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Research Article

Effects of Intermittent Fasting and Sex on Blood Glucose, Free Fatty Acids, and Lipid Profile in Wistar Rats

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Abstract

Intermittent fasting (IF) has been reported to improve metabolic health through its effect on glucose and lipid profile. However, there is limited information on the effects of Intermittent Fasting on free fatty acids and sex related differences. This study was designed to investigate the effects of Intermittent Fasting (IF) on blood glucose, free fatty acids, lipid profile in both male and female Wistar rats and sex related differences. Twenty Wistar rats of both sexes weighing between 170-200g were used in the study. The animals were grouped into four (4) groups (n=5) per group. Groups 1 and 11 were male and female control groups respectively fed *ad libitum* with feed and water while groups 111 and 1V were male and female intermittent fasting groups fasted for 16 hours and fed for 8 hours for 28 days respectively. They had free access to water for 24 hours. At the end of 28 days fasting, blood glucose, free fatty acids and lipid profile were determined. The results of the study showed that intermittent fasting was associated with a significant increase in free fatty acids in female rats ($p< 0.05$) but a non- significant increase in male rats. In both female and male rats, intermittent fasting was associated with a significant decrease ($p< 0.05$) ins blood glucose, total cholesterol, and low-density lipoprotein, but a non-significant decrease in triglyceride compared with control groups in both male and female rats. In conclusion, Intermittent fasting may help to improve blood glucose and maintain a balanced lipid profile in both males and females. However, Intermittent fasting may also raise the level of free fatty acids and the effect might be more pronounced in females.

Keywords

Intermittent Fasting, Sex, Blood Glucose, Free Fatty Acids, Lipid Profile

1. Introduction

Intermittent Fasting (IF) is a practice that enhances metabolic health by reducing inflammation and oxidative stress [1] However, intermittent fasting (IF) involves entirely or partially abstaining from eating for a set amount of time, before eating regularly again. It is a broad term that encompasses a variety of programs that manipulate the timing of eating occasions by utilizing short-term fasts [2]. Fasting has been in therapeutic use since the 5th century by the Greek physician [3]. These physicians consider the abstinence from food as a remedy for certain disease conditions.

Intermittent fasting refers to eating patterns dedicated for periods of time (ranging from 12 h to several days) with consumption of little or no calories [4]. Unlike caloric restriction which requires reduction in total caloric intake without causing malnutrition, intermittent fasting involves fasting for discrete periods of time [5].

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It involves the use of short -term calorie restriction along with a regular daily caloric intake [6]. Meals are only consumed at specific times of the day or week [7]. Intermittent fasting techniques include alternate day fasting, caloric restriction, Ramadan, and periodic fasting. Time-restricted feeding is the most common variant. There are three different ways to use it: 16/8, 18/6 and 20/4. 16:8, consisting of a 16-h fast, and then an 8-h nutritional window. In a stricter approach, the nutritional window can be reduced to 4 h [7]. Another regimen consists of a 24-hour fast followed by a 24-hour eating session, which is done two or three times each week. There are two alternatives 5:2 or 4:3. In the 5:2 regimen, caloric restriction is used for two days a week, and a regular diet for 5 days. Fasting periods are described as consuming 400-600 kcal per day. Most people separate their fasting days [8]. IF diet has been reported to help with weight loss and glycemic control for 12 weeks [9]. Studies in mice showed that intermittent fasting maintains healthy circadian rhythm that regulates metabolic processes [10]. Moreover, in mice and drosophila, time restricted eating has been reported to prevent glucose intolerance, fatty liver, and dyslipidemia [11-13].

Recent studies in humans have shown that time-restricted eating patterns help to maintain healthy metabolism. Some of the reported beneficial effects of intermittent fasting include reduction in energy intake, body weight, body fat, blood pressure, blood glucose, total glyceride, glucose tolerance and inflammatory markers [14-18].

