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Original Article

Beneficial effects of intermittent fasting on lipid profile and histotexture of heart in swiss albino mice

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A B S T R A C T

Intermittent fasting (IF) is a form of time restricted eating which has gained popularity in recent years and proposed benefits include the improvement of lipid profile and the body weight loss, has gained considerable scientific and popular repercussion. The present study was conducted to investigate the effect of IF on lipid profile and histomorphological changes of heart in mice. A total of 18 Swiss Albino Mice (*Mus musculus*), 28-35 days old with an average body weight of 26.2 ± 1 gm were randomly divided into three groups. Group A was considered as control (n=6) and fed on standard mice pellet and fresh drinking water. Group B was considered as 14 hours fasting group (n=6), kept fasting for 14 hrs. and Group C was considered as 18 hours fasting group (n=6), kept fasting for 18 hrs. At the end of the experiment, blood and tissue were collected for biochemical and histomorphological examination. Data showed that Total cholesterol, Low Density Lipoprotein (LDL) level ($p<0.05$) and Triglyceride level ($p<0.01$) were significantly lower whereas High Density Lipoprotein (HDL) was highly significant ($p<0.01$) in 14 hours and 18 hours fasting group than control group. Histopathological studies of heart revealed that normal nuclei and no architectural changes were observed in myocardium in both control and fasting groups. It can be concluded that intermittent fasting may have promoting action on heart health as its limits the development of cardiovascular diseases by significantly increasing the HDL level that is known as good cholesterol for health.

Introduction

The intermittent fasting (IF) diet is getting popularity and considered as less restrictive than conventional methods of calorie restriction (Barnosky *et al.*, 2014). It involves taking a normal, daily caloric intake with the use of short, strict calorie restriction. There are two basic varieties of the IF diet. The most popular variation is time-restricted feeding. It may be used in three variants: 16/8, 18/6 and 20/4. 16:8, consisting of a 16-h fast, and then an 8-h nutritional window whereas 18:6, included as 18-h abstain from food followed by 6-h feeding and 20:4, comprising of a 20-h fast and supplied food for 4-h. In a more rigorous approach, the nutritional window can be shortened to 4 h (Johnstone, 2014). Another protocol consists of a 24-h fasting period, alternated with a 24-h eating period, repeated two or three times a week. There are two possible systems, 5:2 or 4:3. In the 5:2 system, in which caloric restriction is used for two days a week, and a regular diet for 5 days (Harvie and Howell, 2017). There are three most commonly facts of fasting-caloric restrict (CR), alternate-day fasting (ADF), and dietary restriction (DR) (John *et al.*, 2010). Caloric restrict (CR) means reduction or restriction of kilocalorie (kcal) intake by a certain percentage (typically 20 - 40%) of *ad libitum* consumption (Spindler, 2009). Cardiovascular health includes decreases heart rate (HR) and blood pressure (BP); increases in HR variability; and improvements in left ventricular function, post-exercise recovery of both HR and BP, and flow-mediated vasodilation (Mattson *et al.*, 2005). CR has been shown to decrease fasting glucose and insulin levels, increase insulin sensitivity, decrease body fat percentage, and lower the incidence of diabetes (Fontana *et al.*, 2007 and Masoro *et al.*, 2005). Alternate Day Fasting (ADF) means alternating 24-hours period: during the "feast period," fasters may consume food *ad libitum*; during the "fast period," food consumption is restricted completely and water is allowed *ad libitum*during all times. Cardiovascular

