Influence of Various Intermittent Fasting Regimens on Obese Diabetic Female Rats

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ABSTRACT

This study aims to evaluate the effects of various intermittent fasting (IF) regimens, i.e., time-restricted fasting (TRF), alternate day fasting (ADF), and periodic fasting (PF) on obese diabetic rats. Forty adult male albino rats weighing 150 ± 10 g used in this study. After the first week of adaptation rats were randomly divided into 5 equal groups (n=8). Group 1 was fed on ba basal diet (as a negative control group) (NC); Group 2 was fed on a high-fat diet (obese diabetic only) (as a positive control group), while the other three groups were fed on (HFD + IF regimens, i.e. (TRF, ADF, and PF), respectively. 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 4 weeks. Results revealed that the rats in all the treated groups, exhibited significant weight loss in the TRF, ADF, and PF groups, respectively, as compared to positive groups (obese diabetic group). The blood glucose levels decreased to varying degrees, with the PF group showing the most significant decrease (28.03%), followed by the ADF (15.79%) and TRF (32.22%) groups. The plasma insulin levels and insulin tolerance, leptin concentration, liver and kidney functions in addition to lipid profile were improved. In addition, antioxidants enzymes (CAT, SOD and GP_X) were significantly ($P<0.05$) increased while, significantly reduced MDA as compared to the positive control group. 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.

Keywords: Intermittent fasting, Calorie restriction, Restricted eating, Alternate day fasting, Periodic fasting, Glycemic control, Body weight, obesity, Diabetes.

INTRODUCTION

Obesity is a recognized global disease that continues to be a risk factor for chronic medical conditions **(Mechanick *et al.*, 2020)**. Obesity is a global health issue that affects individuals of all ages, genders, and socioeconomic backgrounds. It is associated with various health risks and can increase the likelihood of developing several chronic conditions **(Xihua and Hong, 2021)**. Type 2 Diabetes: Obesity is strongly linked to insulin resistance, a condition where the body's cells become less responsive to insulin, leading to high blood sugar levels and an increased risk of developing diabetes **(Yohannes, 2020)**. The primary causes of obesity are an imbalance between calorie intake and expenditure, influenced by factors such as genetics, environment, lifestyle choices, and socioeconomic status. Poor dietary habits, a lack of physical activity, sedentary lifestyles, and consuming high-calorie processed foods contribute to weight gain **(Al Kibria, 2019)**.

Type 2 diabetes mellitus (T2DM) is a metabolic disorder that results from insulin depletion and/or failure of tissues to respond to insulin (insulin resistance) and metabolize glucose, which ultimately results in chronic hyperglycemia **(Galicia *et al.*, 2020)**.T2DM is known to disrupt carbohydrate, lipid, and protein metabolism and have severe consequences, including long-term complications in many vital organs **(Hoyer, 2004)**. The global incidence of T2DM is drastically escalating, and its prevalence is estimated to reach more than 200 million by 2035 (International Diabetes Federation, Diabetes Atlas 2015). The progressive effects of T2DM extend beyond the disease manifestation itself, with complications that affect the structure and function of other physiological systems and organs, resulting in cerebrovascular dysfunction, renal failure, visual impairment, sexual dysfunction, and dementia **(Oh *et al.*, 2016 and Cholerton *et al.*, 2016)**.

Intermittent fasting (IF) is indeed a nutritional intervention that focuses on the timing of meals rather than specific food restrictions. It involves cycling between periods of fasting and eating, which can vary in duration and frequency depending on the specific fasting protocol followed **(Kristin *et al.*, 2021)**. Intermittent fasting is practised in many common ways. One such strategy is the 16/8 method, which involves cutting meals to an 8-hour window each day after 16 hours of fasting, alternate-day fasting (alternating between fasting days and normal eating days), and the 5:2 method (eating normally for five days of the week and restricting calorie intake on two non-consecutive days) **(Dong *et al.*, 2020)**. The main principle behind intermittent fasting is to extend the period of time between meals, which can lead to various physiological changes in the body. During the fasting period, the body depletes its glycogen stores and starts utilizing stored fat for energy. This may result in decreased body weight and improved blood lipid profiles and insulin sensitivity, two metabolic health indicators **(Razzak and Naz, 2017)**.

