Effect of High-Fat Diet on Systolic Blood Pressure in STZ-Induced Diabetic Adolescent *Sprague Dawley* Rats

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ABSTRACT

Hypertension and diabetes mellitus are two major contributing factors in cardiovascular disease development. Previous studies have shown that insulin resistance is worse in obese youth than adults. This research compared the effect of diabetes mellitus and high fat diet on systolic blood pressure between the normal, streptozotocin (STZ)-induced only and STZ with high fat diet-induced (HFD) of adolescent rats. The subjects were adolescent male Sprague- Dawley rats aged 3 weeks divided into 3 groups. The STZ-induced group received 35 mg / kg STZ by intraperitoneal injection while the STZ and HFD-induced group received the STZ after 14 days high fat diet treatment. The blood glucose was measured after fasting of 12 hours, 3 days after STZ induction using the glucosimeter. The systolic blood pressure was measured in all groups before the research and 3 days after STZ induction. There was a significant difference in rat systolic blood pressure before the intervention. All groups experienced an increase in systolic blood pressure. Systolic blood pressure increase about 3.00 mmHg (2.14%), 1.83 mmHg (1.45%), and 39.33 mmHg (36.87%) respectively for normal, STZ-induced, and STZ and HFD-induced groups. The increase of systolic blood pressure can occur massively in the STZ-induced diabetic adolescent rats with HFD-induced obesity.

Keywords: Diabetes mellitus, systolic blood pressure, obesity, youth

1. INTRODUCTION

Non-Communicable Diseases (NCD) are the causes that dominate the death rate in Indonesia. Environmental influences, technological advances, and changes in people's lifestyles are the main causes of the increasing incidence of NCD. Based on the results of the 2014 Indonesian Sample Registration System (SRS) survey, stroke is the leading cause of death among other NCD, followed by heart disease and Diabetes Mellitus [1]. Non-Communicable Diseases including diabetes mellitus, not only has become a serious threat to national health but also globally. Diabetes mellitus (DM) is a collection of metabolic diseases characterized by an increase in blood sugar above normal due to impaired insulin secretion, insulin performance, or both [2].

Diabetes mellitus (DM) is a chronic disease characterized by elevated blood sugar that leads to insulin resistance produced by the cell β pancreas. Diabetes can be classified into four categories of clinical type 1 diabetes (due to damage of autoimmune cells β), type 2 diabetes (a defect in insulin secretion by the cell β), diabetes mellitus gestational (diabetes diagnosed during pregnancy), and the type of diabetes types caused by genetic defects, drugs (HIV / AIDS treatment or post organ transplantation) or damage to the exocrine glands of the pancreas leading to impaired insulin secretion which eventually leads to diabetes [3]. Type 2 diabetes mellitus (TD2M) or known as Non-Insulin -Dependent Diabetes Mellitus (NIDDM) is a type of DM due to cell insensitivity to insulin (insulin resistance) and relative insulin deficiency which causes hyperglycemia. This type of DM has the highest prevalence among other types, which covers 90-95% of diabetes cases [4].

Several decades ago, T2DM was still relatively rare in the young age group. However, in the middle of 1990, researchers began to observe an increased incidence of T2DM in children and adolescents in various countries such as the USA, Canada, Japan, Austria, the UK, and Germany [5]. The National Institute for Health and Care Excellence (NICE) United Kingdom defines the early onset of T2DM at a young age is those who have T2DM under the age of 40 years. But practically speaking, the early onset of T2DM can be divided into pediatric and adolescent (≤18 years), adolescents (18-25 years), and young adults (>25 years) [6]. In Indonesia, the number of children affected by T2DM has tended to increase in recent years. In 2011, 65 children suffered from Diabetes Mellitus, an increase of 40% compared to 2009. Thirty-two of them were affected by T2DM [7].

