The Effect of Calorie Restriction on The Growth of Adipose Tissue in Mice

Endanova Nur Hamda*, Irfiansyah Irwadi, Tri Hartini Yuliawati
Faculty of Medicine Universitas Airlangga, Surabaya, Indonesia
*Email: endanovanurhamda@gmail.com

ABSTRACT
The aging process can be slowed by reducing the calorie intake consumed by the body by calorie restriction or fasting. This study aimed to analyze the effect of calorie restriction on adipose tissue in mice. The experimental research design uses 12 months old Rattus Novergicus Strain Wistar, weighing 250–300 grams. The intervention was carried out for 14 days, then the average weight of subcutaneous and visceral fat was measured using Kruskal Wallis analysis and ANOVA. Based on the four treatment groups, the mice fasting and given a high-calorie diet (group 4) had the highest average fat weight value of 4.02 grams in the subcutaneous and 3.55 grams in the visceral area. Meanwhile, in group 1, mice that were not fasting and did not get a high-calorie diet had the lowest average fat weight values, namely 2.14 grams in the subcutaneous area and 2.72 grams in the visceral area. Based on the test results, it was found that there was no difference between subcutaneous (p=0.121) and visceral (p=0.443) fat tissue in all groups. Calorie restriction is beneficial for delaying aging and increasing mammal life span, but intermittent calorie restriction does not affect adipose tissue.

Keywords: adipose tissue; calorie restriction; fasting; mice

ABSTRAK
Proses penuaan dapat diperlambat melalui pengurangan jumlah asupan kalori yang dikonsumsi oleh tubuh berupa restriksi kalori maupun puasa. Penelitian ini bertujuan untuk menganalisis pengaruh restriksi kalori terhadap jaringan adiposa pada hewan coba mencit. Desain penelitian eksperimen dengan menggunakan hewan coba Rattus Novergicus Strain Wistar berumur 12 bulan dengan berat antara 250–300 gram. Intervensi dilakukan selama 14 hari, selanjutnya dilakukan pengukuran rerata berat lemak subkutan dan viseral dengan menggunakan analisis Kruskal Wallis dan ANOVA. Berdasarkan keempat kelompok perlakuan, kelompok mencit yang puasa dan diberikan diet tinggi kalori (kelompok 4) memiliki nilai rata-rata berat lemak yang paling tinggi yaitu 4,02 gram pada bagian subkutan dan 3,55 gram pada bagian viseral. Sedangkan pada kelompok 1 yaitu mencit yang tidak puasa dan tidak mendapatkan diet tinggi kalori, memiliki nilai rata-rata berat lemak paling rendah yaitu 2,14 gram pada bagian subkutan, dan 2,72 gram pada bagian viseral. Berdasarkan hasil uji didapatkan bahwa tidak ada perbedaan antara jaringan lemak subkutan (p=0,121) maupun viseral (p=0,443) pada semua kelompok.
kelompok. Pembatasan kalori bermanfaat untuk menunda penuaan dan meningkatkan rentang hidup pada mamalia, namun pembatasan kalori secara intermitten tidak berpengaruh terhadap jaringan adiposa.

**Kata Kunci:** jaringan adiposa; restriksi kalori; puasa; mencit

**INTRODUCTION**

Since ancient times, humans have tried to prolong their life and have done various ways to prevent the aging process (Masoodi *et al.*, 2015). Aging is a syndrome of destructive, progressive, universal, and irreversible changes. This major problem with the aging process is well known and only requires integrating various models and theories to understand the normal aging process (Vosselman *et al.*, 2013; El Hadi *et al.*, 2019). Public awareness and advances in the medical world have succeeded in increasing life expectancy. Data from the Indonesian Central Bureau of Statistics in 2004 showed that life expectancy in Indonesia only reached the age of 69 years. In 2020 the life expectancy of the Indonesian population increased to 71.4 years for men and 75.27 years for women (Central Bureau of Statistics, 2020). Aging is a complex process and involves many factors (multifactorial) and cannot be negotiated (irreducibly). The changes occur from the molecular level (DNA, protein, lipid) to the cellular and organ levels (Burhans *et al.*, 2019). In terms of endocrinology, during the aging process, there is an increase in adipose tissue levels because the process of breaking down food into energy decreases with aging and decreases in activity in the body (Moreno *et al.*, 2016; Kuda, Rossmeisl, and Kopecky, 2018; Schübel *et al.*, 2018). Aging in humans cannot be prevented, but it can still be slowed by reducing the number of calories consumed by the body (Kuzma *et al.*, 2016; Sipe *et al.*, 2017). Based on research conducted at the University of Wisconsin-Madison shows that one of the things that many people miss is the fact that aging can be slowed down by modifying the calories consumed so that the physiological changes of aging can be longer (Gopalan *et al.*, 2016; Nakhuda *et al.*, 2016).