IF has been shown to have beneficial effects in diabetes, and it has even been suggested as a potential treatment for T2DM **(Gabel *et al.*, 2019 and Alfheeaid *et al.*, 2023)**. However, the underlying mechanisms remain unclear. 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 **(Chen *et al.*,2023)**. In addition, IF decreases hyperglycemia, improves insulin sensitivity, decreases islet hypertrophy, protects β-cell identity and function, and further exerts peripheral benefits in the liver **(Patel *et al.*, 2024)**. 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 **(Anton *et al.*, 2018)**. 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 **(Catenacci *et al.*, 2016)**. This weight loss may lead to increased levels of sensitivity to leptin and adiponectin in patients, thus improving appetite control and reducing chronic inflammation and, thereby, lowering multiple risk factors for T2DM **(Wooten *et al.*, 2022)**.

The aim of the study

This study aimed to evaluate the effects of various intermittent fasting (IF) regimens on obese diabetic rats.

MATERIALS AND METHODS

MATERIALS:

Chemicals: Casein, cellulose, choline chloride powder, and DL- methionine powder were purchased from Morgan Co. in Cairo, Egypt. Streptozotocin (STZ, 99% purity) was supplied by Sigma-Aldrich. All other chemicals used were analytical grade and also obtained from Sigma-Aldrich. Starch, soybean oil and sucrose were obtained from the local market.

Kits for blood analysis were purchased from Alkan Company for Biodiagnostic Reagents, Dokki, Cairo, Egypt.

Animals: 40 adult male rats (Sprague Dawley strain) weighing 150±10 g were obtained from the National Research Center, Dokki, Egypt.

METHODS:

Experimental Design

The experimental animals were done using (n=40) male rats, with body weight (150±10) g. The rats were housed in cages under hygienic conditions, in a temperature-controlled room at 25°C. Rats were fed a basal diet for one week for adaptation. The present study was performed using a basal diet, the American Institute of Nutrition AIN-93G purified rat diet or a high-fat diet (HFD; modified Western diet with 45% fat (animal lipid) for a duration of 4 weeks. **(AIN 1993)**, vitamin and mineral mixtures were prepared as described by **Reeves *et al*., (1993)**. The basal diet provides 2.9 kcal/g (fat constitutes 13% of total energy), and the HFD provides 4.73 kcal/g (fat constitutes 45% of total energy).

The control group received only the basel diet. Hyperglycemia was induced in the other groups by an IM injection of STZ (55 mg/kg b.w.) in freshly made 0.1 M citrate buffer (pH 4.5). To avoid hypoglycemia, these rats were given unrestricted access to standard rats' chow during the night after being injected with streptozotocin in a solution of saccharose (10 g/100 ml). Diabetes was diagnosed 72 h after STZ injection in rats with a fasting blood glucose level of more than 200 mg/dl. This was done with the OneTouch Ultra

Easy Blood Glucose Monitoring System and a glucometer after blood was expressed from the tail vein. Four weeks after STZ injections, the treatments began **(Al Aamri and Ali, 2017)**.

Rats were randomly assigned to different experimental groups, each consisting of 8 rats. The following treatment regimens were followed for the control and experimental groups over a period of 4 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, a group of healthy rats fed a basal diet and represented a control negative group.

Group 2: Obese diabetic rats fed on a basal diet only as a positive control group.

Group 3: In the time-restricted feeding (TRF) group, obese diabetic rats were fed on a basal diet daily for 8 hours a day and fasted during the remaining 16 hours.