Intermittent fasting regimen has been shown to induce metabolic switching from utilizing glucose to fatty acids and fatty acid-derived ketones for energy by body cells [19, 20] Most research on intermittent fasting have been on humans and based on observational studies and randomized clinical trials. There are few reported studies on intermittent fasting, sex and free fatty acids using animal models. This study investigated the effects of IF and sex on blood glucose, free fatty acids, and lipid profile in both male and female rats.

2. Materials and Method

Twenty-four male and female Wistar rats weighed between 150-170g were purchased from the central animal house, College of Medicine, University of Ibadan, Ibadan. They were housed and acclimatized for three weeks in the Department of Physiology animal house, in accordance to University of Ibadan Animal care and use research ethics with institutional ethical regulation. They were allowed free access to feed and water ad libitum. After three weeks of acclimatization, the animals were randomly grouped into four groups with 6 animals per each group.

2.1. Measurement of Blood Glucose Levels

The fasting blood glucose (FBG) was determined immediately at the end of each phase of the study. Blood glucose was estimated by modified glucose oxidase method [21].

2.2. Determination of Free Fatty Acids

Free fatty acids level was determined using modified colorimetric determination of free fatty acids [22].

2.3. Estimation of Lipid Profile

The blood plasma was used for measurement of lipid profile. Total cholesterol (TC), triglyceride (TG), and high-density lipoprotein (HDL) levels were determined by standardized enzymatic colorimetric methods using an assay kit obtained from Fortress Diagnostic®, (United Kingdom). The values of very low-density Lipoprotein (VLDL) and low-density lipoprotein (LDL) were calculated mathematically [23].

VLDL = Triglyceride/5 and LDL = Total Cholesterol - (Triglyceride/5 + HDL)

2.4. Statistical Analysis

Data were presented as Mean ± SEM of the variables measured. Differences in mean values were compared using student's t test and one-way of variance (ANOVA) followed by Tukey post-hoc test using software Prism, version 9.0.0 (Graph-Pad Software Inc. San Diego, CA. USA). Statistical significance was considered at $p<0.05$ level.

3. Results

Effect of 28 Days Intermittent Fasting (IF) on Body Weight in Male and Female Wistar Rats. Intermittent fasting caused non-significant decrease in body weight of male (216.00 ± 8.20 mg/dl) and female (153.00 ± 10.36 mg/dl) animals compared with the control male (240.60 ± 9.13 mg/dl) and female (170.60 ± 6.14 mg/dl) animals (figure 1).

Effect of 28 days Intermittent fasting (IF) on blood glucose level in male and female Wistar rats.

There was significant decrease in the blood glucose level of the male (69.00 ± 3.74 mg/dl) and female (66.60 ± 2.71 mg/dl) animals caused by intermittent fasting compared with the control male (98.40 ± 5.56 mg/dl) and female (100 ± 8.35 mg/dl) animals (figure 2).

Effect of 28 Days Intermittent Fasting (IF) on cholesterol level in male and female Wistar rats.

Intermittent fasting caused significant decrease in cholesterol level of male (109.50 ± 4.29 mg/dl) and female (117.40 ± 3.10 mg/dl) animals compared with the control male (128.70 ± 6.27 mg/dl) and female (134.40 ± 4.86 mg/dl) animals (figure 3).

Effect of 28 days Intermittent fasting (IF) on triglyceride level in male and female Wistar rats.

There was non-significant decrease in triglyceride level

caused by intermittent fasting in both male (101.50 ± 1.42 mg/dl) and female (98.77 ± 1.26 mg/dl) animals compared with the control male (110.80 ± 4.32 mg/dl) and female (102.70 ± 1.80 mg/dl) animals (figure 4).

Effect of 28 Days Intermittent fasting (IF) on High Density Lipoprotein (HDL) level in male and female Wistar Rats.

Intermittent Fasting did not produce significant effect in high density lipoprotein level in fasting male (37.37 ± 1.01 mg/dl) and female (39.65 ± 1.95 mg/dl) animals compared with control male (39.36 ± 0.85 mg/dl) and female (37.37 ± 0.87 mg/dl) animals (figure 5).

