Journal of the Medical Sciences
(Berkala Ilmu Kedokteran)

Volume 51, Number 3, 2019; 195-202
http://dx.doi.org/10.19106/JMedSci005103201902

The effect of long-term high-fat diet in ovariectomized Wistar rat (*Rattus norvegicus*)study on lipid profile, *endothelial nitric oxide synthase*(eNOS) dan *endhotelin*-1 (ET-1) serum

Tantri Analisawati Sudarsono[1*], Denny Agustiningsih[2], Sunarti[3]
[1]Postgraduate Student of Medical Sciences, [2]Department of Physiology, [3]Department of Biochemistry, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia

Submited: 2017-02-01
Accepted : 2019-08-20

ABSTRACT

Accumulation of cholesterol in the blood will cause stiffness in arteries and trigger the formation of atherosclerotic lesions. Estrogen has a role as an antioxidant that can prevent the low density lipoprotein(LDL) oxidation. In menopause with high-fat diet, the decrease of estrogen levels will trigger cholesterol accumulation in the blood lead to endothelial dysfunction mediated by endotelin-1 (ET-1) and nitric oxide synthase (eNOS). This study aimed to investigate the effect of long-term high-fat diet on the lipid profile, serum eNOS and ET-1levels on ovariectomized rat. It was experimental using 28 female Wistar rat divided into 4 groups. Group 1 was ovariectomized mice and given a standard diet (OVX-SD), Group 2 was ovariectomized mice and given a high-fat diet (OVX-HFD), Group 3 was not ovariectomized mice and given a standard diet(SHAM-SD) group, and Group 4 was not ovariectomized mice and given a high-fat diet (SHAM-HFD).Lipid profile of blood samples was measured pre- and post-treatment, whereas serum eNOS and ET-1 levels were measured post-treatment using ELISA method. No significantly difference of lipid profileon OVX-HFD group compared to that OVX-SD was observed. The serum eNOSlevel on OVX-HFD(702.11±68.73 pg/mL) was significantly lower than that OVX-SD (857.18±118.08 pg/mL) ($p<0.05$). However, there was no significantly different of serum ET-1 levelbetween OVX-HFD group (299.14±146.61 pg/mL) compared to that OVX-SD (194.25±102.96 pg/mL) ($p>0.05$). In conclusion, the serum eNOS levelon ovariectomized rat with long-term high-fat diet is lower than that on ovariectomized rat with standard diet.

ABSTRAK

Penimbunan kolesterol dalam darah akan menyebabkan kekakuan arteri dan memicu pembentukan lesi aterosklerotik. Estrogen memiliki peran sebagai antioksidan yang dapat mencegah oksidasi LDL. Pada menopause dengan diet lemak tinggi, penurunan kadar estrogen akan memicu akumulasi kolesterol dalam darah yang menyebabkan disfungsi endotel yang diperantai oleh endotelin-1 (ET-1) dan enzim sintase nitrit oksida (eNOS). Penelitian ini bertujuan mengkaji pengaruh diet tinggi lemak jangka panjang terhadap profil lipid, kadar eNOS dan ET-1 serum pada tikus yang diovariektomi. Penelitian ini adalah penelitian eksperimental menggunakan 28 ekor tikus yang dibagi mennjadi 4 kelompok. Kelompok 1 adalah tikus diovariektomi yang diberi diet standar (OVX-SD), Kelompok 2 adalah tikus diovariektomi yang diberi diet tinggi lemak (OVX-HFD), Kelompok 3 adalah tikus tidak diovariektomi yang diberi diet standar (SHAM-SD) dan Kelompok 4 adalah tikus yang tidak diovariektomi yang diberi diet lemak tinggi (SHAM-HFD). Profil lipid sampel darah diukur sebelum dan sesudah perlakuan, sedangkan kadar eNOS dan ET-1 diukur sesudah perlakuan menggunakan metode ELISA. Tidak terdapat perbedaan nyata profil lipid pada kelompok OVX-HFD dengan OVX-SD. Kadar eNOS serum pada OVX-HFD (702,11±68,73 pg/mL) lebih rendah secara nyata dibandingkan dengan OVX-SD (857,18±118,08 pg/mL) ($p<0,05$). Namun demikian, tidak ada perbedaan nyata kadar ET-1 serum antara OVX-HFD (299,14±146,61 pg/mL) dibandingkan dengan OVX-SD (194,25±102,96 pg/mL) ($p>0,05$).Dapat disimpulkan, kadar serum eNOS pada tikus yang diovariektomi yang diberi diet lemak tinggi lebih rendah dibandingkan dengan tikus yang diovriektomi yang diberi diet standar.

