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Effects of different intermittent fasting regimens on leucocyte parameters, erythrocyte sedimentation rate, and bleeding time in male Wistar rats

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ABSTRACT

Objectives: Hematological parameters are associated with various diseases, including inflammatory conditions and bleeding disorders. Investigating intermittent fasting (IF) impact on these parameters may uncover potential strategies for disease prevention or management. This study evaluated the effects of different IF regimens on erythrocyte sedimentation rate (ESR), bleeding time, and differential white blood cell (WBC) count.

Material and Methods: This animal experimental study conducted between February and July 2022 utilized 50 male Wistar rats randomly assigned into six groups based on their weight. Group 1 (control) was fed normally, experimental groups 2, 3, 4, 5, and 6 were intermittently fasted for seven weeks and a day (50 days) for 12, 18, 24, 36, and 48 hours (h), respectively, intermittently. The groups also contained 6, 8, 8, 8, and 10 animals, respectively. Blood for total and differential WBC counts and ESR were collected and analyzed. IBM SPSS Statistics version 25 was used for data analysis.

Results: There were no statistically significant differences in the total WBC count (cells/mcl), ESR (mm/h), bleeding time (s), eosinophil count ($\times 10^9$ cells/L), basophil count ($\times 10^9$ cells/L), lymphocyte count ($\times 10^9$ cells/L), and monocyte count ($\times 10^9$ cells/L) between the control and the different fasting groups ($P < 0.05$). Neutrophil count showed a significant reduction in groups 2, 4, 5, and 6 ($P < 0.05$). Group 3 was not statistically significant when compared to the control.

Conclusion: It can be deducted from this study that IF has no effect on ESR and bleeding time but has a slight effect on the differential WBC count. Because 36 h and 48 h fast can be extreme, the less strict ones that are 12 h, 18 h, and 24 h IF are considered safe for healthy people.

Keywords: Intermittent fasting, Total white blood cell count, Differential white blood cell count, Erythrocyte sedimentation rate, Bleeding time

INTRODUCTION

Before now, there have been several studies on different blood cells of the body as they play a vital role in our survival. The blood cells, among other cells of the body, are very crucial for our survival as anything that affects their function poses a great threat to human life. Although several studies have been carried out to suggest the best way to prevent abnormalities in these cells as well as improve their performance, there are still individuals that have blood cell

challenges (can be abnormal number or morphology). This study leans toward suggesting efficient as well as possible means of enhancing normal blood cell number as well as morphology. As of recent (majorly due to technological advancement and also improved knowledge on several areas of health), there have been significant behavioral as well as lifestyle changes among people with regard to their approach to health and also their nutrition. These changes can be in the form of the type of food they eat, how often they eat it, how well they rest and avoid stress, and how they take exercise seriously. The reason for this change in lifestyle can be attributed to the widespread speculation that excessive food intake as well as lack of adequate exercise can lead to overweight as well as obesity. Many now eat healthy to stay away from diseases instead of resorting to taking drugs (due to their toxic chemical composition).

Nutrition is the total food intake by an individual. This study tries to check if the number of hourly/daily feeds could play a role in increasing or decreasing hematological parameters: Erythrocyte sedimentation rate (ESR), bleeding time, and total and differential leucocyte count. Blood serves as an important pathological indicator of the health of animals that have been exposed to adverse substances or environment and analyzing the components of blood can give crucial information on animal sickness diagnosis and prognosis. Blood components alter with respect to the health's physiological conditions.[1] These adjustments are beneficial in evaluating how animals react to various physiological circumstances.[2] Hematological parameters encompass factors related to blood and the organs responsible for blood production, playing a crucial role in diagnosing the physiological changes affecting the functional and structural status of animals due to their high sensitivity.[3] A number of alterations in the hematology of the erythrocytes, leucocytes, and coagulation elements have been demonstrated to be connected to comorbidities brought on by obesity.[4] Intermittent fasting (IF) can reduce fat mass and circulating leukocyte levels while decreasing proinflammatory cytokines (interleukin-1, interleukin-6, and tumor necrosis factor) and normalizing the body's systemic inflammatory condition.[5] Fasting has been existing over the centuries and due to its promise as a non-pharmacological technique to enhance health and lengthen lifespan in a variety of scientific interventions, it has remained a focal point.[6]

