Original Research Article

ASSESSMENT OF HEMATOLOGICAL AND SERUM ELECTROLYTES EFFECTS OF INTERMITTENT FASTING ON MICE

Abstract

There is growing interest on trying to explore the importance of fasting to health as it has been practiced as a religious. This study assessed the haematological and serum electrolytes effects of intermittent fasting (IF) on mice. Fifty (50) male mice randomly assigned into five (5) groups A, B, C, D and E (n=10). Group A (control) was fed normal chow *ad libitum*, group B was fasted for 12 hours daily, group C was fasted for 18 hours daily, group D was on 24 hour alternate day fasting, and group E was fed one day out of every three days. After the seven weeks period, they were anesthetized and blood samples collected and analysed for haematological and electrolyte indices. Data obtained were analyzed using IBM SPSS version 25. Our findings revealed no significant differences in the RBC, HGB, HCT, MCV, MCH and MCHC between the different fasting groups and the control. There was increase significantly in group C for Red density width-coefficient of variance (RDW-CV) and group C and E for Red density width-standard deviation (RDW-SD). Platelet count decreased, plateletcrit increased significantly in group C. There were no significant differences in the mean platelet volume (MPV), platelet distribution width (PDW), platelet-large cell count (P-LCC) and Platelet-large cell ratio (P-LCR). Sodium ion decreased significantly in group C and chloride ion decreased significantly in group B, C and E. There were no significant differences in serum potassium ion and bicarbonate ion. In conclusion, 12, 24 and 48 hours IF are safe and do not negatively influence hematological indices and electrolyte levels but 18 hours IF could have a slight negative effect on platelet count, plateletcrit and sodium ion levels.

Keywords: *Intermittent fasting, Diseases, Weight, Electrolytes, Platelet, Red blood cell*

Introduction

In recent times, lifestyle changes and behavioural modifications have been suspected to have long lasting effects on health and personal well-being. The type of the food we eat and its frequency, the quality of sleep we get, and exercise habits could all influence our general body physiology ranging from the cardiovascular, to gastrointestinal, to musculoskeletal, to respiratory to neurophysiology and to defence physiology. Dietary changes is a very important category of lifestyle changes which can help one loss excess weight and reduce the risk of developing diseases.

Lots of interest, time and resources have been channelled towards how to eliminate excess fat, prevent unhealthy weight gain and contain our body weight due to the link associating excess body weight to exacerbation or complication of certain diseases and as a predisposing factor for diseases. One of the useful and practicable ways to prevent and eliminate excess body fats is through fasting. Hippocrates correctly said, “When you are ill and eat, you are giving fuel to the raging sickness within you” [1]. Saint Nikolai of Zicha [2] stated that fasting makes man joyful and courageous whereas, gluttony makes him gloomy and fearful.

The human body has been designed by the Creator to fast and man has advanced at times of food scarcity to become a product of feast and famine [2]. Living organisms generally alternate between periods of feeding and fasting throughout their existence [3]. Humans in particular, often fast during night sleep periods between 6 to 12 hours daily. However, longer periods of fasting has been widely practised for various purposes globally for thousands years [4]. Fasting is described as a wilful or voluntary abstinence from feeding for certain duration [5], while, intermittent fasting (IF) is the pattern of alternating feeding and fasting. Alternate day fasting, routine periodic fasting and daily time-restricted feeding are forms of IF which has been studied as a practice to improve metabolic health and many other physiological and molecular markers of health and longevity [6]. IF as a dieting concept is achieved by restricting food intake for a period of time. It has been an important religious practice for Muslims, Christians and other religions during the Ramadan, Lent and other religious seasons.

The most common researched IF is the Ramadan fasting. Ramadan is the ninth month of the Islamic calendar and is observed as a month of fasting for Muslims adult and about a billion Muslims faithful globally observe this injunction by abstaining from eating, drinking and other physical needs such as smoking and sexual intercourse daily during Ramadan from sunrise to sunset [4,7,8]. In similar vein, Christians globally observe Lent in preparation for Easter. Lent is a 40 days period which begins on Ash Wednesday and ends the night before Easter Sunday. Amongst the activities during the Lent includes fasting, praying, almsgiving etc [9]. Fasting during the Ramadan month is the most popular and extensively studied IF for its numerous health benefits.

