OPEN ACCESS

Indonesian Journal of Human Nutrition

P-ISSN 2442-6636
E-ISSN 2355-3987
www.ijhn.ub.ac.id

Effect of Energy Restriction on Malondialdehyde Levels in Rats

A. Millaty Halifah Dirgahayu[1,2*)], Aminuddin Aminuddin[3], Arif Santoso[4], Nurpudji Astuti Daud[5], Ika Yustisia[6], Irfan Idris[7]

[1] Concentration on Aging and Regenerative Medicine, Postgraduate Program in Biomedical Sciences, Graduate School of Hasanuddin University, Makassar, South Sulawesi
[2.] Departement of Physiology, Faculty of Medicine, University Muslim of Indonesia, Makassar, South Sulawesi
[3] Departement of Clinical Nutrition, Faculty of Medicine, Hasanuddin University, Makassar, South Sulawesi
[4] Departement of Pulmonology, Faculty of Medicine, Hasanuddin University, Makassar, South Sulawesi
[5] Departement of Clinical Nutrition, Faculty of Medicine, Hasanuddin University, Makassar, South Sulawesi
[6] Departemen Biochemistry, Faculty of Medicine, Hasanuddin University, Makassar, South Sulawesi
[7] Departement of Physiology, Faculty of Medicine, Hasanuddin University, Makassar, South Sulawesi

*Correspondence author: A. Millaty Halifah Dirgahayu. E-mail: titydirgahayu@gmail.com

Accepted: January 2022 Reviewed: February 2022 Published: July 2023

ABSTRACT

Elevated malondialdehyde (MDA) levels indicate the occurrence of cell membrane oxidation. Calorie restriction is known to extend life expectancy. This study aims to investigate the effect of calorie restriction on MDA levels in young and old rats. An experimental approach was employed using the Rattus norvegicus Wistar strain as the experimental animals, with a pre-post-test control group design. A total of 28 white rats were included in this study and were divided into four groups. The control groups, Group A and Group B, received standard feed and unrestricted access to distilled water, while the treatment groups, Group C and Group D, were fed with a 40% reduction in calorie intake and distilled water. Data analysis was performed using SPSS 21.0 (SPSS, Inc., Chicago, IL) with a 0.05 significance level. The results showed a difference in MDA level changes between group B (control group) and group D (treatment group) ($p<0.05$). This study concludes that MDA levels are higher in the elderly compared to young rats, and a 40% calorie restriction can reduce MDA levels

Keywords: energy restriction, malondialdehyde, Rattus novergicus

INTRODUCTION

The aging process is a complex physiological mechanism, and numerous theories about the aging process have been proposed by many experts. These theories continue to evolve, but no single theory can comprehensively explain the aging process [1]. However, theories often support and complement one another. One theory that has recently been widely embraced and trusted in understanding the aging process is the free radical theory, initially proposed by Denham Harman in 1956. In modern aging theory, this theory

DOI: https://doi.org/10.21776/ub.ijhn.2023.010.01.6

is called the oxidative stress theory [2,3,4]. Oxidative stress refers to an imbalance between the production of oxidants and the capacity of detoxification by antioxidants, causing functional disturbances at the cellular and molecular level [2,4,5] Free radicals formed due to oxidative stress play a crucial role in causing damage to cell function and cell survival. These free radicals include superoxide anions, hydroxyl, peroxyl, and purine radicals, which are produced during normal cell metabolism. In addition, free radicals are also formed through mitochondrial respiration, auto-oxidation of biomolecules, and environmental pollutants and radiation [6].

Reactive oxygen species (ROS), which are compounds that contain one or more unpaired electrons, have the potential to cause cell damage, including mitochondria. Increased ROS levels result in the cessation of cell replication (replication arrest or replication senescence), leading to impaired cell viability and aging [1,9]. This process gradually affects the structure and function of all systems, which decreases the adaptive capacity and increases mortality and morbidity. Humans naturally have complex antioxidant systems, both enzymatic and non-enzymatic, which synergistically protect cells and organ systems from damage caused by free radicals. Despite this natural antidote system, some free radicals still escape. Moreover, the endogenous antioxidant levels decline with age, exacerbating the impact of oxidative stress [2]

Oxidative stress can be measured by the specific end products of the process (specific end products), as free radicals have a short lifespan in circulation. In the body, free radicals most often attack and destroy unsaturated fatty acids [3]. Lipid peroxidation occurs when lipids react with free radicals, resulting in the formation of lipid peroxides. This lipid peroxide will induce endothelial damage and inflammatory response, inhibit vasodilation, and activate macrophages. The decomposition of lipid peroxide forms several byproducts, including malondialdehyde [MDA]. High concentrations of malondialdehyde indicate the process of cell membrane oxidation [2,4,6].

