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The Bioactive Effects of Intermittent Fasting as a Diet Regime on Obese Rats

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Abstract

Intermittent fasting (IF) is an increasingly popular method of weight loss. The present study conducted to investigate the bioactive effects of intermittent fasting as a diet regime on obese rats. Forty-two adult male albino rats weighting (185± 10 g.). After the first week of adaptation rats were randomly divided into 6 equal groups (n=7). Group 1 was fed on basal diet (as negative control group). Groups of rats (2:6) were fasted 24 hours for 3 non-consecutive day/week. Group 2 was fed on basal diet (as positive control group), group 3 was fed on 20%, 20%, and 60% of energy from fat, protein, and carbohydrate, respectively, group 4 was fed on 30%, 20%, 50% of energy from fat, protein, and carbohydrate, respectively, group 5 was fed on 40%, 20%, 40% of energy from fat, protein, and carbohydrate, respectively, group 6 was fed on 50%, 20%, 30% of energy from fat, protein, and carbohydrate, respectively. Rats weighed twice a week and weight gain was calculated. At the last week of the feeding trial, 3 rats from each group were injected using 0.1 ml formalin (4%) to induce inflammation. The end of experimental period (8 weeks) the rats were euthanized and blood samples were withdrawn for separating the serum were collected for biochemical analysis. Peritoneal fat pad were dissected and weighed. Blood glucose level, insulin concentration, leptin concentration, lipid profile (TC, TG, HDL-c, LDL-c and VLDL-c), liver functions (AST and ALT) and kidney functions (creatinine and uric acid) were determined. The results showed 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, peritoneal fat pad, serum (glucose, insulin, leptin, ALT, AST, uric acid, creatinine, TC, TG, LDL-c, VLDL-c) and significant increase ($P<0.05$) in HDL-c level compared to the control group (–ve). Group of rats were fed 50%f, 20%p, 30%c had best result in weight loss compared other tested groups. In conclusion, IF has beneficial effects even with the continuity of the obesogenic diet and proinflammatory diet in obese rats. IF combination with diet administration of high fat/low carb could be beneficial method for weight loss but has many side effect on health status.

Keywords: Intermittent Fasting, High fat/low carb diet, Obesity, Rats.

1. Introduction

Over the last few decades, the incidence of obesity is increasing which is now recognized as public health issue worldwide. Obesity has been associated with an elevated risk of several types of metabolic syndrome and malignancies (Tzenios, 2023). The World Obesity Atlas (2022) predicted that one billion people globally, including 1 in 5 women and 1 in 7 men, will be living with obesity by 2030. Especially concerning are the countries that feature in both the top 20 rankings for prevalence and number of people living with obesity projections; namely USA, Egypt, Turkey and Saudi Arabia. Over

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the past 30 years, there has been a controversy about the optimal diet composition for weight loss and maintenance. Numerous randomized controlled trials have compared the various diets for the treatment of overweight and obesity based on the assumption that one diet fits all without being able to provide strong evidence for one or the other. In addition, even meta analyses comparing the different diet options have been unable to identity a clear winner (Astrup and Hjorth, 2017). Intermittent fasting (IF) is an increasingly popular method of weight loss, as an alternative to daily caloric restriction (Halpern and Mendes, 2021). Intermittent fasting type as dietary pattern that is based on timed periods of fasting. Two different regimens are alternative day fasting and time-restricted eating (Dong et al., 2020). Short-term intervention studies have shown that intermittent fasting can reduce body weight and fat mass, fasting glucose and insulin levels, and improve insulin sensitivity and lipid profiles (Wilson et al., 2018 and Liu, 2019). Fasting is a common religious practice as well (Halpern and Mendes; 2021). Evidence is accumulating that eating in a 6-hour period and fasting for 18 hours can trigger a metabolic switch from glucose-based to ketone-based energy, with increased stress resistance, increased longevity, and a decreased incidence of diseases, including cancer and obesity (de Cabo and Mattson, 2020).

The combination of HFD and IF could be improvement of the ketosis case, Xiaolin et al., (2019) and 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. Li et al., (2019) found that IF/ HFD promoted white adipose browning and decreased obesity by shaping the gut flora. Gonadal and inguinal fat mass was reduced as a result of IF and HFD groups. Fat cell size was decreased by IF in HFD-fed mice.

