



Effect of Intermittent Fasting in Type 2 Diabetes in Adult Male Albino Rats

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Abstract

Type 2 diabetes mellitus (T2DM) accounts more than 80 % of all diabetic cases. It may present with microvascular and macrovascular complications. The term intermittent fasting has been used to describe various types of caloric restriction for several hours per a day, alternating days or several days. **Methods and materials:** Rats were randomly classified into control group, diabetic group (D) and Diabetes + Intermittent fasting group (D + F) group. Diabetes was induced by receiving high-fat diet (HFD) for 2 weeks followed by intraperitoneal injection of streptozotocin. The rat whose blood glucose was ≥ 200 mg/dl was considered diabetic. 3 days from streptozotocin injection, each rat was deprived from food only for 16 h daily for 28 days (nothing but water). Serum glucose, insulin, oxidative injury markers (MDA and TAC) were measured. **Result:** In D group, it showed significantly elevated serum levels of glucose and MDA and significantly decreased serum levels of insulin and TAC in comparison with control group. While, in D + F group showed significantly decreased serum levels of glucose and MDA and significantly elevated serum levels of insulin and TAC in comparison with D group.

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Introduction

Diabetes mellitus (DM) is a common endocrine disorder. It is characterized by high blood sugar due to insulin deficiency or failure of the body cells to use or respond to the insulin. It is classified into type 1 DM (T1DM) and type 2 DM (T2DM). T2DM represents about 85 % to 95% of diabetic cases (*Mukhtar et al., 2020*).

T2DM leads to complications which affect many organs such as heart, kidney and retina (*Zheng et al., 2018*). CVD is a major cause of morbidity and mortality in diabetic patients. One of CVDs is diabetic cardiomyopathy that's defined as ventricular dysfunction that occurs independently of hypertension or myocardial ischemia in diabetic individuals (*Knapp et al., 2019*).

The intermittent fasting includes various types of caloric restriction for several hours per a day, alternating days or several days. It includes a period in which fasters are allowed to consume food while during the fast period the faster refrains from food consumption but he is allowed to take water all the

time (*Farooq and Ahmed, 2019*).

The aim of the present work is to evaluate the effect of T2DM on heart and oxidative injury and the effect of intermittent fasting on these diabetic changes.

Materials and Methods

Animals and Groups

After exclusion of the dead, the present study was conducted on 30 adult male albino rats, weighing (200-300gm), were selected at the beginning of this study. They were housed with 12:12 hour light/dark cycles for two weeks at room temperature after arrival from the supplier for acclimatization.

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Rats were fed on a standard diet of commercial rat chow until the time of the experiment with alsotap water adlibitum (Shawet et al., 2020).

Experimental Groups

Animals were randomly classified into the following groups: (10 rats each):

1. Control Group.

In which each rat received only vehicle (0.01 M citrate buffer, pH 4.5) (MAGALHÃES et al., 2019).

2. Diabetic Group

In which each rat received high-fat diet (HFD) for 2 weeks containing 18% carbohydrate, 25% protein and 40 % fat to induce T2DM followed by intraperitoneal injection of 35 mg/kg streptozotocin dissolved in freshly prepared 0.01 M citrate buffer at pH 4 (Skovs, 2014) . After 3 days from injection, the blood glucose was measured by a blood sample which was obtained from rat tail. The rat with blood glucose was ≥ 200 mg/dl was considered diabetic (Seedevi et al., 2020).

3. Diabetes + Intermittent Fasting

In which each rat received high-fat diet (HFD) for 2 weeks containing 18% carbohydrate, 25% protein and 40 % fat to induce T2DM followed by intraperitoneal injection of 35 mg/kg streptozotocin dissolved in freshly prepared 0.01 M citrate buffer at pH 4 (Skovs, 2014) . After 3 days from injection, the blood glucose was measured by a blood sample which was obtained from rat tail. The rat with blood glucose was ≥ 200 mg/dl was considered diabetic (Seedevi et al., 2020).

Then after 3 days from streptozotocin injection, each rat was not received any food only for 16 h daily for 28 days (nothing but water) (Belkacemi et al., 2012).