Effect of 28 Days Intermittent Fasting (IF) on Low Density Lipoprotein (LDL) level in male and female Wistar rats.

Intermittent Fasting was associated with a significant decrease in the low-density lipoprotein level of male (47.72 ± 3.78 mg/dl) and female (59.68 ± 3.20 mg/dl) animals compared with the control male (68.80 ± 6.38 mg/dl) and female (77.31 ± 4.71 mg/dl) animals (figure 6).

Effect of 28 Days Intermittent Fasting (IF) on Free Fatty Acids (FFA) Level in Male and Female Wistar Rats.

Intermittent Fasting was associated with a non-significant increase in the level of free fatty acids in male (0.40 ± 0.04 mg/ml) animals but was associated with a significant increase in the level of free fatty acids in female animals (0.40 ± 0.06 mg/ml) compared with control male (0.35 ± 0.08 mg/ml) and female (0.22 ± 0.04 mg/ml) animals respectively (figure 7).

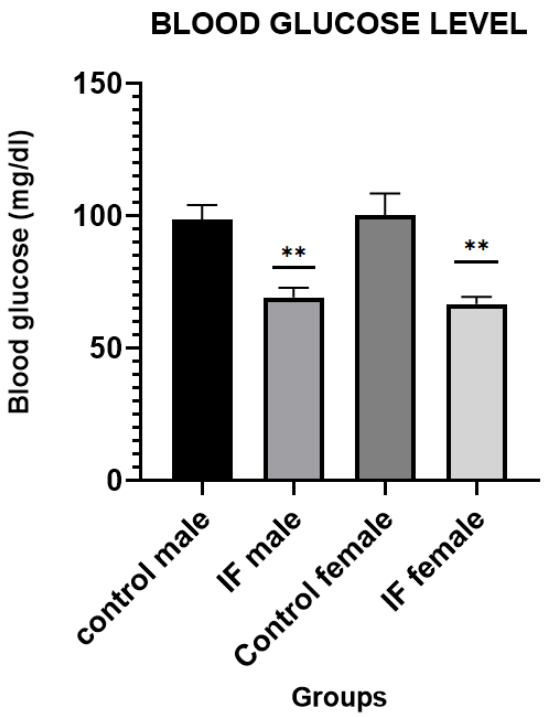

Figure 2. *Effect of 28 Days Intermittent Fasting (IF) on blood glucose Level in male and female Wistar rats. Intermittent Fasting caused significant decrease in blood glucose in both male and female rats. Significant value from control group at ($p < 0.01$) is indicated with asterisks (**).*

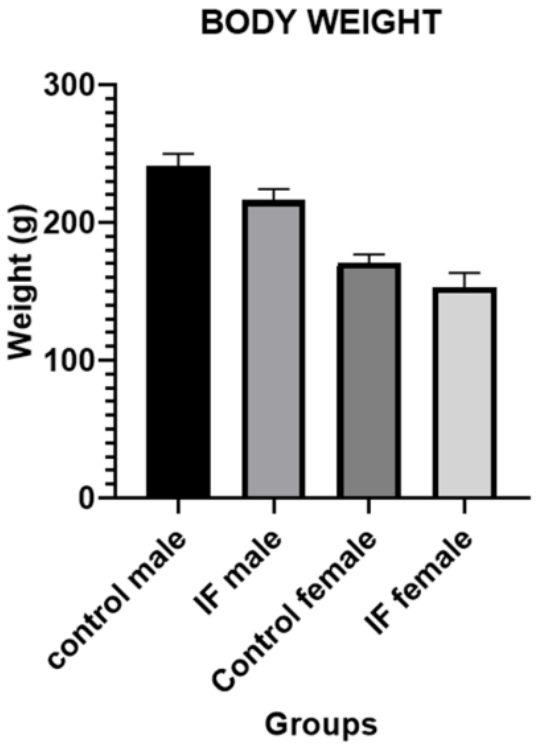

Figure 1. *Effect of 28 days Intermittent fasting (IF) on body weight in male and female Wistar rats. Intermittent fasting (28 days) did not cause significant decrease in body weight in both male and female rats.*