Robertson et al. suggested that caloric restriction can slow oxidative damage in aging animals [7]. Caloric restriction has been studied on various organisms, including flies, worms, fish, and rats, and has been found to maximize life expectancy. Calorie restriction is an act of reducing calories without depriving the individual of nutrients [8]. Research on 20 adult rats over a period of seven weeks conducted by Chuansuo et al. showed a decrease in MDA levels. [9] Similarly, Ilyasova conducted research on humans for two years and found a decrease in oxidative stress levels, as assessed by F2-Isoprotanes levels measured in urine samples.[10]

Harianja in his research entitled "The Effect of Calorie Restriction on Hydrogen Peroxide Levels and Blood Glucose Levels in Old Rats" found that the hydrogen peroxide levels in the control were higher compared to rats receiving calorie restriction treatment.[11]

Based on the aforementioned information, previous studies have investigated the effects of caloric restriction on different organisms, including mice. This prompts researchers to conduct further investigations by measuring levels of malondialdehyde (MDA) before and after caloric restriction treatment in both old and young rats. The selection of these two age groups is based on the theory proposed by Mao et al., which suggests that free radical levels increase with age. Therefore, this study aims to determine whether the effects of caloric restriction on malondialdehyde

DOI: https://doi.org/10.21776/ub.ijhn.2023.010.01.6

levels differ between old and young rats [2]

RESEARCH METHOD

Design/Research Design

This research has received ethical approval with reference number 656/UN4.6.4.5.31/PP36/2021 from Hasanuddin University Health Research Ethics Committee, Faculty of Medicine. The research was carried out in accordance with the research code of ethics.

This research is an experimental study conducted on male white rats Rattus norvegicus Sprague Dawley strain. The study employed a pre-post test with a control group design, comparing the results of observations in the experimental and control groups. The study specifically focused on investigating the effects of treatment on both old and young rats, with one group receiving the treatment and the other group serving as the untreated control.

Data Source

Maintenance and administration of animal interventions were carried out at the Biopharmaceutical Laboratory, Faculty of Pharmacy, University of Hasanuddin, Makassar. Energy restriction procedures were carried out at the Entomology Laboratory, Faculty of Medicine, University of Hasanuddin, Makassar. A 40% calorie restriction was carried out on the experimental animals by modifying their standard feed. The standard feed for experimental animals not subjected to energy restriction was Van Der Vour feed, at a quantity of 15-20 grams/day with moderate water intake. The composition of Van Der Vour's standard feed includes 20% protein, 7% fat, fiber 15-20%, calcium 1%, and phosphorus. Following the energy restriction implementation, the total normal energy requirement was reduced by 40%.

Research Objectives

The research samples used were male white rats (Rattus norvegicus) of the Sprague strain, aged 3-5 months and 12-15 months, with an average weight of 145 grams and 250 grams in healthy condition. These rats were obtained from the Veterinary Laboratory, Faculty of Medicine, Hasanuddin University. The number of samples used was 28 rats. The sample size was determined according to the one-way ANOVA comparison group design.

Prior to the experiment, cage adaptation (acclimatization) was carried out for seven days. During this period, all groups of mice were given a standard feed of approximately 15-20 grams/day and given enough drink. The cages were cleaned every day. To maintain a stable environment, the rats were placed in a room with sufficient air circulation and maintained at standard room temperature (±20-280C) with a humidity level of 50%±10%. The room lighting was set in a 12-hour dark and 12-hour light cycle. The body weight measurement of all groups of rats was recorded every week. A total of 28 rats were divided into four groups, with seven rats per group. Control groups A and B received standard feed, while treatment groups C and D were treated with a 40% energy restriction for seven weeks. The 40% energy restriction is carried out every week after weighing the treatment groups C and D

Development of Instruments and Data Techniques Collection

Body weight was measured every week using an animal scale.