The American Diabetes Association (ADA) in 2017 stated that two out of three people with diabetes mellitus have high blood pressure [8]. Hypertension occurs when the systolic and diastolic blood pressure is higher than the normal limit of blood pressure. Systolic blood pressure is the pressure of contracting blood pumping blood from the left ventricle of the heart by the aortic vessels throughout the body, while diastolic blood pressure is the pressure of relaxing blood pumping blood from the atria to the ventricles [9]. Whether systole and diastole are equally important in

the diagnosis of high blood pressure and heart health monitor. However, most studies show a greater risk of stroke and heart disease is associated with increased systolic pressure compared to diastolic pressure [10].

Hypertension can coincide (comorbid) with diabetes or is a result of the pathogenesis of diabetes. In general, it is estimated that hypertension is found to be twice as common in the diabetic population as in non-diabetics. The relationship with T2DM is that hypertension can make cells insensitive to insulin. Insulin plays a role in increasing glucose uptake in many cells and regulates carbohydrate metabolism so that if insulin resistance occurs by cells, blood sugar levels can also be disrupted. Autonomic nerve dysfunction, activation of the Renin-Angiotensin-Aldosterone system (RAAS), insulin resistance, activation of the sympathetic nerves, endothelial dysfunction, and arterial stiffness are some of the factors known to contribute to the development of hypertension in diabetes. [11].

The adolescent age category is between the ages of 10 and 19. The rat model associated with adolescents to young adults in humans was 3-10 weeks old and had a mean weight of 35- 80 grams [12]. Animal models of T2DM can be induced with and streptozotocin (STZ) and high-fat diets [13]. Streptozotocin (STZ) is a permanent diabetogenic compound produced by gram-positive *Streptomyces achromogenestest* bacteria. Streptozotocin induces diabetes mellitus in animal models by destroying insulin-producing pancreatic beta cells. This partial damage causes the beta cells to secrete less insulin which results in glucose tolerance resembling T2DM in humans [14].

A high-fat diet can promote obesity, hyperinsulinemia, and affect glucose homeostasis leading to compensatory failure by pancreatic beta cells. Obesity is caused more by environmental manipulation than genes, so that modeling the T2DM situation is more accurate than genetic modeling obesity for inducing diabetes. Several studies have reported that a high-fat diet for 2-7 weeks to induce stable insulin resistance [15].

2. METHOD

This study is a true experimental with a pre-post-test control group design and has been ethically approved by Medical and Health Research Ethics Committee (MHREC) Faculty of Medicine Universitas Gadjah Mada with Ref. No KE/FK/0342/EC/2020. The study was conducted at the physiology laboratory of the Faculty of Medicine, Islamic University of Indonesia in January - March 2020. The subjects used were 3 weeks old male *Sprague-Dawley* (SD) rats weighing 35-80 grams in the Physiology Laboratory of the Faculty of Medicine, Islamic University of Indonesia. Selection of *Sprague-Dawley* (SD) rat strain due to the strain of rat that has a relatively rapid metabolic capability that is best suited to be used as research models associated with the disease metabolic such as dyslipidemia and cardiovascular diseases. The rat chosen were male because they could provide stable research results because they were not influenced by the uterine cycle, the hormone estrogen, and pregnancy.

The inclusion criteria 3 weeks old male rat with a weight 35-80 grams, the rat in conditions of healthy and not disabled (have feathers clean, not wet, normal moves). Exclusion criteria rat death or illness during the study, the rat did not reach DM condition, or FBG <126 mg / dL three days after STZ induction in the STZ and STZ-HFD group. The minimum sample size was calculated with the formula Frederer and according to the World Health Organization (WHO) is each treatment group as much as 5 rats, to anticipate the drop-out then each group plus 1 rat, so in this study, was used 18 rat divided into 3 groups, namely:

N: the normal group was healthy rat that were given a normal diet

STZ: the STZ group, namely rats induced by DM by injection of STZ 35 mg/kgBW

STZ-HFD: the treatment group, namely rats induced by DM by injection of STZ 35 mg/kg BW and intervened with a high-fat diet for 14 days

