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Intermittent Fasting Induced Increased White Blood Cells Count and Low Anti-Haemophilic Factor Levels (Factor VIII)

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Abstract

Intermittent fasting is a range of meal timing patterns that involves alternating between periods of voluntary fasting or decreased calorie consumption and periods of normal eating within a specific time frame. Intermittent fasting has been associated with having significant effects on blood related factors such as Anti-haemophilic factor and white blood cells Count. To determine the effect of Intermittent Fasting on Anti-haemophilic factor and White blood cells count. A total of 120 blood samples were collected from sixty 60 apparently healthy adults. Ten milliliters (10mls) of blood samples were collected each during the fasting phase (test) and the non-fasting phase (control) of participants into EDTA and sodium citrate anticoagulated containers and analyzed for anti-Haemophilic factor levels using ELISA and total White Blood Cells count using ERMA Haematology Autoanalyzer PCE-210N (Diamond Diagnostic; Holliston, USA). Data was analyzed using SPSS and Student-t-test was used to compare means. The total white blood cell count was significantly higher ($p < 0.05$) during the fasting phase, Monocytes and Lymphocytes were significantly lower during the fasting phase ($p < 0.05$). Granulocytes were higher during the fasting phase ($p < 0.05$). Factor VIII was significantly lower during the fasting phase ($p < 0.05$). These findings revealed the complex interplay between fasting and blood health. Therefore, intermittent fasting could impact white blood cells parameters by influencing the body immune responses, white blood cells count and factor VIII levels.

Keywords: Intermittent Fasting, White blood cells, Anti Haemophilic factor.

Introduction

Intermittent fasting is a popular dietary practice which refers to a range of meal timing patterns that involve alternating between periods of voluntary fasting or decreased calorie consumption and periods of normal eating within a specific time frame (De Cabo and Mattson, 2019). There are several approaches to intermittent fasting which consist of alternate day fasting (Trepanowski *et al.*, 2017), periodic fasting and daily time-restricted feeding (Tinsley and La Bounty, 2015). Time-restricted feeding is a method that requires restricting food intake to a particular period of the day, typically lasting between 8-12 hours, creating a regular pattern of daily caloric consumption. Studies indicate that time restricted eating could be beneficial for promoting overall health and decreasing obesity (Gabel *et al.*, 2021). Alternate-day fasting is another form of intermittent fasting that requires switching between a 24-hour fast day, during which the individual consumes less than 25% of their typical energy requirements and a 25-hour period of non-fasting (Varady *et al.*, 2022). This method is divided into: complete alternate-day fast (or total intermittent energy restriction), which refers to days during which the individual consumes zero calories, typically referred to as fasting days and modified alternate-day fasting (or partial intermittent energy restriction) this approach permits the intake of up to 25% of an individual's daily caloric requirements on fasting days rather than abstaining from food entirely.

Periodic fasting or whole-day fasting involves any period of continuous fasting that lasts for more than 24 hours, ranging from diets such as 5:2 that involves one or two fasting days per week, to more extreme versions that involves several days or weeks of fasting (De Cabo and Mattson, 2019). On the days designated for fasting, it may be permissible to consume roughly 500 to 700 calories, which corresponds to about 25% of one's normal daily caloric intake, rather than abstaining from food altogether (Mattson *et al.*, 2017).

Intermittent fasting has gained popularity in recent years due to its purported health benefits. Initial findings suggest that intermittent fasting could be a viable method for losing weight and improving metabolic and cardiovascular health, it may also reduce insulin resistance and fasting insulin levels. However, the sustainability of these benefits over the long term has yet to be thoroughly investigated (De Cabo and Mattson, 2019).

