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RESEARCH ARTICLE

The Effect of Fasting on the Concentration of Enzimatic Antioxidants *(Superoxide Dismutase and Glutathione Peroxidase)* in Rats

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ABSTRAK

Latar belakang: Konsumsi kalori yang berlebih dapat meningkatkan kejadian penyakit degeneratif yang dimediasi oleh ROS. Pembatasan kalori, terbukti meningkatkan kadar antioksidan *Superoxide Dismutase* (SOD) dan *Gluthatione Peroxidase* (GPx). Puasa seperti puasa Ramadan (PSPR) merupakan bentuk pembatasan kalori, namun belum jelas apakah berpengaruh terhadap peningkatan kadar SOD dan GPx.

Tujuan: mengetahui efek PSPR terhadap kadar SOD dan GPx.

Metode: dalam *post test only control group design,* sampel sebanyak 24 tikus *Sprague Dawley* jantan berusia 3 bulan, berat badan 250-300 gram, dikelompokkan menjadi 4 kelompok secara random. Kelompok 1 (P-70), 2 (P-100), dan 3 (P-140) dipuasakan selama 6 jam/hari, masing-masing mendapatkan kalori 70%, 100%, dan 140%. Kelompok 4 (C-AL) mendapatkan kalori 100%, diberikan secara *ad libitum.* Hari ke-16 diambil darah dan diperiksa kadar SOD dan GPx dengan metode ELISA. Data dianalisis dengan *one way* ANOVA dilanjutkan *post-hoc* LSD, dengan tingkat signifikansi p<0,05.

Hasil: analisis statistik anova menunjukkan bahwa kadar SOD dan GPx diantara kelompok terjadi perbedaan bermakna, p = 0.000. Hasil uji post hoct kadar SOD (318,64) dan GPx (89.16) pada kelompok P-70, dibandingkan dengan kelompok C-AL (278,60 dan 57,20) lebih tinggi secara signifikan (p = 0,00). Kadar SOD dan GPx kelompok P-70 dibandingkan dengan kelompok P-140 (92,03 dan 48,79), lebih tinggi secara signifikan (p = 0,00). Dibanding dengan kelompok P-100 (296,70 dan 75,71) kadar SOD dan GPx pada kelompok P-70 lebih tinggi bermakna, p = 0.000.

Kesimpulan: Puasa seperti puasa Ramadan dengan jumlah kalori 70% dan 100% selama 15 hari mampu meningkatkan kadar SOD dan kadar GPx pada tikus jantan sprague dawley.

Kata kunci: puasa, *caloric restriction,* SOD, GPx

ABSTRACT

Introduction: *Consumption of excessive calories can increase the incidence of degenerative diseases mediated by ROS. Caloric restriction, have been shown to increase levels of antioxidants superoxide dismutase (SOD) and Gluthatione Peroxidase (GPx). Fasting like Ramadan fasting (FLRF) is a form of calorie restriction, but its effect on levels of SOD and GPx remains unclear.*

Objectives: *to investigate the effect of fasting on levels of SOD and GPx.*

Methods: *in a post-test only control group design, sample of 24 rats Sprague Dawley Rats aged 3-month-old, weighing 250-300 grams, were randomly divided into 4 groups. Group 1 (P-70), 2 (P-100), and 3 (P-140) were fasted for 6 hours/day, each group received of 70%, 100% and 140% calories respectively. Group 4 (C-AL) received 100% calories, ad libitum. Day 16 blood was taken and levels of SOD and GPx were determined by ELISA. Data were analyzed using one-way ANOVAs, followed by post hoc LSD tests, p<0.05 was considered statistically significant.*

Results: *the results showed that the levels of SOD and GPx occur significant differences between the groups, p = 0.000. The test results post hoc SOD (318.64) and GPx (89.16) group P-70, compared with group C-AL (278.60 and 57.20) was significantly higher (p = 0.00). SOD and GPx P-70 group compared with the group P-140 (92.03 and 48.79), significantly higher (p = 0.00). Compared with group P-100 (296.70 and 75.71) SOD and GPx in group P-70 was significantly higher, p = 0.000.*

Conclusion: *Fasting with calorie intake of 70% and 100% for 15 days can increases levels of SOD and GPx in male rats.*