Intermittent fasting regimens are hypothesized to influence metabolic regulation via effects on (a) circadian biology, (b) the gut microbiome, and (c) modifiable lifestyle behaviors, such as sleep. (Ruth et al., 2017).This eating regimen offer promising non pharmacological approaches to improving health at the population level, with multiple public health benefits. So, we call for more research on the subject of the study.

The aim of the study was conducted to investigate the bioactive effects of intermittent fasting as a diet regimeon obese rats

Materials and Methods

Materials

1. **Chemicals:** casein, cellulose, choline chloride, vitamin and mineral constituents were purchased from El-Gomhoriya Pharmaceutical Company, Cairo, Egypt. Starch,butter ,soy oil, and sucrose were obtained from the Egyptian local market.
2. **Experimental animals:** Forty-two adult male albino rats (Sprague Dawley strain), weighing about (185 ± g b.wt.) were obtained from the Laboratory Animal Colony, Agricultural Research Center, Giza, Egypt.

Methods

1. **Diet Preparation:**

   -Diet regime had been formulated to cover the nutrient requirements of the rats following the recommendations of the American Institute of Nutrition (AIN-93M) according to **Reeves et al., (1993).**

   -Groups of rats (3:6) were fed on high fat diet (HFD) comprising 20, 30, 40 and 50% energy from fat, these ratio of fat were prepared interchanged with starch.

   -The source of fat in Reeves had been modified to make it suitable for the diet (butter was used).

2. **Experimental Animal Design:**

   Forty-two adult albino rats were be adapted for one week on AIN-93M basal diet **(Reeves et al., 1993)** before being the seated dietary groups, and received water and diet ad libitum. The room were be lighted on a 12 h light dark cycles. The experiment was be carried out at the animal house at Agricultural Research Center, Giza, Egypt.

After the first week of adaptation, rats were divided into six groups as follows:

Group (1): Rats (n=7) were fed on the basal diet(as negative control group).

Group (2): Rats (n=7) were fasted 24 hours for 3 nonconsecutive day/week and fed on the basal diet(as positive control group).

Group (3): Rats (n=7) were fasted 24 hours for 3 nonconsecutive day/week and fed on high fat diet (HFD) comprising 20%, 20%, and 60% of energy from fat, protein, and carbohydrate, respectively.

Group (4): Rats (n=7) were fasted 24 hours for 3 nonconsecutive day/week and fed on (HFD) comprising 30%, 20%, 50% of energy from fat, protein, and carbohydrate, respectively.

Group (5): Rats (n=7) were fasted 24 hours for 3 nonconsecutive day/week and fed on (HFD) comprising 40%, 20%, 40% of energy from fat, protein, and carbohydrate, respectively.

Group (6): Rats (n=7) were fasted 24 hours for 3 nonconsecutive day/week and fed on (HFD) comprising50%, 20%, 30% of energy from fat, protein, and carbohydrate, respectively.

To avoid any error in the experiment, the diet was tested on a group of mice (before starting the actual duration of the experiment) to determine their acceptance of eating the diet components, especially high-fat ones. All vital signs, external appearance, as well as weight were observed and monitored in order to avoid deaths among the mice.

3. Intermittent Fasting Protocol:-

-Groups of rats (2:6) were fasted 24 hours for 3 nonconsecutive day/week. Food access were controlled by transferring rats daily between cages with or without food.

- All rats had free access to water throughout the study.

-Intermittent fasting was on Sundays, Tuesdays and Thursdays of each week from 2 pm to 2 pm the next day, then, the diet were served so on

4. Biological evaluation:

- During the experiment period (8 weeks), the quantities of diet, which were consumed and/or waste, were recorded every day. In addition, rat's weight were recorded weekly to determine body weight gain and feed efficiency ratio according to **Chapman *et al*., (1959)**.

$$\text{BWG } \% = \frac{final\ body\ weight\ -\ initial\ body\ weight}{initial\ body\ weight} \times 100$$

Feed Efficiency Ratio = Gain weight (g/d) / feed intake (g/d).

$$\text{Relative organ weight } \% = \frac{organ\ weight}{final\ weight} \times 100$$

- **Inflammation examination:** At the last week of the feeding trial, 3 rats from each group were selected for inflammation examination. Rats' paws were injected using 0.1 ml formalin (4%) to induce inflammation according to **Northover and Subramanian, (1962).** The changes in paw thickness after 2, 4 and 6 hours, were measured using skin caliber. The anti-inflammatory effect was assessed by reduction in the thickness of rats' paws.
- **Blood collection:** At the end of the experimental period, the rats were euthanized by prolonged exposure to ether and blood samples were withdrawn for separating the serum by centrifugation at 3000 rpm for 15 min .Serum samples were kept frozen at -70 Ċ till biochemical analyses. The organs (liver, spleen, heart and kidney) were removed, cleaned by saline solution and dried by filter paper then weighted.
- **Peritoneal fat pad:** peritoneal fat pad were dissected from each rat, then were weighed and stored at -20C according to the methods of **Azain et al., (2000).** The percentage of peritoneal fat pad were calculated as following: Weigh of peritoneal fat pad/ Weigh of rats *100.

