



Effect of Dawood Fasting on the Increased Level of Antioxidant Enzymes

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Abstract

BACKGROUND: The incidence of degenerative diseases is increasing. The underlying mechanism for such disease includes rising oxidative stress without correspondingly adequate antioxidants. Intermittent fasting (IF) may stimulate mild oxidative stress with a corresponding increase in antioxidants. Dawood fasting (DF) as an alternative diet similar to IF is normally performed for <24 h (± 14 h) with intermittent intervals of 1st-day fasting, 2nd-day *ad libitum* (AL) diet, 3rd-day fasting, and so on.

AIM: The aim of this study to examine the effect of DF on the changes in superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (Cat) antioxidant enzymes in BALB/c strain mice.

METHODS: A total of 15 BALB/c strain mice were randomly divided into three groups. The AL control (ALC) group was given a standard diet (AIN93) and AL drink every day. The high-fat diet control group was treated with a high-fat diet (24%) and daily AL drink. The DF group fasted on the 1st day (no food or drink) from 17:00 to 07:00, continued every other day, and given a standard feed (AIN93) and AL drink for 8 weeks. Examination of SOD levels in the liver as well as GPx and Cat levels in plasma was carried out using a spectrophotometer. The analysis was conducted using the one-way analysis of variance test followed by Tukey's test (95% confidence level).

RESULTS: The levels of SOD in ALC, DF, and HF groups were $52.86 \pm 5.73\%$, $68.57 \pm 3.7\%$, and $38.57 \pm 4.3\%$, respectively ($p = 0.00$). The GPx levels were 43.06 ± 2.75 U/L, 55.54 ± 2.71 U/L, and 32.26 ± 2.14 U/L, respectively ($p = 0.00$), and the corresponding Cat levels were 5.82 ± 0.63 U/ml, 6.55 ± 0.64 U/ml, and 3.44 ± 0.64 U/ml ($p = 0.00$).

CONCLUSION: The levels of SOD, GPx, and Cat antioxidant enzymes during DF were higher compared to an AL diet and a high-fat diet.

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Introduction

Long-term fasting can raise oxidative stress, which is indicated by increases in reactive oxygen species (ROS) [1], level of saturated fatty acid, and lipid peroxidation [2]. If this condition goes unchecked, it will lead to cell metabolic disorders [3], [4] and damage to deoxyribonucleic acid (DNA), thus resulting in gene mutations [5]. Protein transformation due to oxidative stress can cause protein dysfunction and physiological disorders as well as the development of various degenerative diseases, including hypertension, atherosclerosis, stroke, diabetes mellitus (DM), coronary heart disease, Alzheimer's, and other chronic diseases [6], [7]. Meanwhile, according to the statistical data of the Global Status Report on Non-Communicable Disease from the World Health Organization, by the end of 2008, degenerative diseases have led to the deaths of nearly 36 million people worldwide and are expected to continue to increase by 70% of the global population. It is predicted that by 2030 there

will be 52 million deaths per year in total because of degenerative diseases [8].

A number of degenerative diseases appear through an increased oxidative stress mechanism. Oxidative stress is a phenomenon caused by an imbalance between the production and accumulation of ROS in tissues and the ability of biological systems to detoxify such reactive product [9]. The human body has the ability to deal with oxidative stress using both enzymatic and non-enzymatic antioxidants. Among the enzymatic antioxidants are superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (Cat) [9]. SOD is the main antioxidant enzyme that plays a role in protecting the body from superoxide. Superoxide is catalyzed by SOD to become hydrogen peroxide, then by GPx and Cat to be converted into water [9], [10].

Previous studies have shown that even though long-term fasting can increase oxidative stress. Islamic fasting, which is widely known as intermittent fasting (IF) performed for <24 h followed by iftar (dinner) and sahoor

(pre-dawn meal) before the next fasting day, is able to improve antioxidant levels and thus decrease oxidative stress, instead [11]. This will stimulate the immune system, as indicated by the increased ability of macrophages to kill bacteria [12]. In IF, the oxidative stress imbalance triggers the activation of antioxidant system to detoxify reactive free radicals, resulting in a balance that can ultimately repair cell damage [13]. Other previous studies found that Islamic fasting (Ramadan fasting) indicates neither an increase in oxidative stress [14] nor a change in the levels of pro-oxidants and antioxidants [15].