Keywords: *fasting, caloric restriction, SOD, GPx*

INTRODUCTION

The globalization affects lifestyle changes in developing countries (Swinburn *et al.,* 2004). Lifestyle such as excessive calorie intake, can lead to increased prevalence of degenerative diseases caused by oxidative stress. Oxidative stress can be caused by decreased activity of enzymes such as superoxide dismutase (SOD) and Glutathione Peroxidase (GPx) (Rahman, *et al.,* 2007). To control this, require a reduction in the number of foods by calorie restriction/caloric restriction (CR). Fasting in Ramadan month is one way of calorie restriction with a partial or total of all the food (Trepanowski, 2010). This is in accordance with the word of the prophet Muhammad: "Fast, and

you will be healthy" (Kusumah, 2007). However, up to present, it remains unclear whether the fasting like Ramadan fasting (FLRF) can increase the levels of antioxidants such as superoxide dismutase (SOD) and Glutathione Peroxidase (GPx).

According to National Basic Health Research (Riskesdas 20013) the prevalence of some degenerative diseases in Indonesia remain high. Coronary heart disease showed the prevalence rate of 1,5% in the population aged 15 years or more. The prevalence of stroke was 12,1 per thousand population and Diabetes Mellitus (DM) in the population aged 15 years orabove, was 2,1%. While other data states that the prevalence of tumor/cancer in Indonesia is 1,4 per 1000 population (Balitbangkes, 2013). Various degenerative diseases are associated with oxidative stress cells caused by elevated levels of ROS. Therefore, reducing ROS levels by caloric restriction is an effective way to reduce the incidence of degenerative diseases (Khaled *et al.,* 2011). Various studies have shown that caloric restriction in rats with streptozotocin diabetes induced by showed that CR effective in reducing body weight (BW), blood glucose levels, HbAIC, concentrations of TG, and increases HDL concentrations, however, did not produce a significant effect on the activity of antioxidant enzymatic and concentration malondihaldehid (MDA) (Ugochukwu *et al.,* 2004). Other studies on the effect of calorie restriction on oxidative stress and antioxidants in humans showed no correlation between oxidative stress parameters and changes in body weight, body mass index (BMI) and fat mass (Meydani *et al.,* 2011). Research among 27 men showed that Ramadan fasting could reduce oxidative stress in macrophages (Lahdimawan *et al.,* 2013). Based on his studies of CR, how the CR with FLRF can reduce oxidative stress through increased levels of SOD and GPx remains unknown.

Calorie restriction through fasting will reduce the intake of calories in the body. This would affect the levels of glucose, fats and amino acids in the body. When fasting is done with calorie restriction, by regulating the amount of calories during fasting, it will reduce the number of calories per day, which can decrease the synthesis of Adenosine Triphospat (ATP) and Reactive Oxygen Species (ROS) associated with a decrease in mitochondrial oxidative phosphorylation. On the other hand an increase in levels of antioxidants SOD and GPx enzimatic (Heilbronn, 2003). These events can reduce oxidative stress, inhibits apoptosis, and tissue damage that will reduce degenerative diseases (Rahman, 2007). Objective of this study was to determine the effects of FLRF on levels of SOD and GPx. Calorie restriction through FLRF expected to reduce the incidence of degenerative diseases as a result of oxidative stress that can improve the quality of life.

METHOD

The present study was an analytical randomized trial with *post test only control group design.* The study was conducted in the laboratory of Food and Nutrition Studies Centre, University of Gadjah Mada. A total of 24 male Sprague Dawley (SD) rats aged 3 -month-old, weighing 250-300 grams, were randomly devided into 4 groups. Group 1 (P-70); 2 (P-100); and 3 (P-140); fasted for 6 hour /day starting at 08.00 - 14:00 then given calories as 70%, 100%, and 140% were given with a feeding tube. While group 4 (C-AL) was not fasted and given 100% calories, *ad libitum.* The study was conducted for 15 days and the provision of calories using the converted AIN-93 formula (presented in table 1). Every 1 gram AIN-93m produces 3.6 kcal, so the number of calories was 70% (378 kcal), 100% (540 kcal) and 140% (756 kcal), while the number of calories in a group 4 was 100%. Drinking water is

Table 1. Formulation AIN-93m for rats rearing (Panel, 2007)

INGREDIENTS	(g/kg diet)
Corn starch	465,992
Casein (> 85% protein)	140,000
Dextrinized cornstarch (90-94% tetrasaccharides)	155,000
sucrose	100,000
Soybean oil (no additives)	40,000
fiber	50,000
Mineral mix (AIN-93g-MX)	35,000
Vitamin mix (AIN-93-VX)	10,000
L-cystine	1,800
Choline bitartrate (41.1% choline)	2,500
Tert-Butylhydroquinone (TBHQ), mg	8.0