Keywords:
High-fat diet
ovacriectomy
lipid profile
endothelial nitric oxidesynthase (eNOS)
endothelin-1 (ET-1)

*corresponding author: tan3analisa89@gmail.com

INTRODUCTION

Changes in behavior and an unbalanced high-fat diet cause the increase of blood cholesterol or hypercholesterolemia which lead toatherosclerosis and might coronary heart disease (CHD).[1,2] Atherosclerosis is general term describing any hardening of the arteries due to the accumulation of fat in the artery walls forming plaque accompanied by the formation of fibrous tissue, cell proliferation of smooth muscle in tunica media and change in tunica intima.[1-4] It was reported that hypercholesterolemia causes 4.4 million (7.9%) death of young age, whereas the CHD will be the highest cause of mortality in the word in 2030.[5,6]

One of the risk factors of CHD is dyslipidemia which characterized by a disturbance in the form of lipoprotein metabolism, blood lipid expression changes, namely increased total cholesterol, low density lipoprotein (LDL) and triglycerides, and decrease the expression of high density lipoprotein(HDL) in blood.[7-9] The high expression of total LDL and cholesterol cause the accumulation of cholesterol in cells that accumulated as blood cholesterol.[10] High-fat diet causes stiffness of the walls of blood vessels and is one of the causes of atherosclerosis.[11]

Estrogen has a role as an antioxidant to prevent LDL oxidation process so that the ability of LDL to penetrate the blood vessel wall will be reduced, improved lipid profile (cholesterol) and reduce the risk of CHD.[12] At the menopause, the estrogen production of the body becomes minimal which can affect the lipidmetabolism in the body.[13] Ovariectomized rats were given a high-fat diet showed increased expression of total cholesterol and triglyceride.[14] Hypercholesterolemia triggers adhesion of monocytes and increased free radical formation resulting indeactivation of nitric oxide (NO)which disrupts endothelial cell function.Itincreases vasoconstriction and endothelial permeabilitycausing the LDL easy penetrates the intima.[15] The aim of this study was to investigate the effect of long-term high-fat diet in ovariectomized raton lipid profile, endothelial nitric oxide synthase(eNOS) andendhotelin-1 (ET-1) serum.

MATERIALS AND METHODS

Animals grouping

Twenty eight 28 female Wistar rats aged 6-8 weeks old, 120-150 gobtained from theIntegrated Research and Testing Laboratory (*Laboratorium Penelitian dan Pengujian Terpadu*/LPPT), Universitas Gadjah Mada, Yogyakarta were used in this study.Rats were adapted for seven days atroom temperature ranged of 20-25^{o}C with 12 hours of day and night cycle. Standard feed and drinking water*ad libitum* were provided in the individual cage.

Theratswerethen randomly divided into two groups with 14 rats in each group.The firstgoupwasovariectomized rat (OVX) and second group was non-ovariectomized rat (SHAM). After underwent ovariectomized process and recovery for21 days, sevenrats in groups of OVX and SHAM were dividedbased on the provision of diet. The groups were ovariectomized given standard diet (OVX-SD), ovariectomized given ahigh-fat diet (OVX-HFD), non-ovariectomized given astandard diet (SHAM-SD), and non-ovariectomized given a high-fat diet (SHAM-HFD).

Ovariectomy procedure

Ovariectomy was started by anesthesia using ketamine intramuscular injection of 0.15cc/100gBW under aseptic conditions. Surgery was begun with laparotomy process (OVX group) and dissected (SHAM) by doing a small

incision on the ventral abdominal wall. The ovary was then clamped bilaterally and removed out. Uterine horns, the meeting point between the uterus with fallopian tubes, was tied and uterus was left intact and then the abdominal wall was sutured back. In dissected rat (SHAM group), it was anaesthetized and the abdominal wall was opened in the same way as in the ovariectomy ovaries group without removing it out. The dissected for SHAM group was aimed to give the same stress with OVX group.

