

Research Article

Impact of Intermittent Fasting on Serum Cardiac Biomarkers in Male Wistar Rats

Edith Reuben¹ , Bruno Chukwuemeka Chinko^{2,*} , Nimisoere Peace Batubo¹ ,
Fortune Somiari Amah-Tariah¹ 

¹Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Rivers State University, Port Harcourt, Nigeria

²Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, Port Harcourt, Nigeria

Abstract

Background: Intermittent fasting (IF) is a widely adopted strategy for weight loss; however, it has also been linked to potential risks, including an increased likelihood of cardiovascular complications. This study aimed to investigate the effects of intermittent fasting on cardiac biomarkers in male Wistar rat models. **Methods:** Twenty (20) male Wistar rats (130 g) were used for the study. They were randomly assigned to four (4), consisting of five (5) rats per group. Group I served as the control while groups II, III, and IV served as the experimental groups and intermittently fasted for 6 hours, 8 hours, and 12 hours, respectively, for four (4) weeks. Blood samples were collected to determine cardiac serum biomarkers: troponin-I (cTnI), troponin-T (cTnT), and aspartate transaminase (AST). **Results:** There was a significant increase in cTnI, cTnT, and AST levels among the 12-hour intermittent fasting group (IV) compared to the control group (I), the 6-hour IF (II) and the 8-hour IF (III) groups ($p < 0.05$). Similarly, mean levels of cTnI, cTnT, and AST were significantly elevated among the 8-hour IF group compared to the 6-hour IF group ($p < 0.05$). Expectedly, there was a reduction in the body weight of the rats in all experimental groups compared to the control ($p < 0.01$). **Conclusion:** Prolonged intermittent fasting could pose a risk to cardiac health, necessitating careful consideration and monitoring of the fasting regime.

Keywords

Intermittent Fasting, Cardiac Troponin, Aspartate Transaminase, Biomarkers, Cardiovascular Disease

1. Introduction

Intermittent fasting (IF) is a dietary protocol characterized by alternating cycles of fasting and feeding over a defined period. It involves the deliberate abstention from caloric intake for specific time intervals, ranging from several hours to days, followed by periods of *ad libitum* or controlled food

consumption [1, 2]. It is one of the most widely used weight loss strategies [3, 4] employed in various forms each offering a unique approach to balancing eating and fasting periods. One popular method is alternate-day fasting, where individuals alternate between days of regular eating and days of fast-

*Corresponding author: bruno.chinko@uniport.edu.ng (Bruno Chukwuemeka Chinko)

Received: 19 March 2025; **Accepted:** 31 March 2025; **Published:** 22 April 2025



Copyright: © The Author(s), 2025. Published by Science Publishing Group. This is an **Open Access** article, distributed under the terms of the Creative Commons Attribution 4.0 License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution and reproduction in any medium, provided the original work is properly cited.

ing or significantly reduced calorie intake. Another approach is modified fasting, often referred to as the 5:2 method, which involves eating normally for five days of the week while restricting calorie intake to about 500-600 calories on the remaining two non-consecutive days. Also, there's time-restricted feeding, which focuses on limiting daily eating to a specific window, typically between 8 to 10 hours, and fasting for the remaining 14 to 16 hours each day [5]. Over the years, animal and human studies have shown that the timing of meal intake is as vital as the composition of the meal and caloric quantity in the prevention as well as treatment and prevention of obesity, cancer and its associated comorbidities such as cardiovascular diseases [6, 7]. Intermittent fasting has also been employed as an adjunct therapy or prophylaxis for conditions in which obesity is an important risk factor for the advancement of type II diabetes [1, 8]. In addition, it is utilized by normal-weight individuals and those engaged in physical training to support muscle retention while complementing their exercise regimen [9]. Fasting, when combined with adequate nutrition, serves as an effective strategy for weight loss and has been shown to enhance cardiometabolic health across diverse animal species [10-12].