MATERIAL AND METHODS

Study site

This study was done in the animal house of the Department of Human Physiology, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria. The Wistar rats were acclimatized for a period of 14 days, after which they were exposed to different IF regimens for a duration of 7 weeks and one day. The entire experiment lasted for 50 days.

Materials used for the study

The following materials were used for the experiment: 50 adult male Wistar rats, Iron cages with wire gauze, Water, Weighing balance, Laboratory coat and gloves, feed (Rat Feed, Onitsha, Anambra State), small rope, stopwatch, needle, filter paper, test tubes, ethylenediaminetetraacetic acid (EDTA) bottles, plain bottles, 0.5 mg sodium citrate, capillary tube, small bucket, chloroform, pipettes, pipette pump, conical flask, centrifuge, and blood cell counter (complete blood count [CBC] machine).

Ethical approval

The ethical approval with *Protocol No: 077/07/2021* was obtained from the Faculty of Basic Medical Science Ethical Committee, College of Health Science (CHS), Nnamdi Azikiwe University (NAU), Nnewi campus.

Experimental animals and design

The research was done with 50 adult male Wistar rats weighing between 67 grams (g) and 126 g. The rats were purchased in Abia state and moved to the laboratory vivarium in the Department of Human Physiology, CHS, NAU, Nnewi campus. The weight of each rat was measured with the aid of an analytical weighing balance. They were divided into six groups (1–6) based on their weight, ranging from smallest to highest weight.

The control group was group 1, while groups 2–6 served as experimental groups. Group 2 was exposed to 12 hours (h) of fasting window followed by 12 h of feeding window during the course of the experiment with duration of 50 days; group 3 fasted for 18 h window followed by six h feeding window throughout the experimental duration of 50 days; group 4 was exposed to 24 h of fasting window and 24 h of feeding window during the course of the experiment with duration of 50 days; group 5 fasted for 36 h window and was fed for 12 h window throughout the experimental duration of 50 days; and group 6 was exposed to 48 h of fasting window and 24 h of feeding window throughout the experimental duration of 50 days. After the fasting duration of 50 days, blood was collected for laboratory hematological test procedures.

Experimental procedure

Bleeding time test

The animal was placed on the laboratory table, and then the tail was tied with a small rope (this rope served the same

purpose as a sphygmomanometer). The tip of the tail was punctured with a needle, and as blood was coming out, the tail was dabbed with filter paper at intervals of 5 seconds (no force was applied during the dabbing) until the bleeding stopped.

Collection of blood sample

The animal was first sedated with chloroform that was soaked in cotton wool and placed in a white container. The blood sample was then collected from the sedated animal by the ocular route with the aid of a capillary tube. This sample was poured into an EDTA bottle.

ESR test

2 mL of the collected blood was pipetted into a test tube, and then 0.5 mL of sodium citrate was mixed with the blood in the test tube. The ESR tube, with the aid of the pipette pump, was used to pipette the mixture and was then placed vertically in the ESR stand. This mixture was allowed to stay for an hour, after which the red blood cells (RBCs) settled below, and the plasma was seen upward.

Differential white blood cell (WBC) count

The blood sample was taken to the laboratory where the different WBC type was counted with the aid of the CBC machine.

Statistical analysis

The data collected underwent statistical analysis using IBM SPSS version 25.0. Results were presented as mean ± S.E.M., and the identification of significant differences among group means was conducted through one-way analysis of variance with Tukey *post hoc* test for significance. Significance was attributed to values with $P < 0.05$.