health has been improved by decreasing HR and BP, increasing HR variability, and attenuation of post-infarct chronic heart failure (Ahmet *et al*., 2005; Mager *et al*., 2006). Dietary Restriction (DR) is a reduction of one or more components of dietary intake (typically macronutrients) with minimal to no reduction in total kcal intake. Research suggests that, protein restriction increases maximum of 20% lifespan and this extension may be due to the reduction of the amino acid, methionine (Caro *et al*., 2009). While the body is abstaining from food, the concentration of glucose decreased and glycolysis is inhibited. Glycogen reserves in the liver are consumed and the process of gluconeogenesis is activated, during which fats are consumed. In addition, insulin and IGF-1 (insulin-like growth factor-1) levels are reduced in blood and glucagon levels rise. Fatty acids released from fat cells in the process of lipolysis of triacylglycerol and diacylglycerol are released (Mattson *et al.,* 2018). They are then transported to the liver cells, where they are converted into β-hydroxybutyrate (BHB) and acetoacetate (AcAc) in the β-oxidation process and are further released into the blood and used as a source of energy for body cells, including the brain (Camandola and Mattson, 2017). Such biochemical changes are accompanied by cellular and molecular adaptations of neuronal networks in the brain. The result is an improvement of their functionality and resistance to stress, injuries, and diseases (Mattson *et al.,* 2018). In most of the studies, it was found that Ramadan fasting leads to changes in the metabolic status including blood glucose and lipid (Khafaji *et al*., 2012; Kul *et al*., 2014). It is known that the lipid is influenced by dietary habits, physical factors, the percentage of fat, type of fat saturation, and the percentage of simple sugars in the daily diet (Nagra *et al*., 1998; Furuncuoglu *et al*., 2007). Ramadan fasting showed have effect on lipid profile by increasing HDL and decreasing LDL levels (Abdulrahman *et al*., 2006 and Farshidfar *et al*., 2006). It has been found a significant decrease in serum cholesterol and serum triglycerides (Marbut *et al*., 2005). The concentration of HDL is strongly inversely associated with the risk for atherogenesis and is known to be a protective lipoprotein against coronary heart disease (Miller *et al*., 1975; Gordon *et al*., 1977) by promoting cholesterol efflux from peripheral cells (Barter *et al*., 1993; Glomest *et al*., 1968). Regular fasting can decrease low-density lipoprotein and can improve the way our body metabolizes sugar. This can reduce the risk of gaining weight and developing diabetes, which are both risk factors for heart disease. Indeed, LDL is positively correlated with the risk for atherosclerosis (Brown *et al*., 1986; Shepherd *et al*., 1995). Lifestyle adjustments, i.e., smoking cessation, increasing physical activity, or ensuring proper body weight, reduces the risk of cardiovascular disease. With the growing problem of obesity in the world, diet changes are an important modifiable factor. Along with the growing epidemic of obesity, the search for new and effective dietetic solutions aimed at reducing calories and reducing body mass was initiated (Johnstone, 2014). With that sense, the present experiment has been undertaken with a view to fulfilling the following objectives:

- To determine the effect of intermittent fasting on total cholesterol, triglycerides, HDL and LDL level of mice.
- To investigate the effect of intermittent fasting on histo-morphology of heart.

Materials and Methods

Experimental animals

The experiment was conducted in the Department of Physiology, Bangladesh Agricultural University, Mymensingh, during the period of December 2017 to January 2018. A total of eighteen (18) Swiss albino mice (*Mus musculus*), 28-35 days old with an average body weight of 26.2 ± 1 gm were used. The mice were purchased from International Center for Diarrheal Disease Research, Bangladesh (icddr'b), Mohakhali, Dhaka, and maintained in the Physiology Laboratory with utmost care. In the laboratory, all the mice were maintained in proper hygienic condition.

Experimental laboratory

The laboratory was cleaned and washed with disinfectant and before placing the experimental mice it was left empty for 4 days. All necessary equipment's were arranged properly for proper handling of the animals. Mice were housed in rectangular wooden cages (9×11×7 cubic inches) wrapped with wire mesh. The cages were well ventilated at 28 ± 2ºC and a relative humidity of 70-80% with natural day light. The experimental laboratory was cleaned and washed regularly and treated with disinfectants.

Experimental equipment's

Syringe of 3 ml (blister pack disposable syringe manufactured by JMI and Medical Devices Ltd. Bangladesh) were purchased from local market and used for collection of blood. Hand gloves (manufactured by Tsim's Company Ltd. Chittagong, Bangladesh) used for safety of hands. Eppendrof tubes (manufactured by Netheler and Hinz GmbH Company Ltd., Germany) were purchased from local market and used for storage of serum. Pipettes (manufactured by CAPP, made in Denmark) were used for handling liquid. Diethyl ether (manufactured by Lyondell Basell, North America) was used for anesthesia of mice. Digital weight balance (manufactured by Precision Electronic Instruments Company Ltd., India) for measuring weight.