Rats were kept in a ventilated room with room temperature ranging from 2 0 - 24 °C with a humidity of 60%. Lighting in the enclosure is set 12 hours: 12 hours light-dark cycle. After the acclimatization period, the STZ and STZ-HFD group rats were injected with STZ 35 mg/kg intraperitoneally a single dose [16]. Three days after injection Rats were fasted for 12 hours and then the blood sample through the sinus retro-orbital. The rat blood sample was examined with a glucometer to see the fasting blood sugar (FBG) levels. Rats that reached the condition DM (FBG $\geq$ 126 mg / dL) examined blood pressure systolic her using direct measurement in rats in a non -invasive using a plethysmograph in a conscious state, the arteries are used for the measurement of blood pressure is a rat tail artery. During the intervention period, the normal and STZ group rats were given standard Comfeed AD II feed, while the STZ-HFD group was given high-fat feed for 14 days. The composition of high-fat feed consists of 12% standard feed, 58% beef tallow, 10% quail egg yolk, and 20% high fructose (corn syrup).

The data obtained were then analyzed using SPSS 21 software. Univariate analysis was used to see the distribution of data descriptively on each variable. Researchers used *Shapiro -Wilk normality test* because of the sample size <50. Analysis of the characteristics of the subjects after treatment in the form of changes in fasting blood sugar and body weight between groups were analyzed using *Repeated Measures ANOVA test and Pairwise Comparisons.* Differences of blood pressure before and after treatment of high-fat diets in each group N, STZ, and STZ-HFD were tested using the *Dependent T-test* on normally distributed data and using the *Wilcoxon test* on data that were not normally distributed. While the *General Linear Model Test* was conducted to see the differences in blood pressure before and after treatment between all groups. The data is said to have a significant effect when the *p-value* is <0.05 [17].

3. RESULTS

Table 1. Characteristics of Subjects After Intervention

Variable	Mean ± SD			*p*
	N	STZ	STZ - HFD	
Post- induction fasting blood glucose (mg / dL)	95.67± 18.70[a]	126.00± 11.47[a*]	133.50± 11.93[b*]	0.042[1**]
Post- induction BW (gram)	206.50± 13.23[a]	171.83± 31.85[a]	161.00± 18.40[b]	0.004[1**]

[1]*Repeated ANOVA test,* [a, b)] *different notations showed significant differences in the output of Pairwise Comparisons,* [*] *met the criteria for DM (FBG ≥ 126 mg / dL),* [**] *significant (p<0.05)*
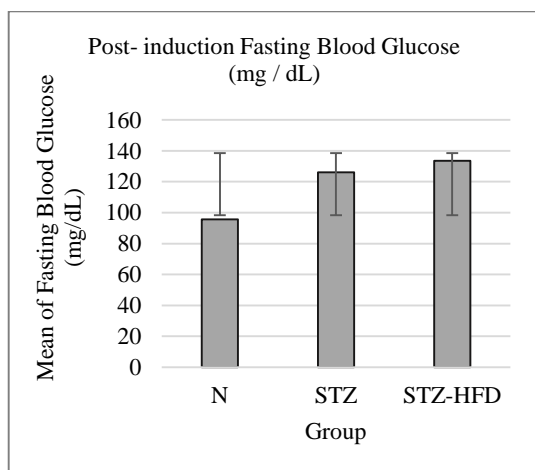
Diagram 1. Post- induction Fasting Blood Glucose (mg / dL)
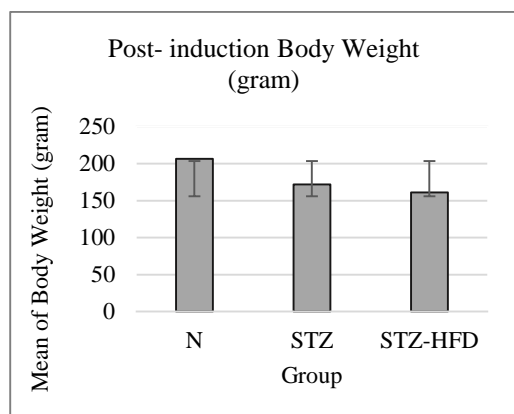
Diagram 2. Post- induction Body Weight (gram)

Table 1., Diagram 1. and Diagram 2. shows an overview of the characteristics of HFD subjects after examination of FBG levels and weight gain before and after STZ injection

and high-fat diet intervention. Based on the results of the *repeated ANOVA test*, there was a significant difference in mean fasting blood glucose in the N, STZ, and STZ-HFD groups after the intervention (p=0.042). In the *Pairwise Comparison*, a significant difference in mean fasting blood glucose was found in the N and STZ-HFD groups (p =0.001).

