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Original Article

COMPARISON OF THE EFFECT OF RAMADAN AND INTERMITTENT FASTING PATTERNS ON TRIGLYCERIDE LEVELS IN ELDERLY MALE WISTAR RATS (*RATTUS NORVEGICUS*)

SAIDATUL FITHRIYAH[1*], NABILA K. CAHYANI[2], NUR MAHMUDAH[3], BUDI HERNAWAN[4]

[1,4]Department of Physiology, Faculty of Medicine, Universitas Muhammadiyah Surakarta, Ahmad Yani Street, Sukoharjo-57169, Central Java, Indonesia. [2]Medical Education Study Program, Faculty of Medicine, Universitas Muhammadiyah Surakarta, Ahmad Yani Street, Sukoharjo-57169, Central Java, Indonesia. [3]Deparment of Histology, Faculty of Medicine, Universitas Muhammadiyah Surakarta, Ahmad Yani Street, Sukoharjo-57169, Central Java, Indonesia

*Corresponding author: Saidatul Fithriyah; *Email: sf945@ums.ac.id

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ABSTRACT

Objective: To compare the effect of Ramadan and intermittent fasting patterns on triglyceride levels in elderly male wistar rats (*Rattus norvegicus*).

Methods: This research is a quantitative experimental study conducted in a laboratory setting, utilizing a posttest-only control group design. A total of 18 male rats aged 24 to 25 mo were divided into three groups, namely, K, P1, and P2, with each group containing of 6 rats. K was control group, P1 group fasted according to the Ramadan fasting pattern, and P2 group fasted according to the intermittent fasting pattern, respectively, for 10 consecutive days. The triglyceride levels were analyzed using a spectrophotometer.

Results: The highest average triglyceride levels after the intervention were found in P1 group compared to other treatment groups; with the mean value of triglyceride levels was 63.50±22.11 mg/dl. In K group, the average of triglyceride level was 46.83±9.45 mg/dl and in P2 group, it was 60.50±26.09 mg/dl. The results of the comparative analysis between groups using the Kruskal-Wallis test showed the significance value was p = 0.341, so there was no significant difference between groups.

Conclusion: Ramadan and intermittent fasting patterns for 10 consecutive days do not have a significant effect on triglyceride levels in elderly male Wistar rats (*Rattus norvegicus*).

Keywords: Ramadan fasting pattern, Intermittent fasting pattern, Triglyceride, Elderly

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INTRODUCTION

World Health Organization (WHO) defines an elderly person as someone aged 60 years or older [1]. Globally, the elderly population reached 1.4 billion in 2022, marking an increase of one billion since 2020. By 2030, one from six people globally will be 60 years old or older. Furthermore, by 2050, the worldwide elderly population is expected to reach 2.1 billion [2]. Indonesia has entered the aging population structure since 2021. In 2022, the elderly accounted for 11.75% of the population. Over the past decade (2010-2022), the percentage of elderly people in Indonesia has grown by at least 4%, translating to an annual increase of approximately 0.3% [3].

In old age, the aging process will occur, which is a complex biological process characterized by a decrease in body function over time and the result is decrease in quality of life [4]. The aging process occurs due to various factors, one of which is changes in lipid metabolism. With age, there is an increase in plasma triglyceride levels, along with an increase in plasma lipoprotein levels. This occurs because of the rate of clearance of plasma triglyceride levels decreases along with a decrease in the activity of the Lipo-Protein Lipase (LPL) enzyme [5].

Increased triglyceride levels in the elderly can be overcome by fasting, which limits food intake for a certain period of time. During the fasting period (12-36 h), triglycerides are converted into fatty acids and glycerol to produce energy [6]. Fasting patterns that can be done include Ramadan and intermittent fasting patterns.