Feeding rat after grouping

OVX-SD rats group and SHAM-SD were given a standard diet, while the rats in the OVX-HFD and SHAM-HFD group were given a high-fat diet. The feed composition which given to the rats was provisions of the American Institute of Nutrition (AIN)-93M.[16] The amount of mice feed needed was 15 g/day/rat.[14]

Blood samples collection

The rats were fasted approximately eight hoursbefore blood sampling. Blood was taken from the retro orbital vein of 1.5 mL in two times which were before and after the treatment was given. The first blood sampling was pre-test for lipid profile measurement and the second was post-test for lipid profile measurement, eNOS and ET-1 expression with ELISA.

Statistical analysis

Data were presented as mean ± standard deviations (SD) and analyzed using ANOVA or Kruskal Wallis test. A p value <0.05 was considered significant. Correlation between lipid profile, the expression of eNOS and ET-1 was tested with Pearson correlation test. Paired t-test or Wilcoxon signed-rank test was used for the analysis of differences ofvariable values before and after the treatment.

RESULT

Lipid profile

Lipid profile measurement were performed twice, before (pre-test) and after (post-test) given treatment diet. Lipid profiles measured were total cholesterol, triglycerides, HDL and LDL (FIGURE 1).

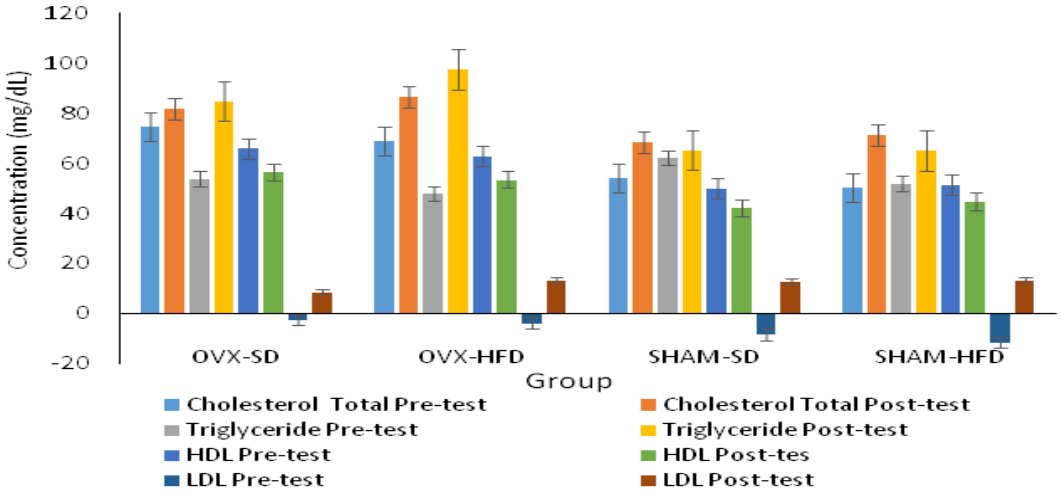

FIGURE1. The bar chart the mean levels ± SD of lipid profile (total cholesterol, triglycerides, HDL and LDL) before and after treatment. OVX-SD: groups of ovariectomized mice and given a standard diet; OVX-HFD: groups of ovariectomized mice and given a high-fat diet; SHAM-SD: groups ofnot ovariectomized mice and given a standard diet; SHAM-HFD: groups ofnot ovariectomized mice and given a high-fat diet.

Total cholesterol

The mean of pre-test total cholesterol of OVX-SD group (74.57±15.40 mg/dL) was highest, whereas the lowestwas SHAM-HFD (50.43±4.24 mg/dL). The mean of pre-test total cholesterol of OVX-HFD group was 68.86±11.39, while for the SHAM-SD group was 54.29±10.19 mg/dL. The mean of post-test total cholesterol of OVX-SD group (86.57±13.21 mg/dL) was highest, whereas the lowest was SHAM-SD (68.43±8.79 mg/dL). The mean of post-test total cholesterol of OVX-SD group was 81.86±13.30 mg/dL, while for the SHAM-HFD group was 71.29±10.16 mg/dL (FIGURE 1).