There was a significant difference in mean body weight between groups after the intervention (p=0.004). There was no difference in mean body weight in the N and STZ groups, but there was a significant difference in mean body weight with the STZ-HFD group (p=0.001).
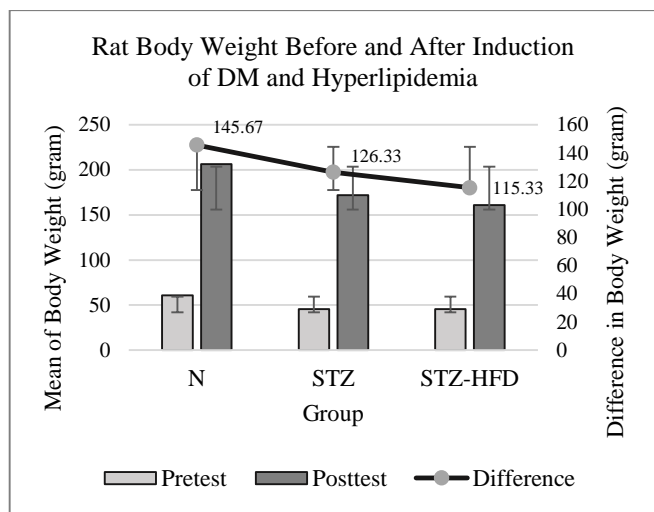
3.1. Rat Weight

During the hyperlipidemia induction period, the treatment group (STZ-HFD) rats were given a high-fat diet for 14 days. The composition of the high-fat diet consists of 12% standard pellets, 58% beef tallow, 10% quail egg yolks, and 20% high fructose (corn syrup). Weighing rat's body weight was carried out in all groups before and after DM induction and high-fat diet intervention using digital scales. The data obtained were then analyzed descriptively using statistical software to determine the mean and range value of each group. The results of weighing the rats are shown in Table 2.

From Table 2. and Diagram 3., it was found that the rat body weight increased in all groups of rats after induction. The largest difference in body weight was in the N or healthy group, while the smallest difference in body weight was in the STZ-HFD group or the group that was induced by DM and hyperlipidemia.

Table 2. Rat Body Weight Before and After Induction of DM and Hyperlipidemia

Group	Mean ± SD		Difference in BW
	BW pretest (gram)	BW posttest (gram)	
N	60.83± 7.39	206.50± 13.23	145.67± 16.86
STZ	45.50± 7.61	171.83± 31.85	126.33± 37.89
STZ-HFD	45.67± 5.75	161.00± 18.40	115.33± 15.06

Diagram 3. Rat Weight Gain Before and After Induction of DM and Hyperlipidemia

3.2. Rat Blood Pressure

The *Dependent T-test* was used to analyze differences between the mean blood pressure systolic each group before and after intervention on the data distributed normally, while the data distribution is not normal, test data are analyzed using *Wilcoxon test*. *General Linear Model test* was used to determine the difference in mean systolic blood pressure between groups before and after the intervention.

Table 3. Analysis of mean systolic blood pressure in group N, STZ and STZ-HFD Pretest and Post test

| Group | Mean ± SD | | | *p* | *p* (Control and Intervention) |
	Pre test (mmHg)	Post Test (mmHg)	Difference		
N	140.00± 21.93	143.00± 38.05	↑3.00± 52.99	0.895[1]	
STZ	125.50± 20.72	127.33± 20.12	↑1.83± 23.54	0.856[1]	0.032[3] **
STZ-HFD	106.67± 24.19	146.00± 5.06	↑39.33± 20.62	0.027[2] **	

[1]*Dependent T test*, [2] *Wilcoxon test*, [3] *General Linear Model test*, ** significant (p <0.05)

From Table 3. and Diagram 4., the high-fat diet intervention showed an increase in systolic blood pressure by 39.33 ± 20.62 mmHg and showed a significant difference in the mean before and after the intervention in the treatment group (STZ-HFD) (p=0.027). Whereas in the normal and STZ groups, there was an increase in the mean systolic blood pressure of 3.00 ± 52.99 mmHg and 1.83 ± 23.54mmHg before and after the intervention but not significant (p=0.895) (p=0.856).