Ramadan fasting offers health benefits in that it can reduce the risk of cardiovascular disease, diabetes, cancer, hypertension, and asthma [16]. In contrast, a systematic review proved that Ramadan fasting has only little effect on the immune system [17], but it can at least become a lifestyle that offers an alternative to the prevention of various diseases [18]. Daily fasting in Islam is exclusively performed during the month of Ramadan, and it is proven that the immune system only undergoes a temporary change. After Ramadan and without fasting, the immune system returns to the same condition as before Ramadan [17]. Therefore, this study aims to examine a different fasting model in Islam named Dawood fasting (DF). DF is performed on the 1st day for approximately 14 h, skipped on the 2nd day, continued again on the 3rd day, and so on. Muslims can perform this fasting model all year round. The fasting includes abstinence from eating, drinking, or smoking, and the recommendation is to start it with having a pre-dawn meal and to end it by eating iftar in the evening. Our previous study showed that although DF indicates no changes in the parameters of lipid profile, hematology [19], and quality of life among the elderly [20], there is a decrease in the oxidative stress and lipid profile among the mice given high carbohydrate and fat diets [21]. The purpose of this study is to prove the effect of DF on the changes in SOD, GPx, and Cat antioxidant enzymes in BALB/c strain mice. The increased antioxidant enzymes after fasting Dawood are expected to support the body in dealing with oxidative stress to prevent degenerative diseases.

Methods

Research design

This study was a true experimental study using randomization with a post-test-only control group design. The experiment was conducted at the Research Laboratory of the Faculty of Medicine, Universitas Islam Indonesia.

Research subjects

The research subjects were male BALB/c strain mice aged 8–10 weeks, weighing 15–30 grams,

healthy with no physical disabilities, and never used as a research subject. The exclusion criteria were mice that died during the treatment, showed inactive movements or refused to eat and drink during the adaptation period, and showed aggressive behavior or appeared not agile and weak. The research subjects were obtained from the Experimental Animal Laboratory of the Faculty of Medicine of Universitas Islam Indonesia.

Determination of sample size

The number of experimental animals used in this study was determined using the formula [22] $E = \text{total number of animals} - \text{total number of groups} = 10-20$. E is a constant within a range of 10–20. This study had 3 groups; therefore, as calculated, the total number of experimental animals to be used in this study should be between 13 and 23 mice with each group consisting of 4–7 mice. This study involved 5 mice as the experimental animal for every group.

Interventions

Before interventions, the mice were put in adaptation in a standard cage for 7 days in a room with a temperature of $23 \pm 2^\circ\text{C}$. The mice were individually caged. The lighting was set to 12 h of daylight-dark cycles. During the adaptation period, *ad libitum* (AL) food and drink were administered daily at 7 am and 5 p.m. After the adaptation, the mice were divided randomly into three groups, each consisting of 5 mice. The normal control group AL control (ALC) was given a standard feed (AIN93) [23] and AL drink on a daily basis. The negative control group high-fat diet control (HFC) was treated with a high-fat diet (24% fat) and given AL drink every day. The fasting group (DF) was made to do DF without drinking and given AIN93 diet and AL drink when not fasting. On the 1st day at 5 pm, the feed was not given, and on the 2nd day, the diet was administered at 7 am and 5 pm. On the 3rd day, the feed was given only at 7 am and so on. Such treatment was administered for 8 weeks.

Sampling

Before the termination, the mice were fasted for 10 h. They were anesthetized with intramuscular injection of 100 mg/kg ketamine. Blood sampling for GPx and Cat examination was done after the mice were anesthetized. The blood samples were taken from the medial canthus of the orbital sinus using a capillary tube. As much as 0.5 mL of blood was collected and put into an Eppendorf tube. The blood was then centrifuged at a speed of 2000 rpm for 20 min to produce serum. In addition, after the abdomen was opened, the liver was taken for SOD examination. Identification of SOD, GPx, and Cat levels was carried out at the Food and Nutrition Laboratory of PAU, Universitas Gadjah Mada.

Ethical clearance

This study has been approved by the Medical and Health Research Ethics Committee of the Faculty of Medicine of Universitas Islam Indonesia through the approval letter No. 48/Ka/Kom.Et/70/KE/IV/2018.

Analysis of SOD levels

The liver was homogenized by being ground and centrifuged. After centrifugation, the liver supernatant was collected and reacted with the SOD reagent of the activity assay kit (Catalog #K335-100) by following the corresponding protocol. The mixture was then incubated on a plate at 37°C for 20 min, and the absorbance was read at a wavelength of 450 nm using a microplate reader.

Analysis of GPx levels

For GPx analysis, as much as 20 μ L serum was taken and added with 200 μ L of 0.1 M phosphate buffer pH 7.0 and then put into a cuvette. The sample was added with 200 μ L of 10 mM reduced glutathione (GSH) and incubated for 10 min at 37°C. After that, it was added with 200 μ L of 1.5 mM NADPH and incubated for 3 min at 37°C. The solution was added with 200 μ L of 1.5 mM H₂O₂, and the absorbance was measured using a spectrophotometer at a wavelength of 340 nm (A₀).