A recent study showed that consuming 25% fewer calories daily could be the key to staying youthful. For women, the intake is equivalent to reducing 500 calories per day, meaning that they still meet the total intake of 1,500 rather than the recommended 2,000 calories per day. Meanwhile, based on research results, men consume 1,875 calories a day, not the 2,500 calories recommended for men to slow down aging that occurs in the body. Fasting diets, such
as the 5:2 diet, where a person limits daily calories twice a week, make people feel fresher and more energetic than those who always consume the same calories for a whole week (Park et al., 2017). Fasting is not a short-term solution because the weight will return after the calorie restriction stops. Based on this, Anderson said the first clinical trial to look at the effectiveness of fasting in a person proves that the method can counteract the signs of aging. This idea first appeared about 80 years in research on rodents (Nakagomi et al., 2015; Barquissau et al., 2018).

Experts identify processes in the body's cells that contribute to an increased risk of various aging-related diseases. Scientists at three locations in the United States revealed that it is not only fasting that can be tolerated in humans (Flachs et al., 2013; Neinast et al., 2015) but also calorie restriction. The population restricted 25% fewer calories a day and showed an increase in biological age of 0.11 years. In comparison, according to dietary guidelines, individuals who consumed calories normally had an average biological age of 0.71 years. Experts say that this first study has paved the way for further research to see how a fasting diet can prevent aging by conducting a calorie restriction test (Haas et al., 2014; Solinas, Borén, and Dulloo, 2015; Hall et al., 2016). The aging process in the body can cause a decrease in the function of human organs, and a decrease in the ability of organs to carry out their functions will cause the body's physiology. The decline in organ function that occurs physiologically and slowly will be accelerated if there are psychological problems in the body, such as stress, anxiety, and depression, so aging will also accelerate (Cowen et al., 2015; Liu et al., 2019). This condition of psychological problems can be caused by several factors, both internal and external, so that in this condition, there is an increase in adipose tissue in the human body. Initially, the increase in adipose tissue occurs because of a decrease in health in the body caused by a decrease in enzymes and the performance of the gastrointestinal and endocrine systems of the body (Desarzens and Faresse, 2016; Davis et al., 2017).

In the aging process, there is an increase in the production of free radicals continuously due to the failure of compensation for body cells. Where free radicals are a by-product of the cellular energy production process, oxidative damage occurs when free radicals attack nucleic acids, DNA, proteins, and fats (Templeman et al., 2018). Free radicals
are highly reactive molecules because they have unpaired electrons in their outer orbitals, so they can react with molecules in the body by binding to these molecules (Alemán et al., 2018; Pardo et al., 2019). According to R. Paul Robertson, oxidative damage in aging animals can be slowed by calorie restriction. Trends in his research argue that calorie restriction is also carried out to slow the aging process. Calorie restriction reduces the number of calories in food without making it undernutrition. A simple intervention is to achieve a diet that contains all the calories and essential vitamins but is significantly limited (30–70%) in the number of calories (Ard et al., 2017). Therefore, researchers are interested in researching the effect of calorie restriction on adipose tissue in experimental animals.

METHOD
This research is experimental research using a true-experimental pre-posttest design. In this design, randomization is carried out, which means that the grouping of the intervention group members is done randomly. This study is an intervention study on experimental rats divided into four groups, namely group 1 (not fasting), group 2 (fasting), group 3 (not fasting and given a high-calorie diet), and group 4 (fasting and given a high-calorie diet). This study used six mice in each group, so the total required sample size was 24 mice. The statistic used is the normality of the distribution tested by Shapiro Wilk because the data is less than 30, the statistical difference test used is determined. If the data distribution is normal, then the ANOVA difference test is used. However, suppose the results of the data distribution are not normal. In that case, the Kruskal Wallis test is then followed by the Least Significant Difference test using the Post Hoc Test to see the significance of the differences between groups. All tests were conducted with SPSS 13.0 with a significance value of 0.05 (p <0.05).

Place and time of research
This research was conducted at the Pharmacology Laboratory, Faculty of Medicine, Airlangga University, Surabaya. The time of research was carried out from May – July 2021. The ethical Clearance number is No.121/EC/KEPK/FKUA/2022.