Haematological indices are important in diagnosing the structural and functional status of animals due to its sensitivity to environmental and physiological changes [10]. Several haematological changes affecting red blood cell (RBCs), white blood cells (WBCs), and coagulation factors have been shown to be linked with obesity associated comorbidities [11]. Wojciak [12] posited that short term fasting significantly decreases iron concentrations in hair and serum as well as levels of ferritin, haematocrit, haemoglobin and red blood cells.

There is growing interest on trying to explore the importance of fasting to health and researchers have identified fasting as a strong non-pharmacological, non-genetic tool to enhance multi-systemic protection against stress and to delay several aspects related to the onset, rate and progression of age-related chronic diseases, mainly due to its anti-inflammatory and antioxidant activities [13]. However, despite established potential health benefits of fasting in ageing-related disease prevention and promoting physiological effects, little has been known about the effect of different fasting duration regimen on haematological indices and immunological function. Therefore, the study will aim to determine the effect of different intermittent fasting regimen on haematological indices, electrolyte levels and immunological function in mice.

IF has been reported to ameliorate diabetic retinopathy and increase insulin sensitivity [14, 15,16]; improve varied indicators of cardiovascular health [17-22]; prevent nephrotoxicity [23]; lower the risk of developing age-related diseases [24-25]; increase lifespan in a number of organisms [24]; good in preventing neurodegeneration in the brain [26-32]; and attenuate and purge precancerous and cancerous cells [24,33-35].

A few studies on IF have been able to evaluate red blood cell parameter and white blood cell parameters among fasters during the month of Ramadan [36-38] but the reports are however conflicting and controversial, hence, the need for this study.

Materials and Methods

Research Design

Animal experimental research design was used for this study.

Experimental Animals

Fifty (50) five to six weeks old Matured male pathogen-free mice weighing between 17-20g were purchased from the Vivarium of Department of Zoology & Environmental Biology, University of Nigeria Nsukka. They were housed in standard polypropylene cages with wood chip bedding at 25 ± 2^0C and 60± 5% relative humidity under a 12-hour light/ 12-hour dark cycle (6am to 6pm Light). Prior to the experiment initiation, they were acclimatized for two week so as to effectively recover from the stress of transport and change in environment. The general health statuses of the mice were evaluated via weight measurement. The polypropylene cages and accessory equipment including feeders and watering devices were washed and autoclaved regularly to keep them clean and free from contamination before use.

Experimental Procedure

The experiment was conducted in the Animal house of the Faculty of Basic Medical Sciences, College of Medicine, University of Nigeria Enugu Campus. After the acclimatization, they were weighed and distributed randomly (n = 10 per group) into Control groups (A) and IF groups (B, C, D and E). To investigate the impact of different IF regimen on haematological and electrolyte indices, the mice in the fasting group were divided into four sub-groups according to the duration of the fasting: 12hr, 18hr, 24hr and 48hr (n =10 per group). Group A (control) was fed normal chow *ad libitum*, group B was fasted for 12 hours daily, group C was fasted for 18 hours daily, group D was on 24 hour alternate day fasting, and group E was fed one day out of every three days. The time for feeding and fasting would be based on group regimen. After the seven weeks of intermittent fasting, fasting was stopped. The animal cages were cleaned every seven days to keep the animals dry and clean throughout the experiments duration. Any individual mouse that died from unknown causes will be excluded from the study.

After the experimental duration, all the mice were anesthetized and blood samples collected via retro-orbital sinus and analysed for haematological and electrolyte indices. Animal care was performed following the experimental guidelines of the U.S. National Institute of Health (NIH) and Institutional Animal Ethics Committee (IAEC) on the care and use of laboratory animals and the protocol will be approved by College of Health Science Research Ethics Committee, University of Nigeria Enugu Campus.

Full Blood Count Testing

The blood will be processed for routine haematological testing immediately after sample collection using the Symex XN-1000 Hematologic Analyser (Symex Corporation) in the Molecular Laboratory of Nnamdi Azikiwe University Awka, Nigeria. The parameters tested included, haemoglobin concentration, hematocrit level, erythrocyte count, leucocyte count, leucocyte differential count (neutrophils, lymphocytes, basophils, monocytes, eosinophils and large unstained cell counts) and platelet count.