DOI: https://doi.org/10.21776/ub.ijhn.2023.010.01.6

Figure 1. Weighing of young animals

Figure 2. Weighing of old animals

Data Technique Analysis

Data processing techniques used the SPSS application program with a significance of ≤ 0.05. The measurement results were presented in the form of narration and tables. The research data were then analyzed statistically using the one-way ANOVA (Analysis of Variance) test method, followed by Bonferonni post hoc test to determine the differences between groups. To compare the pre- and post-treatment data, the Paired Sample T Test was used for normally distributed data and the Wilcoxon test for non-normally distributed data.

.

RESEARCH RESULT

Statistical Test Result for Malondialdehyde (MDA) Levels in Rats

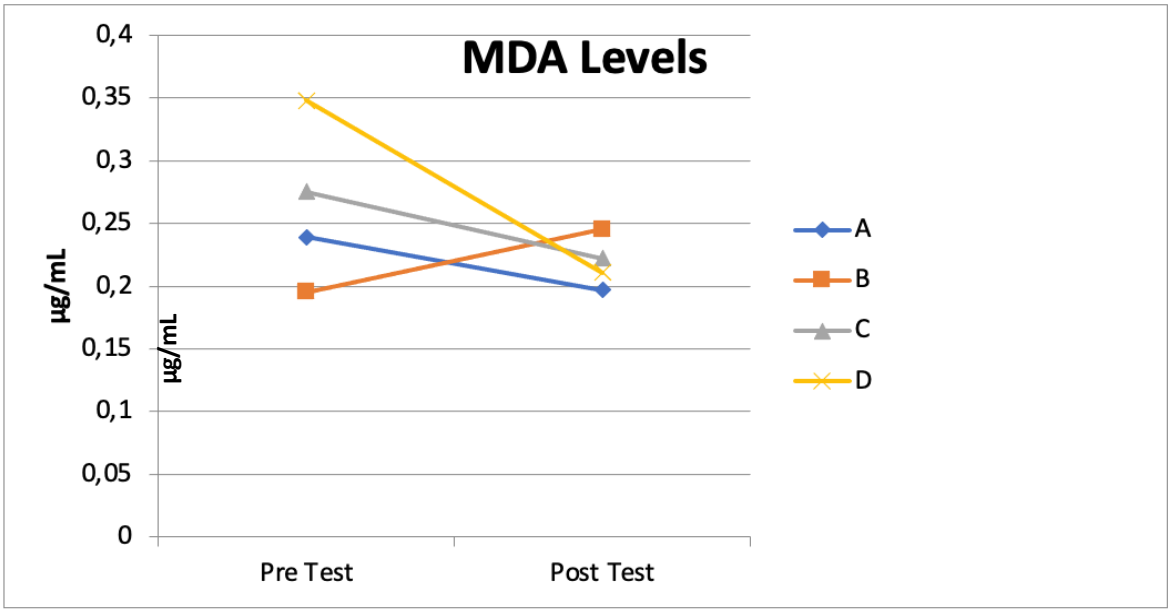
Based on Table 1 and Figure 3, the average change in rat MDA levels decreased in group A (Young Age Control) by 0.042µg/mL with a p-value of 0.162 > 0.05, so it can be concluded that the average MDA level in group A was not significant. In group B (old age control) there was an average increase in MDA levels of 049µg/mL with a p-value <0.05. This indicates a significant difference in the average MDA level in Group B. In group C (Young Age Treatment) the average MDA level decreased by 0.054µg/mL with a p-value of 0.022 <0.05. it can be concluded that the average MDA level in Group C is statistically significant. In group D (Old Age Treatment), the average mean change in MDA levels decreased by 0.137µg/mL with a p-value of <0.001. This suggests a significant difference in the average MDA levels in Group D. (Table 1 and Figure 3)

DOI: https://doi.org/10.21776/ub.ijhn.2023.010.01.6

Table 1. Malondialdehyde (MDA) levels in rats by group

	MDA Levels (Mean±SD)			
Group	Pre Test	Post Test	Perubahan	p
A	0.239±0.086	0.197±0.062	0.042±0.071	0.162
B	0.195±0.044	0.245±0.039	0.049±0.049	**0.039**
C	0.275±0.075	0.222±0.061	0.054±0.047	**0.022**
D	0.348±0.046	0.211±0.028	0.137±0.029	**<0.001**

Figure 3: Malondialdehyde (MDA) Levels in Rats by Group



Based on the paired sample t-test, there was no significant difference in the average malondialdehyde (MDA) levels within the control group (both old and young). Both groups showed a slight increase in MDA levels, with an average change of 0.003 µg/mL and a p-value of 0.876 (>0.05). In contrast, the treatment group (both old and young) demonstrated a significant reduction in MDA levels, with an average change of -0.095 µg/mL and a p-value of <0.001.

