ORIGINAL ARTICLE

Role of High-Fat Diet in Modulating Blood Glucose and Adipokine Responses in Diabetic Rats

Lailatul Muniroh[1,2], Agung Pranoto[3], Sudjarwo[4], Farapti[2], Mahmudah[5]

[1] Doctoral Program of Medical Sciences, Faculty of Medicine, Universitas Airlangga, Surabaya 60132, Indonesia
[2] Department of Nutrition, Faculty of Public Health, Universitas Airlangga, Surabaya 60115, Indonesia
[3] Department of Internal Medicine, Faculty of Medicine, Universitas Airlangga, Surabaya 60132, Indonesia
[4] Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya 60115, Indonesia
[5] Department of Epidemiology, Biostatistics, Population Studies and Health Promotion, Faculty of Public Health, Universitas Airlangga, Surabaya 60115, Indonesia

ABSTRACT

Introduction: The prevalence of diabetes mellitus in the community will reach 1.7% by 2023 and is among the top 10 diseases causing death in Indonesia. Obesity from high-fat diets can contribute to diabetes by altering leptin and adiponectin, which are essential regulators in diabetes mellitus. This study aims to examine the effects of high-fat diets in modulating blood glucose, leptin, and adiponectin levels in diabetic rats. **Materials and Methods:** A Randomized Posttest Control Group Design was used, involving Wistar rats divided into three groups: a control group receiving a placebo (X0), a diabetic group treated with streptozotocin (X1), and a diabetic group treated with both streptozotocin and a high-fat diet (X2). Diabetes was induced using intraperitoneal streptozotocin (60 mg/kg BW) and a high-fat diet administered over 7 days. Blood glucose measured using glucometer, while leptin, and adiponectin levels were assessed using ELISA. **Results:** Statistical analysis with one-way ANOVA at a 95% confidence level (α=0.05) revealed significant differences in blood glucose (p=0.012) between the groups, but no significant differences were found in leptin (p=0.337) and adiponectin (p=0.067) levels. **Conclusion:** The combined streptozotocin and a high-fat diet effectively simulated diabetic conditions by significantly increasing blood glucose levels, though variability in leptin and adiponectin responses suggests a need for further study.

Malaysian Journal of Medicine and Health Sciences (2025) 21(SUPP10):125-129. doi:10.47836/mjmhs.21.s10.25

Keywords: Adiponectin, Blood glucose, Diabetic rats, High fat diet, Leptin

Corresponding Author:
Agung Pranoto, PhD
Email: agung-p@fk.unair.ac.id
Tel :+62-0315023866

INTRODUCTION

The global prevalence of diabetes in 2021 in adults reached 536.6 million people and is expected to continue to increase annually (1). For 2021-2045, the prevalence of diabetes is higher in middle-income countries than in high- or low-income countries (1). Indonesia is a middle-income country where diabetes mellitus is among the top 10 diseases that cause death (2). According to the Indonesian health survey institute report, the prevalence of diabetes mellitus in Indonesian society reached 1.7% in 2023, 50% of which suffered from type 2 diabetes mellitus, the majority of which were suffered by the elderly and type 1 diabetes around 16.9%, the majority of which were suffered by children aged 5-14 years (3).

Diabetes mellitus is a significant risk factor for cardiovascular diseases and is characterized by persistently high blood sugar levels resulting from problems with insulin secretion or function. Over time, this condition causes extensive damage to various organs, particularly the eyes, kidneys, nerves, heart, and blood vessels (4). Adipokines, including leptin and adiponectin, are essential for metabolic regulation, impacting insulin sensitivity and inflammatory processes. Insulin is a primary regulator of adipose tissue function. In people with diabetes mellitus, the body's natural

insulin production is substituted with an external supply, which does not provide the same regulatory control.