Data Analysis

Data are presented as means ± standard error of mean. The differences in body weight, red blood cell parameters, white blood cell counts, platelet parameters and serum electrolyte levels was determined by one-way ANOVA, followed by Tukey's post hoc test for multiple comparisons (IBM SPSS version 25). Our statistically significant value is taken as $p<0.05$.

RESULTS

Prior to the experiment, body weight did not differ between groups. At the end of the experiment, mortality was higher in the control group than the IF groups.

Table 1: Showing the effect of different fasting regimen on mice weight

Weight	Group A Mean±SEM	Group B Mean±SEM	Group C Mean±SEM	Group D Mean±SEM	Group E Mean±SEM
Initial	19.83±1.01	21.63±1.24	21.40±0.97	23.90±1.37	22.50±0.53
Final	36.93±0.42	32.56±0.88	29.70±0.77	36.29±1.13	34.66±1.11

Table 1 show the mean values of the different intermittent fasting groups from week one to week 6. From the result, there is no observable difference of the control group from the experimental groups.

Table 2: Showing the effect of different fasting regimen on Red Blood Cell Parameters

Parameter	Groups	Mean±SEM	ANOVA (Sig)	Post hoc (Tukey)	F-ratio
RBC ($x10^{12}$/L)	A	9.70±0.04	0.680		0.581
	B	9.36±0.15		0.993	
	C	8.91±0.48		0.861	
	D	8.98±0.49		0.898	
	E	8.57±1.07		0.638	
HGB (g/dl)	A	13.30±0.17	0.959		0.153
	B	12.70±0.33		0.992	
	C	12.52±1.29		0.978	
	D	12.98±0.74		0.999	
	E	12.34±1.53		0.954	
HCT (%)	A	48.32±0.50	0.878		0.294
	B	47.28±0.95		0.999	
	C	45.12±3.95		0.953	
	D	46.60±2.60		0.995	
	E	43.92±5.32		0.867	
MCV (fl)	A	49.80±0.32	0.264		1.419
	B	50.48±0.63		0.997	
	C	50.24±2.05		0.999	
	D	53.90±2.03		0.257	
	E	51.50±0.81		0.904	
MCH (pg)	A	13.72±0.12	0.510		0.851
	B	13.58±0.32		0.999	
	C	13.90±0.82		0.998	
	D	14.46±0.28		0.770	

	E	14.46±0.38		0.770	
MCHC (g/dl)	A	27.54±0.16	0.613		0.681
	B	26.92±0.40		0.915	
	C	27.56±0.60		1.000	
	D	27.30±0.83		0.997	
	E	28.10±0.36		0.939	
RDW-CV (%)	A	18.90±0.21	0.001		7.729
	B	19.78±0.69		0.893	
	C	23.58±1.03*		0.001	
	D	19.240.14		0.997	
	E	19.46±0.89		0.978	
RDW-SD (fl)	A	29.70±0.23	0.004		5.575
	B	31.66±0.91		0.218	
	C	33.76±0.71*		0.002	
	D	31.70±0.44		0.202	
	E	32.54±0.64*		0.033	

RBC=Red blood cell, HGB=Hemoglobin, HCT=Hematocrit, MCV=Mean corpuscular volume, MCH=Mean corpuscular haemoglobin, MCHC=Mean corpuscular haemoglobin concentration, RDW-CV=Red density width-coefficient of variance, RDW-SD= Red density width-standard deviation. *= Statistically significant

From Table 2 above showing the results of mean red blood cell count, haemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, red density width-coefficient of variance and red density width-standard deviation for groups A, B, C, D and E, it is observed that there is no significant difference in the mean RBC between group A (Control) and groups B, C, D and E. The result also shows that there is no significant difference in mean haemoglobin concentration between group A and groups B, C, D and E, no significant difference in mean hematocrit between group A and groups B, C, D and E, no significant difference in mean corpuscular volume between group A and groups B, C, D and E, no significant difference in mean corpuscular haemoglobin between group A and groups B, C, D and E, and no significant difference in mean corpuscular haemoglobin concentration between group A and groups B, C, D and E. However, for red density width-coefficient of variance and red density width-standard deviation there was an increase which was statistically significant in experimental group C as compared to group A.