Research tools and materials
The tools used in this research are stirrer rod, measuring cup (pyrex®), beaker (pyrex®), mouse cage, cannula, oven (Memmert®), dropper pipette, rotary evaporator (heidolph®), jar, and analytical balance. (kern®), microscope. The materials used in this study were distilled water, 70% ethanol, mice (Mus musculus), and adipose tissue mice. Mice Observation

DOI: 10.32668/jitek.v10i1.941
Research Instruments used to observe changes in mice are cameras, stationery, glass objects, and microscopes.

**Procedure**

a) Creation of a calorie restriction and dosage program

The calorie restriction program limits caloric intake in experimental animals by applying several interventions to each group to determine the resulting differences. Calorie restriction is done by giving the effect of fasting in mice with intermittent differences in time. The group was divided into four groups, namely group 1 (not fasting), group 2 (fasting), group 3 (not fasting and given a high-calorie diet), and group 4 (fasting and given a high-calorie diet).

b) Selection and preparation of experimental animals

The test animals used were 24 healthy male mice (Mus musculus) with a body weight of 20-30 grams. Previously, mice were acclimatized for one week, which aims to condition the animals in a laboratory atmosphere and relieve stress due to transportation. Mice were divided into four treatment groups, and each group consisted of 6 male mice determined randomly.

c) Observation

Before observing, it is better to observe the experimental animals before being given treatment. It aims to determine changes in symptoms after being treated by comparing symptoms or behavior before treatment. Then after 24 hours, it was observed again and counted the number of mice that died in each group. Observations were continued until day 14. Observation criteria included: The number of animals that died in each test group.

**RESULTS AND DISCUSSION**

After the calorie restriction intervention in the four treatment groups, the average fat weight was measured in the subcutaneous and visceral sections.

<table>
<thead>
<tr>
<th>Group</th>
<th>Subcutaneous fat (grams; mean±SD)</th>
<th>Visceral fat (grams; mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not fasting</td>
<td>2.14 ± 1.17</td>
<td>2.72 ± 1.1</td>
</tr>
<tr>
<td>Fast</td>
<td>3.84 ± 2.11</td>
<td>3.39 ± 1.24</td>
</tr>
<tr>
<td>Not fasting + high-calorie diet</td>
<td>2.68 ± 1.39</td>
<td>3.48 ± 0.60</td>
</tr>
<tr>
<td>Fasting + high-calorie diet</td>
<td>4.02 ± 1.21</td>
<td>3.55 ± 0.80</td>
</tr>
</tbody>
</table>
Table 1 shows that of the four groups, the group of mice that were fasting and given a high-calorie diet (group 4) had the highest average fat weight value of 4.02 grams in the subcutaneous and 3.55 grams in the visceral area. Meanwhile, in group 1, mice that were not fasting and did not get a high-calorie diet had the lowest average fat weight values, namely 2.14 grams in the subcutaneous area and 2.72 grams in the visceral area.

Table 2. Test the effect of calorie restriction on subcutaneous and visceral fat tissue in mice (n=24)

<table>
<thead>
<tr>
<th>Group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subcutaneous fat (back)</td>
<td>0.121</td>
</tr>
<tr>
<td>Visceral fat (testicles)</td>
<td>0.443</td>
</tr>
</tbody>
</table>

Test the effect of calorie restriction on fat tissue in mice using the Kruskal Wallis test for groups with abnormal data distribution, namely subcutaneous fat group data, and the ANOVA test in groups with normal data distribution, namely visceral fat group data. Based on the results of the two tests, it was found that there was no difference between subcutaneous (p=0.121) and visceral (p=0.443) fat tissue in all groups. Calorie restriction did not affect mice's subcutaneous fat content (back). Calorie restriction is a beneficial dietary regimen for delaying aging and increasing mammal life span. However, intermittent calorie restriction did not affect adipose tissue, circulating biomarkers (glucose metabolism, lipid metabolism, and inflammation as well as adipokines and steroid hormones), body weight, or volume of subcutaneous fat tissue (Schübel et al., 2018).

Fat is a normal body component that is stored in adipose tissue. Body fat consists of subcutaneous fat, visceral fat, and ectopic fat. In addition to functioning as an energy storage system, fat also serves as protection from the cold and the dangers of everyday impacts. Adipose tissue produces a wide variety of compounds, which affect many body functions, including appetite, fertility, neurodevelopment and plasticity, inflammatory response, and hormones, including insulin. The excess fat accumulation that interferes with health can lead to obesity (Masoodi et al., 2015). Subcutaneous fat is fat or adipose tissue just below the skin layer by 50%, which contains not only fatty tissue but also blood vessels and nerves. Subcutaneous fat is a shock absorber, which helps protect the
skin against trauma and stores energy. Leptin levels and expression were higher in subcutaneous fat than in visceral fat. In addition, the size of subcutaneous fat is directly proportional to plasma leptin levels. Thus, sample obesity could be caused by increased subcutaneous fat via increased leptin secretion. It explains that plasma leptin levels are more influenced by total body fat than subcutaneous fat (Cowen et al., 2015).