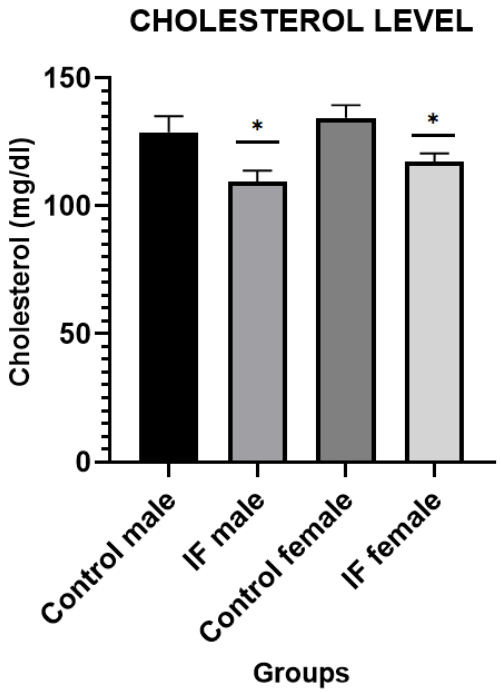

Figure 3. *Effect of 28 Days Intermittent Fasting (IF) on cholesterol level in male and female Wistar rats. Intermittent Fasting caused significant decrease in cholesterol level in both male and female rats. Significant value from control group at ($p < 0.05$) is indicated with asterisk (*).*

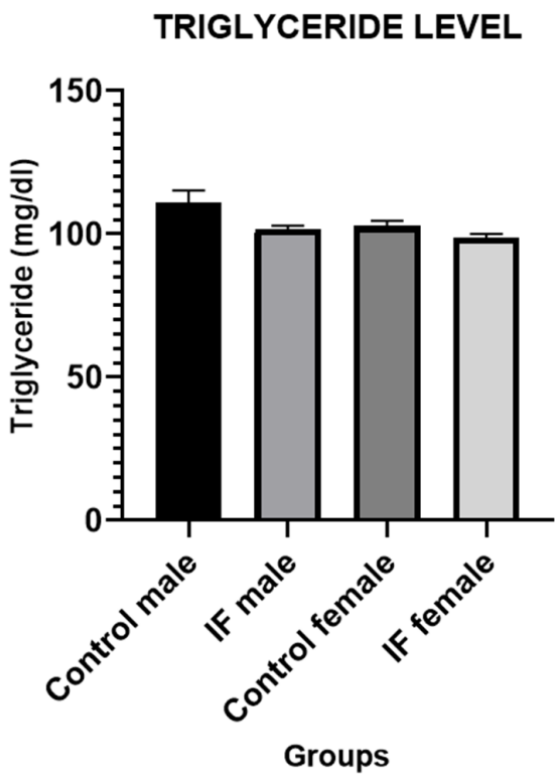

Figure 4. *Effect of 28 Days Intermittent Fasting (IF) on triglyceride level in male and female Wistar rats. Intermittent Fasting did not cause significant effect in triglyceride level in both male and female rats.*