Experimental design

Before being used in the experiment, mice were kept for 15 days to adjust in the new environment. Then, the mice were randomly divided into three groups. Group A was considered as control (n=6) and fed on standard mice pellet and fresh drinking water. Group B was considered as 14 hours fasting group (n=6), kept fasting for 14 hrs and fed on standard mice pellet and water after fasting. Group C was considered as 18 hours fasting group (n=6), kept fasting for 18 hrs and fed on standard mice pellet and water after fasting. For fasting, feed and water were taken out from the cage of mice every evening at 6 pm. In the following morning feed and water were given in the cage of group B at 8 am and group C at 12 pm. But in the control group feed and water were present at all time in the cage. The experiment was conducted for two weeks.

Management practices

The mice cages were kept on a well-ventilated room. In order to prevent spoilage, feeds were kept in air tight poly packed bag. The feed was supplied daily to the mice and fresh drinking water was made available. Mice cages were cleaned regularly with proper hygienic measure and sanitary measures were during the experimental period. Commercial mice pellet was purchased from the local market near Bangladesh Agricultural University, Mymensingh-2202.

Collection of blood and organ

At the end of experimental period, blood samples were collected by sacrificing the mice. The mice were placed in an airtight container with diethyl ether presoaked cotton. They were checked for unconsciousness. The mice were taken out and the blood was collected directly from the heart by a sterile syringe. About 1.5 to 2 ml blood was collected and transferred to another tube without anticoagulant for serum preparation. The organ (heart) was collected and transferred to 10% neutral buffer formalin.

Preparation of serum

The blood containing tubes were placed in upright slanting position at room temperature for 6 hours. They were then incubated overnight in the refrigerator (4ºC). The serum samples were separated by centrifugation and collected by using 200 µl pipettes. About 0.4 to 0.5 ml serum was collected from each mouse. Serum samples were stored in capped tube at -20ºC for biochemical analysis.

Serum biochemical studies

The lipid profile (total cholesterol, triglycerides, HDL and LDL) were analyzed in Health Care Center, Bangladesh Agricultural University, Mymensingh-2202.

Histomorphological study and photomicrography

The heart from each group of mice were collected after completely removal of blood by perfusion with phosphate buffered saline and kept in 10% neutral buffered formalin for 15 days. The well-fixed tissues were processed, sectioned and stained as per standard procedure (Robert *et al.*, 2014) in the Department of Surgery and Obstetrics, Bangladesh Agricultural University, Mymensingh -2202. The stained slides were observed under Optka Vision Lite 21 and photographs of the characteristic findings were recorded.

Statistical analysis

Data were continuous and normally distributed. One-way analysis of variance (ANOVA) was used to determine the effect of different parameters (Steel and Torrie, 1980). The data was placed and stored in Microsoft Excel- 2016 and imported to the software IBM SPSS Statistics 20 for analysis. Descriptive statistics analysis was done to measure the mean, standard deviation and standard error and p value of different parameters. Because of using multiple comparisons, the corrected p value was calculated adjusted at 0.01 and 0.05 considered for level of significance.

Results and Discussion

Effects of intermittent fasting on lipid profile in mice

Intermittent fasting (IF) has gained considerable scientific and popular repercussion, being introduced as a feeding method under certain conditions in the clinical practice. Studies that elaborate pathways created on the basis of the animal experiments may lead to overestimation of IF regarding biochemical markers, such as the traditional lipid profile including high-density lipoprotein (HDL), low-density lipoprotein (LDL), total cholesterol and triglycerides. (Martin *et al.*, 2006).

Effect of intermittent fasting on total cholesterol in mice

Total cholesterol concentration in control group, 14 hrs. and 18 hrs. fasting groups were (185.33 ± 16.76 mg/dl), (129.67 ± 3.53 mg/dl) and (139.67 ± 4.07 mg/dl) respectively. Total cholesterol was significantly ($p<0.05$) decreased in 14 hrs (129.67 ± 3.53 mg/dl) and 18 hrs (139.67 ± 4.07 mg/dl) fasting groups (Table 1 and Figure 1) compare to control group. The findings showed close agreement with the findings of Haghdoost *et al.* (2009) and Abbas *et al.* (2003) and they found that serum lipid levels such as low-density lipoprotein (LDL), total cholesterol (TC), triglycerides (TG) and anthropometric parameters, body weight and body mass index all were reduced. This might be due to break down of body fat for energy as the fasting groups were lacking of food and water.