5. Biochemical Analysis of Serum:

Collected serum was used for determination of:

A. Blood Glucoselevel was determined according to **(Arita et al.1999).**

B. Insulinconcentration was determined according to **(Steffens, 1970).**

C. Leptin concentrationwas determinedaccording to**(Turner, et al., 2010).**

D. Lipid Profile: Serum total cholesterol (TC) was calorimetrically determined according to

Allain et al., (1974) and triglycerides (TG) according to **Wahlefeld (1974)**. Low-Density Lipoprotein (LDL-c) and VLDL-C will be calculated according to **Friedewald's formula (Friedewald et al.,1972).**

E. Liver function: Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined according to the method of **Bergmeyer *et al*., (1978)**.

F. Kidney function: Serum uric acid and creatinine levels were estimated as described by **Lorentz and Brendt (1967)** and **Agbafor etal., (2015),** respectively.

6. Statistical Analysis:

Results were expressed as the mean ± standard error (x± SE). Data were statistically analyzed for variance "ANOVA" test at P ≤ (0.05) using SPSS statistical software, version 20 according to **Armitage and Berry, (1987)**.

Results and Discussion:-

Body weight loss (BWL %), feed intake (FI) and peritoneal fat pad were recorded in **Table (1)**. The results showed that group 2 that fed on basal diet and fasted 24 hours for 3 nonconsecutive day/week had a significant decrease (P< 0.05) in body weight, feed intake and peritoneal fat pad compared to the negative control group. The mean value of BWG was 8.45g in control positive versus to 19.30g in control negative group. These results were in harmony with several researches by **Varady et al., (2009)**, **Marinho et al. (2019) and Liu et al., (2019)** who showed that IF 24 hours for 3 nonconsecutive day/week caused significantly decrease (P< 0.05) in body weight, feed intake and peritoneal fat pad compared to the negative control group.

The other treated groups of rats from (3:6) were fed on high fat diet (HFD) comprising 20,30.40 and 50 energy from fat combination with IF had a significant increase (P< 0.05) in body weight, feed intake and peritoneal fat pad compared to control positive group. The best result for decreasing in body weight, feed intake and peritoneal fat pad was group of rats were fed 50% F, 20% P, 30% C compared with other tested groups. These results agreed with those reported by **Xiaolin et al., (2019), Liu et al., (2019) and Park et al., (2020)** founded that a significant (P≤0.05) decrease in body weight, feed intake and peritoneal fat pad for the group of rats fed on (HFD) combination with fasting. Liu et al., 2019 found that HFD-IF (HFD; 43% fat) (IF; 24-hour fast on 3 nonconsecutive days per week) displayed reduced energy intake and weight loss compared to the control group. In the same line **Hu et al., (2020)** showed that the concept of 'fasting physiology' refers to changes in cellular responses due to restriction of food intake, so that cells rely less on glucose and more on ketone body-like carbon as fuel sources, which is reported to improve glucose homeostasis, mitochondrial function, and DNA repair. Furthermore, these metabolic changes are reported to consume fat, causing weight loss. **Joaquim et al., (2022)** suggested that weights loss might be due to IF that also modulate of the circadian rhythms of hormones like insulin or leptin, among others, which levels change in conditions of food abundance and deficit. On the other hand **Zarrinpar et al., (2014)** showed that IF decreased abundances of several obesogenic microbes, and increased the portion of presumed obesity-protective bacteria. Moreover, the intestinal flora appeared to interact with host clock genes and regulate the host's circadian rhythm **(Parkar et al., 2019)**.

Marinho et al. (2019) confirmed that IF has been suggested as a nutritional intervention for the treatment of obesity. 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.