General Linear Model test showed a significant difference in mean systolic blood pressure between groups before and after the intervention (p=0.032).

Diagram 4. Systolic blood pressure in group N, STZ and STZ-HFD Pretest and Post test
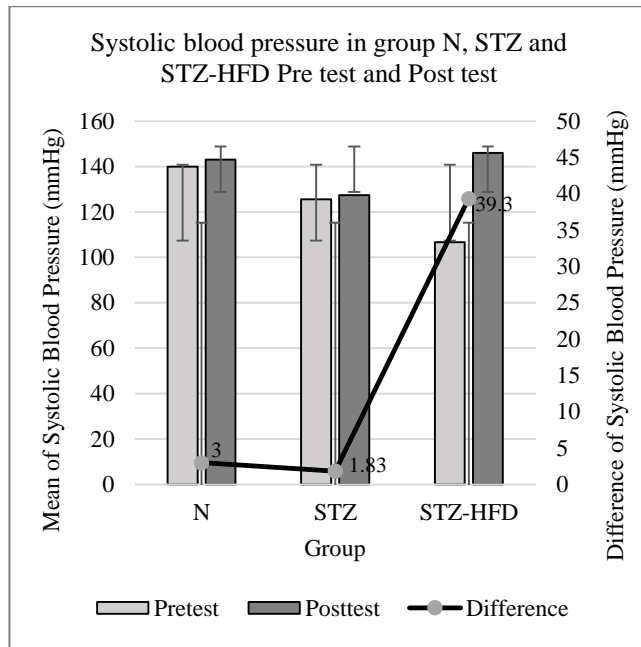
4. RESULTS

Diabetes Mellitus conditioning was carried out on rats in the STZ and STZ-HFD groups by injecting STZ 35mg/kgBW. Rats are said to have diabetes if their fasting blood glucose (FBG)levels are ≥126 mg / dL [18]. The results of DM induction in the STZ and STZ-HFD groups showed the FBG level are ≥126 mg / dL so that it met the DM requirements in rats. The basic mechanism that causes DM in experimental animals induced by STZ is glucotoxicity of pancreatic β cells. STZ contains deoxyglucose molecules that are highly reactive and exert cytotoxic effects directly on pancreatic β cells. He recognized the GLUT 2 receptor on the plasma membrane of β cells so that it could reach its main target, namely pancreatic β cells [18]. Four main mechanisms are related to STZ cytotoxic activity in inducing rat to experience DM conditions, namely; 1) DNA methylation of cellular components, 2) Release of NO (Nitric Oxide), 3) Formation of reactive oxygen species (ROS), and oxidative stress and 4) Inhibition of O- GlcNAcase [14, 20].

When STZ enters the cell, it decomposes to form metabolic methyl nitrosourea are highly reactive and cause DNA damage that results in pancreatic β cell necrosis due to the depletion of cellular energy reserves. The PARP enzyme (poly ADP -ribose polymerase) is activated to repair damaged DNA, but excessive stimulation of this DNA repair mechanism has an impact on reducing NAD + and ATP as cellular energy stores. The depletion of cellular energy stores ultimately leads to beta-cell necrosis [14, 20, 21]. STZ also causes the accumulation of NO (Nitrite Oxide)

