Original Research

EFFECT OF LONG-TERM KETOSTATIC DIET IN MICE SERUM ADIPONECTIN

Hamidah Kurniasari1, Purwo Sri Rejeki2, Hartono Kahar3, Sri Sunarti4

1Medical Program, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia
2Department of Physiology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia
3Department of Clinical Pathology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia
4Geriatric Division of Internal Medicine Department, Universitas Brawijaya, Malang, Indonesia

ABSTRACT

Ketogenic diet is a popular diet to reduce weight gain quickly. This diet has become a lifestyle. The ketogenic diet has been reported to affect adiponectin, although it is still contraindicated. Adiponectin is a biomarker for a metabolic disease that plays an important role as a protective factor for cardiovascular disease and increases insulin sensitivity. This study aimed to determine the long-term effect of ketogenic diet on adiponectin in mice. This study was an experimental laboratory study with a randomized posttest-only control group design. Fourteen male mice aged 2-3 months (20-30 g) were divided randomly into SD (n=7, standard diet) and KD (n=7, ketogenic diet), given a diet for eight weeks and ad libitum. Bodyweight was measured pre- and post-intervention, whereas adiponectin was measured post-intervention using ELISA. Significant difference of weight gain (∆) on SD (12.00±6.26) g, KD (1.29±7.41) g with p<0.005. There was a significant difference of serum adiponectin on SD (0.082±0.014) µg/ml and KD (0.096±0.008) µg/ml with p<0.005. This study showed ketogenic diet-induced higher serum adiponectin and slower weight gain. There was no correlation between the difference in body weight and serum adiponectin (p>0.005).

Keywords: Adiponectin level; ketogenic diet; weight gain; obesity

INTRODUCTION

The ketogenic diet is a diet strategy with a composition of high fat, low carbohydrates, and sufficient protein, which is more popular than other diets (Li et al. 2020, Walczyk & Wick 2017). This diet can overcome overweight and obesity quickly (Castellana et al. 2020). Li et al. (2020) stated that the ketogenic diet effectively treats epileptic seizures, metabolic disorders, tumors, autosomal dominant polycystic kidney disease, and neurodegeneration. In addition to
its usefulness for non-pharmacological therapy, this diet has become popular because it is healthier than currently recommended (Kirkpatrick et al. 2019). The public increasingly recognizes ketofastosis lifestyle in Indonesia with number of users in 2016. Ketofastosis uses ketogenic diet and fasting to maintain health (Fatimah & Husniawati 2019).

The use of ketogenic diet has been reported to affect adiponectin. Adiponectin is a hormone produced by adipose tissue and is a biomarker of metabolic disease (Fang & Judd 2018, Li et al. 2020). Adiponectin decreased significantly in obese patients (negatively correlated with BMI), type 2 diabetes mellitus (DMT2) patients (regardless of BMI), and coronary artery disease (CAD) patients. Adiponectin is a significant cardiovascular disease protective factor, because it improves insulin sensitivity, improves postprandial glucose and lipid metabolism, and also has anti-inflammatory, anti-atherogenic, and anti-angiogenic properties (Balsan et al. 2015, Monda et al. 2020).

The effect of the ketogenic diet on adiponectin is known to be contraindicated (Asrih et al. 2015, Monda et al. 2020, Partsalaki et al. 2012, Sena et al. 2017). Even though the use of ketogenic diet is already widespread in Indonesia (Fatimah & Husniawati 2019), because it can prevent overweight and obesity. The preventive effects resulting from ketogenic diet through adiponectin, such as protection against cardiovascular disease and increasing insulin sensitivity, may also contribute to the use of this diet. Therefore, a study explored the long-term effect of a ketogenic diet to increase serum adiponectin in mice.

MATERIALS AND METHODS

It was an experimental laboratory study with a randomized posttest-only control group design using fourteen male mice, DDY strains, aged 2-3 months, 20-30 g, as subjects. The subjects were acclimatized for one week, given a standard diet ad libitum. For the next eight weeks, the control group (SD) was given a standard diet (n=7) and the ketogenic group (KD) was given a ketogenic diet (n=7), ad libitum.