The combination of HFD and IF could be improvement of the ketosis case, **Xiaolin et al., (2019) and 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. **Li et al., (2017)** found that IF/ HFD promoted white adipose browning and decreased obesity by shaping the gut flora. Gonadal and inguinal fat mass was reduced as a result of IF and HFD groups. Fat cell size was decreased by IF in HFD-fed mice**.**

Table (1): Effect ofIntermittent Fasting on Body Weight Gain (BWG), Feed Intake (FI) and Peritoneal Fat Pad of Obese Rats

Parameters / Groups	BWG%	Peritoneal Fat Pad (g)	FI (g/d/rat)
G1:(-ve Control)	19.30±0.34ab	4.79±0.14a	25
G2:(+ve control)	8.45±0.31e	2.50±0.08d	21
G3	20.24±0.67a	3.56±0.08b	22
G4	17.38±0.43bc	3.19±0.14bc	19
G5	15.40±0.40c	2.83±0.15cd	17
G6	11.66±0.51d	2.75±0.16cd	15

*Mean values are expressed as means ± SE. Mean values at the same column with the same superscript letters are not statistically significant at P<0.0

Data recorded in **Table 2** showed that the intermittent fastingfor24 hours nonconsecutive day/week caused significantly decreased (P<0.05) in serum concentration of glucose, insulin and leptin hormone compared to negative control group. Clinical studies have also shown the benefits of intermittent fasting in metabolic regulation.

Marinho et al. (2019) and **Hoddyet al., (2016)** conducted on obese subjects where 8-week alternate-day fasting (24 h of fasting) regimen was followed, participants had a reduction in glucose, insulin, leptin, and fat mass.

The groups that treated with IF and fed on HFD comprising 20, 30. 40 and 50 energy from fat had a significant decrease (P< 0.05) in serum concentration of glucose, insulin and leptin hormone compared to positive control group.

The group of rats which fed on (50% F, 20% P, 30% C) had the best effect concerning the activity of glucose, insulin and leptin level. **Marinho et al., (2019)**, **Liu et al., (2019**) **andPark 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. On the other hand **Esteve et al., (2009) and Marinho et al., (2019)** showed that hyperleptinemia might be connected to insulin resistance and inflammation**,** and a 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.

Marinho et al. (2019)showed that the combination with HFD and IF decreased insulin resistance by attenuating hepatic insulin signaling and lowering glycogen phosphorylase expression despite decreased fat mass in young male rats. So, IF might prevent (or diminish) diabetes by increasing the sensitivity of insulin receptor signaling such that insulin more readily stimulates glucose uptake by muscle and liver cells.

Table (2): Effect of intermittent fasting on serum glucose,insulin and leptin on obese rats

Parameters / Groups	Glucose ng/ml	Insulin Uu/ml	Leptin ng/ml
G1:(-ve Control)	87.06±0.66a	22.87±0.52a	5.14±0.04a
G2:(+ve control)	81.34±1.00b	20.16±0.60ab	4.71±0.21ab
G3	77.06±0.66bc	19.59±0.82b	4.28±0.12bc
G4	76.63±0.68bc	19.02±0.57b	4.16±0.03bc
G5	75.20±0.26c	18.87±0.57b	4.02±0.14bc
G6	74.49±0.50c	18.45±.75b	3.87±0.18c

*Mean values are expressed as means ± SE. Mean values at the same column with the same superscript letters are not statistically significant at P<0.05.

As shown in in **Table 3** rats were fed on basal diet and fasting for24 hours had significantly (P<0.05) decrease in serum levels of TC ,TG ,LDL,VLDL and increase in HDL level compared to (control –ve). **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. **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.

The treated groups with fastingfor24 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. These results are in the same line with **Marinho et al. (2019)** who found that group of rats were fed on HFD (50% fat) had significantly (P<0.05) increase in serum level of TC,TG,LDL,VLDL and decrease the HDL level in serum compared to the group fasted only.

The best improvement and the best result groups of rats were fed 20% fat compared with other tested groups. The improvement in the serum level of lipids at these percentages (20%) compatible with studies by**Abbasi et al., (2020**) and **Ahmed et al., (2021)**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.**Shin et al., (2018)** found that intermittent fasting improved dyslipidemia for rats fed a high-fat diet compared to ad- libitum. **Wilson et al., (2018) and Abbasi et al., (2020)** 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.