Cardiac biomarkers are important heart health indicators used in diagnosing and monitoring cardiovascular diseases [13, 14]. The cardiac myocyte is the sole source of troponin I (cTnI) and troponin T (cTnT) and provides a specific means for detecting myocardial injury and necrosis and other serum cardiac biomarkers including aspartate transaminase (AST), creatine kinase (CK-MB), B-type natriuretic peptide (BNP), and c-reactive protein (CRP), lactate dehydrogenase etc. [15, 16]. The discovery of elevated enzymatic biomarkers, such as AST, in cardiovascular diseases like myocardial infarction marked a pivotal advancement in the use of biomarkers for diagnosing cardiovascular conditions [17, 18]. However, the discovery of cardiac troponins (cTnI and cTnT) marked a breakthrough in cardiology, owing to their exceptional specificity and sensitivity in detecting myocardial injury [13, 17, 18].

While intermittent fasting is an effective intervention for preventing and managing obesity, it also acts as a stressor that induces hormesis. This process enhances cellular resistance and provides protective benefits to various tissues, including the heart [3, 19]. Previous research has highlighted that intermittent fasting improves several cardiometabolic parameters: blood pressure, glucose, cholesterol, and adiponectin levels [7, 20-23]. However, a recent study found that individuals adhering to an 8-hour time-restricted eating schedule, a form of intermittent fasting, had a 91% increased risk of cardiovascular disease-related mortality [24]. With some conflicting reports on the health benefits of intermittent fasting, there is a lack of data specifically investigating its effects on cardiac biomarkers. The present study aims to investigate the impact of intermittent fasting (time-restricted feeding) on cardiometabolic health by evaluating cardiac biomarkers (cTnI, cTnT and AST) in male Wistar rat models. This re-

search will explore intermittent fasting as a hormetic stressor and identify potential optimal IF protocols for improving cardiovascular health.

2. Materials and Methods

2.1. Experimental Animals

Twenty (20) male Wistar rats (110 -130 g) were sourced from the animal house of the Department of Pharmacology, College of Health Sciences, University of Port Harcourt, Nigeria. They were allowed to acclimate themselves to the new location within the animal house for two (2) weeks. The Wistar rats were provided with the standard rat chow and water *ad libitum*. The animals were housed in groups of five rats per cage in a controlled environment that adhered to standard animal husbandry conditions: 30-50% humidity, 12-hour light/dark cycle, and room temperature (25 °C).

2.2. Research Design and Fasting Protocol

The male Wistar rats were randomly assigned to four (4) groups of five (5) animals each. Group I served as the control group and was provided with normal rat chow and distilled water *ad libitum* while groups II, III and IV served as the experimental groups and were subjected to intermittent fasting (IF) protocols with varying fasting durations: 6 hours, 8 hours, and 12 hours, respectively. The fasting animals were allowed unlimited access to water during their fasting window. During their respective feeding windows, all IF groups (II, III and IV) were given normal rat chow and distilled water *ad libitum* for 18, 16 and 12 hours respectively. The intermittent fasting lasted for twenty-eight (28) days.

2.3. Weight Measurement and Sample Collection

The body weight of the Wistar rats was monitored weekly using a digital weighing scale (AINODIS, Xiamen Aiduomei, China). At the end of the experiment, the animals were anaesthetized using intraperitoneally administered ketamine (50mg/kg) and blood was collected from the animals via cardiac puncture.

2.4. Laboratory Assay

Serum cardiac troponin I (cTnI), T (cTnT) and aspartate aminotransferase (AST) levels were assayed using standard laboratory test kits (Elabscience, China). Blood samples collected into dry sample bottles were centrifuged at 3000 rpm for ten (10) minutes and the supernatant serum was collected using a micropipette and stored at 4 °C pending laboratory assay.