The composition of the standard diet was 20% protein, 12% fat, and 62% carbohydrate. The composition of the ketogenic diet was 30% protein, 60% fat, and 0% carbohydrate.

This study was approved by the Research Ethics Committee, Faculty of Medicine, University Airlangga No. 256/EC/KEPK/FKUA/2020. The study took nine weeks at the Biochemistry Animal Laboratory, Faculty of Medicine, Universitas Airlangga.

Bodyweight measurement

Bodyweight was measured at pre- and post-intervention using Harnic HL-3650 Heles Digital Scale which maximizes 5 kg and division graduation of 1 g.

Adiponectin measurement

The cardiac puncture procedure was used to collect blood samples 24 hours after the last meal. Blood samples were centrifuged at 4000 rpm for 5 minutes to obtain serum samples. Serum adiponectin were measured post intervention using an Enzyme-Linked Immunosorbent Assay (ELISA) kit, catalog No E-EL-M0002. The specification was sensitivity up to 9.38 pg/mL and detection range 15.63-1000 pg/mL.

Statistical analysis

Data analysis was performed using Statistical Package for Social Sciences (SPSS) program version 16. Data were presented in numbers and percentages. Numerical data were presented in the mean (standard deviation) and (standard error) if the data were normal. The Shapiro Wilk test was used to determine normality. Independent t-test was used to determine mean difference for normal distribution and Mann Whitney test was used for abnormal distribution. Pearson Correlation was used to determine correlation between bodyweight and serum adiponectin level. The statistical significance was p<0.05.

RESULTS

Fourteen male mice, DDY strains, aged 2-3 months, 20-30 grams were divided into control group (SD) and ketogenic group (KD). Pre- and post-intervention bodyweight was normally distributed (p>0.05). Since there is a connection between pre- and post-intervention bodyweight, a difference of bodyweight analysis was performed. The differences of body weight were not normally distributed (p<0.05). Furthermore, Mann-Whitney comparative test was performed. The characteristics of the body weight of subjects reported at Table 1. Adiponectin of KD (0.096±0.008 µg/ml) was reported significantly different than SD (0.082±0.014 µg/ml) with p-value 0.035 (Figure 1). The difference of body weight and adiponectin were tested using Pearson’s correlation. The p-value of 0.403 was assumed that difference of body weight and serum adiponectin in the standard and ketogenic diet had no relationship.
Table 1. Characteristics body weight of subjects

<table>
<thead>
<tr>
<th></th>
<th>SD (7)</th>
<th>KD (7)</th>
<th>Comparative test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-intervention</td>
<td>24.29±3.64</td>
<td>26.71±2.87</td>
<td>0.19</td>
</tr>
<tr>
<td>Post-intervention</td>
<td>36.29±6.75</td>
<td>28.00±9.49</td>
<td>0.08</td>
</tr>
<tr>
<td>Difference (∆)</td>
<td>12.00±6.26</td>
<td>1.29±7.41</td>
<td>0.02*</td>
</tr>
</tbody>
</table>

Abbreviation: SD=Control Group; KD=Ketogenic Group

Ketogenic diet consists of a high fat, low carbohydrate, and sufficient protein (Li et al. 2020). In condition with lack of carbohydrates, body will carry out gluconeogenesis to provide adequate energy. When endogenous glucose production does not meet body's needs, ketogenesis begins to take over to provide an alternative energy supply (Sherrier & Li 2019). The absence of compensation in low glucose conditions causes a decrease in insulin and an increase in glucagon, which leads to increased ketogenesis (Widiatmaja et al. 2021). Ketogenesis results in ketone bodies: acetyl CoA, β-hydroxybutyrate (βHB), and acetone, which lead to ketosis (Paoli 2014).