Table (3): The effect of intermittent fasting on serum of total cholesterol (TC), triglyceride (TG), high density Lipoprotein cholesterol (HDL-C), low density Lipoprotein cholesterol (LDL-C) and very Low density Lipoprotein cholesterol (VLDL-C)

Parameters / Groups	T. Cholesterol	Triglycerides	HDL-C	LDL-C	VLDL-C
					mg/dl
G1:(-ve Control)	158.22±0.39a	135.08±0.73a	52.40±0.98b	78.70±1.20a	27.01±0.14a
G2:(+ve control)	123.42±0.45e	105.03±0.57d	63.30±1.70a	39.12±1.96e	21.00±0.11d
G3	129.34±0.32d	112.13±0.48c	47.73±0.42bc	59.18±0.44c	22.42±0.09c
G4	133.61±0.42c	115.07±0.64c	44.43±0.48cd	66.16±0.77b	23.01±0.12c
G5	137.91±0.52b	121.47±0.47b	43.19±.49d	70.42 ±.55ab	24.29±0.09b
G6	139.65±0.41b	120.47±0.81b	41.67±0.42d	73.88±0.55a	24.09±0.16b

*Mean values are expressed as means ± SE. Mean values at the same column with the same superscript letters are not statistically significant at P<0.05

Data presented in **Table 4** revealed that the intermittent fasting (24 h of fasting nonconsecutive day/week) caused significantly decreased (P<0.05) in serum concentration of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) when compared to negative control group.**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.

On the other hand, HFD groups had a significant increase (P< 0.05) the activity of AST and ALT enzyme in serum compared to the control fasting group. **Ismail et al., (2022**) a demonstrated that HFD for 2 weeks caused significantly increased in serum liver enzyme levels. However, Islamic fasting - as a model for intermittent fasting- improved AST, ALT level but were not statistically significant. The result showed also that group of rats which fed on 20% F had the best effect concerning the activity AST and ALT level compared with other tested groups. **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 (4): The effect of intermittent fasting on serum aspartate aminotransferase (AST) and alanin aminotransferase (ALT) on obese rats

Parameters Groups	AST (µ /L)	ALT (µ /L)
G1:(-ve Control)	101.68±0.54c	6.67±0.12bc
G2:(+ve **control)**	96.39±0.82d	5.69±0.18c
G3	105.11±1.23bc	6.96±0.22b
G4	107.39±1.02ab	7.24±0.21b
G5	108.69±0.18ab	7.53±0.11b
G6	110.11±0.28a	8.10±0.25a

*Mean values are expressed as means ± SE. Mean values at the same column with the same superscript letters are not statistically significant at P<0.05

Results in **Table 5** showed the effect of intermittent fasting on uric acid, creatinine and urea with obese rats. Data revealed that rats were fed on basal diet combination with intermittent fasting had significantly ($P \leq 0.05$) decreased serum uric acid, creatinine and urea compared with control negative group. The obtained results were in harmony with research by **Gouda and Aljuhani, (2023)** who revealed that intermittent fasting caused reduction and improvement in level of uric acid from 9.3±0.1 to 3.6±0.2 mg/dl, Creatinine 0.9±0.11 to 0.72±0.2 mg/dl. **Shahhat et al., (2022)** concluded 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.

Furthermore, groups of rats (3:6) were fed on high fat diet (HFD) comprising 20,30.40 and 50 energy from fat combination with IF had a significant increase (P< 0.05) in serum uric acid, creatinine and urea compared to group of rats were fed on basal diet and fasted. These results are in accordance with previous study by **Ismail et al., (2022)** who reported that HFD administration for 2 weeks caused increased urea and creatinine levels significantly. **Marinho et al., (2019)** showed that the enhanced formation of inflammatory markers associated with increased serum uric acid and creatinine, which may decrease adiponectin. The result showed also that group of rats which fed on 20% F had the best effect concerning the uric acid and creatinine level compared with other tested groups.

Ismail et al. 2022 who used the 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. **Hatori et al., (2012)** studied that IF, even without reducing caloric intake, could improve cell functions and prevent metabolic diseases in mice fed a high-fat diet.

Results in **Table 6** showed the effect of IF on formalin-induced inflammation for different times on the paw's thickness of obese rats. Combination with IF/basal diet caused significantly decreased (*P<0.05*) levels inflammation for different times (2, 4 and 6hrs) on the paw's thickness compared with control negative group. This result agree with that recorded by **Liu et al., (2019)** who studied that IF decreased altered genes involved in NLRP3 inflammasome pathway in group of mice fed a chow. **Rocha et al., (2002)** reported that IF decreased levels of proinflammatory cytokines Interleukin-1 beta (IL-1β), Interleukin- 6 (IL6) and Interleukin-8 (IL-8) in

rats with ulcerative colitis. In the study by **Unalacak et al., (2011**) a demonstrated that interleukin (IL)-2 and IL-8 and TNF-α levels were decreased after the period of Ramadan in eutrophic and obese.