2.4.1. Determination of Serum Troponin I and T

A micro-ELISA plate pre-coated with antibodies specific to Mouse TNNT3/cTn-I and cardiac troponin T (cTnT/TNNT2) was utilized. Serum samples were added to the wells, enabling the target antigens to bind to the immobilized antibodies. A biotinylated detection antibody specific for Mouse TNNT3/cTn-I and cardiac troponin T (cTnT/TNNT2) was then introduced, followed by the addition of an Avidin-Horseradish peroxidase (Avidin-HRP), conjugate. The plate was incubated to facilitate antibody-antigen interactions, after which unbound components were removed through washing. A substrate solution was added to the wells, producing a blue colour in wells containing Mouse TNNT3/cTn-I and cTnT/TNNT2. The reaction was stopped using a stop solution, which changed the colour from blue to yellow. The optical density (OD) of each well was measured spectrophotometrically at 450 ± 2 nm, with the OD values being directly proportional to the concentration of mouse troponin I and T in the samples.

2.4.2. Determination of Serum Aspartate Aminotransferase (AST)

To prepare the Elisa standard well plates, 5 μ L of Buffer solution to each well followed by decreasing amounts of substrate solution (20, 18, 16, 14, and 12 μ L) to wells A to E, respectively. Finally, increasing amounts of Sodium pyruvate (0, 2, 4, 6, and 8 μ L) were added to the same wells. Similarly, 20 μ L of pre-heated substrate solution and 5 μ L of serum sample were added to the sample wells while only 20 μ L of pre-heated substrate solution to each control well. The con-

tents of the wells were mixed thoroughly and allowed to incubate at 37 °C for 30 minutes after which 20 μ L of chromogenic agent to each well. For control wells, 5 μ L of sample was added after adding the chromogenic agent. This was mixed using a microplate reader for 10 seconds and incubated at 37 °C for 20 mins. Using a multichannel pipette, 200 μ L of Alkali working solution was added to each well and thoroughly mixed using a microplate reader for 10 seconds. The plate was allowed to stand at room temperature for 15 minutes after which the optical density (OD) of each well was read at 510 nm using a microplate reader. The OD values to calculate the concentration of AST in the samples.

2.5. Ethical Consideration

The animals were cared for and handled in full compliance with the most stringent ethical guidelines established for the use of animals in scientific research [25, 26]. The study protocol was approved by the Research Ethics Committee of the University of Port Harcourt with approval number UPH/CEREMAD/REC/MM107/054.

2.6. Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics version 26. All values were expressed as mean \pm standard error of the mean (SEM). Differences between the two groups were analyzed using the one-way analysis of variance (ANOVA), followed by a Tukey post hoc analysis. A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant.

3. Results

Table 1. The Effect of Intermittent Fasting (IF) on some Cardiac Biomarkers.

Parameters	Control (n=5)	6 Hour IF (n=5)	8 Hour IF (n=5)	12 Hour IF (n=5)
cTnI (pg/ml)	0.18 \pm 0.02	0.13 \pm 0.01	0.23 \pm 0.03 ^b	0.38 \pm 0.05 ^{a,b,c}
cTnT (pg/ml)	19.20 \pm 3.15	10.58 \pm 1.16	22.48 \pm 1.19 ^b	33.08 \pm 3.30 ^{a,b}
AST (U/L)	23.00 \pm 2.68	30.40 \pm 6.53	50.40 \pm 5.44 ^b	71.00 \pm 0.94 ^{a,b,c}

All values are expressed as mean \pm standard error of the mean (SEM)

^a $p < 0.05$, significant change when compared with the control,

^b $p < 0.05$, significant change when compared with the 6hr IF group

^c $p < 0.05$, significant change when compared with the 8hr IF group.

Table 1 presents the effects of different protocols of intermittent fasting (IF) on Troponin I, Troponin T, and Aspartate Transaminase (AST). The data show that Troponin I levels in the control group were 0.18 \pm 0.02 pg/ml. A non-significant decrease was observed after 6 hours of IF (0.13 \pm 0.01 ng/ml). However, Troponin I levels increased significantly at 8 hours