Ramadan fasting is a fast performed by Muslims by refraining from food intake, drinks, and everything that cancels the fast from dawn to sunset in a period of 12-20 h for 29-30 days [7]. When the body is deprived of carbohydrates, blood glucose levels decrease and glucagon is released to increase lipolysis and gluconeogenesis. Glucagon stimulates lipolysis by enhancing the activity of Hormone-Sensitive Lipase (HSL) and Adipose Tri-Glyceride Lipase (ATGL) in adipose tissue, which then break down triglycerides into glycerol and fatty acids [8]. Ramadan fasting also decreases leptin levels and increases adiponectin levels in the body. Leptin is one type of adipokine hormone secreted by adipose cells to regulate energy homeostasis and modulate satiety when energy reserves in the body are sufficient by sending signals to the hypothalamus to suppress food intake and stimulate energy expenditure. In a fasting state, when energy reserves in the body are reduced, leptin levels will decrease and cause an increase in appetite. Adiponectin is an adipokine hormone secreted by adipose tissue that one of its functions is regulating the inflammatory process through activating the AMP-activated Protein Kinase (AMPK) pathway by Adiponectin Receptor 1 (AdipoR1), which increases fatty acid breakdown and reduces inflammatory effects [9]. In the fasting state, the level of adiponectin in the body increases, which causes an improvement in lipid metabolism by increasing the process of fatty acid oxidation through the expression and activity of Peroxisome Proliferator-Activated Receptor-Alpha (PPAR-α) and upregulating acetyl-Co-A, thereby reducing triglyceride stores in the body [10]. Research conducted by Dinata *et al.* (2023) showed that fasting during the month of Ramadan (for 30 days) carried out consecutively in middle-aged adults (>40 years) was proven to reduce triglyceride levels (before fasting was 186.50±75.12 mg/dl and after fasting was 113.65±43.91 mg/dl) [11].

Intermittent fasting is a dietary method that involves abstaining from caloric intake for specific periods. There are three main approaches to intermittent fasting: Time-Restricted Feeding (TRF), Alternate-Day Fasting (ADF), and modified fasting [12, 13]. This fasting pattern offers various health benefits, primarily by activating the process of intracellular lipolysis, which breaks down triglycerides into glycerol and fatty acids. In the intracellular lipolysis pathway, triglycerides are hydrolyzed by lipolytic enzymes: ATGL catalyzes the conversion of triglycerides to Di-Acyl Glycerol (DAG), HSL transforms DAG into Mono-Acyl Glycerol (MAG), and Mono-Glyceride Lipase (MGL) completes the process by breaking down MAG into Free Fatty Acids (FFAs) and glycerol. During fasting, the activity of ATGL and HSL increases, enhancing intracellular lipolysis and providing FFAs as an energy source for the body. This mechanism mobilizes stored fat in adipocytes for energy use during fasting, leading to a reduction in triglyceride levels [14]. In a study conducted by Gabel *et al.* (2018) showed that intermittent fasting with the TRF method carried out at the age of 25-65 years can reduce triglyceride levels, from before to after fasting, were 105±11 mg/dl to 93±9 mg/dl [15].

Previous studies have examined the benefits of fasting on triglyceride levels in young adults and middle adulthood; however, direct comparisons between the effects of Ramadan and intermittent fasting patterns in the elderly is still very limited. The lack of researches that examining the effects of Ramadan and intermittent fasting patterns on triglyceride levels in the elderly is the basis for researchers to conduct this research.

MATERIALS AND METHODS

Material

This research was a quantitative laboratory experiment utilizing a posttest-only control group design, where the dependent variable (triglyceride levels) was measured after the intervention, and the posttest results were compared across groups. The research design (posttest-only) was chosen to prevent mortality of the subjects after the pretest conducted. Eighteen elderly male Wistar rats (*Rattus norvegicus*) aged 24-25 mo, with body weight ranging from 250-350 gs, obtained from the Biomedical Laboratory of the Faculty of Medicine, Universitas Muhammadiyah Surakarta. Rats at this age (24-25 mo) are equivalent to humans around 60 years old [16]. Male Wistar rats were chosen for this study due to their relatively fast metabolic rates, making them more sensitive to research on body metabolism. Additionally, male rats are unaffected by hormonal fluctuations and they share similar environmental, genetic, and aging responses to humans [17].

This research was conducted at the Biomedical Laboratory of the Faculty of Medicine, Universitas Muhammadiyah Surakarta (UMS) and has passed the ethical feasibility test by the Health Research Ethics Commission of Dr. Moewardi Hospital Surakarta with ethical clearance number 2.533/X/HREC/2024 on October 28, 2024, for the treatment of Ramadan and intermittent fasting patterns as well as sampling data and measurement of triglyceride levels in Wistar male rats (*Rattus norvegicus*).