Anti Haemophilic Factor

Coagulation factor VIII (also known as Anti-haemophilic factor) is a glycoprotein primarily produced in liver cells (hepatocytes), but it is also synthesized in the kidneys, endothelial cells, and lymphatic tissue. In the bloodstream, it forms a non-covalent complex with von Willebrand factor (Vehar *et al.*, 1984). vWF plays a crucial role in safeguarding factor VIII from premature breakdown by proteases and facilitates its transport to areas of endothelial damage. Factor VIII and von Willebrand factor (VWF) form a noncovalent complex while circulating in the body. VWF is a glycoprotein that acts as a carrier for factor VIII. When factor VIII is bound to VWF, its half-life lasts between 8 to 12 hours. Factor VIII plays a crucial role in the internal clotting process. The genetic locus for factor VIII is situated on the X chromosome at position Xq28. A mutation in this specific gene, responsible for encoding coagulation factor VIII, leads to a hereditary bleeding disorder known as haemophilia A. This mutation primarily arises in male germ cells. The mutation's impact results in either a reduced or absent production of factor VIII, or the synthesis of an abnormal protein (Hong *et al.*, 2007). To maintain proper blood clotting, a minimum of 25% of factor VIII

activity is necessary. Individuals with symptomatic haemophilia generally have factor VIII levels at around 5% of the normal level. The severity of the disease is classified as severe when the level is below 1%, moderate when it ranges from 1% to 5%, and mild when it exceeds 5%. The accepted range for factor VIII is considered to be between 55% and 145% of the normal level (Pagana *et al.*, 2019).

White Blood Cells

White blood cells, also known as Leukocytes, are a diverse group of nucleated cells found in the circulatory system. They are an essential part of the immune system and play a major role in defending the body against infections and foreign invaders. The normal range of WBC is 4000-11000 cells/ μ L of blood which may vary slightly based on factors such as age, gender and health status.

There are several types of white blood cells, each with distinct functions in immune response. The main types of white blood cell include:

- Neutrophils-these are the most abundant type of white blood cells, and they are the body's first line of defense against foreign pathogens. They are responsible for phagocytosis, the process of engulfing and destroying bacteria and other pathogens. Neutrophil count above $7.5 \times 10^9/L$ is termed neutrophilia (Estridge *et al.*, 2000).
- Lymphocytes- there are 2 types of lymphocytes, the B and T lymphocytes. T lymphocytes play a major role in adaptive immunity, while B lymphocytes is responsible for the production of antibodies and immune responses. Lymphocytosis indicates an increased number of lymphocytes.
- Monocytes- these cells are involved in phagocytosis and can differentiate into macrophages when they enter tissues, helping to clear debris and dead cells.
- Eosinophils- primarily involved in defense against parasitic infections and mediating allergic responses.
- Basophils- they are the white blood cells that participate in inflammatory responses and releases histamine which mediates allergic reactions (Pierre, 2002).

Table 1: Reference range for Differential White Blood Cells (Pagana *et al.*, 2019).

Parameters	SI unit	Reference range
Neutrophils	Cells/ μ L	2500-8000 (55-70%)
Lymphocytes	Cells/ μ L	1000-4000 (20-40%)
Monocytes	Cells/ μ L	100-700 (2-8%)
Eosinophils	Cells/ μ L	50-500 (1-4%)
Basophils	Cells/ μ L	25-100 (0.5-1%)

Potential Mechanism of Action

The mechanism underlying the effects of intermittent fasting on anti-haemophilic factor and white blood cells count are not yet fully understood, however several potential mechanisms have been proposed. One of the potential mechanisms is autophagy, a process that involves the degradation and recycling of damaged cells which plays a major role in maintenance of cellular homeostasis (Chowdhury *et al.*, 2019). It has been suggested that Intermittent fasting may enhance autophagy, leading to improved coagulation and homeostasis. Another potential mechanism is inflammation, fasting has been shown to decrease the levels of pro-inflammatory cytokines such as interleukin-6 and tumor necrosis factor, which are known to play a role in haemophilia and other bleeding disorders (Kaynak *et al.*, 2018). By reducing inflammation, fasting may improve the coagulation profile and reduce the risk of developing haemophilia or other bleeding disorders.

Materials and Method

Study Population and Design

This is a longitudinal study involving sixty (60) apparently healthy adults both male and female aged 18-30 years in Ovia North East LGA of Benin City. The study was divided into two phases: the fasting phase (test phase) and non-fasting phase (control phase). During the fasting phase, participants were made to fast or deprived of food and water for 12 hours while the control phase involved maintaining regular eating pattern.

Ethical Consideration

Ethical approval was Obtained from the Health Research Ethics committee, College of Medical Sciences, University of Benin, Benin City, Edo state with Approval number CMS/REC/2023/

406. A properly structured questionnaire was administered to every participant to obtain basic health, demographic details and lifestyle eating patterns. Only participants who gave informed consent were recruited into the study.