Group 4: In the alternate day fasting (ADF) group, obese diabetic rats were fasted on alternate days, where they fed on a basal diet made up of exciting 24 hours followed by a 24-hour dinner period by substituting daily fasting. They fed on a basal diet on the remaining days.

Group 5: In the periodic fasting (PF) group, (alternate-day fasting and the 5:2 method); obese diabetic rats were fed on a basal diet for five consecutive days of the week and restricted calorie intake on two non-consecutive days.

Nutritional evaluation:

The biological evaluation of the diet was carried out by determination of feed intake, body weight gain percent (BWG %) and feed efficiency ratio (FER) according to **Chapman, (1959)** using the following equation:

$$\textbf{BWG}\ \% = \frac{\text{Final body weight} - \text{Initial body weight}}{\text{Initial body weight}} \times 100$$

FER = Weight gain (g) / Feed intake (g)

Blood Collection and Serum Separation:

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 28-day intervention period. At the end of the experimental period (28 days), rats were fasted overnight, then the blood was collected under slight ether anesthesia. Serum was separated by centrifugation at 2500 rpm for 20 min. The obtained serum was used immediately for routine laboratory investigation.

Biochemical Analysis:

Glucose was determined according to **Trinder, (1959)** and insulin was determined according to **Matthews *et al.*, (1985)**.

Liver Function:

Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured according to (**Bergmeyer *et al.*, 1978)**, Alkaline phosphates (ALP) was determined according to **Belfield and Goldberg (1971)**. Serum bilirubin was measured by **Weissman *et al.*, (1950).**

Kidney function: Serum urea **(Kaplan, 1984)**, uric acid **(Patton and Crouch, 1977)** and creatinine were measured according to **(Murray, 1984)**.

Serum Lipid Profile:

Serum total cholesterol (TC) **(Richmond, 1973)**, triglycerides (TG) **(Wahlefeld, 1974)**, and high-density lipoprotein (HDL) **(Albers *et al.*, 1983)** were determined. Meanwhile, low density lipoprotein (LDL) and very low-density lipoprotein (VLDL) were calculated according to **Fridewald *et al.*, (1972)**.

$$\textbf{LDL-c} = \textbf{TC-[HDL-c} + \textbf{(TG/5)]}$$
$$\textbf{VLDL-c} = \textbf{TG/5}$$

Antioxidant Enzymes

The serum level of malondialdehyde (MDA) was calculated to measure lipid peroxidation and was determined according to **Draper and Hadley (1990).** Glutathione (GSH) was measured by methods of **Moin, (1986).**

Statistical analysis:

Statistical analysis was performed using the SPSS computer program (Graph pad software Inc, San Diego, CA, USA). One-way analysis of variance (ANOVA) followed Duncan's multiple tests was done. $P \leq 0.05$ was significant **(Armitage and Berry, 1987)**.

RESULTS AND DISCUSSION

As shown in **Table (1)** there has been no significant variation in IBW among all groups. Regarding FBW, +ve control group had a significant ($p<0.05$) increase in the final body weight (FBW) as compared to the –ve group. Restricted feeding (TRF), alternate day fasting (ADF) and periodic fasting (PF) caused a significant decrease in FBW as compared to the positive control group.

As regards to BWG%, there was a significant increase in the positive (+ve) control group in comparison to the negative (-ve) control group. The results show the TRF, ADF, PF groups had a significant decrease in BWG% compared with the positive control group. The results show a significant decrease in BWG% between groups fed on TRF,ADF, PF group. The best outcomes had been found in the group fed on restricted feeding (TRF) groups. Control negative groups had FI (g/d/rat) lower than that of the positive control group, according to outcomes.

Regarding FER, the primary value of the negative control group had been significantly lower than that of the positive control group at $p < 0.05$. The

TRF, ADF, and PF diet groups had had a significant decrease ($p < 0.05$) in the mean value of FER as compared to the positive control group. A significant decrease had shown up in mean values of FER for rats that fed on TRF diet groups and was considered the best result compared to positive group . It was noticed that the group fed on TRF had the highest in decrese records for FBW, BWG%, FI and FER.