Leptin and adiponectin exert contrasting effects on insulin resistance and subclinical inflammation. Leptin promotes inflammation and insulin resistance (5), while adiponectin has anti-inflammatory effects, reducing proinflammatory markers. An imbalance between leptin and adiponectin often linked to high-fat diets, increases the risk of diabetes and cardiovascular disease (6). The leptin-adiponectin ratio is closely link to metabolic syndrome and energy homeostasis issues (7).

Obesity is another risk factor for diabetes, which can be induced in lab animals through chemicals like streptozotocin, a substance that damages insulin-producing cells and causes hyperglycemia (8). This study aims to examine the effects of high-fat diets in modulating blood glucose, leptin, and adiponectin levels in diabetic rats.

MATERIALS AND METHODS

Research's design and sample

This research received ethical approval from the Health Ethics Commission of the Faculty of Public Health, Universitas Airlangga (No.546/EA/KEPK/2018). The study employed a Randomized Posttest Control Group design. Male Wistar rats (Rattus norvegicus), aged 2–3 months, weighing 150–200 grams, and deemed physically fit, were used as the subjects. The animals were obtained from the Animal Implementing Unit, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia. The sample size consisted of 8 rats per group, for a total of 24 rats across 3 groups. Dropouts were defined as cases where a rat died or experienced severe, unintended, or incurable side effects during the study period.

Treatment of experimental and outcome measurement

The rats in the control group and treatment group X1 were given a standard pellet diet and water ad libitum, while treatment group X2 was given a high-fat diet (HFD) and water ad libitum. The composition of the high-fat diet was 50% Rat Bio feed pellets, 25% wheat flour, 2% cholesterol, 0.2% cholic acid, 5% pork oil, 17% water. They underwent a one-week acclimatization period to ensure their adaptation to the experimental environment. Diabetes was induced in the two experimental groups using different methods. This study used a single high dose injection of streptozotocin (STZ) 60 mg/kg BW injected intraperitoneally in rats of groups X1 and X2. Group X2 underwent a high fat diet (HFD) for seven days before receiving the same dose of STZ to induce type 2 diabetes while group X1 was only given STZ alone to induce type 1 diabetes. Reference to this method comes from previous research conducted by Akbarzadeh et al. (2007) (9)

On the eighth day, all groups of rats were sacrificed, blood samples were taken to measure glucose, leptin, and adiponectin levels. The rats were not fasted prior to blood collection so the normal blood glucose threshold using the Random Plasma Glucose (RPG), which is less than 200 mg/dL. Blood glucose was measured using a glucometer (EasyTouch® GCU), EasyTouch® blood glucose test strips, while leptin and adiponectin levels were measured using Enzyme-Linked Immunosorbent Assay (ELISA) at the Institute of Tropical Disease, Universitas Airlangga. This study involved three experimental groups: (1) X0, the control group, which received placebo treatment, (2) X1, the diabetes group induced with STZ only, and (3) X2, the diabetes group induced with a combination of HFD and STZ injections. This grouping allowed comparative analysis of diabetes induction methods and their effects on key metabolic markers.

Statistical Analysis

The data were analyzed with a One-Way ANOVA, and significant findings were further evaluated at a 95% confidence level ($p \leq 0.05$). In addition, the Least Significant Difference (LSD) test was performed on blood sugar, leptin and adiponectin levels to see the difference in the means of two different treatment groups marked (a-b) for 2 significantly different groups.

RESULTS

Blood Glucose Level

In this study, there were notable differences in blood glucose levels among groups, with the X2 group (STZ + HFD) showing the highest levels and the X0 group showed lowest levels (Table I). ANOVA results indicated significant differences in blood glucose levels across groups ($p=0.012$). Further testing using LSD showed that there was a significant difference between X0 and X2 and between X1 and X2, but not between X0 and X1.

Leptin level

Table II indicates that while the X2 group had the highest average leptin level among all groups, there was no statistically significant difference in average leptin levels across the groups ($p=0.337$). Further testing using the LSD also found no significant difference between the two differently treated groups.