in pancreatic β cells which inhibits aconitase enzyme activity and then causes disturbance of substrate oxidation and ATP production. The increase in NO results in stimulation of the formation of guanyl cyclase and cGMP (cyclic guanosine monophosphate) which are responsible for cell death [14, 20]. The accumulation of NO and other free radicals such as superoxide (O2-), hydroxide (OH-), peroxynitrite (ONOO-) causes oxidative stress [14, 22] Inhibition of the enzyme O- GlcNAcase (OGA) by STZ metabolites also leads to the accumulation of harmful glycosylated proteins and activation of stress pathways that induce cell apoptosis [14]. Oxidative stress accompanied by an imbalance of antioxidants and reduction of β cell mass causes β cell dysfunction resulting in the resulting insulin insufficiency [19, 23]. The lack of insulin production which functions as a regulator of glucose balance in the body increases the concentration of glucose circulating in circulation. Glucose concentration that exceeds the requirement will cause hyperglycemia. Hyperglycemia causes a decrease in the sensitivity of insulin receptors to the target tissue resulting in insulin resistance and an increase in blood glucose levels that occur continuously. These two conditions, β cell dysfunction, and insulin resistance play an important role in the formation of hyperglycemia conditions in T2DM [23].

Insulin resistance is known to occur physiologically during the transition between puberty and adulthood. Puberty is thought to be one of the main pathophysiological factor which causes differences in insulin sensitivity between adolescents and adults [24]. In healthy adolescents, there is a transient 50% decrease in insulin sensitivity when they enter puberty. As compensation, insulin secretion increases and triggers hyperglycemia which can be exacerbated by genetic, epigenetic, and lifestyle factors. Just like pregnancy, puberty is also a time of high risk for the development of DM in susceptible individuals [25]. Growth hormones, estrogens, and androgens also altered fat accumulation can affect insulin sensitivity. Low levels of HDL, which function to increase the disposal of glucose through skeletal muscle, are also one of the factors that influence insulin resistance in adolescence [26].

Based on Table 1., after the repeated ANOVA test was carried out there was a significant difference in the level of FBG in rats between groups, while the Pairwise Comparisons output showed a significant difference in mean FBG between groups N and STZ-HFD. This was because the N group rats were not injected with STZ so that FBG levels are classified as normal. In the STZ and STZ-HFD groups, there was no significant difference in FBG because the rat had diabetes.

The data in Table 2. shows the mean difference impairment successive BW from the group N, STZ and STZ-HFD before and after the intervention. This difference shows the increase in the bodyweight of rat along with the growth of rat. The highest increase in body weight occurred in group N while the lowest increase in weight was in group STZ-HFD. Based on repeated ANOVA tests, there was a significant difference in weight between groups after the intervention. Pairwise comparison showed that there was

only a significant difference in the mean weight of the N and STZ-HFD groups. This was because the STZ-HFD group experienced insulin resistance which was exacerbated by the provision of a high-fat diet which caused hyperlipidemia. So that the increase in body weight in the STZ-HFD group is lower than STZ who experience DM without being accompanied by hyperlipidemia.

Table 3. shows that after the intervention, there was an increase in the mean systolic blood pressure in all groups but only group STZ-HFD had a significant increase in mean systolic blood pressure. Systolic blood pressure is the blood pressure when the heart contracts, pumping blood from the left ventricle of the heart by the aortic vessels throughout the body, while diastolic blood pressure is the blood pressure when the heart relaxes pumping blood from the atria to the ventricles. High systolic blood pressure can indicate the occurrence of hypertension in a person which is then followed by an increase in diastolic blood pressure.