Blood Sample Collection and Storage

At the end of all experiments, all rats were sacrificed by decapitation and blood samples were obtained from jugular vein. Blood samples were collected in tubes then left to clot at room temperature and centrifuged at 3000 rpm for 15 min in a cooling centrifuge (Hettich centrifuge). The supernatant serum was withdrawn into labeled eppendorf tubes and then stored at - 20 °C for estimation of glucose by spectrophotometer using glucose oxidase colorimetric kit (Spinreact.A./S.A.U. Ctra.Santa Coloma, rat, no. 7 E-

17171), insulin level by enzyme-linked immunosorbent assay kit (ELISA, Calbiotech; USA, CA rat no. E-EL-R2462), malondialdehyde (MDA) level (MDA; Biodiagnostic, Dokkai, Egypt; cat. no. MD2522), total antioxidant capacities (TACs) by using colorimetric assay kit (Biodiagnostic; cat. no. TA23522).

Results

The Changes in Serum Levels of Glucose and Insulin in Different Experimental Groups

Figure 1 and 2 show significant increase in serum glucose level and significant decrease in serum insulin level in diabetic group in comparison with control group. While, intermittent fasting produced significant decrease in serum glucose level and significant increase in serum insulin level in (D + F) group in comparison with (D) group.

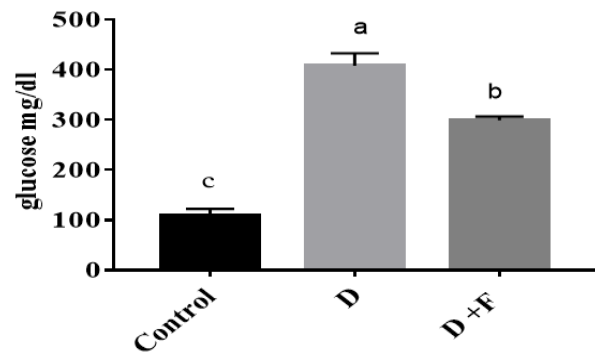


Figure 1. Shows serum glucose levels different experimental groups

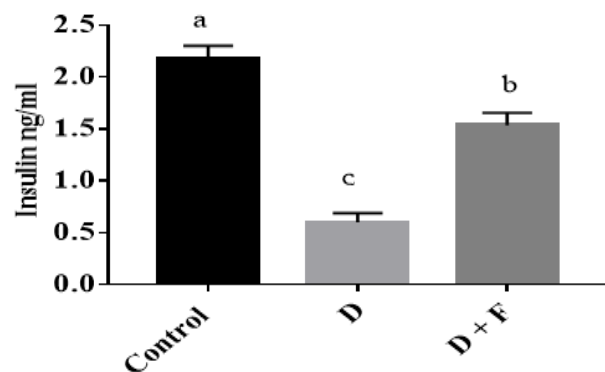


Figure 2. Shows serum insulin level different experimental groups

The Changes in Serum Levels of Oxidative Markers (Malondialdehyde (MDA) and Total Antioxidant (TAC) in Different Experimental Groups

Figure 3 and 4 show that serum levels of MDA was significantly increased and TAC was significantly decreased in diabetic group in comparison with



control group. While, intermittent fasting treatment produced significant decrease in serum MDA levels and significant increase in TAC in (D + F) group in comparison with (D) group.