Adiponectin level

Table III illustrates that the treatment group had decreased adiponectin levels, with X2 showing the lowest levels. Despite this reduction, an ANOVA test indicated no significant differences in adiponectin averages across

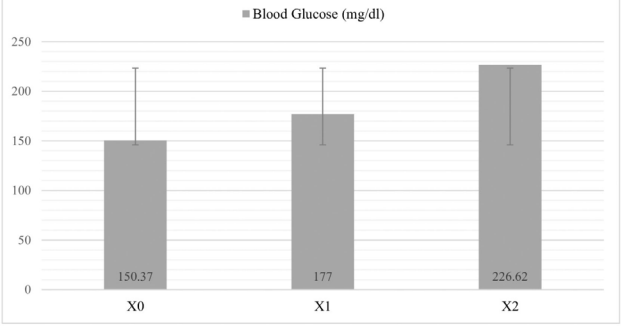

Figure 1: The average of glucose level

Table I: The average glucose levels (mg/dl) in the control and treatment groups

Groups	n	Mean±SD	Min	Max	p
X0	8	150.37±15.07a	130	175	0.012*
X1	8	177.00±27.78a	145	230	
X2	8	226.62±74.63b	152	359	

*p<0.05 indicates a significant difference between the groups.
Different letters (a-b) indicate a significant difference (p<0.05) in the same column.

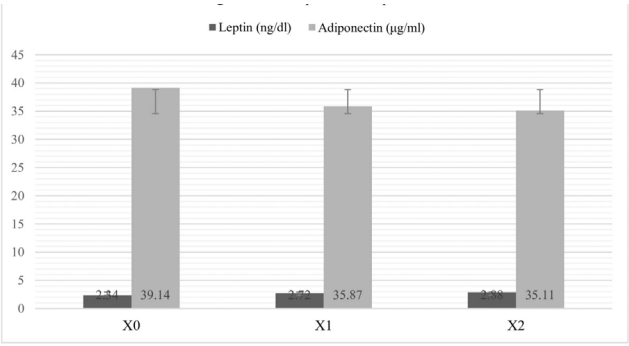

Figure 2: The average between leptin and adiponectin levels

Table II: The Average leptin level (ng/ml) in the control and treatment groups

Groups	n	Mean±SD	Min	Max	p
X0	8	2.34±0.47a	1.88	3.11	0.337
X1	8	2.72±0.68a	2.03	4.05	
X2	8	2.88±0.93a	2.43	4.71	

*p<0.05 indicates a significant difference between the groups.
Different letters (a-b) indicate a significant difference (p<0.05) in the same column.

Table III: The average adiponectin level (µg/ml) in the control and treatment groups

Groups	n	Mean±SD	Min	Max	p
X0	8	39.14±1.79a	35.93	41.09	0.067
X1	8	35.87±4.51	28.65	40.89	
X2	8	35.11±3.48b	29.06	39.00	

*p<0.05 indicates a significant difference between the groups.
Different letters (a-b) indicate a significant difference (p<0.05) in the same column.

the three groups (p=0.067). Further testing using LSD only between groups X0 and X2 that there is a significant difference.

DISCUSSION

Blood Glucose Level

Induction with streptozotocin (STZ) combined with a high-fat diet can cause type 2 diabetes by increasing blood glucose, leptin, and adiponectin levels. An increase in blood glucose is an indicator of diabetes, which often appears asymptomatic. Type 1 diabetes is characterized by autoimmune damage to β cells, leading to insulin deficiency. Without insulin, glucose cannot enter cells, resulting in hyperglycemia and metabolic disruptions.

In type 1 diabetes, insulin deficiency leads to uncontrolled lipolysis and an increase in plasma free fatty acids, which hinders glucose metabolism in peripheral tissues and disrupts glucose utilization. This deficiency also reduces the expression of genes essential for normal insulin function, like glucokinase in the liver and GLUT4 in tissues (10), with GLUT4 facilitating glucose transport. Consequently, type 1 diabetes disrupts the metabolism of glucose, fat, and protein.