Table (5): Effect of intermittent fasting on serum uric acid, creatinine and urea on obese rats

Parameters / Groups	Uric Acid mg/dl	Creatinine mg/dl	Urea mg/dl
G1:(-ve Control)	7.00±0.03d	0.88±0.01bc	15.44±0.30bc
G2:(+ve control)	6.54±0.12cd	0.81±0.01c	14.73±0.16c
G3:	7.11±0.18cd	0.90±0.02bc	15.44±0.16bc
G4	7.68±0.0.29bc	0.94±0.01bc	16.01±0.21bc
G5	8.11±0.27b	0.96±0.02b	16.58±0.35b
G6	9.26±0.20a	1.01±0.06a	19.58±0.88a

*Mean values are expressed as means ± SE. Mean values at the same column with the same superscript letters are not statistically significant at P<0.05.G=Group of rats, F=Fat, P=Protein, C=Carbohydrate.

Marinho et al. 2019 and **Wan et al., 2010** showed that IF increased levels of adiponectin is a potent anti-inflammatory agent, a protein hormone secreted from adipose tissue that modulates several metabolic processes, including glucose regulation and fatty acid oxidation and is an independent risk factor for metabolic syndrome. **Vendelbo et al., (2014)** found that one of the cellular reactions to fasting is the initiation of the autophagy-lysosome system. A nutritionally fasted state deactivates the signaling of mammalian target of rapamycin complex 1 pathway, thereby initiating ketogenesis and autophagy and decrease inflammation.

While, there was a significant increase in paw's thickness of rats were fed HFD with IF compared to control fasted group. The result showed also that group of rats which fed on 20% F had the best effect concerning the inflammation level compared with other tested groups. **Liu et al., 2019** a demonstrated that intermittent fasting improved and ameliorated adipose tissue inflammation and fibrosis in HFD-fed mice.

Hu et al., (2020) Fasting enables organisms to enter alternative metabolic phases, which set the stage for improved metabolic traits and healthy lifespan in animals. This biological process can be attributed to waste evacuation and restoration of a stable internal microenvironment. The concept of 'fasting physiology' refers to changes in cellular responses due to restriction of food intake, so that cells rely less on glucose and more on ketone body-like carbon as fuel sources, which is reported to improve glucose homeostasis, mitochondrial function, and DNA repair **(De Cabo and Mattson, 2020).** In addition, it stimulates autophagy, stem cell renewal, stress resistance, and suppresses inflammation as well as providing a more diverse gut flora. Furthermore, during fasting, defenses against oxidative and metabolic stress, and removal of damaged molecules are also enhanced **(Mattson et al., 2018).**

Table (6): The effect of intermittent fasting on formalin-induced inflammation for different times on the paw's thickness (mm) of obese rats

Groups / Parameters	Parameters as Mean ± SD of paws thickness (mm) after induced for:		
	2 Hours	**4 Hours**	**6 Hours**
G1:(-ve Control)	3.43±0.03c	2.92±0.03c	2.77±0.03b
G2:(+ve control)	2.42±0.03d	2.06±0.02d	1.77±0.03c
G3	3.71±0.20c	3.21±0.18bc	2.91±0.16b
G4	4.14±0.18bc	3.50±0.18abc	3.05±0.19b
G5	4.57±0.15ab	3.78±0.11ab	3.34±0.21b
G6	5.00±0.22a	4.21±0.25a	3.77±0.03a

*Mean values are expressed as means ± SE. Mean values at the same column with the same superscript letters are not statistically significant at P<0.05.

Conclusion:

The findings from the present study lead us to conclude that IF has beneficial effects even with the continuity of the obesogenic diet and proinflammatory diet in rats. The beneficial effects of IF on glucose metabolism, the liver function, the kidney function, lipid profile and inflammation on obese rats. IF consider beneficial method of weight loss for obese person. Diet administration of high fat/ low carb may be beneficial combination with intermittent fasting in decreased weight gain.

Recommendations:

Despite the good results achieved by intermittent fasting combination with a high-fat diet on body weight, we do not recommend using this diet for more than two months in rats' life, which is equivalent to about six months of humans' life, to avoid negative effects.

Conflicts of interest

There is not conflict of interest is any influence on author that could sway his or her judgment or decisions concerning the investigator's or author's research, such as the valid reporting of data or its analysis or interpretation.

Formatting of funding sources

Personal Formatting of funding sources.

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