The one-way ANOVA statistical test revealed no significant difference in the average change of MDA levels between the young and old age groups. The average change in MDA levels was a decrease of 0.048 µg/mL in the young age group and a decrease of 0.043 µg/mL in the old age group. The p-value was 0.884 (> 0.05), indicating no significant difference.

Comparing group A (Young Age Control) and group C (Young Age Treatment), both showed an average decrease in MDA levels of 0.042 µg/mL and 0.054 µg/mL, respectively. The p-value obtained was 0.974 (>0.05), suggesting no significant difference in the average MDA levels between the two groups.

However, there was a significant difference in the changes in MDA levels between group B (Old Age Control) and group D (Old Age Treatment). Group B exhibited an average increase in MDA

DOI: https://doi.org/10.21776/ub.ijhn.2023.010.01.6

levels by 0.049 µg/mL, while group D showed an average decrease in MDA levels by 0.137 µg/mL. The obtained p-value was < 0.001, indicating a significant difference in the average MDA levels between the two groups.

Table 2. Differences in Changes of Malondialdehyde (MDA) Levels in Model Rats Between Groups

Group	Difference of MDA Levels (Pre test-Post test)		
	n	Mean ±SD	p Value*
A (Young Age Control)	7	0.042±0.071	0.013
B (Old Age Control)	7	0.049±0.049	
A (Young Age Control))	7	0.042±0.071	0.974
C (Young Age Treatment)	7	0.054±0.047	
A (Young Age Control)	7	0.042±0.071	0.011
D (Old Age Treatment)	7	0.137±0.029	
B (Old Age Control)	7	0.049±0.049	0.005
C (Young Age Treatment)	7	0.054±0.047	
B (Old Age Control)	7	0.049±0.049	<0.001
D (Old Age Treatment)	7	0.137±0.029	
C (Young Age Treatment)	7	0.054±0.047	0.029
D (Old Age Treatment)	7	0.137±0.029	
Young Age	14	0.048±0.058	0.884
Old Age	14	0.043±0.104	
treatment	14	0.095±0.057	0.001
Control	14	0.003±0.075	

Based on the results of the one-way ANOVA statistical test, there was no significant difference in the average change of MDA levels between old and young age groups. In the young age groups, there was an average decrease in MDA levels of 0.048 µg/mL, while in the old age group, there was a decrease in MDA levels of 0.043 µg/mL. The obtained p-value was 0.884 (> 0.05), indicating that the difference in average MDA levels was not significant.

Comparing the changes in MDA levels between group A (Young Age Control) and group C (Young Age Treatment), both groups showed an average decrease in MDA levels of 0.042 µg/mL and 0.054 µg/mL, respectively. The p-value obtained was 0.974 (> 0.05), indicating that the difference in average MDA levels was not significantly different. However, there was a significant difference in changes in MDA levels between group B (old age control) and group D (old age treatment). Group B exhibited an average increase in MDA levels of 0.049 µg/mL, while group D showed an average decrease in MDA levels of 0.137 µg/mL. The obtained p-value was < 0.001, indicating a significant difference in the average MDA levels between the two groups.

DISCUSSION

Effect of Energy Restriction on Malondialdehyde (MDA) Levels in Rats

The study results showed changes in the malondialdehyde (MDA) levels in rats after 7 weeks of energy restriction. In Group A (Young Age Control), there was a decrease in the average MDA levels. However, in Group B (Old Age Control), Group C (Young Age Treatment), and Group D (Old Age Treatment), there was an increase in the average MDA levels. Specifically, in the control group (both old and young), there was an average increase in MDA levels

DOI: https://doi.org/10.21776/ub.ijhn.2023.010.01.6

of 0.003 µg/mL, while in the treatment group (both old and young), there was an average decrease in MDA levels of 0.095 µg/mL.