Methods

Eighteen male Wistar rats aged 24-25 mo with the body weights of 250-350 gs were used in the present study. The rats were habituated to the experimental room for seven days prior to experimental treatments. The rats were randomly assigned into three groups consisting of six rats per group. Sample size calculation in this research using resource equation approach. From the calculation, the results obtained that the minimum and maximum sample required was 5-7 rats each group to maintain the Degree of Freedom (DF) in the range of 10-20 [18]. So, 6 rats each group are sufficient for this study. K group was the control group, that was not fasted, had unlimited access to food and drink both during day and night. This group given food and drink ad libitum. P1 group was the group with Ramadan fasting pattern treatment (13:11), which includes fasting period for 13 h (16,00-05,00 Waktu Indonesia Barat (WIB)) and feeding period for 11 h (05,00-16,00 WIB). P2 group was the group with intermittent fasting pattern treatment (16:8), which includes a fasting period for 16 h (05,00-21,00 WIB) and a feeding period for 8 h (21,00 – 05,00 WIB), according to the method studied by Moro *et al.* (2016) [19]. Rats are nocturnal animals, so the time of treatment given based to this cycle. During the Ramadan fasting period, rats did not consume any food and drink, while for the intermittent fasting period, rats were allowed to consume non-calorific drinks. The treatments for all groups were carried out for 10 consecutive days. Because 10 days in rat is equal to 1 mo in humans [16].

They were housed in separate well-ventilated cages (1 rat per cage) under standard conditions of naturally 12 h light/dark cycles and free access to pellets and water. Animal cages were made of polycarbonate measuring 40x25x20 cm, with a cage lid in the form of a stainless-steel wire lid equipped with a drinking bottle and feeding container. The bedding materials (cage base substrate) were wood shavings and the cages were cleaned every day. Rat feed was in the form of pellets with the AD II brand produced by PT. Japfa Comfeed Indonesia.

After 10 days fasting intervention, the rats were weighed again and blood was taken through the retroorbital vein to determine the triglyceride levels after the intervention. Before the blood sampling taken, all groups were fasted for approximately 8 h without restrictions on drinking non-caloric drinks. The blood obtained was placed into a 1.5 ml microtube and centrifuged at 5000 rpm for 10 min, to take the serum. Triglyceride levels in serum measured using a spectrophotometer UV-1800 produced by Shimadzu Japan. The procedures of triglyceride measured principles was use the enzymatic colorimetric method. Measure absorbance at 540 nm using the spectrophotometer, then calculate triglyceride concentration using the formula:

$$\text{Triglyceride (mg/dl)} = \frac{\text{(Absorbance of sample)}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$$

The data of triglyceride level was analyzed using one-way ANOVA procedure. Statistical significance was set at a probability value of $p<0.05$. All statistical analyses were carried out using statistical software application.

RESULTS AND DISCUSSION

Result

Table 1 presents the data of the rat body weight before and after fasting intervention of the three group. It was found that there was an increase in Body Weight (BW) of rats in all treatment groups, both in the control, Ramadan, and intermittent fasting group.

Table 1: Mean BW (g) of rats before and after intervention

Groups	Mean BW before fasting±SD	Mean BW after fasting±SD
K	311.3±12.91	329.5±12.91
P1	296.6±23.23	306.8±23.23
P2	305.6±24.15	337.5±24.15

K = control; P1 = Ramadhan fasting treatment; P2 = intermittent fasting treatment, BW: body weight; g: g; SD: standard deviation.

Based on the table 1, the highest average body weight after fasting was the intermittent fasting group, which is 337.5±24.15 g.

Table 2 presents the data of the triglyceride level of the three groups. All groups had triglyceride levels within normal limit (26-145 mg/dl), but all intervention groups have higher tryglyceride levels. Group with the highest mean triglyceride levels was the Ramadan fasting group with the mean triglyceride levels 63.50±22.11 mg/dl. The results of the Shapiro-Wilk test showed that the significance value of K and P2 group was>0.05, while the P1 group, the significant value was <0.05. The results suggesting the data were not normally distributed. Because of it, the homogeneity test was not performed and the data were analyzed using the Kruskal-Wallis test to evaluate the post-test results between groups.

Kruskal-Wallis of these data showed there was no significant main effect of groups in the level of triglyceride.