These results were in harmony with several researches by **Alfheeaid, et al., (2023)** obtained that the rats in all the groups exhibited significant weight loss (46.4%, 31.0%, and 33.9% in the TRF, ADF, and PF groups, respectively). **Abou Bakr et al., (2024)** showed rats were fed 50%f, 20%p, 30%c, fasting (24 h of fasting non-consecutive day/week) combination with basal diet caused a significant decrease ($P<0.05$) in weight gain, feed intake, compared to the control group (–ve). These results agreed with those reported by **Xiaolin et al., (2019) and Park et al., (2020)** found that a significant ($P\leq0.05$) decrease in body weight, feed intake and peritoneal fat pad for the group of rats fed on (HFD) combination with fasting. Also, **Marinho et al. (2019) ; Liu et al., (2019)** found that HFD-IF (HFD; 43% fat) (IF; 24-hour fast on 3 nonconsecutive days per week) caused significantly decrease ($P< 0.05$) in body weight, feed intake compared to the negative control group.

Different mechanisms may explain the effects of IF on metabolism. The organism uses fat pad depots for energy during IF, decreasing adipose tissue and softening the inflammatory profile. Also, caloric restriction positively affects metabolism, helping especially prediabetes and insulin-resistant patients without any pharmacologic approach possibly because of Forkhead Box A genes **(Marinho et al. 2019)**. The combination of HFD and IF could be be an improvement of the ketosis case, **Lichtash et al., (2020)** found the combination of HFD and every-other-day fasting which has been developed for improvement of the ketosis, could accelerate ketosis, indicating its stronger ketogenic ability.

Table (1): Effect of intermittent fasting on body weight status of obese diabetic rats

Parameters / Groups	IBW g	FBW g	FI (g/d/rat)	BWG%	FER
Control (-Ve)	186.8±1.77[a]	233.4±1.43[b]	20	24.96±0.77[b]	0.083±0.002[b]
Control (+Ve)	187.4±1.87[a]	251.6±1.74[a]	21	34.27±0.74[a]	0.109±0.002[a]
TRF	186.8±1.39[a]	119.4±0.92[e]	11	-36.08±0.21[e]	-0.145±0.001[d]
ADF	186.2±1.74[a]	146.6±1.93[c]	13	-21.27±0.54[c]	-0.072±0.001[c]
PF	187.4±1.07[a]	140.6±102[d]	15	-24.96±0.68[d]	-0.074±0.001[c]

Data are expressed as mean ± SE.
Means with different superscript letters in the column are significantly differences at ($P < 0.05$).

As shown in **Table (2)** results exhibited the effect of intermittent fasting on leptin, blood glucose, insulin hormone, and insulin tolerance of obese diabetic rats. STZ injection significantly increased mean leptin and glucose values in the control positive group compared to the control negative group according to results. While leptin and glucose levels had been decreased significantly at ($p<0.05$), in restricted feeding (TRF), alternate day fasting (ADF) and periodic fasting (PF) diet groups as compared to the positive control group. Restricted feeding (TRF) diet groups had shown significant reduction and best outcomes in leptin and glucose as compared to positive control group. It was observed that highest glucose reduction had been recorded in the group fed on TRF by 32.22%. These results agreed with those reported by **Marinho et al., (2019)** showed that significant decrease of leptin was seen in the HF-IF animals, probably linked with the improvement of carbohydrate metabolism and expression of inflammatory markers in this group. 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.

These results agreed with those reported by **Alfheeaid, et al., (2023)** showed that 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. In the same line **Hsu et al., (2021)** demonstrated that TRF with 18 h of daily fasting improved glycemic levels in diabetic rats. Additionally, a study by **Antoni et al., (2017)** reported that IF, a form of TRF, had beneficial short- and medium-term effects on glucose and lipid homeostasis.