Triglycerides

The mean of pre-test triglycerides of SHAM-SD group (62.29±21.18 mg/dL) was highest, whereas the lowest was OVX-HFD (48.00±8.20 mg/dL). The mean of pre-test triglycerides of SHAM-HFD group was 52.00±14.28, while for the OVX-SD group was 53.86±12.09 mg/dL.The mean of post-test triglycerides of OVX-HFD group (97.57±31.05 mg/dL) was highest, whereas the lowest was SHAM-HFD (65.14±24.28 mg/dL). The mean of post-test triglycerides of OVX-SD group was 81.86±45.55 mg/dL, while for the SHAM-SD group was 65.29±15.56 mg/dL (FIGURE 1).

HDL

The mean of pre-test HDL of OVX-SD group (66.00±10.50 mg/dL) was highest, whereas the lowest wasSHAM-SD (50.14±6.47 mg/dL). The mean of pre-test HDL of OVX-HFD group was 62.86±10.25, while for the SHAM-HFD group was 51.57±7.85 mg/dL.The mean of post-test HDL of OVX-SD group (56.43±12.18 mg/dL) was highest, whereas the lowest was SHAM-SD (42.29±6.73 mg/dL). The mean of post-test HDL of OVX-HFD group was 53.57±9.27 mg/dL, while for the SHAM-HFD group was 44.86±7.06 mg/dL (FIGURE 1).

LDL

The mean of pre-test LDL of OVX-SD group (-2.20±12.90 mg/dL) was highest, whereas the lowest was SHAM-HFD (-11.54±7.38 mg/dL). The mean of pre-test LDL of OVX-HFD group was -3.60±18.71, while for the SHAM-SD group was -8.31±8.31 mg/dL.The mean of post-test LDL ofOVX-HFD group (13.49±8.87 mg/dL)was highest, whereas the lowest was OVX-SD (8.46±14.98 mg/dL). The mean of post-testLDL of SHAM-SD group was 13.09±5.40 mg/dL, while for the SHAM-HFD group was 13.40±7.12 mg/dL (FIGURE 1).

The difference pre-post test lipid profile

The mean difference of lipid profile(total cholesterol, triglycerides, HDL and LDL) between pre-test and post-test was not significantly different ($p>0.05$) as presented in FIGURE 2.

