The Effect of Long-Term High Protein, Low Carbohydrate, and Low Fat Diet in CKMB Enzymes and Troponin T in Male Wistar Rats

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Abstract. Obesity is one of the diseases caused by unhealthy eating patterns and diets. An unhealthy diet can increase cholesterol levels in the blood which will then accumulate on the inner walls of blood vessels, causing atherosclerosis. This study aims to determine the effect of long-term high-protein, low-carbohydrate and low-fat diet on heart function by examining male CKMB & Troponin T levels of Rattus Norvegicus. This research was conducted experimentally in a laboratory with a Post-Test Control Design Group research design. The number of samples was 20 male wistar rats and divided into two groups (standard feed group) and the TPRKRL diet (high protein, low carbohydrate, low fat diet group). Blood was examined in the Hasanuddin University medical research center laboratory using the ELIZA method. The analysis used to determine the difference between the two groups was the Maan-Whitney test. The results showed that the macronutrient composition of the high-protein diet had significantly higher protein content and percentage and lower carbohydrates (protein 78.25% vs. 15.25%; CHO 10.5% vs. 52.2%) compared to the standard feed composition. Meanwhile, post-intervention blood and organ Troponin T levels in the treatment group were 25.45 ± 1.80 and 1022.52 ± 447.89 pg/ml, respectively. And post-intervention blood and organ Troponin T levels in the control group were 23.99 ± 1.24 and 1117.56 ± 324.44 pg/ml. The TPRLLK diet was able to significantly reduce body weight compared to the standard diet and the TPRLLK diet did not increase the enzyme markers of heart damage.

Keywords: CKMB, Troponin T, TPRLLK.

A. INTRODUCTION

The World Health Organization (WHO) states that obesity is one of the top 10 conditions at risk worldwide and one of the top 5 conditions at risk in developing countries. Worldwide, more than 1 billion adults are overweight and more than 300 million are obese. In the future the obesity epidemic will hit countries in the Asian continent (Dasthi et al. 2006). Asian people are generally smaller in body shape than the population in Western countries, but have a higher visceral fat composition is one of the important factors for the increase in cardiovascular disease in Asia (Joshi et al. 2019). In Indonesia, an estimated 210 million Indonesians in 2000, the number of overweight people is estimated at 76.7 million (17.5%) and obese patients number more than 9.8 million (4.7%) (Rahayu, 2018).

The results of the study prove that there is a close relationship between obesity and risk factors for cardiovascular disease, especially CHD. In Indonesia, coronary heart disease ranks first as the cause of all deaths, namely 16% in the 1992 Household Health Survey (SKRT). In 1995, it increased to 18.9% (Paoli, 2014). Coronary heart disease is a problem in the coronary arteries consisting of acute coronary syndrome and angina pectoris. Acute coronary syndrome consists of AMI and unstable angina pectoris. Infarction/STEMI and acute myocardial infarction without ST segment elevation (Non STEMI) (Darmawan et al., 2018).
Causes Habits behind the large number of deaths from cardiovascular disease in the world are smoking, lack of exercise, the habit of drinking alcoholic beverages, and unhealthy eating patterns (Gershuni, Yan & Medici, 2018). An unhealthy diet can increase cholesterol levels in the blood which will then accumulate on the inner walls of blood vessels, causing atherosclerosis. For this reason, dietary modifications are carried out by reducing or replacing unhealthy food intake into foods that contain lots of fiber. One of the popular diet patterns in recent times is a high-protein diet for weight loss, especially in obese patients (Ellenbroek et al. 2014). With weight loss in obese patients, eating will reduce the risk of CKD. High-protein diets are becoming increasingly popular where this diet can have a dual effect, namely feeling full and losing weight (Pesta & Samuel, 2014).