Outcomes also showed that there had been a significant decrease ($p < 0.05$) in the value of insulin and insulin tolerance in the control positive group as compared to the control negative group. Restricted feeding (TRF), alternate day fasting (ADF) and periodic fasting (PF) diet groups had shown significant increases in insulin and insulin tolerance as compared to the control positive group. The highest increasing of insulin was recorded at ADF by 63.09%. These results were in harmony with several researches by **Abou Bakr et al., (2024)** showed rats fasting (24 h of fasting non-consecutive day/week) which fed on (50% F, 20% P, 30%C) combination with basal diet caused a significant decrease (P<0.05) in serum (glucose, insulin, and leptin level. **Alfheeaid, et al., (2023); Joaquim et al., (2022); Park et al., (2020)** found IF combination with HFD caused decreased in glucose, insulin and leptin levels. **Xiaolin et al., (2019)** suggested that the combination of HFD (65%Kcal from fat) and every-other-day fasting improvement of the ketosis.

Table (2): Effect of intermittent fasting on Leptin, blood glucose, insulin hormone and Insulin Tolerance of obese diabetic rats

Parameters / Groups	Leptin ng/mL	Glucose mg/dl	Glucose reduction (%)	Insulin uIU/ml	Insulin Increment (%)	Insulin Tolerance mmol/L-1 min1
Control (-Ve)	10.10±0.38[b]	91.83±0.35[e]	-	1.79±0.05a	-	0.049±0.001a
Control (+Ve)	15.73±0.47[a]	168.11±0.90[a]	-	0.55±0.02e	-	0.015±0.0007e
TRF	7.49±0.19[d]	113.49±0.43[d]	**32.22**	1.03±0.03d	**46.91**	0.035±0.001b
ADF	8.99±0.18[c]	141.57±0.59[b]	**15.79**	1.49±0.02b	**63.09**	0.019±0.001d
PF	8.19±0.17[cd]	120.98±0.67[c]	**28.03**	1.23±0.02c	**55.57**	0.025±0.001c

Data are expressed as mean ± SE.
Means with different superscript letters in the column are significantly differences at (P < 0.05).

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 obese 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 **(Salama et al., 2022)**.

Outcomes recorded at **Table (3)** illustrated the effect of intermittent fasting on serum liver function of obese diabetic rats. There was a significant increase at (p<0.05) in the mean value of AST, ALT and ALP in the control positive group as compared to the control negative group. Rats had a significant decrease at ($p<0.05$) in mean values of liver function levels, when they fed on restricted feeding (TRF), alternate day fasting (ADF) and periodic fasting (PF) diet, as compared to the control positive group. The best outcomes were recorded at the group fed on restricted feeding (TRF) in AST and ALP levels, while the best outcomes were recorded at the group fed on periodic fasting (PF) in ALT.

These results were in harmony with several researches by **Abou Bakr et al., (2024)** showed rats fasting (24 h of fasting non-consecutive day/week) which fed on (50% F, 20% P, 30%C) combination with basal diet caused a significant decrease ($P<0.05$) in serum ALT, AST level compared to the control group (–ve). **Alfheeaid, et al., (2023); Ismail et al., (2022) and Shin et al., (2018)** reported that IF improve the liver enzyme in serum and improve liver damage index compared to ad libitum. **Marinho et al. (2019)**a demonstrated that IF lessens markers of injury and treats steatosis in the liver elevated levels of ALT and AST observed in HFD (only) were controlled by HFD combination with IF.

Table (3): Effect of intermittent fasting on Liver Function of obese diabetic rats

Parameters / Groups	AST (μ /L)	ALT (μ /L)	ALP mg/dL
Control (-Ve)	22.05±0.58e	31.13±0.71d	116.58±0.69d
Control (+Ve)	38.98±0.33a	51.93±0.50a	162.39±0.74a
TRF	26.21±0.49d	34.93±0.51c	137.26±0.94c
ADF	32.18±0.52b	37.73±0.86b	158.98±1.09b
PF	30.58±0.27c	32.42±0.38cd	155.78±0.93b

Data are expressed as mean ± SE.
Means with different superscript letters in the column are significantly differences at ($P < 0.05$).