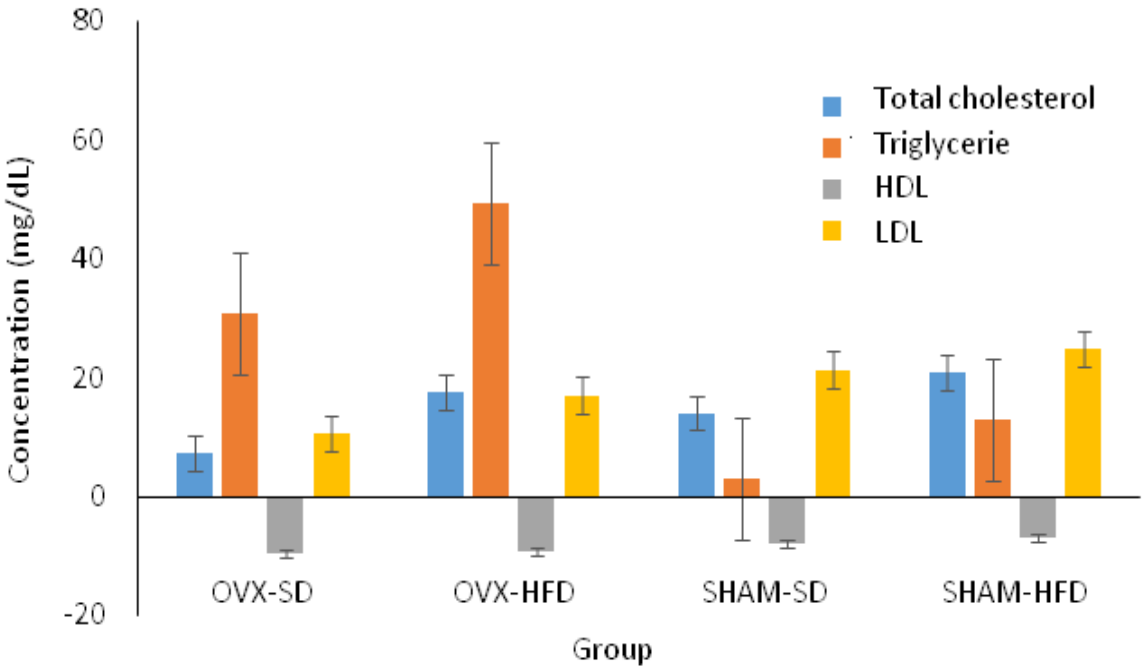

FIGURE 2. The mean differenceof lipid profile (total cholesterol, triglycerides, HDL and LDL)between pre-test and post-test. OVX-SD: groups of mice were ovariectomized and given a standard diet; OVX-HFD: groups of mice were ovariectomized and given a high-fat diet; SHAM-SD: groups of mice were not ovariectomized and given a standard diet; SHAM-HFD: groups of mice were not ovariectomized and given a high-fat diet.

Expression of eNOS and ET-1

Significantly difference between groups was observed in the expression of eNOS ($p<0.05$). The highest eNOS expression were found in OVX-SD group (857.18±118.08 pg/mL) followed by OVX-HFD group (702.11±68.73 pg/mL), SHAM-SD group (666.93±223.19 pg/mL) and the lowest was SHAM-HFD (635.96±93.26 pg/mL). The highest ET-1 expression was found in OVX-HFD group (299.14±146.61 pg/mL) followed by SHAM-SD group (257.46±117.75 pg/mL), SHAM-HFD group (232.9 ±105.31 pg/mL) and the lowest was OVX-SD (194.25±102.96 pg/mL). However, there was no significantly difference between groups in the expression of ET-1($p>0.05$).

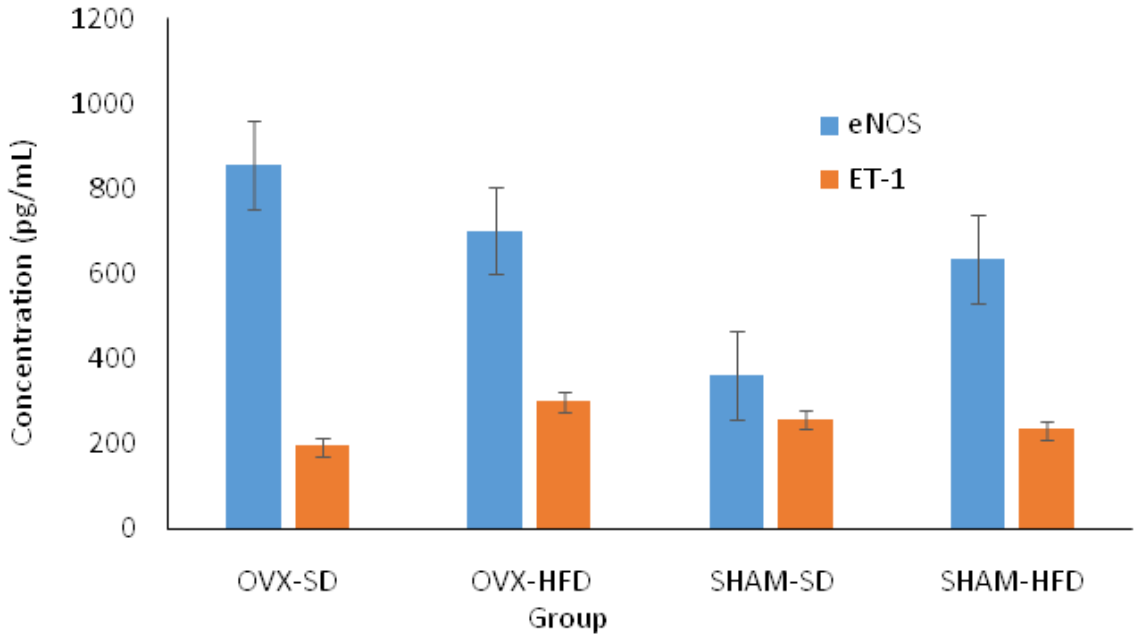

FIGURE 3. The expressionsof eNOS and ET-1. OVX-SD: groups of mice were ovariectomized and given a standard diet; OVX-HFD: groups of mice were ovariectomized and given a high-fat diet; SHAM-SD: groups of mice were not ovariectomized and given a standard diet; SHAM-HFD: groups of mice were not ovariectomized and given a high-fat diet.