In Table I, the result showed no difference in glucose levels was observed between the control (X0) and streptozotocin (X1) groups. This can occur because the administration of streptozotocin only damages pancreatic beta cells through the Glut-2 transporter gene which causes DNA alkylation and activation of PARP proteins into NAD+ proteins, thus reducing ATP production and inhibiting insulin production, producing free radicals and DNA damage so that pancreatic beta cells undergo necrosis (11). However, streptozotocin does not directly increase blood glucose levels because STZ acts like a switch that shuts down insulin production by destroying pancreatic beta cells (12). Without insulin, glucose levels will rise, but STZ itself does not directly add glucose to the blood.

Differences in glucose levels were observed between X0 and X2, and X1 and X2. There was a significant difference between the blood glucose levels of the X0 and X1 groups and the X2 (STZ+HFD) group. The significant increase in blood glucose levels after induction with STZ and HFD is likely due to the combined effects of these interventions on insulin resistance and beta-cell destruction in the pancreas. Streptozotocin is known to selectively damage insulin-producing beta cells, leading to reduced insulin secretion and hyperglycemia. When coupled with a high-fat diet, which exacerbates insulin resistance, this results in significantly elevated blood glucose levels.

Leptin level

Leptin links obesity with an increased risk of cardiovascular disease by helping to regulate food intake and weight, which may prevent insulin resistance in type 1 diabetes. A study discovered that elevated leptin levels are associated with body mass index and insulin resistance in diabetes (13), while another study noted that leptin resistance can occur alongside increased insulin levels (6).

There was no significant difference in the mean leptin

levels among the three groups. This was due to the lack of time of administration of the high-fat diet which was only given for 1 week. In a previous study, Wistar rats only increased body weight if the HFD was given for 1 month, this increase in body weight was caused by an increase in body fat mass which then affected leptin production in the body (14).

Leptin in obese patients reduces pancreatic beta cell receptor response, leading to increased insulin secretion and, eventually, hyperinsulinemia and resistance to both leptin and insulin. A study found positive correlation between body mass index (BMI), blood glucose, HbA1c, cholesterol, triglycerides, insulin, and leptin levels, with leptin serving as a predictor of insulin resistance syndrome (15). In type 1 diabetes, where insulin is insufficient, leptin acts in the brain to regulate glucose. This is because leptin suppresses liver glucose production, increases tissue glucose uptake, and helps normalize hyperglycemia, functioning independently of insulin (16). Leptin deficiency impacts insulin resistance and related conditions like lipodystrophy and lipolysis. Insulin promotes fat storage and suppresses fat breakdown, so its deficiency can lead to excessive fat mobilization, reducing leptin levels. Overall, leptin and insulin work together to balance glucose and fat metabolism.

Adiponectin level

Adiponectin is an adipokine with anti-inflammatory and insulin-sensitizing properties, typically decreased in obesity and insulin resistance. Adiponectin as antidiabetic effects arise through multiple mechanisms, including increased fatty acid oxidation, decreased endoplasmic reticulum stress, enhanced insulin signaling, improved mitochondrial function and insulin secretion, reduced liver glucose production, and increased glucose uptake and metabolism in liver and muscle.