Results recorded at **Table (4)** illustrated the effect of intermittent fasting on serum kidney function of obese diabetic rats. There was a significant increase ($p<0.05$) in the mean value of urea, creatinine and uric acid in the control positive group as compared to the control negative group. These results agreement **(Ma *et al.*, 2022; Negm, (2020)** observed that injection with STZ significantly increase ($P\leq0.05$) the level of urea and creatinine, compared to the control negative group.

Rats had significant decrease at ($p<0.05$) in mean values of kidney function levels, when they fed on restricted feeding (TRF), alternate day fasting (ADF) and periodic fasting (PF) diet, as compared to the control positive group. The best outcomes were recorded at the group fed on restricted feeding (TRF) in urea, creatinine and uric acid levels. These results were in harmony with several researches by **Abou Bakr et al., (2024)** showed rats fasting (24 h of fasting non-consecutive day/week) which fed on (50% F, 20% P, 30%C) combination with basal diet caused a significant decrease ($P<0.05$) in serum uric acid, creatinine compared to the control group (–ve).

The obtained results were in harmony with research by **Gouda and Aljuhani, (2023); Alfheeaid, et al., (2023)** who revealed that intermittent fasting caused reduction and improvement in level of uric acid, creatinine. **Shahhat et al., (2022)** showed that the intermittent fasting of rats with ulcerative colitis significantly ($P < 0.05$) decreased both uric acid and creatinine serum levels as compared to the control group. These results are in accordance with previous study by **Ismail et al., (2022)** who reported that Islamic fasting models as model for intermittent fasting reported that, there was a decrease in uric acid and creatinine levels in the fasting treated groups, this finding indicates the role of IF "Islamic fasting models" improvement to lower uric acid and creatinine although complexity of the diet "high-fat-high fructose" induced rats.

Table (4): Effect of intermittent fasting on Kidney Function of obese diabetic rats

Parameters / Groups	**Urea** (mg/dl)	**Creatinine** (mg/dl)	**Uric Acid** (gm/dl)
Control (-Ve)	23.32±0.38e	0.67±0.03e	2.78±0.05d
Control (+Ve)	38.75±0.32a	1.70±0.01a	4.60±0.12a
TRF	29.22±0.73d	0.85±0.09d	3.08±0.04c
ADF	36.05±0.45b	1.33±0.05b	4.04±0.05b
PF	32.08±0.75c	0.95±0.01c	3.94±0.07b

Data are expressed as mean ± SE.
Means with different superscript letters in the column are significantly differences at (P < 0.05).

Outcomes recorded at **Table (5)** illustrated the effect of intermitting fasting on serum lipid profile (TC, TG, HDL.C, LDL-C & VLDL-C) in obese diabetic rats. Mean value of (TC, TG, LDL-C & VLDL-C) in the positive control groups had significantly higher (p<0.05) than in the negative control group. These results agreement with **Negm and El-Soadaa, (2020); El-Soadaa, & Negm, (2019)** observed that HFD fed on significantly increase (P≤0.05) the level of lipid profile compared to the control negative group.