Table. 2. The mean of body weight, Total Calories, levels of SOD and GPx

VARIABLE	GROUP P- 70 (n = 5) ($\bar{x}$ ± SD)	P-100% (n = 5) ($\bar{x}$ ± SD)	P-140% (n = 5) ($\bar{x}$ ± SD)	C-AL (n = 5) ($\bar{x}$ ± SD)	*P*
BW after fasting	(290.80 ± 5.63)	(292.00 ± 8.45)	(308.20 ± 4.44)	(298.00 ± 6.67)	
Σ Calories (kcal)	378	540	756	358.20	0.00
SOD (U/mL)	(318.64 ± 6.99)	(296.70 ± 5.29)	(92.03 ± 3.91)	(278.60 ± 4.29)	
Shapiro-Wilk	0.53	0.30	0.34	0.92	
Levene test					0.51
one way ANOVA					0.00
GPx (U/mL)	(89.16 ± 4.60)	(75.71 ± 5.95)	(48.79 ± 7.03)	(57.20 ± 7.04)	
Shapiro-Wilk	0.00	0.32	0.31	0.31	
Levene test					0.74
one way ANOVA					0.00
Pearson Correlation r = 0.74					0.00

provided *ad libitum* at 14:00 to 8:00 o'clock for group 1-3, while there was no time restriction for group 4. Blood samples were taken on day 16 for level of SOD and GPx assessment by ELISA. Data were analyzed by one way ANOVA followed by post-hoc LSD with a significance level of <0.05. This study was approved by the ethics committee of Medical Faculty of Sultan Agung Islamic University Semarang.

RESULTS

After being fasted like Ramadan fasting for 16 days, the rats were subjected to levels of SOD and GPX, the result is presented in Table 2.

In accordance with Table 2, the results of this study indicate that there is a difference in SOD and GPx among the groups.

Levels of SOD

The highest SOD levels was found in the group of fasting with 70% (318.64 U/mL) calories intake, followed by fasting with 100% (296.70 U/mL) calories intake, and *ad libitum* group (278.60 U/mL). The lowest levels of SOD (92.03 U/mL) was found in in the fasting group with of 140% calories intake. *One-way* ANOVA showed significant differences in SOD levels between groups, p = 0.000 (Table 2).

Post-hoc LSD test on SOD showed that the levels of SOD in group P-70 was significantly higher (p = 0.00) compared with that of in group C-AL. SOD in group P-70 was was significantly higher (p = 0.00) compared to that of in P-140. SOD in group P-70 was significantly higher (p = 0.00) compared with that of in group in P-100. SOD in P-100 group was significantly higher (p = 0.00) compared with that of C-AL group. SOD in the P-100 was significantly higher (p = 0.00) compared to that of P-140 group. SOD in the P-140 group was significantly lower (p = 0,00). compared to that of in the group C-AL. GPx levels in group P-70 was significantly higher (p = 0.00) compared to that of in group C-AL *(Figure 1. The Concentration of SOD and GPx (A))*.

