# EFFECT OF LONG-TERM KETOGENIC DIET IN MICE SERUM ADIPONECTIN

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**Submission date:** 19-Jun-2023 02:08PM (UTC+0500)
**Submission ID:** 2118934608
**File name:** NAS-11.pdf (280.39K)
**Word count:** 2948
**Character count:** 16408

**Original Research**

# EFFECT OF LONG-TERM KETOGENIC DIET IN MICE SERUM ADIPONECTIN

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## 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 (Δ) 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*

## ABSTRAK

*Diet ketogenik merupakan diet yang populer untuk menurunkan berat badan dengan cepat. Diet ini sudah menjadi gaya hidup. Diet ketogenik dilaporkan mempengaruhi kadar adiponektin, meskipun masih menjadi polemik. Adiponektin merupakan biomarker penyakit metabolik yang berperan penting sebagai faktor protektif penyakit kardiovaskular dan meningkatkan sensitivitas insulin. Penelitian ini bertujuan untuk mengetahui pengaruh jangka panjang diet ketogenik terhadap kadar adiponektin mencit. Penelitian ini merupakan penelitian eksperimental laboratorium dengan rancangan randomized posttest-only control group design. Empat belas mencit jantan usia 2-3 bulan, berat badan (20-30 g), dibagi secara acak menjadi SD (n=7, diet standar) dan KD (n=7, diet ketogenik) yang diberi diet selama delapan minggu, ad libitum. Berat badan diukur pada pra dan pasca intervensi, sedangkan kadar adiponektin diukur pada pasca intervensi menggunakan ELISA. Terdapat perbedaan signifikan pada peningkatan berat badan (Δ) kelompok SD (12.00±6.26) g, KD (1.29±7.41) g dengan p<0.005. Selain itu, terdapat perbedaan bermakna pada kadar adiponektin serum SD (0.082±0.014) g/ml dan KD (0.096±0.008) g/ml dengan p<0.005. Studi ini menunjukkan peningkatan adiponektin serum dan penambahan berat badan yang lebih lambat pada kelompok diet ketogenik. Tidak ada hubungan antara selisih berat badan dengan kadar adiponektin serum (p>0.005).*

***Kata Kunci:** Kadar adiponectin; diet ketogenic; penambahan berat badan; obesitas*

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pISSN:2355-8393 ● eISSN: 2599-056x ● doi: 10.20473/fmi.v58i1.30223 ● Fol Med Indones. 2022;58: 28-32
● Submitted 22 May 2021 ● Revised 2 Jan 2021 ● Accepted 20 Jan 2022 ● Published 5 Mar 2022


## 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 $\mu$g/ml) was reported significantly different than SD (0.082±0.014 $\mu$g/ml) with p-value 0.035 (Figure 1). The difference of bodyweight and adiponectin were tested using Pearson's correlation. The p-value of 0.403 was assumed that difference of bodyweight and serum adiponectin in the standard and ketogenic diet had no relationship.

Table 1. Characteristics body weight of subjects

| Bodyweight (g) | SD (7) | KD (7) | Comparative test |
|---|---|---|---|
| Pre-intervention | 24.29±3.64 | 26.71±2.87 | 0.19 |
| Post-intervention | 36.29±6.75 | 28.00±9.49 | 0.08 |
| Difference (Δ) | 12.00±6.26 | 1.29±7.41 | 0.02* |

Abbreviation: SD=Control Group; KD=Ketogenic Group

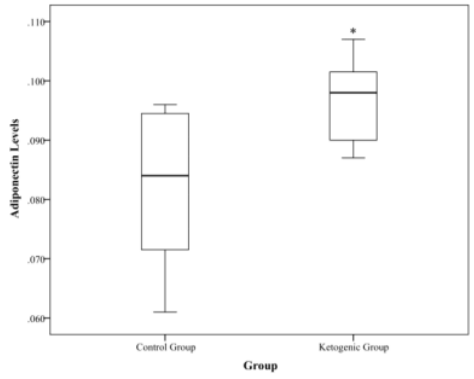


Figure 1. Serum adiponectin levels significantly increased in ketogenic group (p=0.035)

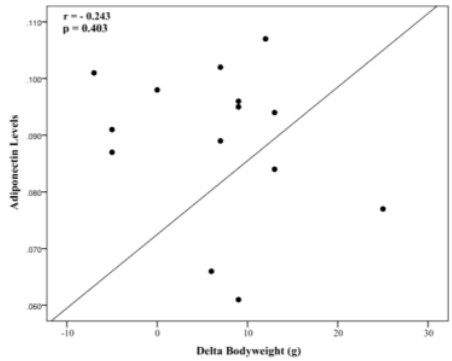


Figure 2. Serum adiponectin levels were not correlated with difference of bodyweight

## DISCUSSION

Ketogenic diet consists of a high fat, low carbohydrate, and sufficient protein (Li et al. 2020). In benzopyrene-induced mice, ketogenic diet with a fat has protein ratio of 60 : 30 that can reduce weight gain (Utami et al. 2021). The high-fat diet group with a composition of 30% protein, 60% fat, and 0% carbohydrate experienced the most weight loss and had the least amount of visceral fat compared to other diet groups (Syahraya 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) (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 $\mu$g/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-Activated 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.

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