Leptin is a peptide hormone secreted from adipose tissue whose secretion is directly proportional to the mass of adipose tissue. The main functions of leptin in the hypothalamic nervous system are decreased food consumption and upregulation of thermogenesis and energy expenditure through the activation of sympathetic nerves. This function is mediated by α melanocyte-stimulating hormone (α-MSH) at high leptin levels and binds to melanocortin 3 and 4 receptors (MC3R and MC4R) which play a role in the regulation of blood pressure, heart rate, and sodium reabsorption in the kidneys. In the condition of obesity, despite high circulating leptin levels, the expected metabolic actions such as a reduction in appetite and increased energy expenditure do not occur. As a result, leptin resistance triggers an increase in sympathetic nerves and changes in endothelial NO expression which causes an increase in blood pressure. Insulin resistance caused by DM can be exacerbated by a decrease in the hormone adiponectin caused by obesity. The adiponectin hormone functions as a regulator of insulin sensitivity and prevents the formation of foam cells which is a process of atherogenesis. Insulin resistance, low adiponectin levels, increased leptin, angiotensinogen, serum glucose, free fatty acids, and proinflammatory and inflammatory molecules trigger vascular and systemic inflammation which results in endothelial dysfunction. Vasodilator (decreased NO) and vasoconstrictor (increased Endothelin-1, protein kinase VCAM-1, ICAM-1, and E-Selectin) imbalance results in endothelial dysfunction leading to thickening and stiffening of the arteries. Continuous activation of the sympathetic nerves causes vasoconstriction of blood vessels which ultimately increases blood pressure. Increased blood pressure is also triggered by an imbalance in salt and potassium levels which causes an increase in the volume of body fluids. It can also cause narrowing of the arteries, which in turn raises blood pressure [27].

The occurrence of hypertension in people with Type II DM in adolescents involves many interrelated path mechanisms. Adolescence itself is a time that is prone to insulin sensitivity disorders and can be worsened by the presence of genetic factors, lifestyle, and hormonal changes

that occur during the transition from adolescence to adulthood. Along with lifestyle changes and increasingly varied types of diets favored people, the consumption of a high-fat diet is now becoming one of the main phenomena that is frequently and easily found on the child's age groups - children, adolescents, and young adults. Thus, diet is one of the important factors that must be considered both in supporting growth and preventing diseases that are susceptible to appearing in people at that age. Other factors such as regular physical activity, healthy environmental conditions, good stress management, and education about diseases are factors that can be taken to prevent hypertension and metabolic syndrome in children, adolescents, and young adults.

5. CONCLUSION

Giving a high-fat diet (High Fat Diet) influence significantly on blood pressure systolic rat model of juvenile diabetes mellitus type II-induced Streptozotocin. There was an increase in blood pressure in the N, STZ, and STZ-HFD groups after the high-fat diet intervention was carried out, but only the P group had significant values for changes in systolic blood pressure before and after the intervention.

REFERENCES

[1] Indonesian Ministry of Health Information Center, "Indonesian Health Profile," Jakarta: Ministry of Health Republic of Indonesia, 2016.

[2] American Diabetes Association (ADA), "Diagnosis and Classification of Diabetes," Diabetes Care January 1, vol: 27, 2010.

[3] Damasceno , DC, Netto, AO, Iessi , IL, Gallego, FQ, Corvino, SB, Dallaqua , B., Sinzato , YK, Bueno, A., Calderon, IM, & Rudge, MV, "Streptozotocin-induced diabetes models: pathophysiological mechanisms and fetal outcomes," BioMed research international, 819065, 2014.

[4] American Diabetes Association (ADA), "Diagnosis and Classification of Diabetes Mellitus. Diabetes Care,". Jan: 34 (suppl 1): S62-S69, 2014.

[5] Temneanu , OR, Trandafir , LM, & Purcarea , MR, "Type 2 diabetes mellitus in children and adolescents: a relatively new clinical problem within pediatric practice," Journal of medicine and life, 9 (3), 235–239, 2016.

[6] Htike ZZ, Webb D, Khunti K, Davies M. Emerging epidemic and challenges of Type 2 diabetes in young adults. Diabetes Manag; 5 (6): 473–483, 2015.

[7] Batubara JRL, Tridjaja B, Pulungan AB., "Handbook of Child Endocrinology," 1st ed. Jakarta: IDAI, pp: 162-3, 195, 2010.

[8] American Diabetes Association, "Standards of Medical Care in Diabetes 2017". Vol. 40. USA: THERE, 2017.

[9] Cooper- DeHo ff R.M. , Gang Y, Handberg EM,Bavry AA, Denarjo SJ, Bakris GL, et al., "Tight blood pressure control and cardiovascular outcome among hypertensive patients with diabetes and coronary artery disease," JAMA; 304 (1): 61-8, 2010.