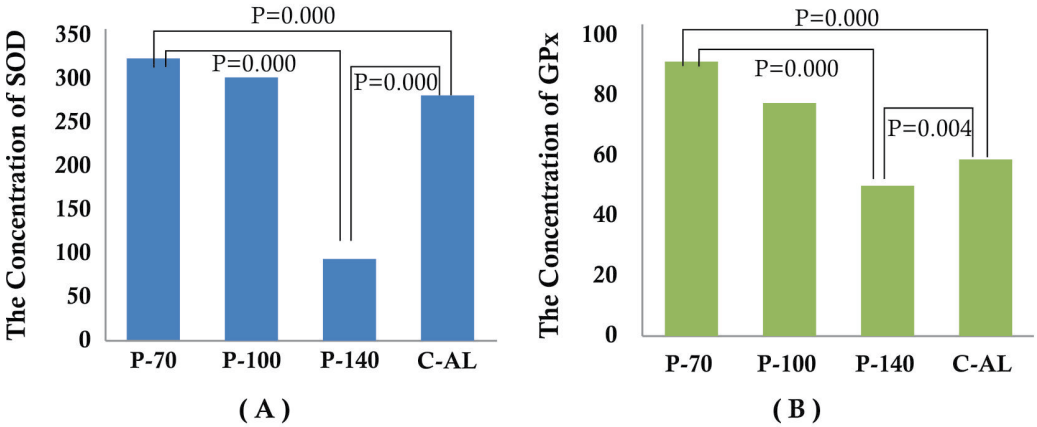

Figure 1. The Concentration of SOD and GPx

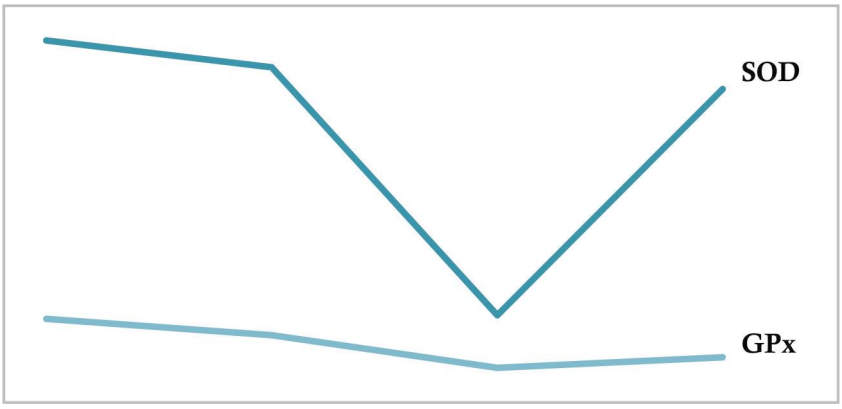

Figure 2. Correlation Between SOD and GPx

Levels of GPx

The highest GPx levels found in the fasting group with 70% calorie (89.16 U/mL) intake, followed by fasting calorie counts 100% (75.71 U/mL), *ad libitum* group (57.20 U/mL, and the lowest levels of GPx (48.79 U/mL) in the fasting group with calorie counts 140%. Test results for *one-way* ANOVA showed a significant difference in the levels of GPx between groups, p = 0.000.

The results of *post-hoc* LSD test on levels of GPx showed that levels of GPx the P-70 group compared with the group P-140, significantly higher, (p = 0.000). GPx levels in group P-70 when compared with the P-100 group was significantly higher (p = 0.000). GPx levels in P-100 group compared with the group C-AL also significantly higher (p = 0.00). GPx levels in group P-100 compared to the fasting group P-140 also significantly higher (p = 0.00). While levels of GPx in fasting P-140 group was significantly lower (p = 0.04), compared with group C-AL *(Figure 1. The Concentration of SOD and GPx (B)).*

The correlation between the levels of SOD and GPx levels

To determine a correlation between increased levels of SOD and high levels of GPx Pearson correlation test was applied. The results showed that there was a strong positive correlation (r = 0.74) and significant (p = 0.00) between the levels of SOD by GPx. These results suggest that elevated levels of SOD will be followed by increased levels of GPx (Figure 2).

DISCUSSION

The results in this study showed that the group with FLRF 70% calori intake had higher levels of SOD (318.64) and GPx (89.16), followed by fasting with 100% calorie intake. When compared with *ad libitum* group, the levels of SOD and GPx were still higher. The results of this study supports previous results by Jayrone & Parimal (2012), showing that a diet with caloric restriction of 80% for 2 weeks in rats SD, were able to significantly reduce levels of MDA and increase antioxidants and decreases oxidative stress in all tissues (Jayrone & Parimal, 2012). In this study, rats were fasted like Ramadan fasting with CR 70% and CR 100% for 15 days can increase SOD and GPx. This illustrates that this study are consistent with previous research showing that fasting with calorie restriction of 70% - 100% has been shown increase the levels of SOD and GPx, while calorie restriction up to 80% have been shown to repair oxidative stress.

Reduction in calorie intake during fasting will cause a decrease in the rate of energy metabolism so that the oxygen consumption is also reduced, and this is helpful to the decline in the production of toxic reactive oxygen species (ROS). Antioxidants are substances that neutralize free radicals (Noori, 2012). Decrease oxidants (ROS) as a result of fasting due to decreased production of ROS, especially oxygen radicals of complexes I and III in the mitochondria oxidative phosphorylation reactions (Noori, 2012). Decreased levels of ROS will be followed by an increase in the relative levels of antioxidants SOD and GPx resulting in a balance of antioxidants with a more stable oxidant, as a result of oxidative stress and cell damage can be prevented. This study also confirm other studies showing that 40% CR in adult rats could increase the activity of SOD so as to prevent oxidative stress (Guyton, 2006).