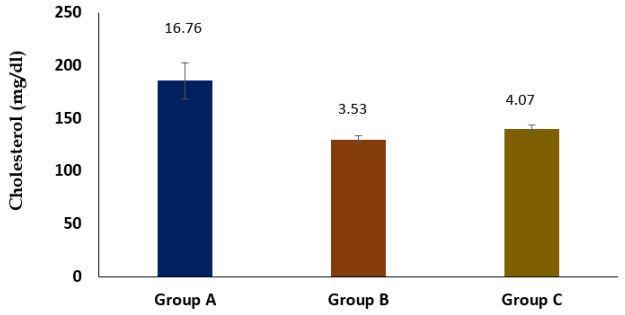

Figure 1: Effect of intermittent fasting on total cholesterol. The superscript value above the bars indicates standard error

Effect of intermittent fasting on triglycerides in mice

Triglyceride level was significantly lower ($p<0.01$) in 14 hrs (153.67 ± 11.14 mg/dl) and 18 hrs (172.33 ± 09.61 mg/dl) fasting group than in control group (362.67 ± 13.42 mg/dl) (Table 1 and Figure 2). This might be due to broken down of body fat into fatty acid in the liver which leads to decline triglycerides level in fasting groups. The present finding is closely related with the findings of Huda *et al.* (2009) and Wissam *et al.* (2008) and reported that serum glucose and triglycerides and plasma total cholesterol were significantly decrease in Ramadan compared to before Ramadan.

Table 1. Effects of intermittent fasting on lipid profile in mice

Parameters	Mean ± SE			Level of significance	P- value
	Group A (Control)	Group B (14 hour fasting)	Group C (18 hour fasting)		
Total Cholesterol (mg/dl)	185.33±16.76	129.67±3.53	139.67±4.07	*	0.018
TG (mg/dl)	362.67±13.42	153.67±11.14	172.33±09.61	**	0.000
HDL (mg/dl)	68.00±1.15	81.33±0.67	86.00±2.00	**	0.000
LDL (mg/dl)	50.13±11.21	16.60±4.88	20.87±2.23	*	0.032

**= Highly Significant at $p<0.01$ *= Significant at $p<0.05$ NS= Non significant

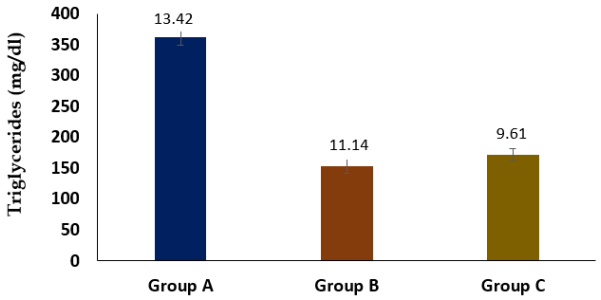

Figure 2: Effect of intermittent fasting on triglycerides. The superscript value above the bars indicates standard error.

Effect of intermittent fasting on high density lipoprotein (HDL) in mice

High Density Lipoprotein (HDL) in 14 hrs fasting group (86 ± 02 mg/dl) and 18 hrs fasting group (81.33 ± 0.67 mg/dl) were increased which was highly significant ($p<0.01$) than in control group (68 ± 1.15 mg/dl) (Table 1 and Figure 3). The present study related with the previous study of Mohsen *et al.* (2015) who found that Ramadan fasting was associated with significantly increase in high-density lipoprotein cholesterol (HDL), and decrease in low-density lipoprotein cholesterol (LDL) and total cholesterol (TC) by decreasing the saturated fatty acid from adipose tissue via lipolysis which leads increasing HDL. Ramadan fasting also lowers body weight, body fat percentage and body mass index (BMI). Calorie restriction with adequate nutrition may have beneficial effects on molecular factors.

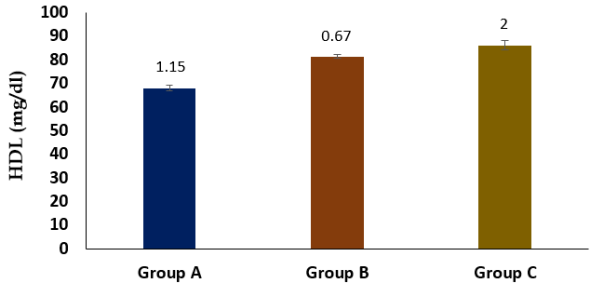

Figure 3: Effect of intermittent fasting on HDL. The superscript value above the bars indicates standard error.