[10] LeWine , H., "Which blood pressure number is important?" https://www.health.harvard.edu/staying-healthy/which-blood-pressure-number-is-important [accessed on July, 26th 2020]

[11] Cho NH , Whiting D, Guariguata L, Montoya PA, Forouhi N, Hambleton I, et al., "IDF Diabetes Atlas," 6th ed. Guariguata L, Nolan T, Beagley J, Linnenkamp U, Jacqmain O, editors. International Diabetes Federation, 2013.

[12] Sengupta P., "The Laboratory Rat: Relating Its Age With Human's," International journal of preventive medicine, 4 (6), 624–630, 2013.

[13] Banerjee, S, "Characterization of Cognitive Impairment in Type 2 Diabetic Rats'," pharmaceutical-sciences, January 2017.

[14] Goud et al., "Streptozotocin-A Diabetogenic Agent in Animal Models," International Journal of Pharmacy and Pharmaceutical Research, 2015.

[15] Vatandoust, "Novel High-Fat Diet Formulation and Streptozotocin Treatment for Induction of Prediabetes and Type 2 Diabetes in Rats", Adv Biomed Res; 7: 107, 2018

[16] Pramaningtyas, M., Utoro, T., Purwono, S., & Agustiningsih, D, "Changes on aortic diameter and number of Endothelin-B receptor in aortic endothelium of diabetes mellitus rat model after exercise," Bangladesh Journal of Medical Science, 15(3), 381-384, 2016.

[17] Dahlan, M. Sopiyudin, "Statistics for Medicine and Health", Jakarta: Indonesian epidemiology, 2014.

[18] Konsue , A., Picheansoonthon, C. , Talubmook , C., "Fasting blood glucose levels and hematological values in normal and streptozotocin-induced diabetic rats of Mimosa pudica L. extracts," Pharmacogn J., 9 (3): 316, 2017.

[19] Wu, J., & Yan, LJ, "Streptozotocin-induced type 1 diabetes in rodents as a model for studying mitochondrial mechanisms of diabetic β cell glucotoxicity," Diabetes, metabolic syndrome and obesity: targets and therapy, 8, 181–188, 2015.

[20] Eleazu , CO, Eleazu , KC, Chukwuma, S., & Essien, UN, "Review of the mechanism of cell death resulting from streptozotocin challenge in experimental animals, its practical use and potential risk to humans," Journal of diabetes and metabolic disorders, 12 (1), 60, 2013.

[21] Lenzen S., "The mechanisms of alloxan- and streptozotocin-induced diabetes," Diabetologia , 51 (2), 216–226, 2008.

[22] Rajarajeswari , N., & Pari, L., "Antioxidant role of coumarin on streptozotocin-nicotinamide-induced type 2 diabetic rats," Journal of biochemical and molecular toxicology, 25 (6), 355–361, 2011.

[23] Cerf ME, "Beta cell dysfunction and insulin resistance," Frontiers in endocrinology, 4, 37, 2013.

[24] Arslanian , S., Kim, JY, Nasr, A., Bacha, F., Tfayli , H., Lee, S., & Toledo, F, "Insulin sensitivity across the lifespan from obese adolescents to obese adults with impaired glucose tolerance: Who is worse off ?". Pediatric diabetes, 19 (2), 205–211, 2018.

[25] Nadeau, KJ, Anderson, BJ, Berg, EG, Chiang, JL, Chou, H., Copeland, KC, Hannon, TS, Huang, TT, Lynch, JL, Powell, J., Sellers, E., Tamborlane , WV , & Zeitler, P., "Youth-Onset Type 2 Diabetes Consensus Report: Current Status, Challenges, and Priorities." Diabetes care, 39 (9), 1635–1642, 2016.

[26] Cree-Green, M., Triolo , TM, & Nadeau, KJ, "Etiology of insulin resistance in youth with type 2 diabetes." Current diabetes reports, 13 (1), 81–88, 2013.

[27] Kotsis , V., Stabouli , S., Papakatsika , S., Rizos , Z., & Parati , G. "Mechanisms of obesity-induced hypertension." Hypertension research: official journal of the Japanese Society of Hypertension, 33 (5), 386– 393, 2010.