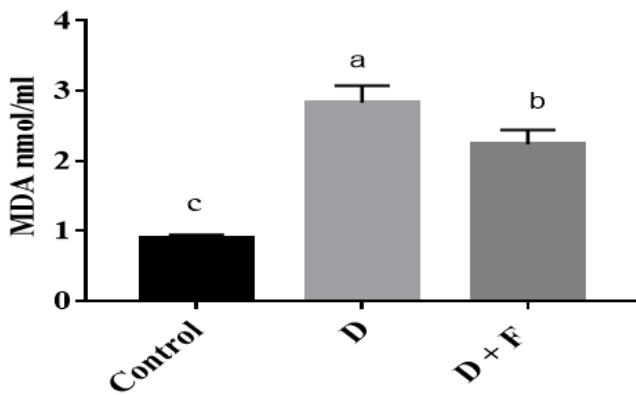


Figure 3. Shows serum MDA level different experimental groups

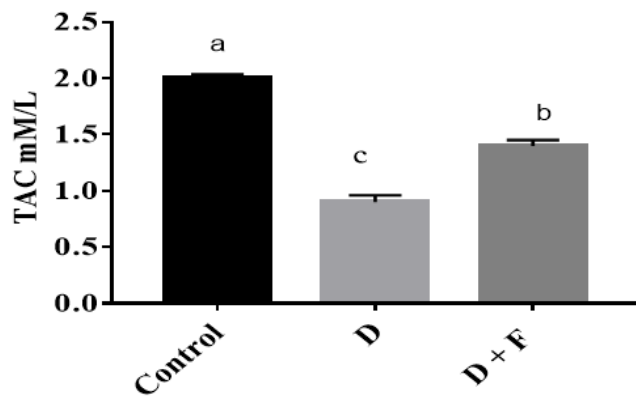


Figure 4. Shows serum TAC level different experimental groups

Discussion

Type 2 diabetes mellitus (T2DM) is a progressive disease that is characterized with significant morbidity and mortality. It leads to four million deaths all over the world (Kalra and Sahay, 2020).

The widely used animal model of type 2 diabetes is the high fat diet (HFD) followed by streptozotocin (STZ) injection. It accelerates the time-course of type 2 diabetes by initiating a state of obesity-associated insulin resistance during the period of HFD feeding. Then, injection of STZ leads to decrease in pancreatic beta cell mass which occurs due to interruption of a number of important cellular processes which finally culminate in DNA damage and cell death (Premilovac et al., 2017).

The data of the current study showed significant increase in blood glucose level in diabetic group in comparison to control group. This could be as

a result of decrease glucose uptake in peripheral tissues. Also, these may be due to increase hepatic gluconeogenesis through increasing uptake of gluconeogenic precursors and increasing the activity of gluconeogenic enzymes (Cersosimo et al., 2018). Furthermore, defected insulin action leads to FFA oxidation which produce gluconeogenic precursors such as glycerol (da Silva Rosa et al., 2020).

This hyperglycemia leads to oxidative stress that decreases insulin biosynthesis and secretion. Also, there are apoptotic β -cell death, impaired K_{ATP} channel, mitochondrial dysfunction and reduction in expression and/or activities of several insulin gene transcription factors such as pancreatic and duodenal homeobox-1 (PDX-1) (Yaribeygi et al., 2020). This may explain decreased insulin in diabetic group.

The data of the current work showed significantly lower serum glucose level and significantly higher level of serum insulin in D+F. IF decreases blood glucose through adiponectin secretion that suppress both glycogenolysis and gluconeogenesis by reducing the rate-limiting enzymes for hepatic glucose production such as glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxy kinase (PEPCK) (Yanai and Yoshida, 2019).

The data of the present work revealed markers of oxidative stress in diabetic group in the form of significant increase in serum level of MDA and significant decrease in serum level of total antioxidant than control group. These were resulted from either abnormal glucose metabolism or lipid metabolism. In hyperglycemic conditions, there is excessive production of superoxide anion radical (O_2^-) which suppresses the body antioxidant systems and so induce oxidative stress and damage nuclear DNA as well as other biomolecules (Oguntibeju, 2019). Also, in hyperglycemia, the generation of fructose leads to formation of advanced glycation product (AGEs) which generate oxidative stress by binding with its receptor RAGE thereby causing oxidative damage through nonenzymatic glycation (Julius and Hopper, 2019).

The data of the current study showed significant decrease in serum level of MDA and significant increase in serum level of total antioxidant in IF treated group than diabetic group. IF induces other transcriptional mechanisms that reduce oxidative stress. It activates the transcription factor, nuclear factor erythroid related factor-2 (NRF2) which induces many antioxidant enzymes including



superoxide dismutase-2 and catalase (**Mattson, 2019**).

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