Differences in Level Changes Malondialdehyde (MDA) in Inter-Group Model Rats

There were significant differences in the average malondialdehyde (MDA) levels observed in each group. In Group A (Young Age Control), there was an average decrease in MDA levels by 0.042 µg/mL, while in Group C (Young Age Treatment), there was an average decrease in MDA levels by 0.054 µg/mL. Furthermore, there was a difference in the changes in MDA levels between Group B (Old Age Control) and Group D (Old Age Treatment), with Group B showing an average increase in MDA levels of 0.049 µg/mL, and Group D showing an average decrease in MDA levels of 0.137 µg/mL.

Based on the analysis results, it can be concluded that energy restriction has an impact on MDA levels in rats, both at a young age and an old age. At a young age, the body has a natural ability to produce antioxidants, which help counteract the production of free radicals from both internal and external sources. In a healthy state, the body has mechanisms, such as enzymatic antioxidants (SOD) and Vitamin E, to maintain a balance between MDA levels and antioxidants.

A study by Hofer and Riordan has shown that caloric restriction, leading to weight loss, can significantly reduce oxidative damage to DNA and RNA in white blood cells, as well as improve left ventricular diastolic function (12). MDA, as the end product of lipid peroxidation, can serve as an indirect measure of lipid peroxidation accumulation. While experimental animal studies have shown a relationship between Superoxidase (SOD) and MDA with age, further research and epidemiological studies are needed to explore the associations of SOD or MDA with causes of death in the older age group (2).

CONCLUSION

Based on the findings of the study, it can be concluded that implementing a 40% energy restriction for seven weeks resulted in a reduction of malondialdehyde (MDA) levels in both young and old rats.

ACKNOWLEDGEMENT

The authors would like to thank all individuals who participated in this research, and also the staff at Hasanuddin University and the University of Muslim Indonesia for their assistance.

REFERENCES REFFERENCES

1. Briant T, Weinert, Timiras PS. Physiology Of Aging; Invited Review: Theories Of Aging', J ApplPhysiol, vol. 95. 2003. 1706–16
2. Mao C, et al, Associations between superoxide dismutase, malondialdehyde and all causemortality in older adults : a community-based cohort study', BMC Geriatrics, 2019. 104.
3. Harman D, The Free Radical theory of aging, Antioxid redox signal. 2003. 557-61

4. Winarsi H. Pembentukan Senyawa Oksigen Reaktif dan Radika Bebas: Antioksidan Alami & Radikal Bebas, Penerbit Kanisius, Yogyakarta. 2007

5. Pazil SNB. Perbandingan Aktivitas Antioksidan Ekstrak Daging Pisang Raja (Musa Aab "Pisang Raja") Dengan Vitamin A, Vitamin C, Dan Katekin Melalui Penghitungan Bilangan Peroksida. 2009.
6. Singh Z, et al. Use of malondialdehyde as biomarker for assessing ocidative stress in different disease pathologies : a review, Iran J Public Health, vol. 43. 2014. 7-16.

7. Robertson RP, et al.Perspective in Diabetes. Glucosa Toxicity in B-Cell : type 2 diabetes. Good Radicals gone bad and the glutathion connection, Diabetes, vol. 52. 2003. 581-7.
8. Anderson RM. The Calory Restriction paradigm. 2015 (Diunduh 11 Maret 2021). Available from: Biochemistr Society, http://www.biochemist.org
9. Chuan Suo et al. Effects of short- term calorie restriction on testicular morphology and antioxidant capacity in male rats, Animal husbandry and veterinary medicine. 2017.
10. Ilyasova et al. Effects of 2 years of caloric restriction on oxidative status assessed by urinary F2-isoprotanes:b the Calerie 2 randomized clinical trial, Aging Cell. 2018.17.
11. Harianja E, Widijanti A, Arsana PM, & Handono. Pengaruh restriksi kalori terhadap kadar hidrogen peroksida dan kadar glukosa darah pada tikus tua, Indonesian Journal of Clinical Pathology and Medical Laboratory, vol. 14, no. 1. .2018. 24-27.
12. Most J, Tosti V, Redman L, Fontana L. Calorie Restriction in Humans: an update, Ageing Research Review. 2016.

DOI: https://doi.org/10.21776/ub.ijhn.2023.010.01.6