Table 3: Showing the effect of different fasting regimen on Platelets

Parameter	Groups	Mean±SEM	ANOVA (Sig)	Post-hoc (Tukey	F-ratio
PLT (x10^9/L)	A	1106.60±30.30	0.023		3.608
	B	736.60±131.40		0.188	
	C	592.20±49.3*		0.033	
	D	695.60±82.24		0.119	
	E	997.00±193.97		0.959	
MPV (g/dl)	A	6.16±0.07	0.112		2.147
	B	7.14±0.19		0.228	
	C	7.32±0.67		0.113	

	D	7.12±0.06		0.245	
	E	6.70±0.14		0.751	
PDW (%)	A	15.54±0.08			
	B	15.68±0.08	0.612	0.999	
	C	15.68±0.30		0.999	0.682
	D	15.86±0.07		0.982	
	E	16.42±0.88		0.582	
PCT (fl)	A	0.70±0.02			
	B	0.52±0.09		0.383	
	C	0.37±0.01*	0.028	0.025	3.399
	D	0.59±0.07		0.811	
	E	0.66±0.11		0.989	
P-LCC (pg)	A	38.00±4.21			
	B	46.00±9.86		0.993	
	C	65.00±24.60	0.703	0.632	0.548
	D	45.40±13.53		0.995	
	E	45.80±4.49		0.994	
P-LCR (g/dl)	A	3.42±0.30			
	B	6.08±0.74		0.973	
	C	12.12±6.82	0.368	0.313	1.135
	D	7.88±0.64		0.846	
	E	5.16±0.97		0.994	

PLT=Platelet, MPV=Mean platelet volume, PDW=Platelet distribution width, PCT=Plateletcrit, P-LCC=Platelet-large cell count, P-LCR=Platelet-large cell ratio, *= Statistical significant

From the result in Table 3, there is no statistically significant difference in the MPV, PDW, P-LCC and P-LCR between group A and groups B, C, D and E. The result from Table 3 shows a decrease in PLT in experimental groups (B, C, D and E) which was only statistical significant in group C. The result also shows decrease in PCT in the experimental groups (B, C, D and E) which was only statistically significant in group C.

Table 4: Showing the effect of different fasting regimen on Serum Electrolyte Levels

Parameter	Groups	Mean±SEM	ANOVA (Sig)	Post-hoc (Tukey)	F-ratio
	A	162.82±7.64			
	B	151.42±3.80		0.282	
Sodium ion-Na^+ (mE/L)	C	144.48±0.70*	0.041	0.027	3.050
	D	148.02±1.97		0.098	
	E	152.44±0.80		0.369	
	A	7.38±1.66			
	B	6.36±0.52		0.959	
Potassium ion (K^+)	C	6.04±0.24	0.564	0.898	0.759
	D	8.05±1.52		0.991	
	E	5.91±0.54		0.864	
Bicarbonate ion	A	24.58±1.75	0.384		1.100
	B	20.20±0.24		0.298	

(HCO_3^-)	C	22.48±1.45		0.869	
	D	21.56±1.54		0.644	
	E	22.84±2.07		0.928	
Chloride ion (Cl^-)	A	122.59±2.90	0.004		5.442
	B	114.00±2.30*		0.025	
	C	111.30±0.70*		0.002	
	D	114.98±1.18		0.055	
	E	113.98±1.06*		0.024	

*=statistically significance

Data in Table 4 presents the mean values of the serum electrolytes of Groups A, B, C, D and E respectively. From the Table, there was decrease in serum sodium ion which was significant only in group C relative to the control. There was also decrease in chloride ion which was significant in groups B, C and D respectively in relative to the control group. There were no significant differences between the mean serum levels of potassium ion and bicarbonate ion of the experimental groups (B, C, D and E) as compared to the control (A).

DISCUSSIONS

Research on intermittent fasting is growing in popularity globally. Few evidences have shown the adaptive effects of physiological fasting in promoting health and in the treatment and prevention of metabolic diseases [39]. This study appears to be the first study to investigate the effects of different intermittent fasting regimen on haematological parameters, and electrolytes level in mice. Lifestyle modifications have been found to have significant impact on human health, decreasing the risk for cardiovascular related issues, inhibiting growth of malignant conditions and impacting ageing. On the effects of different intermittent fasting regimen on mean mice weight, the results showed no statistically significant difference. Although other reports had declared decrease in body weight gain during fasting period [3,40-41], it is unclear as to why we did not record a significant decrease following extended period of intermittent fasting.