(0.23 \pm 0.03 ng/ml; $p < 0.05$) compared to the 6-hour IF group and further increased significantly at 12 hours (0.38 \pm 0.05 ng/ml; $p < 0.05$) compared to the control, 6-hour, and 8-hour groups. Troponin T levels exhibited a similar trend, with a slight decrease at 6 hours (10.58 \pm 1.16 pg/ml) followed by a significant increase at 8 hours (22.48 \pm 1.19 pg/ml; $p < 0.05$)

compared to the 6-hour group). The highest level was observed at 12 hours (33.08 ± 3.30 pg/ml), which was significantly elevated compared to the control ($p < 0.05$), 6-hour ($p < 0.05$), and 8-hour groups ($p < 0.05$). Similarly, AST levels showed a progressive increase with fasting duration. The control group had an AST level of 23.00 ± 2.68 U/L, which increased moderately at 6 hours (30.40 ± 6.53 U/L), though this change was not statistically significant. However, AST levels rose significantly at 8 hours (50.40 ± 5.44 U/L; $p < 0.05$ compared to the control and 6-hour fasting group) and peaked at 12 hours (71.00 ± 0.94 U/L), with significant increases compared to the control ($p < 0.05$), 6-hour ($p < 0.05$), and 8-hour groups ($p < 0.05$). Overall, intermittent fasting led to a time-dependent increase in cardiac biomarkers, with significant elevations observed at 8 and 12 hours of fasting compared to the control and the 6-hour fasting protocol.

Table 2. The Effect of Intermittent Fasting (IF) on Body Weight in Wistar Rats.

Groups	Pre-Weight (g)	Post-Weight (g)
Control	126.60 ± 1.08	154.20 ± 2.03
6 Hour IF	125.20 ± 0.86	135.20 ± 1.20^a
8 Hour IF	126.20 ± 0.58	134.00 ± 1.45^a
12 Hour IF	126.80 ± 1.06	138.40 ± 1.44^a

All values are expressed as Mean \pm SEM

^a $p < 0.05$, when compared with the control

Table 2 shows the effect of intermittent fasting (IF) on body weight in experimental groups. There was no significant difference in the pre and post-experimental body weights among the control animals. In contrast, all IF groups had a significant decrease in post-intervention weights compared to the control group ($p < 0.05$). The 6-hour fasting group had a mean post-intervention weight of 135.20 ± 2.63 g, the 8-hour fasting group recorded 134.00 ± 2.24 g, and the 12-hour fasting group showed a slightly higher post-weight of 138.40 ± 3.20 g.

4. Discussion

Intermittent fasting (IF) has gained significant attention for its potential benefits on metabolic and cardiovascular health. Cardiac biomarkers are commonly used in clinical practice in diagnosing and monitoring cardiovascular diseases [13, 14]. The cardiac troponins T and I (cTnT and cTnI) have been shown to have higher specificity and sensitivity as markers for diagnosing myocardial injury as well as indicators of heart abnormalities and lesions [16] while aspartate transaminase (AST) is among the first cardiac biomarkers though non-specific biomarker found in both liver and cardiac tissue,

and often used as an indicator of cellular damage [16, 27].

The present study observed a slight reduction in troponin I and troponin T levels among the 6-hour IF group when compared to the control and a significant increase in troponin I and troponin T among the 8 and 12-hour IF groups when compared to the control. The 12-hour IF group was significantly higher when compared with the control, 6 and 8-hour IF groups ($P < 0.05$). These findings suggest that intermittent fasting of short duration as seen in the 6-hour group may exert minimal myocardial stress. Although intermittent fasting has been associated with cardiovascular benefits [23, 28], prolonged fasting periods (>8 hours) could potentially contribute to subclinical myocardial strain, as evidenced by the progressive rise in cardiac biomarkers in this study. Extended fasting may trigger mild myocardial stress due to increased catecholamine release and metabolic shifts [29]. The increase in troponin levels in prolonged fasting states may be attributed to enhanced fatty acid oxidation and metabolic adaptations, which place additional stress and inflammation on the cardiomyocytes [30-32]. However, a few other studies demonstrated the beneficial effect of IF on the heart. For example, Wan et al [22] observed that IF protected the myocardium against ischemia-induced cell damage and inflammation in rats by increasing adiponectin levels in rats with myocardial infarction. Also, IF has been shown to reduce heart size and myocardial infarction in young rodents [21] while another study indicated a reduced left ventricular myocyte hypertrophy and collagen interstitial fraction when IF is combined with exercise [33].