In addition, a high-protein diet can improve physical performance, body composition, and better health. A high-protein diet has been recorded to have reached its level of popularity since the 70s through the Atkins diet which has successfully helped many people lose weight quickly (Setiowati, 2015). On the other hand, the new study also adds to the long list of previous studies that reveal that consuming high and excess protein is not ideal and can increase the risk of heart disease. Several previous studies conducted by (Masrika et al., 2020) & (Arafah et al., 2020) regarding a high-protein diet that is able to lose weight significantly. that there is no impact on the heart (cardiac ischemia) (Kephart et al. 2018).

The application of a high protein diet has an impact on reducing the portion of other macronutrients, namely carbohydrates and fats (Wlodarek, 2019). Several studies conducted to reduce the portion of other macronutrients such as the ketogenic diet (Arsyad et al., 2020) found that this diet was able to reduce body weight and blood sugar. Subsequent studies have shown that the use of trans fats is associated with all-cause and cardiovascular and coronary heart deaths (Kosinski & Jornayvaz, 2017). Furthermore, the researchers also found a study conducted by (Zhang et al., 2020) where the study examined how the effect of a high protein and high fat diet on the heart, especially plaque formation by reviewing mTOR activating macrophages, this study explains why protein has an unfavorable impact on the heart (Brouns, 2018).

This makes researchers interested in trying to examine a high-protein diet which, according to several previous studies, is able to lose weight significantly by combining a low-fat and low-carbohydrate diet which will be associated with enzymes that are markers of heart damage, namely CKMB and Troponin-t. In this study, researchers will use a diet with a composition of high protein (>70%), low fat (10-20%) and low in carbohydrates (<10%). Researchers used male rats, Rattus Norvegicus, which will be analyzed for effects on the heart with parameters namely Enzyme Creatine kinase Myocardial band, Troponin T.

**B. METHOD**

The research location is at the Biochemistry Laboratory, Faculty of Medicine, Hasanuddin University. The inspection of standard feed and diet feed high in protein, low in carbohydrates, low in fat will be carried out at the Animal Food Chemistry Laboratory, Faculty of Animal Husbandry, Hasanuddin University. The research population is healthy rats (no dull and bald hair, and active movement), male sex, about 3 months old, weight 250-300 grams. The number of samples was 20 male wistar rats. The sample allocation in this study used the Simple Random Sampling method.

Before starting the treatment, the experimental animals were adapted in cages for 7 days to have a uniform way of life and food. The health of the mice was monitored daily and body weights were weighed at the end of each week. Mice were placed individually in metabolic cages, fed AD2 feed for the standard diet group, then TPRKRL feed for the
TPRKRL diet group, and drinking water was given ad libitum. The cage environment is made so that it is not humid. Cleared every day, adequate ventilation, room temperature ranging from 28 to 32°C, and sufficient lighting.

The TPRKRL diet feed is made from natural ingredients, namely > 70% protein, <10% carbohydrates and 10-20% fat according to the formulation obtained from Nutrisurvey. All ingredients (liquid) are mixed using a mixer and then frozen. The solidified material was then pulverized and shaped into balls to obtain a uniformly mixed TPRKRL diet feed. The feed was then checked for its proximate content at the Animal Feed Chemistry Laboratory, Faculty of Animal Husbandry, Hasanuddin University according to a predetermined composition.

Blood samples were taken at week 8 after treatment. First, the mice were made unconscious before the blood drawing process. Starting with ether poured on a handful of cotton and put in a jar, followed by mice and waited until the mice weakened. After that, the mice were removed and placed on a table that had been covered with plastic and then positioned on their back. The rats' tails were cleaned using an alcohol swab and then blood was drawn intravenously using a 3 and 1cc syringe.

The blood that has been obtained is then put into an Eppendorf tube and then allowed to stand for a few minutes. The blood in the tube was then centrifuged at 3000 rpm for 10 minutes to obtain serum. The serum obtained was then transferred to a labeled microtube, then the levels of CKMB and Troponin-T were examined using a spectrophotometer. The results of the examination are then recorded.

The analysis used to test the difference in mean levels of variables in the two unpaired groups (control group and treatment group) was an unpaired T-Test with a significance level of 5% (p<0.05) if the data were normally distributed. The analysis was then carried out with a comparability test using the Mann-Whitney test if the data were not normally distributed.