Table 2: Mean triglyceride levels (mg/dl) after intervention

Groups	Mean±SD
K	46.83±9.45
P1	63.0±22.11
P2	60.50±26.09
Results of Kruskal-Wallis: Groups: df = 2, p = 0.341	

K = Control, P1 = Ramadhan fasting treatment, P2 = Intermittent fasting treatment, SD: Standard deviation, df: Degree of freedom, p: Significance value.

DISCUSSION

One factor that might cause weight gain was stress that may be experienced by the rats during the research period (we just found out it, after the intervention was complete). It can be caused by the rats were kept in cages separately from other. Because rats were animals that live in groups, when separating them from their group would cause the rat become stressed [20].

Social isolation in rats has been linked to weight gain through complex physiological and behavioral mechanism. Social isolation raises plasma corticosterone levels, a stress-related glucocorticoid that contributes to hyperphagia (excessive eating) and weight gain. This hormone interferes with normal appetite control and energy metabolism. While isolation leads to a decrease in ghrelin (an appetite stimulant), it paradoxically increases food intake due to corticosterone counteracting the effects of ghrelin. At the same time, muscle loss occurs through increased myostatin (a muscle growth inhibitor), which reduces lean body mass despite weight gain. Rodents isolated and fed high-fat diets show increased fat accumulation, insulin resistance, and systemic inflammation, which worsen obesity-related metabolic conditions [21, 22].

Stressful conditions in rats can cause increased secretion of the hormone cortisol/corticosterone. Cortisol is a stress hormone produced by the adrenal cortex that functions to regulate the immune system and control blood sugar. Excessive secretion of cortisol can cause inflammation in tissues, especially in adipose tissue. Inflammation that occurs in adipose tissue can cause increased accumulation of visceral fat. Accumulation of visceral fat can cause weight gain [23].

Corticosterone influences lipid metabolism in the body by elevating triglyceride levels through the upregulation of genes associated with lipid synthesis, such as Sterol Regulatory Element-Binding Protein 1 (SREBP1), Fatty Acid Synthase (FAS), and Glycerol-3-Phosphate Acyl-Transferase (GPAT), and decreased expression of genes associated with beta-oxidation of fatty acids, such as Carnitine Palmitoyl Transferase (CPT) and Acyl-CoA Oxidase (ACOX) genes [24]. The response of rats to stress causes hyperphagia behavior, where rats consume more food in stressful situations as a response to anxiety or discomfort. Thus, even though the rats were treated with fasting when entering the feeding period, the rats tend to consume large amounts of food (hyperphagia) to replace the energy lost during fasting and as a response to the stress experienced. As the result, the body weight of rats will increase and cause an increase in triglyceride levels in the body [25, 26].

From the results of this study, the highest increase in body weight was obtained in the intermittent fasting group. This is because in intermittent fasting, the fasting period was done when the rats were in the resting phase (morning-afternoon) and the feeding period is done when the rats are active (night). Circadian rhythms affect the gastrointestinal tract (GIT) in various functions, including digestive function, nutrient absorption, and secretion of enteroendocrine hormones that are important for regulating metabolism. Circadian rhythms in digestion are largely influenced by the pattern of food intake, which serves as the main circadian *zeitgeber* (time marker) in the GIT. Meal timing also influences the secretion of intestinal hormones, namely incretin hormones such as Glucagon-Like Peptide-1 (GLP-1) produced by enteroendocrine l cells mainly found in the small intestine and colon and Glucose-dependent Insulinotrophic Polypeptide (GIP), which is a hormone secreted by small intestinal K cells that plays a role in increasing insulin secretion. The digestive phase in rats was active at night when there was food intake into the body. During this phase, enzymes that digest nutrients are increased, such as the enzyme disaccharidase, which is an enzyme that plays a role in carbohydrate digestion, enzymes involved in the breakdown of proteins and lipids also increase during the feeding period. Besides enzymes, there are several transporters whose expression increases during the feeding period, such as peptide (peptide cotransporter 1) and triglyceride transporters as well as cholesterol transporters (apolipoprotein B and intestinal microsomal triglyceride transfer protein) [27].

This study used elderly male Wistar rats (*Rattus norvegicus*) as research subjects where rats are nocturnal animals. Thus, the intermittent fasting period of rats is carried out in the morning-late afternoon and for the period of eating rats is carried out at night, where at night food is digested and absorbed optimally. At the same time, due to it stress resulting in the accumulation of nutrients (glucose, fatty acids, and amino acids) in the body and form adipose tissue, resulting in increased body weight.