Analysis of Cat levels

For Cat analysis, 60 μ L of serum was taken and reacted with 2.70 mL of 50 mM sodium carbonate buffer and added with 0.04 unit of xanthine oxidase. After 30 min, the absorbance was measured at a wavelength of 560 nm. The blank solution was obtained from the solution prepared for the blood samples, that is, the phosphate buffer saline containing 11.5 g/L KCl. The Cat activity was calculated using the equation $[1 - (\text{Absorbance of sample solution} / \text{absorbance of blank solution})] \times 100\%$.

Statistical analysis

The Shapiro-Wilk normality test was employed to examine the distribution of numerical data, while the variance was analyzed using the Levene's test. One-way analysis of variance (ANOVA) test was used to determine differences in SOD, GPx, and Cat levels among the groups. If there were statistically significant differences, the *post-hoc* test was employed. The confidence level of 95% was applied and the differences were considered significant if the $p < 0.05$.

Results

Before the interventions, the randomly grouped mice were first measured for the weights to ensure that they were in the same condition at the beginning of the study. The results of one-way ANOVA test showed no difference in the mean body weight among the groups before the treatment ($p > 0.05$).

SOD levels

The analysis of SOD levels in mice from the liver tissue indicated that the highest was found in the DF group (68.57 \pm 3.7%) followed by the ALC group and the HFC group (Figure 1). The one-way ANOVA test resulted in $p < 0.05$ ($p = 0.000$), indicating significant differences in the mean SOD levels among the three groups. The *post-hoc* test (Tukey's test) also found significant differences between the groups.

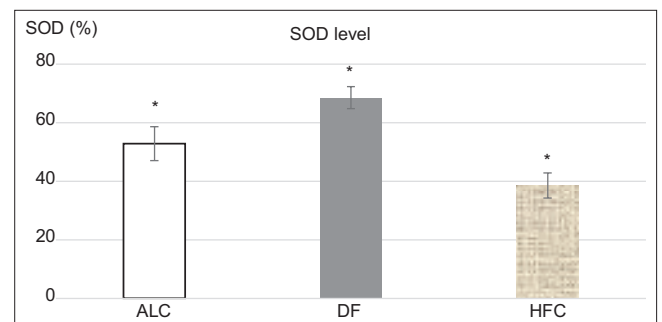


Figure 1: Effect of Dawood fasting administered for 8 weeks on hepatic superoxide dismutase activity. The data are presented as mean \pm standard deviation ($n = 5$). Significant differences were determined using a one-way analysis of variance followed by the *post-hoc* Tukey's test. * $p < 0.05$ versus the ad libitum control diet and high-fat control diet

GPx levels

The highest mean GPx levels were found in the DF group (55.54 \pm 2.71 U/l) followed by the ALC group and HFC group with a p value = 0.000. These findings marked the significant differences in the mean GPx levels among the treatment groups. The *post-hoc* test (Tukey's test) also showed that the mean GPx levels were significantly different between the groups (Figure 2).

Cat levels

The group of mice that received DF treatment had the highest mean Cat levels (6.55 \pm 0.64 U/mL) followed by the ALC and HFC groups with $p = 0.000$. The Tukey's test obtained significantly different mean Cat levels between the DF group and the ALC and HFC groups (Figure 3).

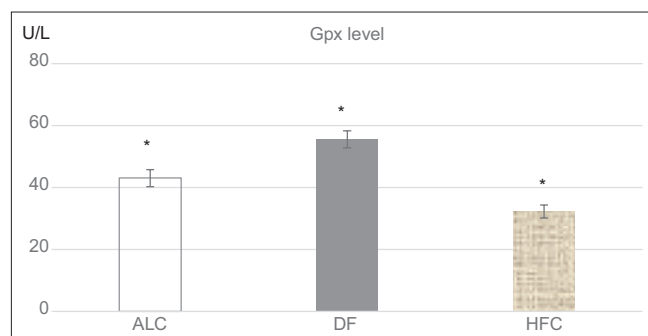


Figure 2: Effect of Dawood fasting for 8 weeks on plasma glutathione peroxidase levels. The data are presented as the mean \pm standard deviation. Significant differences were determined by the one-way analysis of variance test followed by post-hoc Tukey's test, * $p < 0.05$ versus the ad libitum control diet and high-fat control diet

Discussion

The statistical analysis showed that the mean levels of SOD antioxidant in the DF group were significantly higher than those in the ALC and HFC groups. Our previous study found that DF could reduce the level of oxidative stress through the malondialdehyde (MDA) marker compared to the AL diet [21]. A previous study of IF for 24 h also indicated an increase in SOD levels and a decrease in MDA levels in the liver of rats induced with alcoholic fatty liver disease and in the kidney of DM rats compared to that of DM-induced rats with AL diet [24]. Similarly, such finding was shown in the hippocampus of rats induced with cerebral hypoperfusion [25] and in the spleen of rats with a lymphoma risk, thus making it feasible to reduce the incidence of lymphoma [26].