A study found reduced adiponectin levels in the treatment group, which had been given a HFD with STZ, compared to the control, indicating a lower antidiabetic function (17). Adiponectin interacts with receptors on cells, activating intracellular pathways via molecules like AMPK and PPARs that are critical for fat and carbohydrate metabolism (18). Although adiponectin levels decreased in the treatment group, no significant differences were found in the average adiponectin levels between the three groups. Mitochondrial dysfunction can stress the endoplasmic reticulum, reducing adiponectin transcription and leading to lower adipokine production, contributing to insulin resistance. Adiponectin helps protect beta cells, which face loss in diabetes patients due to free fatty acids and cytokines (19). Leptin and adiponectin serum in the blood also tend to be stable in contrast to insulin. Compared with adipokine levels, the leptin and adiponectin ratios are better at diagnosing the occurrence of the metabolic syndrome (20), which is characterized by an increase glucose levels in the blood. Therefore, the ratio can be used as an additional indicator for the occurrence of obesity complications such as insulin resistance and endothelial dysfunction.
In this study, there was no significant difference in mean adiponectin levels between the three groups of rats. This was also due to the short duration of high-fat diet feeding so that it did not have an impact on the body condition of the rats. A study showed contrasting results with a period of 42 days of HFD feeding (21).

CONCLUSION

The study results suggest that high-fat diet induction causes changes in blood glucose levels in the treatment group. Although blood glucose levels differed across the groups, leptin and adiponectin levels did not show significant differences between the control and treatment groups. It is necessary to develop a method for further research regarding the administration of HFD for at least 30 days or more so that there is a significant increase in fat mass that affects the average leptin and adiponectin levels of rats.

ACKNOWLEDGEMENTS

This research was funded by the Ministry of Research, Technology, and Higher Education of Indonesia. The authors wish to express their gratitude to the Institution of Research and Innovation at Universitas Airlangga for their support in completing this project No. 2484/UN3.1.10/PT/2018. The authors declare that there are no conflicts of interest related to the preparation of this manuscript.

REFERENCES

1. Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, et al. IDF Diabetes Atlas: Global, Regional and Country-Level Diabetes Prevalence Estimates for 2021 and Projections for 2045. Diabetes Res Clin Pract. 2022;183:109119. Available from: 10.1016/j.diabres.2023.110945.
2. World Health Organization. Health Data Overview for The Republic of Indonesia [Internet]. Jenewa; 2024 May [cited 2025 Feb 9]. Available from: https://data.who.int/countries/360
3. Badan Kebijakan Pembangunan Kesehatan. Indonesian Health Survey 2023 in Numbers. Available from: https://www.badankebijakan.kemkes.go.id/hasil-ski-2023/
4. American Diabetes Association. American Diabetes Association. 2022. Diabetes Overview. Available from: https://diabetes.org/diabetes
5. Vilarico-Garcна T, Polonio-Gonzбlez ML, Pйrez-Pйrez A, Ribalta J, Arrieta F, Aguilar M, et al. Role of Leptin in Obesity, Cardiovascular Disease, and Type 2 Diabetes. Int J Mol Sci. 2024;25(4):1–21. Available from: 10.3390/ijms25042338.