Aspartate Transaminase (AST) is a non-specific enzyme found in cardiac, hepatic, and skeletal muscle tissues, and its elevation is often indicative of tissue damage or increased metabolic activity damage [16, 27, 34]. The progressive increase in mean AST levels with fasting duration, as observed in the present study, suggests potential hepatic involvement and metabolic stress during prolonged fasting. This elevation may be attributed to increased lipolysis and gluconeogenesis, which are hallmark physiological and metabolic adaptations to fasting. These processes place additional demands on the liver, potentially leading to cellular stress and the subsequent release of AST into the bloodstream [35]. The mean AST showed a time-dependent increase that was significant in the 12-hour IF group, which is consistent with the findings by Harahap et al [36] though the fasting period was for 16-20hrs. However, AST levels decreased in another study where the difference was attributed to energy restriction and subsequent reduction in weight and tissue adiposity [37]. It is worth noting that these cardiac biomarkers do not show the aetiology or pathogenesis of the disease. However, they characterize the final stage, and their concentrations in the blood correlate with the size of the cardiac injury [38].

The study revealed that the control group experienced a significant increase in body weight over the experimental period, whereas all intermittent fasting (IF) groups exhibited significantly lower post-experimental weights ($p < 0.05$). This

underscores the effectiveness of IF in reducing and maintaining body weight. These findings are consistent with previous studies, which have demonstrated that IF mitigates weight gain by modulating metabolic processes such as lipolysis, insulin sensitivity, and energy expenditure [39, 40]. Additionally, IF promotes increased fat oxidation, contributing to improved metabolic efficiency and potentially reduced weight gain over time [41-44]. Despite the marked reductions in body weight across all IF groups, the 12-hour fasting group had a slight increase in their final weight compared to the 6-hour and 8-hour IF groups. This could be due to a potential threshold effect, which occurs during prolonged fasting durations leading to adaptive compensatory mechanisms such as increased energy effectiveness and reduction in basal metabolic rate [44]. This suggests that fasting interventions, particularly shorter durations such as 6-hour and 8-hour fasting periods are more likely to be effective in preventing excessive weight gain compared to extended fasting periods such as the 12-hour IF protocol.

5. Conclusion

This study demonstrates that intermittent fasting (IF) for 12 hours led to elevated levels of cardiac biomarkers (troponin T, troponin I, and AST) in Wistar rats. While intermittent fasting is widely advocated for its weight loss and cardioprotective benefits, our findings suggest that prolonged fasting durations (>8 hours) should be approached with caution. These results highlight the need for careful consideration and monitoring of fasting regimens, particularly in individuals with pre-existing cardiovascular diseases or metabolic disorders, to mitigate potential risks associated with extended fasting periods.

Abbreviations

IF	Intermittent Fasting
cTnI	Cardiac Troponin I
cTnT	Cardiac Troponin T
AST	Aspartate Transaminase
CK-MB	creatine kinase, MB-type
Avidin-HRP	Avidin-Horseradish Peroxidase
BNP	natriuretic Peptide, B-type
CRP	C- Reactive Protein
TNNI3	Troponin I3, Cardiac Type
TNNT2	Troponin T2, Cardiac Type
OD	Optical Density
ANOVA	Analysis of Variance
SEM	Standard Error of the Mean

Author Contributions

Edith Reuben: Conceptualization, Funding acquisition, Investigation, Methodology, Writing – original draft

Bruno Chukwuemeka Chinko: Conceptualization, Supervision, Validation, Writing – review & editing

Nimisoere Peace Batubo: Funding acquisition, Project administration, Resources

Fortune Somiari Amah-Tariah: Supervision, Validation

Conflicts of Interest

The authors declare no conflicts of interest.