RESULTS

The result obtained in Table 1 reveals no statistically significant difference in the mean total WBC in all the groups in comparison with the control.

The result obtained in Table 2 reveals no statistically significant difference in the mean lymphocytes, monocytes, basophils, and eosinophils of all the groups in comparison with the control. The neutrophil count, however, showed a statistically significant decrease in all groups except for the group that fasted 18 hours.

The result obtained in Table 3 reveals no statistically significant change in the mean ESR of the experimental groups in comparison with the control.

The result obtained in Table 4 reveals no statistically significant change in the mean bleeding time of the experimental groups in comparison with the control.

DISCUSSION

Implementing lifestyle changes has proven to exert a substantial influence on human health, reducing the likelihood of cardiovascular issues, impeding the development of malignant conditions, and influencing the aging process. Globally, there is an increasing interest in research on IF, with emerging evidence indicating its adaptive physiological effects in promoting health and in the therapeutic and preventive approaches to metabolic diseases.[7] This study investigated the impact of different IF regimens on total and differential WBC count, ESR, and bleeding time.

From the findings of the study, the total WBC count was not statistically changed in healthy mice exposed to different IF regimens for a long period of time. This is in line with Shawky *et al.* as well as Fidianingsih *et al.,* who observed no significant change in total WBC count.[8,9] Attarzadeh *et al.* and Nematy *et al.* recorded an increase in total white cell count.[10,11] However, Nasiri *et al.* observed a decreased total WBC count, which contradicts my findings.[12] No statistically significant difference was observed in the eosinophil, basophil, and monocyte count of all the groups exposed to IF in comparison to the control, which agrees with the report of Roy and Bandyopadhyay, who observed no significant change in all the WBC differential parameters following fasting.[13] It was also observed that IF has no

Table 1: Comparison between the mean total WBC count (cells/mcl) of the control and experimental groups.

Parameter	Groups	Mean±SEM	ANOVA sig	*Post hoc* (Turkey)	F-ratio
WBC count	1	2.87±1.99	0.255		1.522
	2	2.87±1.99			
	3	2.70±1.99			
	4	4.40±1.99			
	5	1.30±1.99			
	6	4.43±1.99			

ANOVA: Analysis of variance, WBC: White blood cell, SEM: Standard error of the mean

Table 2: Comparison between the mean differential WBC count ($\times 10^9$ cells/liter) of the control and experimental groups.

Parameters	Groups	Mean±SEM	ANOVA sig	*Post hoc* (Turkey)	F-ratio
Lymphocyte count	1	8.550±1.850	0.063		3.939
	2	4.200±0.900			
	3	4.000±0.400			
	4	3.700±0.200			
	5	4.200±1.600			
	6	1.950±0.550			
Neutrophil count	1	0.350±0.050	0.000*		29.800
	2	0.200±0.000		0.015*	
	3	0.400±0.000		0.559	
	4	0.200±0.000		0.015*	
	5	0.200±0.000		0.015*	
	6	0.100±0.000		0.001*	
Monocyte count	1	0.296±0.074	0.415		1.183
	2	0.296±0.000			
	3	0.260±0.110			
	4	0.150±0.000			
	5	0.186±0.036			
	6	0.186±0.036			
Basophil count	1	0.004±0.001	0.536		0.900
	2	0.004±0.001			
	3	0.004±0.002			
	4	0.002±0.00			
	5	0.003±0.001			
	6	0.003±0.001			
Eosinophil count	1	0.100±0.025	0.536		0.900
	2	0.088±0.013			
	3	0.088±0.038			
	4	0.050±0.000			
	5	0.063±0.013			
	6	0.063±0.013			

ANOVA: Analysis of variance, WBC: White blood cell, SEM: Standard error of the mean, *Indicates significant difference in relation to the Control Group1

Table 3: Comparison between the mean ESR (mm/h) of the control and experimental groups.