Inclusion Criteria

- Individuals with no medical history of coagulation or other blood related disorders.
- Participants who were willing to participate in the study, able to comply with the intermittent fasting protocol for the duration of the study.
- Participants aged between 18-30 years.

Exclusion Criteria

- Individuals who are unable to comply with the study protocol, including the intermittent fasting regime.
- Individuals with a history of liver disease or other chronic medical conditions that has potential to interfere with the study results.
- Individuals below < 18 years.

Sample Preparation

Sample Collection

Under aseptic condition, 10ml of Blood Samples was collected from participants at baseline and at the end of the intervention period into EDTA and sodium citrate anticoagulated containers and labelled appropriately. Venous blood (4.5ml) was dispensed into 0.5ml of sodium citrate anticoagulated container in a ratio of 1:9, and the remaining volume of blood was used for total WBCs count.

Sample Size Determination

The sample size for this study was determined using the prevalence value from previous studies

on coagulation disorders, sample size was determined using the sample size expression (Araoye, 2004).

$$n = Z^2 pq / d^2$$

Where;

- n = the desired sample size.
- d = permissible error (0.05).
- Z = the standard normal deviation, usually set at 1.96.
- p = the proportion in the target population estimated to have a particular characteristic. In this case, a reasonable estimate will be 0.08 (8%).
- q = 1 - p = 0.92
- $n = 1.96^2 \times 0.08 \times 0.92 / 0.05^2$
0.283/0.0025
= 113

Therefore, 120 participants were recruited for this study.

Laboratory Analysis

Total and Differential White Blood Cell Count

White blood cell count was analyzed using the three parts ERMA Haematology Auto analyser PCE-210N (Diamond Diagnostic; Holliston, USA). Calibration and standardization of the equipment, processing and analysis of the samples were done strictly according to the manufacturer's instructions.

Antihæmophilic Factor (Factor VIII) Analysis

Antihæmophilic factor was analyzed by Enzyme Linked Immunosorbent Assay using the Elabscience Human F8 (Coagulation Factor VIII) ELISA kit.

Principle

Strip wells are pre-coated with sheep polyclonal antibody to human factor VIII. Plasma samples are diluted and added to the wells, The Factor VIII antigen present binds to coated antibody. After washing away unbound material, Horseradish Peroxidase-labelled sheep detecting antibody is applied and allowed to bind to the captured Factor VIII antigen. The wells are again washed and a solution of TMB (peroxidase substrate Tetramethylbenzidine) is applied and allowed to react for a period of time. A blue color develops which changes to yellow upon

terminating the reaction with acid. The color formed is measured spectrophotometrically in a microplate reader at 450nm. The absorbance is directly proportional to the concentration of Factor VIII in the sample.

Procedure

- One hundred microlitres (100µL) of plasma sample was added to the wells and incubated at 37°C for 90 minutes. The wells were washed three times with 300µL wash buffer and excess was removed by gently tapping the plate on a paper towel.
- One hundred microlitres (100µL) of primary antibody was added to each well and incubated at 37°C for 60 minutes. The wells were washed three times with 300µL wash buffer and excess was removed by gently tapping the plate on a paper towel.
- One hundred microlitres (100µL) streptavidin-HRP was added to each well and centrifuged at 37°C for 30minutes. The wells were washed 3 times with 300µL wash buffer and excess was removed by gently tapping the plate on a paper towel.
- One hundred microlitres (100µL) of TMB substrate solution was added to each well and incubated for 15 minutes at 37°C.
- Fifty microlitres (50µL) of HCL Stop solution was added and the absorbance in the wells was measured at 450nm.

Statistical Analysis

Data obtained from this research was presented and analysed using IBM statistical packages for social sciences (SPSS) Software version 21.0. The student's t-test was used to compare means and results were expressed in means ± SEM, bar chart and line graph as it may occur. A p-value of less than 0.05 (p < 0.05) was considered statistically significant.