References

- [1] Hailu KT, Salib K, Nandeesh SS, Kasagga A, Hawrami C, Ricci E, Hamid P. The effect of fasting on cardiovascular diseases: a systematic review. *Cureus*. 2024; 16(1). <https://doi.org/10.7759/cureus.53221>
- [2] Longo VD, Di Tano M, Mattson MP, Guidi N. Intermittent and periodic fasting, longevity and disease. *Nature aging*. 2021; 1(1): 47-59. <https://doi.org/10.1038/s43587-020-00013-3>
- [3] Chijiokwu EA, Nwangwa EK, Oyovwi MO, Naiho AO, Emojewwe V, Ohwin EP, et al. Intermittent fasting and exercise therapy abates STZ - induced diabetotoxicity in rats through modulation of adipocytokines hormone, oxidative glucose metabolic, and glycolytic pathway. *Physiological Reports*. 2022; 10(20): e15279. <https://doi.org/10.14814/phy2.15279>
- [4] Welton S, Minty R, O'Driscoll T, Willms H, Poirier D, Madden S, Kelly L. Intermittent fasting and weight loss: Systematic review. *Canadian Family Physician*. 2020; 66(2): 117-25.
- [5] Ayudia EI, Harahap H, Irfannuddin I. The Effect of Intermittent Fasting Diet on Kidney Function. *International Journal of Islamic and Complementary Medicine*. 2020; 1(2): 65-70. <https://doi.org/10.55116/IJIM.V1I1.9>
- [6] Olamoyegun MA, Ajao FO, Iyedupe MO. Effect of 4-h time restricted feeding on body weight, leptin concentration and lipid profile in healthy non-obese male Wistar rats. *Journal of Obesity and Overweight*. 2021; 7(1): 1-12.
- [7] G̃ınbatar N, Bulduk B. The effect of Intermittent fasting on the growth and ghrelin hormone in rats feeding on a standard diet. *Journal of Contemporary Medicine*. 2022; 12(4): 570-3. <https://doi.org/10.16899/jcm.1123443>
- [8] Patikorn C, Roubal K, Veettil SK, Chandran V, Pham T, Lee YY, et al. Intermittent fasting and obesity-related health outcomes: an umbrella review of meta-analyses of randomized clinical trials. *JAMA network open*. 2021; 4(12): e2139558-e. <https://doi.org/10.1001/jamanetworkopen.2021.39558>
- [9] Malinowski B, Zalewska K, W̃sierska A, Sokołowska MM, Socha M, Liczner G, et al. Intermittent fasting in cardiovascular disorders—an overview. *Nutrients*. 2019; 11(3): 673. <https://doi.org/10.3390/nu11030673>
- [10] Danladi CD, Serakinci N, Ivanovich BA, Girich M. Intermittent Fasting Improves Some Serum Proteins: a Study on Rats Model. *Research Square* 2023; PREPRINT (Version 1). <https://doi.org/10.21203/rs.3.rs-2481031/v1>