Intracellular lipids can accumulate from ingestion of food or can be synthesized from Acetyl-CoA and Malonyl-CoA resulting from the catabolism of different substrates. In conditions where body mass does not increase in average fat, excess calories are stored as triglycerides in white adipocyte tissue. However, caloric excess that occurs continuously over a long period can lead to the deposition of triglycerides and other more toxic lipids in non-adipose tissue, leading to lipotoxicity and inflammation (Solinas, Borén, and Dulloo, 2015). A high-calorie diet can influence the mean in subcutaneous fat tissue in the percentage and frequency of certain interventions. In addition, the individual's weight and sex factors also affect the type of fat reserves owned by the body. In addition, measuring the average amount of fat also needs to consider the number of research subjects included. It is because research on a limited number of subjects may not be relevant given the large variability of some physical criteria.

Calorie restriction did not affect mice's visceral fat content (around the testes). Moderate calorie restriction in the adult group did not selectively reduce visceral adipose tissue (VAT) levels. However, it led to beneficial improvements in total body composition, cardiometabolic risk, and quality of life without reducing physical function or increasing the risk of side effects (Ard et al., 2018). Although the results of other studies explain that nutritional factors influence weight gain in an individual. Nutrients are nutrients found in food and consumed by individuals. Nutritional factors will affect the percentage of body fat and are believed to be associated with various metabolic disease risks (Haas et al., 2014). Calorie restriction can be achieved by reducing the quantity of food a person consumes or by reducing the frequency of eating by fasting. During fasting, the frequency of eating will decrease, which was originally three times a day. Now it is done two times a day, namely at dawn (towards the morning) and iftar (towards the evening). This shows that
the calorie intake consumed when fasting is lower than when not fasting (Akbar and Jumsa, 2016). Calorie restriction reduces the number of calories by 20-40% of the normal number of calories needed in a day. Reducing the amount of caloric intake within this limit can reduce the risk of cardiovascular disease by reducing cholesterol levels, triglycerides, blood pressure, and thickness of the carotid artery intima (Templeman et al., 2018).

Visceral fat is fat that is stored in the adipose tissue of the abdomen (abdominal area), also known as organ fat or intra-abdominal fat. Fat stored in adipose tissue (functions to store fat) is usually in the form of triglycerides. Visceral fat in the abdominal cavity surrounds and encloses organs in the human body, such as the heart, stomach, liver, kidneys, and intestines. Belly fat is primarily semi-fluid. Excess visceral fat is also associated with type 2 diabetes, insulin resistance, and other obesity diseases (Ard et al., 2017). A decrease in visceral fat levels can be done with calorie restriction. However, things that need to be considered in restrictions are the percentage of restrictions used and the duration of the intervention. Based on the results of previous studies, it is known that the intervention of calorie restriction by 25% for six months can reduce BMI by 10%. A significant decrease occurred in basal temperature, saturated fat, visceral adipose tissue, subcutaneous adipose tissue, adipose cell size, and increased insulin sensitivity (Windasari, 2018).

The research found that the difference in treatment in the four groups through fasting intervention and the provision of a high-calorie diet did not affect the average visceral fat weight in mice. It can be influenced by the percentage of restrictions given and the length of intervention on the research subject. Changes in the mean visceral fat tissue can also be influenced by other factors, including age, gender, and physical activity undertaken by the individual.

**CONCLUSION**

Restriction or restriction of calories in mice did not cause a significant difference in the weight of subcutaneous fat or back fat. The highest mean weight of subcutaneous and visceral fat was found in the group with fasting treatment and a high-calorie diet. Meanwhile, the lowest mean fat weight was found in the non-fasting group and those not given a high-calorie diet. Restriction or caloric restriction in mice did not cause a significant difference in visceral fat weight or the fat portion in the area around the testes.
ACKNOWLEDGEMENT

The authors thank the Faculty of Medicine, Airlangga University, Surabaya.

REFERENCES


DOI: 10.32668/jitek.v10i1.941


DOI: 10.32668/jitek.v10i1.941