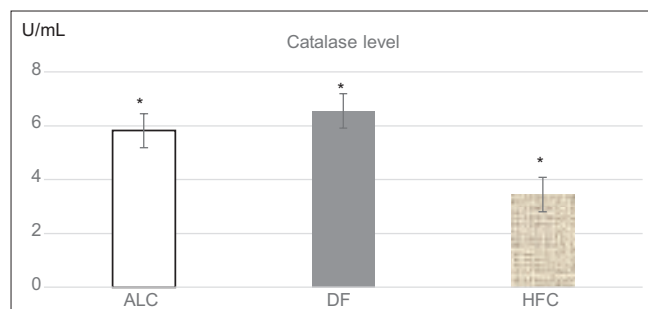


Figure 3: Effect of Dawood fasting for 8 weeks on plasma catalase levels. The data are presented as the mean \pm standard deviation. Significant differences were determined by the one-way analysis of variance test and followed by the post-hoc Tukey's test, * $p < 0.05$ versus the ad libitum control diet and high-fat control diet

This study also found higher levels of GPx and Cat in DF compared to those in the ALC and HFC groups. Previous studies of 24-h IF also showed the same results [27], [28], and it was the same finding in 6-h IF [29]. IF can increase SOD, GPx, and Cat levels because of the adaptation response mechanism [30]. While fasting, the body will break down the adipose tissue into free fatty acids. The beta-oxidation of free fatty acids in the mitochondria of liver tissue will produce ketone objects as a result of metabolites that

cause mild oxidative stress. However, this condition will be followed by the activation of NFE2-related factor 2 which functions as a transcription of antioxidant and detoxification genes, thereby reducing oxidative stress [31]. In addition to Manganese-SOD, there are chaperone proteins and proliferator-activated receptor gamma coactivator 1 α that deal with DNA damage and regeneration of mitochondria and organelles through the mechanism of autophagy [30]. Fasting can lead to high oxygen (O₂) demand due to increased metabolism, but it is compensated by a rise in bioenergy [32]. This shows that fasting has an effect on increasing hormesis, which is the ability to adapt and neutralize numerous endogenous challenges, thereby improving the survival rate [33].

However, other studies of 24-h IF interventions found no differences in the GPx and Cat levels in the liver, heart, and skeletal muscle; even in the brain, it showed a decrease [32]. This is because fasting can actually raise oxidative stress because the body lacks energy and has to process gluconeogenesis, thus accumulating free radicals [34]. The high oxidative stress can disrupt the antioxidant system, reduce antioxidant enzymes such as GPx, thereby leading to increased oxidative stress such as lipid peroxidase and MDA [35], the risk of severe oxidative damage [36], and an effect on kidney functions [37]. Fasting has a different meaning from the terms starvation or malnutrition. Fasting for <24 h does not cause starvation. Starvation without subsequent refeeding will activate degradation of lipids and protein catabolism in the tissue along with increasing nitrogen as a result of a stressful condition which is not supported by adequate antioxidants. Such condition as fasting for 5 consecutive days without adequate provision of nutrients leads to decreasing antioxidants and increasing free radicals, thereby triggering oxidative stress [38].

This study proves that DF model can actually stimulate an increase in antioxidants. A previous study found that increased antioxidants are able to protect lymphocyte cell membranes from the oxidation caused by oxidative stress as well as increase the immunoglobulin (Ig) IgM and IgG. This is because antioxidants have the ability to improve interleukin formation in the body and stimulate immunomodulator activation. Therefore, with an improvement in the components of the immune system, one of the defense mechanisms of the body against antigens can function appropriately and is able to reduce the risk of disease [39].

In contrast, the levels of SOD, GPx, and Cat antioxidant enzymes in the high-fat diet group are lower, and this is identical to the findings of a previous study [40]. A high-fat diet causes excessive oxidative stress, resulting in an imbalance in antioxidant demand and subsequently leading to inflammation. At the beginning of a high-fat diet, the antioxidant enzymes increase, but the longer the high-fat diet is performed, the higher the oxidative stress and the lower the

antioxidant enzymes will be [41]. DF is expected to become an alternative lifestyle to prevent imbalanced oxidative stress, which can further prevent various degenerative diseases that have recently become more prevalent. However, this research has a limitation in that it is conducted on experimental animals.

Conclusion

The model of DF can become an alternative to food restriction to improve levels of SOD, GPx, and Cat antioxidant enzymes.

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