Fasting group with 140% calorie intake have the lowest levels of SOD. It is a implies that excessive caloric consumption can improve energy metabolism rate so that the production of ROS will increase the impact on the activity of SOD and GPx leading to damage cell membranes and DNA mutations (Baynes, 2003). By comparing the levels of SOD and GPx in the group of P-140 and P-70 and 100 on the results of this study, the cellular oxidative stress can be reduced or even prevented by fasting like fasting Ramadan with

CR of 70% and CR 100% per day. However further researchs is needed to prove that PLRF with 70% and 100% CR can reduce oxsidative stress indicated by decreased levels of MDA and 8OHdG.

The highest levels found in the group GPx fast with 70% calorie intake, followed by fasting group with 100%, calorie intake, the *ad libitum* group, and the lowest levels of GPx in the fasting group with 140% calorie intake. Pevious studies have shown that 40% CR conducted for 9 weeks in wistar rats causes an increase in the activity of GPx (Ugochukwu, 2004). This present study differs in term of the fact that CR of 70% and 100% can increase GPx in 2 weeks, so it can be concluded that FLRF with calorie reduction up to 70% and 100% per day was shown to significantly increase the levels of GPx and SOD in SD rats.

The study is limited for its uncontrolled food in *ad libitum* group. Initially, this group was expected to consume as much energy as 540 kcals, but in fact adlibitum group can only consume 358.20 Kcal calories for 15 days. Although the number of calories in the *ad libitum* group was lower than the fasting group of 70% and 100%, but levels of SOD and GPx in *ad libitum* group remained higher than 70% of the fasting group. These results suggest that by fasting like Ramadan fasting still can increase the levels of SOD and GPx, while eating and drinking adlibitum has been shown to unable to increase the levels of SOD and GPx, although it has a number of lower calorie intake, it can be concluded that FLRF independently have a role to boost antioxidant levels both SOD and GPx, however more studies are needed.

The results of this study also illustrates that the total calorie intake in the fasting group with CR 70% and groups fasting with 100% CR, although higher than the *ad libitum* group, has a lower body weight compared with *ad libitum* groups (data not shown). The results supports studies conducted on male Wistar rats aged 3 month old given CR 60% having a lower body weight than the rats received feed *ad libitum* (Ugochukwu *et al.,* 2004). Similarly, adult humans undergoing fasting with various compositions CR also showed a decrease in body weight (Varady, 2011). These results suggest that the rats SD fasted as Ramadan fasting with CR 70% and 100% cause a significant reduce in body weight. The factors of the increase in body weight in at *ad libitum* group were likely caused by food intake, absorption macronutrients, and of course the continues energy supply compared to fasting group, so overall calorie intake indicated by glucose levels in adlibitum was higher (Baynes, 2003). However further studies are still needed.

On the other hand, the decline in the body weight on FLFR is caused by decrease in the amount of adipose tissue. According to the literature during fasting, body weight tend to decline due to loss of adipose tissue caused by a decrease in caloric intake by 30% of the total calorie intake before the fasting and changes in the types of activities during fasting (Baynes, 2003). Fasting includes two phases fasting state in which absorption of carbohydrates takes place 3-6 hours after sahur meal and fasting timein which is the sugar levels decrease occurring six hours after eating sahur meal until breakfasting (Baynes, 2003). Fasting time phase has been associated with cause of significant body weight loss, considering that in this phase 50% the supply of energy for the body's metabolism is derived from fat catabolism. On the other hand, despite fasting, a high calorie intake will not cause decline in body weight. Evidence exists showing that the number of calories in fasting group with CR with 140% calorie intake had the highest body weight compared to other groups. This is because the daily caloric intake exceeds energy output (Nix, 2009). With time, excess daily calories will result in a significant reduction.

The results also showed that there was a strong positive correlation between increased levels of SOD and GPx levels. This suggests that increased levels of SOD will lead to increase in the levels of GPx. The results of this study supports previous published reports showing that SOD, the enzyme that converts oxygen radicals from phosphorylation oxidative mitochondria into hydrogen peroxide (H2O2), followed by increased catalase to convert H2O2 to H2O and O2. In addition to catalase, GPx also plays a role in changing the toxic H2O2 into non toxic H2O and O2. Besides converting H2O2 into H2O and O2, GPx also reduces or regenerate oxidized gluthation (GSH) is to reduced gluthation wih the help of enzymes gluthation reductase, so GPx continue to function as an antioxidant (Kefer JC *et al.* 2009).

CONCLUSION

Fasting like Ramadan Fasting with 70% and 100% calorie intake for 15 days can increase the levels of SOD and GPx expected to prevent oxidative stress and degenerative diseases.

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