In this study, we observed no significant difference in the RBC, HGB, HCT, MCV, MCH and MCHC between the different fasting groups in comparison with the control. This finding is in line with the reports of Akrami et al. [42], Plumelle et al. [43] and Turan et al. [44] who observed no statistically significant difference between fasting and postprandial levels of these haematological parameters. However, the findings of this study disagrees with the report of Nematy et al. [45] who observed higher RBC count following Ramadan fasting and Naderi et al. [46], and Koscielnak et al. [47] who posited that fasting decreased RBC counts, Hb and HCT. The study also disagrees with the findings of Attarzadeh [37] and Ahmed [38] who reported increase significantly for Hb and HCT in Ramadan type intermittent fasting. There was increase significantly in group fasted for 18 hours for Red density width-coefficient of variance (RDW-CV) and groups fasted intermittently for 18 and 48 hours for Red density width-standard deviation (RDW-SD). Ahmed [38] similarly reported an increase in RDW-CV and RDW-SD during Ramadan fast. High RDW may be indicative of nutrient deficiency such as a deficiency of iron, folate, or vitamin B-12. This translates that exposure to intermittent fasting without specialized nutrient rich diet intake at feeding intervals could predispose one to having high red cell distribution width.

From our findings, we found out that there was decrease in the platelet count of the fasting groups which was only significant in the group fasted intermittently for 18 hours. Akrami et al. [42], Koscielnak et al. [47] and Turan et al. [44] similarly reported that fasting decreased platelet count after Ramadan fasting. There was increase in plateletcrit value which was significant only in group fasted intermittently 18 hours daily for seven weeks. Our findings also showed that there is no significant difference in the mean platelet volume (MPV), platelet distribution width (PDW), platelet-large cell count (P-LCC) and Platelet-large cell ratio (P-LCR) between the control group and fasting groups. Recent researches have suggested that platelet may have diagnostic and prognostic roles use in certain diseases [48]. Our finding identified decrease platelet count and increase in plateletcrit in an IF group and Gao et al. [49] suggested that PCT is nonlinearly correlated to platelet count.

The findings for serum electrolytes showed a slight decrease in sodium ion level and chloride ions. This decrease in sodium ion is significant in the group fasted for 18 hours and the decrease in chloride ion is only significant in groups fasted intermittently for 12, 18 and 48 hours respectively. Findings reported in literature on influence of fasting on sodium and chloride ion levels have been inconsistent and contrasting. Sedaghat et al. [50] similarly found a significant decrease in sodium ion following Ramadan fasting while; Kenenisa et al. [40] reported that sodium ion levels increased however insignificantly and chloride ion increased significantly following Ethiopian Orthodox Christians fasting. There was no significant difference in serum potassium ion and bicarbonate ion between the control and fasting groups. Literature on serum potassium ion during fasting is however conflicting. Sedaghat et al. [50] indicated that serum potassium ion decreased during Ramadan fasting. In a conflicting report, Kenenisa et al. [40] found that serum potassium ion increased following Ethiopian Orthodox Christian fasting.

Conclusion

This study thus concludes that different intermittent fasting regimen does not impact RBC results but may affect its distribution width, does not impact MPV, PDW, P-LCC and P-LCR but 18 hours IF could decrease platelet count and plateletcrit. IF for 18 hours was shown decrease sodium ion concentration and chloride ion concentration. Our study have demonstrated that 12, 24 and 48 hours IF are safe and do not negatively influence hematological indices and electrolyte levels but 18 hours IF could have a slight negative effect on platelet count, plateletcrit and sodium ion levels. However, a larger study using specific nutrient rich diet and extended duration are required to duplicate our findings is recommended.

Consent

Not applicable

Ethical Approval

The study protocol was approved and conducted in accordance with the rule for animal care as stipulated by the College of Medicine Ethics Committee, University of Nigeria Enugu Campus, Enugu, Nigeria (050/09/2021) which is in compliance with the experimental guidelines of the U.S. National Institute of Health (NIH) and Institutional Animal Ethics Committee (IAEC) on the care and use of laboratory animals.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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