Rats had significant decrease at (p<0.05) in mean value of TC, when fed on restricted feeding (TRF), alternate day fasting (ADF) and periodic fasting (PF) diet, as compared to the control positive group. No significant difference (p<0.05) in TC level among tested levels of restricted feeding (TRF), alternate day fasting (ADF) and periodic fasting (PF) groups. The best results were recorded at the periodic fasting (PF) groups of TC, LDL-c. The best result had been recorded at TRF diet groups of VLDL-c. On the other hand, it was observed a significant reduction at ($p<0.05$) in the mean value of HDL-C of the control positive group as compared to the control negative group. As compared to control positive group, rats which had been fed on restricted feeding (TRF), alternate day fasting (ADF) and periodic fasting (PF) diet groups had significant increase at ($p<0.05$) in mean value of HDL-C as compared to the control positive group. These results were in harmony with several researches by **Abou Bakr et al., (2024)** showed the treated groups with fasting for 24 hours and fed on high level of fat (20,30,40 and 50) had significantly ($P<0.05$) increase TC,TG,LDL,VLDL levels and decrease the HDL level in serum compared to positive control group. **Ismail et al., (2022)** reported that IF improve the lipid profile, diminishing plasma levels of TG, TC and the accumulation of hepatic TG, and liver steatosis independent of the diet. **Negm, (2023); Ahmed et al., (2021)** conducted that intermittent fasting could be adopted as a lifestyle intervention for the prevention, management and treatment of cardiovascular disorders by raising the sub-optimal HDL. These results are consistent with the study conducted by **Abbasi et al., (2020)** who showed that IF caused decrease in TC,TG,LDL,VLDL levels and enhance HDL level in serum when rats were fed on diet content of 20% from fat. These results are in the same line with **Marinho et al. (2019)**; **Shin et al., (2018) ; Wilson et al., (2018)** showed that IF/HFD caused a decrease in LDL,TC and increase in HDL compared to the control groups. These results demonstrate the effectiveness of IF despite the simultaneous intake of high-fat diet. 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 **(Kolb et al., 2021; Kersten, 2022)**.

Table (5): Effect of intermittent fasting on Lipid Profile of obese diabetic rats

Parameters / Groups	TC mg/dl	TG mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl
Control (-Ve)	138.60±0.81c	91.85±0.40e	32.50±0.50a	87.29±0.41c	18.37±0.08e
Control (+Ve)	183.95±1.91a	121.35±1.10a	23.13±0.49e	136.55±0.77a	24.27±0.22a
TRF	164.85±0.49ab	98.78±1.30d	26.93±0.24b	118.15±0.54ab	19.75±0.46d
ADF	161.67±0.38b	114.22±0.95b	23.04±0.48d	115.78±0.39ab	22.84±0.10b
PF	159.90±0.68b	108.80±0.68c	25.41±0.37c	112.72±0.38b	21.67±0.13c

Data are expressed as mean ± SE.
Means with different superscript letters in the column are significantly differences at ($P < 0.05$).

As shown in **Table (6)** the effect of intermittent fasting on antioxidant enzymes of obese diabetic rats is illustrated. The current study indicated that the level of oxidative stress parameters such as MDA increased in obese diabetic rats and some antioxidant parameters such as CAT, SOD and GP_X, decreased when compared to the control group. In many studies, oxidative stress has been demonstrated to participate in the progression of diabetes, which plays an important role in diabetes, including impairment of insulin action and the increased complication incidence rate **(Asmat *et al.*, 2016)**. Thus, oxidative stress seems to be more worrying about metabolic disorders, especially diabetes **(Ceriello *et al.*, 2016)**.

In contrast, in the present study, rats fed on restricted feeding (TRF), alternate day fasting (ADF) and periodic fasting (PF) diet groups increased the antioxidants enzymes (CAT, SOD and GP_X) but significantly reduced MDA in comparison to the +ve control group. The best outcome had been recorded at TRF diet groups in antioxidants enzymes (CAT, SOD and GP_X). 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 **(Wang and Wu, 2022; Vasim et al., 2022)**. Indeed, studies utilize ketogenic diets, which lead to the elevation of ketone bodies serving as the main source of energy fuel. **Bendridi et al., (2022); Garcia et al., (2020)** 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 **(Alfheeaid, et al., (2023); Min et al., 2018; Polidori et al., 2018)**.