From the Kruskal-Wallis test, we can conclude there was no significant difference between group (p>0.05). The highest mean triglyceride levels in Ramadan fasting group occurs because during the fasting period of Ramadan, there is a total restriction of food and beverage intake, which has a direct impact on appetite and hunger during fasting, which increases gradually and peaks at the time of breaking the fast. This has the potential to cause unbalanced consumption of food eaten (eating large portions) when breaking the fast [28]. The results of this study are in line with the results of research conducted by Mirmiran *et al.* (2019) revealed that there was an increase in triglyceride levels in adult men and women after Ramadan fasting (for 30 days with a fasting duration of 8-14 h). This increase was due to an increase in plasma FFAs concentration released from adipose tissue during fasting [29].

The response of rats to stress causes hyperphagia behavior, where rats consume more food in stressful situations. This condition can mask the true effects of fasting. Chronic stress triggers the activation of the Hypothalamic-Pituitary-Adrenal (HPA) axis, which results in increased levels of Gluco-Corticoids (GCs), such as cortisol. These hormones play a dual role; they can increase appetite during stress recovery but may suppress it during acute stress episodes. For example, while some people may seek "comfort foods" rich in sugar and fat as a response to stress, others may experience a reduced appetite under similar stress conditions. Stressed individuals often tend to choose highly palatable foods that are calorie-dense and high in sugar and fat, even when they are not physically hungry. This tendency is more prominent in those who are overweight or obese, suggesting that stress may worsen pre-existing irregular eating habits. The preference for such foods can lead to higher caloric consumption, which may obscure the body's natural physiological responses to stress [30].

Fasting also elevates the number of orexin neurons, which are crucial in boosting appetite, particularly under energy-deficient conditions like fasting. Orexin neuron activation is associated with increased food-seeking and consumption behaviors, especially during energy shortages. Beyond direct regulation of feeding behavior, orexin also influences the motivation to eat in emotional or stressful situations tied to hunger. This highlights orexin's role in integrating emotional and metabolic signals. The feeding behavior of rat is influenced by specific neurons in the hypothalamus. Neurons that produce Melanin-Concentrating Hormone (MCH) and orexin in the Lateral Hypothalamus Area (LHA) are closely linked to feeding regulation. MCH plays a key role in controlling feeding behavior, with its activity increase during fasting, as indicated by the phosphorylation of cAMP Response Element-Binding protein (CREB) via the P2X7R gene, which enhances the desire to eat [31].

During the fasting period on Ramadan, there is a change between eating patterns that are shifted during the day, which results in changes in circadian rhythms, especially changes in the hormone melatonin which decreases due to changes in eating patterns that occur during the Ramadan fasting pattern [32]. Melatonin plays a role in shifting energy metabolism towards the use of fat through the process of lipolysis, if melatonin levels decrease there will be a decrease in lipolysis, which causes fat accumulation in the body. Decreased melatonin levels also lead to decreased expression of Un-Coupling Protein 1 (UCP1) in Brown Adipose Tissue (BAT) which plays a role in reducing lipid stores in the body. This process is mediated by the Fibroblast Growth Factor 21 (FGF21) hormone, which plays a role in increasing energy metabolism and lipolysis. With decreased levels of FGF21 and decreased expression of UCP1 protein during Ramadan fasting pattern, it causes an increase in lipid levels in the body [33].

The results of this study showed that the measurement of triglyceride levels after the intervention in the K group was 46.83±9.45 mg/dl, in the P1 group the mean value was 63.50±22.11 mg/dl, and in P2 group the mean value was 60.50±26.09 mg/dl. Normal triglyceride levels in rats are 26-145 mg/dl [34], so that the results of these measurements, both the Ramadan and the intermittent fasting patterns group have higher mean triglyceride levels than the control group. Meanwhile, the group with the highest mean triglyceride levels was in the Ramadan fasting group.

The results of this study also prove that in intermittent fasting there was also an increase in triglyceride levels due to the increased body weight of rats and the resulting accumulation of ectopic fat, which increases triglyceride levels in the body [35]. The results of this study are also in line with the results of research by Harahap *et al.* (2023) proved that there was an increase in triglyceride levels after intermittent fasting (fasting for 24 h by consuming 20-25% of calories). Triglyceride levels before fasting were 70.33 mg/dl and after fasting, increased to 84.25 mg/dl [36].