Effect of intermittent fasting on low density lipoprotein (LDL) in mice

Low Density Lipoprotein (LDL) which was significantly ($p<0.05$) decreased in 14 hrs. (16.60 ± 4.88 mg/dl) and in 18 hrs. fasting group (20.87 ± 2.23 mg/dl) than in control group (50.13 ± 11.21 mg/dl) (Table 1 and Figure 4). This finding was closely agreeable with the finding of Katerina *et al.* (2003) and Ziaee *et al.* (2006) and found that low level of LDL. There is an important function of HDL which carries the body cholesterol and triglycerides to liver for degradation and elimination from the body (Sembulingam *et al.* 2016). So high level of HDL leads to low level of LDL in the body by breaking down of bad cholesterol in liver.

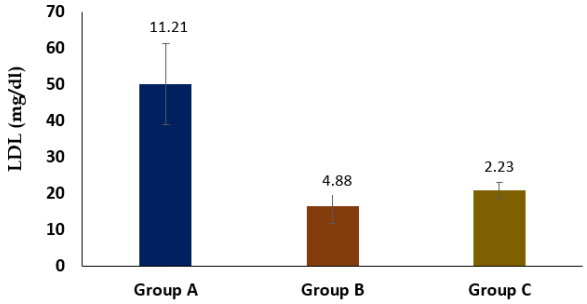

Figure 4: Effect of intermittent fasting on LDL. The superscript value above the bars indicates standard error.

Effect of intermittent fasting on histomorphological alterations in heart

Histopathology of heart was done to evaluate the health status of heart. There was non-specific changes were found in the heart of all groups. Normal nuclei and no architectural changes were observed in myocardium in both control and fasting groups (Figure 5). It was different from the work of Adrianus *et al.* (2011) who reported that there was accumulation of myocardial triglycerides (TG) and lipid droplets in cardiomyocytes due to excess breaking down of fat in heart of due to fasting in mice. The short duration of our study and variation of individual mice health status may be the causal factors for disagree with Adrianus *et al.* (2011) findings and those can be interfere with the synthesis and degradation of lipids.

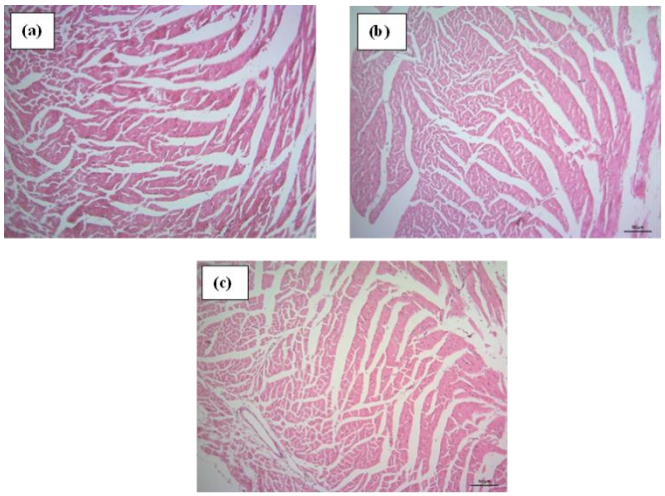

Figure 5: Photomicrographs showing histomorphology of heart (10X), (a) control group; (b) 14 hrs fasting group; (c) 18 hrs fasting group in mice.

Conclusion

The intermittent fasting limits many risk factors for the development of cardiovascular diseases and therefore the occurrence of these diseases. It can be concluded from the present study that intermittent fasting may be beneficial as HDL significantly increase but cholesterol, triglycerides, LDL significantly decrease in fasting groups. The results obtained from this study demonstrated that, fasting may not affect oxidative stress or cellular damage and may have positive effects on lipid profile and heart in healthy subjects. So, intermittent fasting is important for the improvement of one's physical health. However, individuals' current health

and situation should be considered before commencing the fasting. Finally, the results provided in this study will be the basis for future investigations on health benefits of fasting.

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Authors' contribution
MK Islam designed the experiment, and T Saha performed the experiment. KM Sujan analyzed the data and wrote the draft. KM Sujan, Z Haque and MK Islam critically revised the manuscript.

Conflict of interest
The author declares that no conflict of interest exists.

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