- [11] Dote-Montero M, Sanchez-Delgado G, Ravussin E. Effects of intermittent fasting on cardiometabolic health: an energy metabolism perspective. *Nutrients*. 2022; 14(3): 489. <https://doi.org/10.3390/nu14030489>
- [12] Varady KA, Cienfuegos S, Ezpeleta M, Gabel K. Cardiometabolic benefits of intermittent fasting. *Annual review of nutrition*. 2021; 41(1): 333-61. <https://doi.org/annurev-nutr-052020-041327>
- [13] Thupakula S, Nimmala SSR, Ravula H, Chekuri S, Padiya R. Emerging biomarkers for the detection of cardiovascular diseases. *The Egyptian Heart Journal*. 2022; 74(1): 77. <https://doi.org/10.1186/s43044-022-00317-2>
- [14] Qureshi A, Gurbuz Y, Niazi JH. Biosensors for cardiac biomarkers detection: A review. *Sensors and Actuators B: Chemical*. 2012; 171: 62-76. <https://doi.org/10.3390/jpm12121942>
- [15] Subbaiah GV, Mallikarjuna K, Shanmugam B, Ravi S, Taj PU, Reddy KS. Ginger treatment ameliorates alcohol-induced myocardial damage by suppression of hyperlipidemia and cardiac biomarkers in rats. *Pharmacognosy Magazine*. 2017; 13(Suppl 1): S69. <https://doi.org/10.4103/0973-1296.203891>
- [16] Jbrael YJ, Hamad BK. Ameliorating impact of coenzyme Q10 on the profile of adipokines, cardiomyopathy, and hematological markers correlated with the glucotoxicity sequelae in diabetic rats. *Plos one*. 2024; 19(1): e0296775. <https://doi.org/10.1371/journal.pone.0296775>
- [17] Nair SM, Pareek A, Jamali MC. Assessment of Biochemical Markers for Early Detection and Monitoring of Cardiovascular Diseases: Myocardial Infarction and Heart Failure. *Quality Assurance*. 2024; 15(1): 288-95. <https://doi.org/10.25258/ijpqa.15.1.43>
- [18] Omran F, Kyrou I, Osman F, Lim VG, Randeva HS, Chatha K. Cardiovascular biomarkers: lessons of the past and prospects for the future. *International Journal of Molecular Sciences*. 2022; 23(10): 5680. <https://doi.org/10.3390/ijms23105680>
- [19] Home BD, Muhlestein JB, Anderson JL. Health effects of intermittent fasting: hormesis or harm? A systematic review. *The American journal of clinical nutrition*. 2015; 102(2): 464-70. <https://doi.org/10.3945/ajcn.115.109553>
- [20] Brennan L, McNulty B. New technology in nutrition research and practice. *Proceedings of the Nutrition Society*. 2017; 76(3): 173-4. <https://doi.org/10.1017/S0029665116002986>
- [21] Carvalho MR, Mendonça MLM, Oliveira JM, Romanenghi RB, Morais CS, Ota GE, et al. Influence of high-intensity interval training and intermittent fasting on myocardium apoptosis pathway and cardiac morphology of healthy rats. *Life Sciences*. 2021; 264: 118697. <https://doi.org/10.1016/j.lfs.2020.118697>
- [22] Wan R, Ahmet I, Brown M, Cheng A, Kamimura N, Talan M, Mattson MP. Cardioprotective effect of intermittent fasting is associated with an elevation of adiponectin levels in rats. *The Journal of nutritional biochemistry*. 2010; 21(5): 413-7. <https://doi.org/10.1016/j.jnutbio.2009.01.020>
- [23] Francis L, Young J, Lara J. The impact of intermittent fasting on body composition and cardiovascular biomarkers: a systematic review and meta-analysis. *Proceedings of the Nutrition Society*. 2017; 76(OCE2): E42. <https://doi.org/10.1017/s0029665117000982>
- [24] Chen M, Zhong VW. Abstract P192: Association Between Time-Restricted Eating and All-Cause and Cause-Specific Mortality. *Circulation*. 2024; 149(Suppl_1): AP192-AP. https://doi.org/10.1161/circ.149.suppl_1.P192
- [25] Albus U. *Guide for the care and use of laboratory animals (8th edn)*: SAGE Publications Sage UK: London, England; 2012. <https://doi.org/10.1258/la.2012.150312>
- [26] Council NR. *Guide for the care and use of laboratory animals*. 2010.
- [27] Danese E, Montagnana M. An historical approach to the diagnostic biomarkers of acute coronary syndrome. *Annals of translational medicine*. 2016; 4(10): 194. <https://doi.org/10.21037/atm.2016.05.19>
- [28] Cheung K, Chan V, Chan S, Wong MMH, Chung GK-K, Cheng W-Y, et al. Effect of intermittent fasting on cardiometabolic health in the Chinese population: a meta-analysis of randomized controlled trials. *Nutrients*. 2024; 16(3): 357. <https://doi.org/10.3390/nu16030357>
- [29] Longo VD, Mattson MP. Fasting: molecular mechanisms and clinical applications. *Cell metabolism*. 2014; 19(2): 181-92. <https://doi.org/10.1016/j.cmet.2013.12.008>
- [30] Lopaschuk GD, Ussher JR, Folmes CD, Jaswal JS, Stanley WC. Myocardial fatty acid metabolism in health and disease. *Physiological reviews*. 2010; 90(1): 207-58. <https://doi.org/10.1152/physrev.00015.2009>
- [31] Wang Y, Zhao R, Wu C, Liang X, He L, Wang L, Wang X. Activation of the sirtuin silent information regulator 1 pathway inhibits pathological myocardial remodeling. *Frontiers in Pharmacology*. 2023; 14: 1111320. <https://doi.org/10.3389/fphar.2023.1111320>
- [32] Young EA, Cantu TL, Harris MM. Gastrointestinal and cardiac response to refeeding after low-calorie semistarvation. *The American journal of clinical nutrition*. 1989; 50(5): 922-9. <https://doi.org/10.1093/ajcn/50.5.922>
- [33] Hall AR, Karwi QG, Kumar S, Dongworth R, Aksentijević D, Altamimi TR, et al. Fasting increases susceptibility to acute myocardial ischaemia/reperfusion injury through a sirtuin-3 mediated increase in fatty acid oxidation. *Scientific Reports*. 2022; 12(1): 20551. <https://doi.org/10.1038/s41598-022-23847-w>
- [34] Chinko BC, Pughikumo DT. Haematological and Hepatorenal Alterations Induced by Potash (Akanwu) on Male Wistar Rats. *International Blood Research & Reviews*. 2023; 14(1): 38-46. <https://doi.org/10.9734/ibrr/2023/v14i1300>
- [35] Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arco-raci V, et al. Oxidative stress: harms and benefits for human health. *Oxidative medicine and cellular longevity*. 2017; 2017(1): 8416763. <https://doi.org/10.1155/2017/8416763>

