations after the six-week experimental intervention. Values are expressed as mean ± SEM. *$p < 0.05$, ns = not significant ($p > 0.05$). NC, normal control; DC, diabetic control; TRF, time-restricted feeding; ADF, alternate day fasting; PF, periodic fasting (n = 6 for each group).

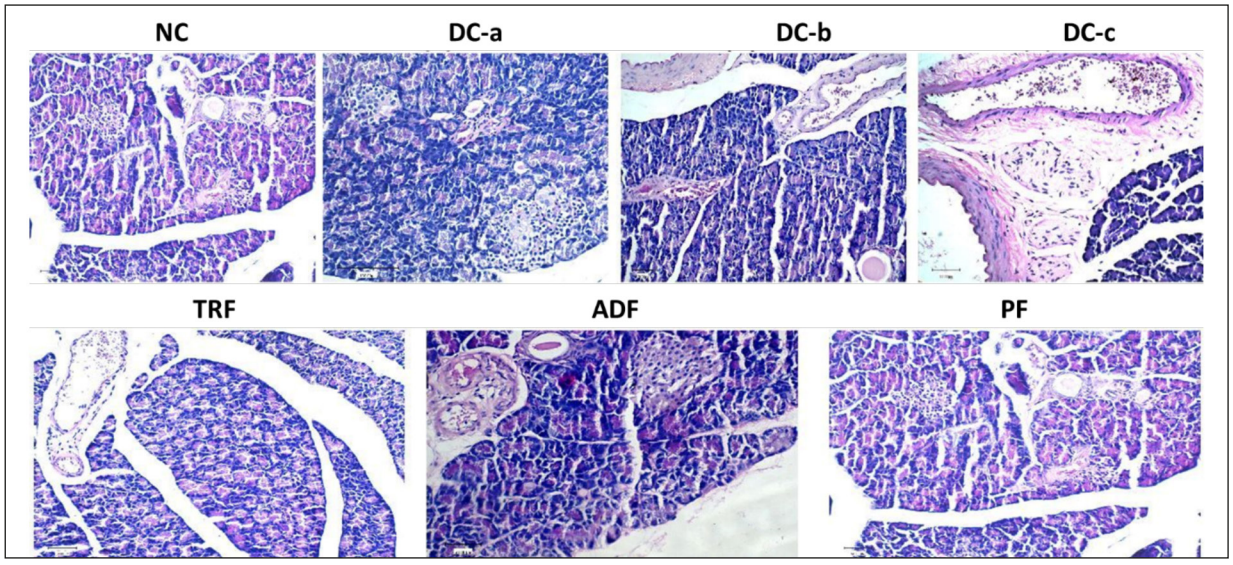

Figure 8. Histological micrographs of pancreatic sections after the six-week experimental intervention. Magnifications, 200x. NC, normal control; DC, diabetic control; TRF, time-restricted feeding; ADF, alternate day fasting; PF, periodic fasting.

neutrophil phagocytic activity, phagocytic index, and brain neurotransmitters (serotonin and norepinephrine), but these parameters were not examined in the present study. Thus, future research should investigate how different IF regimens may affect parameters related to body fluids balance and neurotransmitters.

Reduced energy intake through IF may lead to long-term reductions in insulin production, as observed in this study when the IF groups were compared with the NC group. Further, the increase in plasma insulin levels found in the diabetic rats in this study is consistent with earlier findings, which showed that IF boosted insulin levels and improved glucose tolerance by enhancing β-cell mass[34,41]. A comparison of leptin levels revealed that while leptin levels were increased in the ADF and PF groups, the increase was significant only in the ADF group. Further, it decreased in the TRF group. This could be attributed to prolonged fasting and starvation, as this group consumed only 50% of their daily calorie intake during the feeding period, i.e., 8 h. Therefore, TRF may not be an ideal IF regimen for diabetic rats, as lower leptin levels increase appetite and reduce energy expenditure. On the other hand, ADF and PF may have helped to maintain energy and prevent excessive fat loss through increased leptin levels. However, their effects need to be confirmed and clarified in the long term.

In this study, we did not observe significant effects of the various IF regimens on the lipid profiles of the experimental groups. However, previous animal studies[42,43] have reported improved lipid profiles in rats fed a high-fat diet following IF. This improvement may be attributed to the lipolysis process, which involves decreased insulin levels and fasting that leads to the release of stored fat from adipose tissue into the bloodstream[44,45]. This effect was probably not observed in our study because of insulin concentrations in the IF groups being restored to near-normal levels.

Consistent with other studies[46-49], we observed an increase in the ketone body β-HBA in all the fasting groups. This suggests that some of the health benefits of IF may include reduction of inflammation and amelioration of metabolic disorders such as T2DM and obesity[50-52]. Indeed, studies utilizing ketogenic diets, which lead to the elevation of ketone bodies serving as the main source of energy[53,54] fuel, have shown to induce therapeutic effects such as reducing oxidative stress and improving insulin sensitivity. Thus, the elevated ketone bodies found in IF groups, as a result of energy restriction, may have contributed to enhancing glycemic control[55-57].

Limitations

To the best of our knowledge, this study is the first to examine the effects of various forms of IF on different metabolic markers. However, it has certain limitations. First, the study did not investigate the underlying mechanisms behind the observed results. Additionally, body composition,

liver lipid metabolism, glucose control mechanisms, and measurements of adipose tissue (both visceral and subcutaneous fat) were not considered. Nevertheless, energy intake is an important factor associated with body weight control and metabolic regulation and has been rarely considered and accounted for in IF studies. This is an important strength of this study, as calorie consumption and food intake were strictly controlled according to the IF protocol of each group.

Conclusions

The study findings highlight the significant potential of various IF regimens in counteracting the negative impact of diabetes, particularly by enhancing insulin sensitivity. The findings demonstrated that all the IF strategies were promising in terms of weight loss. However, while all the regimens led to significant weight loss in diabetic rats, they exhibited varying effects on glycemic control and associated metabolic parameters. This implies that the appropriate IF regimen should be selected with caution and be based on individual conditions. Moreover, the intricate mechanisms of dietary strategies need to be fully understood in diabetic rats, and therefore, more extensive research is needed to compare the long-term effects of various IF regimens and their influence on weight management and diabetes markers.

Conflict of Interest
The authors declare that they have no conflict of interests.

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Authors' Contributions
HA conceptualization, HA, and AH designed the study. HA and FA authored, analyzed, interpreted the data, and prepared the manuscript. HA and FA conducted animal experiments. FA, AZ, AA. and AH contributed to laboratory assessments. All authors have read and agreed to the published version of the manuscript.

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Data Availability
The data presented in this study are available on request from the corresponding author.

Ethics Approval
All experiments in the present study were approved by the Institutional Animal Ethics Committee, the Deanship of Scientific Research, and the College of Pharmacy at Qassim University, Saudi Arabia [Approval ID 2020-CP-12] for project number 10138-cavm-2020-1-3-I.

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