C. RESULT AND DISCUSSION

1. Body Weight of Mice in the Standard Diet Group and the TPRLRK Group

The figure shows the average body weight of the Wistar strain rats in the standard diet group and the TPRLRK diet group for 8 weeks. This shows that there is an increase in body weight of rats in the standard diet group before and after the intervention of 27.7 gr. In the TPRLRK diet group, the body weight of the wistar strain rats before the intervention was 262.90 11.789 while after the intervention was 146.40 13,468. It can be seen that there was a decrease in body weight of rats in the TPRLRK diet group before and after the intervention of 116.5 g.

The macronutrient composition of the high-protein diet had significantly higher protein content and percentage and lower carbohydrates (78.25% vs. 15.25% protein; 10.5% vs. 52.2% CHO) compared to the standard feed composition (Table 4). The number of calories on the high-protein diet and the standard diet per gram of food was 4.18 and 3.32 kCal, respectively. The two types of diets showed quite similar results with respect to energy intake, giving the impression that feeding behavior was not influenced by food aversion. The average daily caloric intake in both groups can be seen in Table 1.

<table>
<thead>
<tr>
<th>Diet</th>
<th>I</th>
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<th>IV</th>
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<th>VI</th>
<th>VII</th>
<th>VIII</th>
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<td>82.08</td>
<td>82.62</td>
<td>78.48</td>
<td>72.44</td>
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<td>81.27</td>
<td>81.92</td>
<td>87.72</td>
<td>80.13</td>
<td>81.51</td>
<td>74.76</td>
<td>79.42</td>
</tr>
</tbody>
</table>

Source: Data proceed
2. Levels of CKMB and Troponin T

Figures A and B show that post-intervention blood and organ Troponin T levels in the treatment group were 25.45 ± 1.80 and 1022.52 ± 447.89 pg/ml, respectively. And Troponin T blood and organ levels post-intervention in the control group 23.99 ± 1.24 and 1117.56 ± 324.44 pg/ml Although there was no significant difference in CKMB concentrations in each treatment between the two, it was seen that the levels of CKMB enzyme in the organs in the experimental group given the high protein diet was slightly lower than the control group.

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Levels of (A) Blood CKMB & Troponin T and (B) Organ CKMB & Troponin T in the Standard Diet Group (n=10) and Control Group (n=10) Measured at the end of Week 8; Data are Presented as Mean ± SEM; ***P < 0.001; Unpaired t Test

Source: Data proceed

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Levels of (A) Blood (CKMB & Troponin T) (B) organs (CKMB & Troponin T) in the High Protein Diet Group (n=10) and Control Group (n=10) as Measured at the end of Week 8; Data are Presented as Mean ± SEM; ***P < 0.001; Unpaired t Test

Source: Data proceed

3. Discussion

Weight

A high protein diet has been recognized as an effective strategy to achieve satiety, a process that is believed to be able to reduce energy intake and fat mass, as well as in an effort to achieve weight loss (Kosinski & Jornayvaz, 2017). widely used to treat obesity and treat chronic diseases such as metabolic syndrome and type 2 diabetes mellitus (Pesta & Samuel, 2014) This study tested and analyzed 2 groups of male Wistar rats fed a standard diet or a high protein diet for 8 weeks, then assessed the response of CKMB and Troponin T enzymes in the heart to long-term high-protein diet There are several mechanisms that are thought to underlie protein stimulation having a greater thermal effect than carbohydrates and fats, which in turn increases the process of food-induced heat energy production /diet-induced thermogenesis (DIT) (Party & Samuel, 2014). This high value of DIT also triggers an
increase in energy expenditure as a result of the activation of food processing steps that mainly involve absorption, transport and protein metabolism. As a result, the DIT effect of protein affects energy homeostasis and increases satiety. Second, the process of breaking down proteins into amino acids triggers gluconeogenesis, which is the process of forming new glucose molecules from non-carbohydrate materials (Westerterp-Plantenga et al., 2006). This metabolic process generally occurs in the liver, and it has been suggested that high protein intake increases the rate of regulation of important enzymes involved in gluconeogenesis such as glucose-6-phosphatase (G6P) and phosphoenolpyruvate carboxykinase (PEPCK) (Chalik et al., 2014a). Activation of the hepatic gluconeogenesis process will maintain plasma glucose levels which will later be used to produce energy so that weight loss is achieved (Pesta & Samuel, 2014). On the other hand, experimental animals fed standard diets experienced significant weight gain due to excess formation of glucose molecules from carbohydrates (Masrika et al., 2020).