6. Lypez-Jaramillo P, Gymez-Arbelбez D, Lypez-Lypez J, Lypez-Lypez C, Martнnez-Ortega J, Gymez-Rodrнguez A, et al. The Role of Leptin/Adiponectin Ratio in Metabolic Syndrome and Diabetes. Horm Mol Biol Clin Investig. 2014;18(1):37–45. Available from: 10.1515/hmbci-2013-0053.
7. Zhao S, Kusminski CM, Scherer PE. Adiponectin, Leptin and Cardiovascular Disorders. Circ Res. 2021;128(1):136–49. Available from: 10.1161/CIRCRESAHA.120.314458
8. Kumar R, Arora V, Ram V, Bhandari A, Vyas P. Hypoglycemic and Hypolipidemic Effect of Allopolyherbal Formulations in Streptozotocin Induced Diabetes Mellitus in Rats. Int J Diabetes Mellit. 2015;3(1):45–50. Available from: https://doi.org/10.1016/j.ijdm.2011.01.005
9. Akbarzadeh A, Norouzian D, Mehrabi MR, Jamshidi Sh, Farhangi A, Verdi AA, et al. Induction of Diabetes by Streptozotocin in Rats. Indian Journal of Clinical Biochemistry. 2007;22(2):60–4. Available from: 10.1007/BF02913315
10. Li M, Chi X, Wang Y, Setrerrahmane S, Xie W, Xu H. Trends in Insulin Resistance: Insights Into Mechanisms and Therapeutic Strategy. Signal Transduct Target Ther. 2022;7(1):1–25. Available from: 10.1038/s41392-022-01073-0
11. Nahdi AMTA, John A, Raza H. Elucidation of Molecular Mechanisms of Streptozotocin-Induced Oxidative Stress, Apoptosis, and Mitochondrial Dysfunction in Rin-5F Pancreatic β -Cells. Oxid Med Cell Longev. 2017;2017(ID 7054272):1–15. Available from: 10.1155/2023/8974960
12. Kim J, Shin SH, Kang JK, Kim JW. HX-1171 Attenuates Pancreatic B-Cell Apoptosis and Hyperglycemia-Mediated Oxidative Stress Via Nrf2 Activation in Streptozotocin-Induced Diabetic Model. Oncotarget. 2018;9(36):24260–71. Available from: 10.18632/oncotarget.24916
13. Uslu S, Kebapэi N, Kara M, Bal C. Relationship between Adipocytokines and Cardiovascular Risk Factors in Patients with Type 2 Diabetes Mellitus. Exp Ther Med. 2012;4(1):113–20. Available from: 10.3892/etm.2012.557
14. Marques C, Meireles M, Norberto S, Leite J, Freitas J, Pestana D, et al. High-Fat Diet-Induced Obesity Rat Model: A Comparison between Wistar and Sprague-Dawley Rat. Adipocyte. 2016;5(1):11–21. Available from: 10.1080/21623945.2015.1061723
15. Zhao Y, Li H. Association of Serum Leptin and Insulin Levels Among Type 2 Diabetes Mellitus Patients: A Case-Control Study. Medicine (United States). 2022;101(41):E31006. Available from: 10.1097/MD.0000000000031006
16. German JP, Thaler JP, Wisse BE, Oh-I S, Sarruf DA, Matsen ME, et al. Leptin Activates A Novel CNS Mechanism for Insulin-Independent Normalization of Severe Diabetic Hyperglycemia. Endocrinology. 2011;152(2):394–404. Available from: 10.1210/en.2010-0890
17. Wang X, Zhang S, Li Z. Adipokines in Glucose and Lipid Metabolism. Adipocyte. 2023;12(1):1–14. Available from: 10.1080/21623945.2023.2202976
18. Da Silva Rosa SC, Liu M, Sweeney G. Adiponectin Synthesis, Secretion and Extravasation from Circulation to Interstitial Space. Physiology. 2021;36(3):134–49. Available from: 10.1152/physiol.00031.2020
19. Koenen M, Hill MA, Cohen P, Sowers JR. Obesity, Adipose Tissue and Vascular Dysfunction. Circ Res. 2021;128(7):951–68. Available from: 10.1161/CIRCRESAHA.121.318093
20. Ayina CNA, Endomba FTA, Mandengue SH, Noubiap JJN, Ngoa LSE, Boudou P, et al. Association of the Leptin-to-Adiponectin Ratio with Metabolic Syndrome in a Sub-Saharan African Population. Diabetol Metab Syndr. 2017;9(1):66. Available from: 10.1186/s13098-017-0265-6
21. Roy JR, Janaki CS, Jayaraman S, Periyasamy V, Balaji T, Vijayamalathi M, Veeraraghavan VP, Krishnamoorthy K, Prasad M. Carica Papaya Reduces High Fat Diet and Streptozotocin-Induced Development of Inflammation in Adipocyte Via IL-1β/IL-6/TNF-A Mediated Signaling Mechanisms in Type-2 Diabetic Rats. Current Issues in Molecular Biology. 2023;45(2):852.Available from: https://doi.org/10.13040/IJPSR.0975-8232.14(12).5643-54