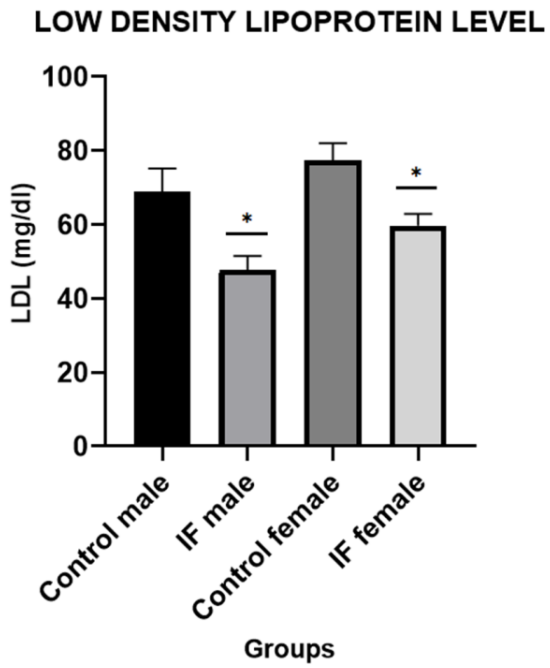

Figure 6. *Effect of 28 Days Intermittent Fasting (IF) on Low Density Lipoprotein (LDL) Level in male and female Wistar rats. Intermittent Fasting caused significant decrease in Low Density Lipoprotein (LDL) level in both male and female rats. Significant value from control group at ($p < 0.05$) is indicated with asterisk (*).*

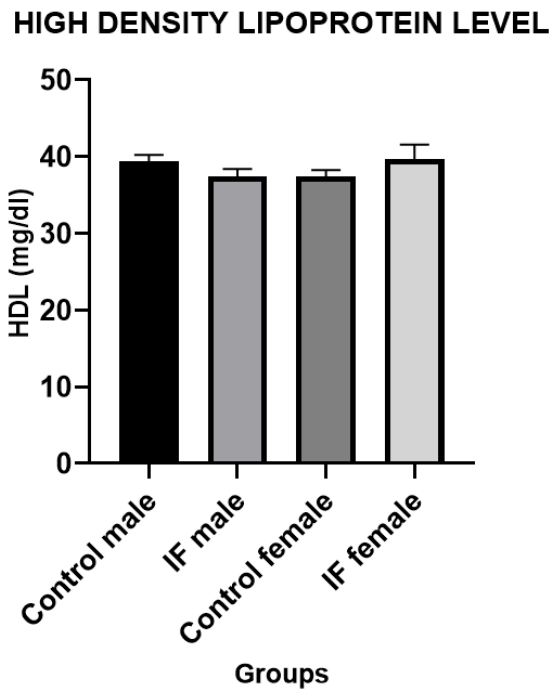

Figure 5. *Effect of 28 Days Intermittent Fasting (IF) on High Density Lipoprotein (HDL) level in male and female Wistar rats. Intermittent Fasting did not cause significant effect in high density lipoprotein (HDL) Level in both male and female rats.*

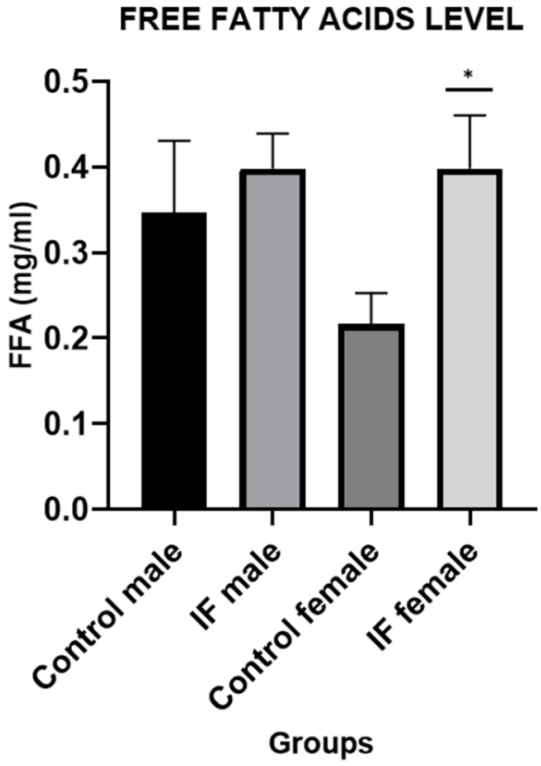

Figure 7. *Effect of 28 Days Intermittent Fasting (IF) on Free Fatty Acids (FFA) Level in male and female Wistar rats. Intermittent Fasting caused significant increase in Free Fatty Acids (FFA) level in female rats and insignificant decrease in male rats. Significant value from control group at ($p < 0.05$) is indicated with asterisk (*).*