- [36] Harahap H, Ayudia EI, Kusdiyah E. The Effect of Intermittent Fasting (Time Restriction Feeding) on Body Weight, Aspartate Transaminase and Alkaline Transaminase in Sprague Dawley Rats. The 3rd Green Development International Conference, 2020. Universitas Jambi. 87.
- [37] Faris M, Jahrami H, Abdelrahim D, Bragazzi N, BaHamam A. The effects of Ramadan intermittent fasting on liver function in healthy adults: a systematic review, meta-analysis, and meta-regression. *Diabetes research and clinical practice*. 2021; 178: 108951. <https://doi.org/10.1016/j.diabres.2021.108951>
- [38] Upadhyay RK. Emerging risk biomarkers in cardiovascular diseases and disorders. *Journal of lipids*. 2015; 2015(1): 971453. <https://doi.org/10.1155/2015/971453>
- [39] Soeters MR, Lammers NM, Dubbelhuis PF, Ackermans M, Jonkers-Schuitema CF, Fliers E, et al. Intermittent fasting does not affect whole-body glucose, lipid, or protein metabolism. *The American journal of clinical nutrition*. 2009; 90(5): 1244-51. <https://doi.org/10.3945/ajcn.2008.27327>
- [40] Antoni R, Johnston KL, Collins AL, Robertson MD. Effects of intermittent fasting on glucose and lipid metabolism. *Proceedings of the Nutrition Society*. 2017; 76(3): 361-8.
- [41] Harvie MN, Pegington M, Mattson MP, Frystyk J, Dillon B, Evans G, et al. The effects of intermittent or continuous energy restriction on weight loss and metabolic disease risk markers: a randomized trial in young overweight women. *International journal of obesity*. 2011; 35(5): 714-27. <https://doi.org/10.1038/ijo.2010.171>
- [42] Patterson RE, Sears DD. Metabolic effects of intermittent fasting. *Annual review of nutrition*. 2017; 37(1): 371-93. <https://doi.org/10.1146/annurev-nutr-071816-064634>
- [43] Morales-Suarez-Varela M, Collado Sanchez E, Peraita-Costa I, Llopis-Morales A, Soriano JM. Intermittent fasting and the possible benefits in obesity, diabetes, and multiple sclerosis: a systematic review of randomized clinical trials. *Nutrients*. 2021; 13(9): 3179. <https://doi.org/10.3390/nu13093179>
- [44] Kersten S. The impact of fasting on adipose tissue metabolism. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*. 2023; 1868(3): 159262. <https://doi.org/10.1016/j.bbalip.2022.159262>