Ketone bodies production activates *hypothalamic ventromedial nucleus*, which is directly related to satiety, suppressing appetite, and leads to weight loss (Monda et al. 2020). A previous study revealed that people with ketogenic diet felt significantly less hungry (p=0.014) (Gershuni et al. 2018). Ketogenic diet participants tend to maintain lean body mass with a decrease in preferential fat mass, regardless of exercise (Gershuni et al. 2018). There was no statistically significant difference in daily energy expenditure between ketogenic and standard diets (Hall 2019). After 12 weeks of ketogenic diet, there was a decrease in bone volume fraction, cancellous bone trabecular number, cortical thickness, total cross-sectional area in the periosteal envelope, and cortical bone area in the tibia and humerus, while trabecular separation increased (Ding et al. 2019).

Monda et al. (2020) used twenty obese subjects consisting of 10 women and men aged 20 to 60 years who were fed a very low-carbohydrate ketogenic diet (VLCKD) for eight weeks. Adiponectin was found to be 10.8±1.2 g/ml at the beginning of the study. Adiponectin became 25.55±1.3 µg/ml with p value<0.001 after the intervention of VLCKD (Monda et al. 2020). Adiponectin increased significantly after VLCKD intervention. Another study demonstrated a significant difference in adiponectin between the control and high-fat diet groups (HFD). Sena et al. (2017) fed 12-month-old male Wistar rats HFD (40% triglycerides and 10% carbohydrates) and a standard diet (5% triglycerides and 45% carbohydrates) for four months. At the end of the study, adiponectin in the standard diet group was 43.99±6.0 g/ml and 46.15±4.37 g/ml in the HFD group with p<0.05. This previous study demonstrates that exposing rodents to ketogenic diet for four months can increase adiponectin level compared to standard diet. The results of this study were in accordance with previous research, where there was significant difference in serum adiponectin between KD and SD.

Decreased ROS and improved mitochondrial function are the effects of carbohydrate restriction which
induces stress response proteins. By reducing coenzyme Q, ketone bodies induce a decrease in free radical production (Veyrat-Durebex et al. 2018). The increased mitochondrial biogenesis function also increases adiponectin synthesis (Fang & Judd 2018). The ketogenic diet produces βHB which induces adiponectin secretion through G Protein-Coupled Receptor 109A (GPR109A) (Li et al. 2020, Plaisance et al. 2009). Fatty acids activate peroxisome Proliferator-Activatetd Receptor Alpha (PPARα), and it inhibits pro-inflammatory cytokines including IL-6 and Tumor Necrosis Factor Alpha (TNFα) (Veyrat-Durebex et al. 2018). TNFα and IL-6 can inhibit adiponectin gene expression and adiponectin secretion from 3T3-L1 adiposity (Fang & Judd 2018). The inflammatory cytokine TNFα also interferes with Fibroblast Growth Factor 21 (FGF21) through β-Clotho regulation. A ketogenic diet induces hepatic insulin resistance and increases the level of FGF21, increasing adiponectin secretion production by targeting adipose tissue and mediating systemic effects (Asrih et al. 2015).

Sena et al. (2017) explain that adiponectin has pleiotropic action which improves endothelial dysfunction through reducing production ROS, promoting coupling and activity of Endothelial Nitric Oxide Synthase (eNOS), increasing NO availability, and inhibiting JNK pro-inflammatory kinase. Adiponectin has anti-inflammatory, anti-atherogenic, and anti-angiogenic functions (Monda et al. 2020). Adiponectin stimulates oxidation of fatty acid in muscle by increasing the expression of molecules involved in fatty acid transport (CD36), their combustion (acetyl Co-A oxidase), and energy dissipation through an increased expression of type-2 release protein (Uncoupling Protein 2/UCP-2) (von Frankenberg et al. 2017).

CONCLUSION

A long-term ketogenic diet, consisting of 30% protein, 60% fat, and 0% carbohydrate, induced higher serum adiponectin, and slowed down weight gain, although there was no correlation between weight gain and serum adiponectin levels.

ACKNOWLEDGMENT

This study was funded by the Directorate of Research and Community Development, Deputy of Research and Development, Ministry of Research and Technology, The National Research and Innovation Agency, Indonesia. The authors would like to express their gratitude to Universitas Airlangga for hosting the experiment.

REFERENCES