4. Discussion

The results of this study showed that IF improved blood glucose levels and lipid profile in both male and female rats. This is consistent with previous studies [24-26] in which different types of IF have been reported to improve lipid profile. The significant decrease observed in glucose level in the present study is similar to the study of Gottardt et al [27] in which IF carried out for 4weeks was reported to cause significant decrease in blood glucose, glycated hemoglobin levels and insulin sensitivity compared with the control. During fasting, the level of blood glucose decreases, and glycolysis is inhibited. Glycogen reserves in the liver are depleted and the process of gluconeogenesis is activated [4]. This may explain the mechanism by which IF lowers blood glucose level.

The findings of the present study in which IF caused significant reduction in total cholesterol, LDL-cholesterol are in agreement with the report of [28] which reported that IF was effective in improving total cholesterol, LDL-cholesterol but had no significant effects on HDL-C concentration. In the present study IF caused an insignificant decrease in triglyceride level. However, this contrasts the reports that IF caused significant reduction in triglyceride level [29, 30]. Another notable observation in the present study is the significant increase in level of free fatty acids caused by intermittent fasting. This might explain the mechanism by which IF lowers and improves lipid profile. This increased free fatty acids observed during IF may contribute to the increased fatty acid oxidation reported during intermittent fasting [31]. According to Santos and Macedo (2018) [32], IF increases expression of peroxisome proliferator-activated receptor-α (PPAR-α) and peroxisome proliferator-activated receptor γ (PPAR-γ) coactivator 1α in the liver, leading to increased fatty acid oxidation and production of Apo A and decreased synthesis of Apo B. As a result, there is a reduction in hepatic production of LDL-C and VLDL while increasing fatty acid oxidation. Intermittent fasting also, has been reported to induce metabolic changes resulting in metabolic switching from utilizing glucose to fatty acids and fatty-acid derived ketones [19, 20] as a result, triglyceride is broken down into fatty acids and glycerol, the fatty acids are then converted into ketones in the liver [33, 34] When glucose is depleted during fasting, the body begins to use ketones produced from the breakdown of fatty acid [34, 35]. Fatty acids and ketone bodies become the primary sources of energy in cells and tissues during fasting [4, 35]. The transition is called intermittent metabolic switching. These biochemical changes lead to adaptations of neuronal networks in the brain and improved function and resistance to stress, injuries and diseases [34].

The findings in the present study in which IF caused significant increase in free fatty acids might explain probably the mechanism through which IF induces metabolic switching from utilizing glucose to fatty acids and fatty acids derived ketones [20]. Also, it is worth noting that in this study IF caused significant increase in free fatty acids in female rats compared with the male rats in which there was insignificant increase in free fatty acids. This shows probably that IF caused sex difference in the fasting levels of free fatty caids in rats. This is consistent with previous report in humans [36]. Sex difference in substrate utilization and oxidation during fasting has been reported in humans [36]. Women have been reported to have higher lipolytic rate and higher plasma free fatty acids levels than men during fasting [37]. This sex difference in physiologic response to fasting may explain the significant increase in free fatty acids in female rats compared with the male rats observed in the present study.

5. Conclusion

The present study showed that 28 days intermittent led to a reduction in blood glucose level, total cholesterol level, and LDL-cholesterol level in both male and female Wistar rats. Meanwhile, intermittent fasting caused a significant increase in free fatty acids in female rats but not in male rats. Findings suggest that intermittent fasting may improve metabolic health and lipid profile. Also, the potential impact of intermittent fasting on free fatty acids might be sex dependent.

Author Contributions

Isehunwa Grace Olufunmilayo: Conceptualization, Data curation, Funding acquisition, Investigation, Project administration, Resources, Supervision, Validation, Methodology, Writing – review & editing, Writing – original draft

Ibitoye Aishat Adenike: Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Resources, Visualization

Conflicts of Interest

The authors declare no conflicts of interest.

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