The results of weight loss following the administration of a high-protein diet have been reported by several previous short and long-term studies, both in animal and human models. A study by (Arafah et al., 2020) reported that a group of mice fed a high-protein diet (50% protein) showed a significant increase in satiety and weight loss. In addition, a study by J (Zhang et al., 2020) also reported similar results, but was followed by a significant decrease in fat tissue and hepatic lipid accumulation in a group of rats fed a high-protein diet for 21 days.

**CKMB**

CKMB is an isoenzyme of creatine kinase (CK) which is found in various myocardial and skeletal tissues about ±20% (Sri Rahayu, 2018). The normal level of CKMB is <25 U/L (Chalik et al., 2014b). Our results showed that blood CKMB levels were low compared to post-intervention organ CKMB levels in the treatment group, namely 34.10 U/L and 243.67 pg/ml. Meanwhile, blood CKMB levels in the standard diet group were lower than organ CKMB levels in the standard diet, namely 36.49 U/L and 336.09 pg/ml. However, when compared with levels of organ CKMB enzymes in the standard diet group and the TPRLRK diet, it was seen that levels of organ CKMB in the standard diet group were higher at 336.09 pg/ml and levels of organ CKMB in the TPRLRK diet group were 243.67 pg/ml. This is because the Wistar sample in the standard diet group is categorized as obese, thus affecting the heart muscle by several mechanisms, the presence of obesity itself is associated with hemodynamic and metabolic factors that can cause changes in the structure and function of the myocardium that can affect left ventricular mass and further hypertension associated with obesity increases the work of the heart and stimulates heart enlargement (Ujiani, 2013). Based on these data, it can be seen that blood and organ CKMB levels in both the TPRLRK Diet group and the standard diet were above the normal level, which was < 25 U/L.

**Troponin T**

The high levels of CKMB in each group indicate a sign that there has been an injury to the myocardium. Theories suggest that serum CKMB levels are an important indicator of myocardial necrosis (Kosinski & Jornayvaz, 2017). At the time of injury to the myocardium, cardiac troponins are immediately released by the cells of the myocardium and enter the circulation, resulting in the appearance of acute troponins in the serum. In healthy people troponin T is undetectable or detectable in very low levels in serum. Troponin T blood levels increase within 4 hours after myocardial damage. Cardiac troponins have good sensitivity in detecting myocardial damage from the onset of damage to several days later (10-14 days). Another advantage of this cardiac troponin is that it can show the presence of small damage
to the myocardium (microscopic zone). In this regard, because CKMB levels in each group are >25 U/L, it will affect Troponin T levels in each group. The results of our study showed that blood troponin levels in the standard diet treatment were lower at 25.4566 pg/mL when compared to troponin T levels in the TPRLRK diet, which was 23.99 pg/mL. Meanwhile, the results showed that Troponin T levels in the Standard Diet treatment were lower at 1022.52 pg/mL when compared to Troponin T levels in the TPRLRK Diet treatment, which was 1117.56 pg/mL.

D. CONCLUSION

Based on this study, it can be concluded that the administration of a long-term high protein diet for 8 weeks in experimental animals in the TPRKRL diet group: (1) was able to significantly reduce body weight compared to the control group; (2) Does not increase the Enzyme Marker of Heart Damage. The need for further research using more samples and a longer duration of the study to assess the pattern of changes in body weight in experimental animals. It is necessary to add research variables, especially enzymes and other cardiac proteins that have the potential to have a direct relationship with the heart.

REFERENCES