Table (6): Effect of intermittent fasting on antioxidants enzymes of obese diabetic rats

Parameters / Groups	MDA ng/ml	CAT U/ml	SOD U/ml	GPx U/ml
Control (-Ve)	118.78±0.52e	2.83±0.15a	136.26±1.07a	15.99±0.79a
Control (+Ve)	402.25±0.68a	0.95±0.05c	38.23±0.40d	4.10±0.05d
TRF	355.81±0.91d	1.67±0.68b	94.76±0.35b	10.87±0.54b
ADF	382.41±1.55b	1.43±0.19b	91.31±0.31c	7.55±0.32c
PF	364.13±1.66c	1.31±0.04b	92.56±0.61bc	8.56±0.27c

Data are expressed as mean ± SE.
Means with different superscript letters in the column are significantly differences at ($P < 0.05$).

Conclusion:

The study findings highlight the significant potential of various IF regimens in counteracting the negative impact of obese diabetic rats. 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 obese 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 obese 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.

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تأثير أنظمة الصيام المتقطع المختلفة على إناث الفئران البدينة المصابة بالسكرى

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الملخص العربي

تهدف هذه الدراسة إلى تقييم آثار أنظمة الصيام المتقطع (IF)، وهو الصيام المقيد زمنيًا (TRF)، والصيام المتقطع يومًا بعد يوم (ADF)، والصيام الدوري (PF)، على فئران مصابة بالسمنة والسكر. أُجريت الدراسة على أربعين فأرًا بالغًا من الذكور الألبينو، بوزن (١٥٠ ± ١٠ جرام). بعد الأسبوع الأول من التكيف، قُسِّمت الفئران عشوائيًا إلى خمس مجموعات متساوية (عددها ٨). تلقَّت المجموعة الأولى نظامًا غذائيًا أساسي (كمجموعة ضابطة سالبة) (NC)؛ خضعت المجموعة الثانية لنظام غذائي غني بالدهون (كمجموعة ضابطة إيجابية) (مصابون بالسمنة والسكر فقط)، بينما خضعت المجموعات الثلاث الأخرى لنظام غذائي غنى بالدهون مع تطبيق نظام الصيام المتقطع (TRF، ADF، وPF)، على التوالي. تم احداث الإصابة بمرض السكر من النوع الثاني في جميع المجموعات، باستثناء المجموعة الضابطة السالبة، عن طريق الحقن العضلي بالستربتوزوتوسين (٥٥ مل جم/كجم). تم تطبيق تدخلات الصيام المتقطع لمدة ٤ أسابيع. أظهرت النتائج أن الفئران في جميع المجموعات، باستثناء المجموعات الضابطة السالبة، أظهرت فقدانًا ملحوظًا في الوزن في مجموعات TRF وADF وPF، على التوالي) مقارنةً بالمجموعات الضابطة الموجبة (المصابون بالسمنة والسكر). انخفضت مستويات السكر في الدم بدرجات متفاوتة، حيث أظهرت مجموعة TRF أكبر انخفاض (٣٢,٢٢٪)، تليها مجموعتا PF (٢٨,٠٣٪)، ADF ١٥,٧٩ %). تحسنت كلا من مستويات الأنسولين في البلازما وتحمل الأنسولين، وتركيز اللبتين، ووظائف الكبد والكلى ومستوى الدهون، كما ارتفعت إنزيمات مضادات الأكسدة (CAT، وSOD وGPx) بشكل ملحوظ (P<0.05)، بينما انخفضت بشكل ملحوظ مستويات MDA مقارنةً بالمجموعة الضابطة الموجبة. تشير نتائج الدراسة إلى أنه على الرغم من أن بروتوكولات الصيام المتقطع أدت إلى فقدان وزن الجسم، إلا أنها أظهرت تأثيرات متفاوتة على التحكم في نسبة السكر في الدم وغيرها من المؤشرات الأيضية.

الكلمات المفتاحية: الصيام المتقطع، تقييد السعرات الحرارية، تناول الطعام المقيد، الصيام يومًا بعد يوم، الصيام المتقطع، التحكم في نسبة السكر في الدم، وزن الجسم، السمنة، مرض السكري.