Results

Table 1 shows the comparison of Mean±SD of White Blood Cells Count and Factor VIII concentration. Total white blood cell count was significantly higher (p<0.05) during the fasting phase (5.53 ± 1.34) when compared with the non-fasting phase (4.22 ± 1.95). Monocytes and Lymphocytes were significantly lower during the fasting phase (8.42 ± 1.95, 51.60 ± 7.89)

when compared with the non-fasting phase (12.42 ± 3.26 , 58.04 ± 15.41) ($p < 0.05$), while granulocyte was higher during the fasting phase (39.98 ± 7.80) when compared with the non-

fasting phase (27.20 ± 11.54) ($p < 0.05$). Factor VIII was significantly lower during the fasting phase (64.46 ± 24.02) when compared with the non-fasting phase (85.93 ± 31.34) ($p < 0.05$).

Table 2. Mean Comparison of White Blood Cell Parameters Among Participants During Fasting and Non-Fasting Phase

Parameters	Random (N=60)	Fasting (N=60)	P value
White Blood Cells ($\times 10^3/\mu\text{l}$)	4.22 ± 1.95	5.53 ± 1.34	0.005
Lymphocytes (%)	58.04 ± 15.41	51.60 ± 7.89	0.049
Monocytes (%)	12.42 ± 3.26	8.42 ± 1.95	< 0.001
Granulocytes (%)	27.20 ± 11.54	39.98 ± 7.80	< 0.001

Values are shown as Mean \pm SD, $p < 0.05$ are considered significant

Table 3. Mean Comparison of Anti Haemophilic Factor Among Participants During Fasting and Non-Fasting Phase.

Parameters	Random (N=60)	Fasting (N=60)	P value
Factor VIII (%)	85.93 ± 31.34	64.46 ± 24.02	0.0001

Values are shown as Mean \pm SD, $p < 0.05$ are considered significant

Discussion

In this study, there was a significant increase in white blood cell count following intermittent fasting temporarily due to dehydration. This increase suggests a potential activation of the immune system which resulted in an increased production of white blood cells, possibly as a response to the physiological stress which accompanies fasting. However, the reduction in the lymphocyte and monocyte count that are important components of the immune system, which is also acute agrees with Nagai *et al.* (2019) which revealed that fasting downregulates the gene CXCL 13- CXCR5 pathway of B cells of the lymphocytes in the bone marrow and also down regulates CCL2-CCR2 gene pathway in monocytes of the bone marrow. This is also in agreement with the work done by Rangan *et al.* (2019). This study disagrees with a study carried out by Roy *et al.* (2017) who noted a decrease in white blood cell count after intermittent fasting, however he also reported a decrease in lymphocyte count which agrees with our study. Granulocytes are often described as major key players in the body's defense against infections, and their increase might be attributed to the increased immune

response triggered by fasting. This finding highlights the connections between dietary patterns and immune functions.

Antihaemophilic factor, also known as factor VIII, is an important factor in the process of blood clotting. This protein is essential for preventing excessive bleeding by helping to form blood clots when needed, such as in response to injuries (Kruse *et al.*, 2018). In this study, there was a significant reduction in anti-haemophilic factor (factor VIII) levels after intermittent fasting. Other studies have reported a reduction in clotting factors after intermittent fasting for example, Sarraf *et al.* (2000) reported reduction in factor VII after intermittent fasting. These findings hold potential clinical implications, especially for individuals suffering from deficiency of Factor VIII who depend largely on sufficient levels of factor VIII for proper blood clotting. The reduction in factor VIII could be attributed to various factors, including altered liver function during intermittent fasting, changes in clotting cascade dynamics, or indirect effects on intrinsic and extrinsic pathways of coagulation.

Conclusion

The results from this study revealed increased white blood cell count, decreased lymphocyte and monocyte counts, elevated granulocyte count and decreased anti-haemophilic factor (factor VIII). These findings reveal the complex interplay between fasting and blood health. Therefore, intermittent fasting could impact certain blood parameters by influencing the body's immune responses, some blood cell counts and clotting factors.

Recommendations

Regular monitoring of blood parameters should be recommended for individuals practicing intermittent fasting to detect any adverse changes in immune function. A balanced diet should be emphasized during fasting periods to support overall blood health and maintain adequate levels of essential nutrients. Further research should be conducted to explore the long-term effects of intermittent fasting on blood parameters and immune responses.

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