The rise in triglyceride levels despite theoretical activation of lipolysis, can be attributed to several interrelated physiological mechanisms: Insulin is a powerful antilipolytic hormone, and resistance to it can diminish the effectiveness of lipolysis. In individuals with insulin resistance, even if lipolysis is theoretically activated, the body may struggle to effectively mobilize fatty acids for energy, leading to elevated triglyceride levels due to impaired clearance and metabolism of circulating lipids. Regarding lipolysis and triglyceride dynamics, studies have shown that while stimulated lipolysis (e. g., through catecholamines) can be enhanced, basal lipolysis (the rate of lipolysis during fasting or resting states) is positively correlated with triglyceride levels. This indicates that increased basal lipolytic activity doesn't always result in efficient fat utilization, potentially leading to higher circulating triglycerides. The liver also plays a crucial role in conditions like diabetes or metabolic syndrome, where it can increase the production and secretion of Very Low-Density Lipoproteins (VLDL), which are high in triglycerides. This overproduction by the liver can occur regardless of peripheral lipolysis rates, contributing to elevated plasma triglycerides even when lipolytic activity is high. Additionally, increased circulating FFAs from lipolysis can lead to insulin resistance and alter lipid metabolism pathways, further worsening dyslipidemia. Elevated FFAs can stimulate hepatic triglyceride production while hindering their clearance from circulation, resulting in higher triglyceride levels [37, 38].

In this study, the duration of fasting undertaken was for 10 days on the body's metabolism, especially on adipose tissue. Fasting causes changes in adipose tissue morphology by reducing the size of White Adipose Tissue (WAT), which functions to store energy reserves by storing triglycerides in the body. Acute fasting also causes an increase in the amount of BAT through the process of 'browning' in WAT by increasing the expression of the UCP1 gene, which is a marker of BAT activation and reducing lipid stores in the body. Acute fasting improves lipid metabolism by reducing the expression of FAS and Acetyl-CoA Carboxylase (ACC) genes and increasing the expression of genes associated with lipolysis (HSL and ATGL) [39].

This study used elderly male Wistar rats (*Rattus norvegicus*); along with increasing age, there are changes in body metabolism, which include increased plasma triglyceride levels, decreased plasma triglyceride clearance, decreased LPL activity, decreased adipose tissue lipolysis, and increased ectopic fat accumulation [23, 41]. Elevated triglyceride levels in the elderly are associated with reduced plasma triglyceride clearance. In the triglyceride clearance process, LPL plays an important role to hydrolyse triglycerides from triglyceride-rich lipoproteins (chylomicrons and VLDL) and release fatty acids to be absorbed by adipose tissue and muscle tissue. With age, LPL activity tends to decrease resulting in decreased clearance of triglycerides from the circulation and increased plasma triglyceride levels. Adipose tissue lipolysis, triglycerides are broken down into fatty acids, which are then released into the bloodstream. Adipose tissue lipolysis occurs after activation by adrenergic receptors (catecholamines); the activity of lipase enzymes (ATGL and HSL) decreases with age leading to decreased lipolysis activity and increased visceral fat accumulation in aging, which in turn increases plasma triglyceride levels [41].

CONCLUSION

Based on the research findings presented it can be concluded that Ramadan and intermittent fasting patterns do not have a significant impact on triglyceride levels in elderly male Wistar rats (*Rattus norvegicus*). Further research are need to increase the number of samples size for each group in order to strengthen adequacy of the results of statistical analysis and determine the effect of Ramadan and intermittent fasting patterns on triglyceride levels in elderly male Wistar rats (*Rattus norvegicus*) with placed 2 or 3 rats per cage (pair-housing), so that the rats will not be stressed. It is also recommended to measuring stress biomarkers, so that the results obtained are indeed the result of fasting, not the result of confounding factors such as stress (biased results). The stricter calorie control during fasting and measuring food consumption are also need to be considered so that it can provide more significant results.

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AUTHORS CONTRIBUTIONS

Sa'idatul Fithriyah was responsible for conceptualization, supervision, methodology, review, and writing the original draft. Nabila K. Cahyani contributed to conducted experiments, performed data analysis, writing the original draft, and editing. Nur Mahmudah and Budi Hernawan also played a role in writing review and editing.

CONFLICT OF INTERESTS

The author's declare no conflict of interest.

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