Parameters	Groups	Mean±SEM	ANOVA sig	*Post hoc* (Turkey)	F-ratio
ESR	1	3.00±0.58	0.602		0.750
	2	2.67±0.33			
	3	3.33±0.33			
	4	4.00±1.00			
	5	3.67±0.33			
	6	3.33±0.33			

ANOVA: Analysis of variance, ESR: Erythrocyte sedimentation rate, SEM: Standard error of the mean

statistically significant difference in lymphocyte count of all the groups in comparison with the control, which supports the finding of Roy and Bandyopadhyay, who observed no significant change in lymphocyte count following fasting.[13] In contrast to our study finding, Makedou *et al.* reported a significant increase in lymphocyte count, and Nasiri *et al.* noticed a significant decrease in lymphocyte count.[14,15] On the effects of different IF regimens on the neutrophil count, it was observed that neutrophil count was significantly decreased in all the IF groups, barring group 3 in comparison to the control. This finding disagrees with the report of Ahmad, who recorded a significant increase in neutrophil count during the Ramadan fast in healthy females.[16] The decrease in neutrophil count observed in this study may be due to a reduction in inflammation, which may lead to decreased demand for neutrophils or part of the complex immune response to the physiological stress of fasting. In addition, it is possible that protein depletion may, in the

Table 4: Comparison between the mean bleeding time (seconds) of the control and experimental groups.

Parameters	Groups	Mean±SEM	ANOVA sig	*Post hoc* (Turkey)	F-ratio
Bleeding time	1	110.00±13.23	0.110		2.297
	2	170.00±13.23			
	3	125.00±30.41			
	4	130.00±10.00			
	5	95.00±20.00			
	6	100.00±13.23			

ANOVA: Analysis of variance, SEM: Standard error of the mean

later phase of fasting, impairs bone marrow activity. Under the stimulus of acute infection, the number of neutrophils increased regardless of the duration of the preceding fast.

The study further revealed that there was no significant difference between the ESR of the experimental groups and the control, which is in line with the findings of Askari *et al.* and Yucel *et al.* but disagrees with Mohammadzade *et al.* and Nasiri *et al.,* whose findings recorded decrease and increase in ESR, respectively.[12,17-19] The reason why the ESR does not change in our study after fasting is not entirely clear, but it is suspected that ESR is not affected by fasting-satiety status.[18] These variations in previous findings may be due to several factors that affect ESR, such as specific gravity between RBCs and plasma, presence of rouleaux formation, and ratio of red cells to plasma.[20]

In addition, this study shows no statistically significant difference between the bleeding time of the experimental groups and the control. Although much work has not been done on bleeding time and fasting, the finding of Aybak *et al.* contradicts with this study's finding as they reported that Ramadan fasting led to an increase in bleeding and coagulation time, though this increase was still within the physiological limit.[21] This increase, according to Aybak *et al.,* was due to a reduction in the response of platelets to different aggregating agents (collagen, ADP, and adrenaline).[21]

CONCLUSION

This study delved into the effects of different IF regimens on various hematological parameters in healthy mice. While the total WBC count remained statistically unchanged, the study revealed a significant decrease in neutrophil count, possibly indicative of reduced inflammation or a complex immune response to the physiological stress of fasting. Interestingly, no significant alterations were observed in eosinophil, basophil, monocyte, lymphocyte counts, ESR, or bleeding time across experimental groups when compared to the control. These findings align with certain previous studies but also highlight discrepancies in the existing literature, emphasizing the need for further exploration of the intricate relationship between IF and hematological parameters. Future research should aim to elucidate the underlying mechanisms contributing to the observed variations and expand our understanding of the broader implications of IF on human health.

Ethical approval

The research/study approved by the Faculty of Basic Medical Science Ethical Committee, at Nnamdi Azikiwe University, Awka. Nigeria, number 077/07/2021, dated 21/07/2021.

Declaration of patient consent

Patient's consent not required as there are no patients in this study.

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Conflicts of interest

There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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