DISCUSSION

Estrogen has an important role in the regulation of energy homeostasis. Estrogen plays a role in the metabolism of glucose, lipids, regulate fat distribution as well as interact with adipose signal.[17] Estrogen increases lipoprotein receptor which causes a decrease in the concentration of LDL.[10] Estrogen is also considered a hormone that maintains the cardiovascular system in women.[18]In general, the all four groups showed the increase in total cholesterol, triglycerides and LDL level followed by decreases HDL. Although it was not significantly different ($p>0.05$). This indicates that the administration of high-fat diet has an influence on lipid profile in ovariectomized rats, although statistically not significant. The protective effect of estrogen through indirect protection mechanism in this study was not confirmed as indicated no change in the plasma cholesterol expression such us reducing LDL and increasing HDL. The increase of LDL expression in postmenopausal women also lead to increased incidence of atherosclerosis by 50% when compared to premenopausal.[17,10]

The serum eNOS level on ovariectomized mice and given a high-fat diet was significantly lower than that ovariectomized mice and given standard diet ($p<0.05$). However, there was no significantly difference between the both groups in the expression of ET-1 ($p>0.05$). The decrease of serum eNOS level may be caused by the decrease serum estrogen level which lead to decrease eNOS activity. Estrogen will be captured by the receptor in endothelium vascular and vascular smooth muscle cells, and estrogen will act as a vasodilator. Estrogen provides non-genomic effects due to the increased in NO releasewhich is largely mediated

by the activation of eNOS and increases the speed of endothelial vasodilation.[18]

CONCLUSION

In conclusion, long-term high-fat diet on ovariectomized rat does not affect the profile lipid. However, it can decrease the serum eNOS level but does not affect the serum ET-1 level.

ACKNOWLEDGEMENTS

The authors would like tothanks the Director of the Study Program of the Basic Medical and Biomedical Sciences, the lecturers and staff of the Department of Physiology, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta for theirvaluable advices and assistances.

REFERENCES

1. Kalim. Peranan Statin pada Sindroma Koroner Akut. Jakarta: Bagian Kardiologi FK UI – RSJN Harapan Kita, 2005.
2. Hanslet L. Kolesterol perlu anda ketahui. Jakarta: Kesaint Blanc, 2007.
3. Falk E. Pathogenesis of atherosclerosis. J Am Coll Cardiol 2006; 47(8 Sppl):7-12. https://doi.org/10.1016/j.jacc.2005.09.068
4. Poredos P. Oxidative stress and atherosclerosis. Europ Health Cardiol J 2007; 6(6):1-3.
5. Ross R. Atherosclerosis--an inflammatory disease. N Engl J Med 1999; 340(2):115-26. https://doi.org/10.1056/NEJM199901143400207
6. Katzung BG. Farmako Dasar dan KlinisEd. 8. Jakarta: Salemba Medika, 2002.
7. Falk E. Pathogenesis of atherosclerosis. J Am Coll Cardiol 2006; 47(8s1):c7-c12. http://doi.org/10.1016/j.jacc.2005.09.068
8. Carey VJ, Bishop L, Laranjo N, Harshfield BJ, Kwiat C, Sack FM. Contribution of high plasma triglicerides and low high-density lipoprotein cholesterol to residual risk of coronary heart disease after establishment of low density lipoprotein cholesterol control. Am J Cardiol 2010; 106:757-63. http://doi.org/10.1016/j.amjcard.2010.05.002.
9. Clearfield MDO. The National Cholesterol Education Program Adult Treatment Panel III Guidelines. JAOA Supl2003; 103(1 suppl):S1-5.
10. Gruber CJ, Tschugguel W, Schneeberger C, Huber JC. Production and actions of estrogens. N Engl J Med 2002; 346(5):340-52. https://doi.org/10.1056/NEJMra000471
11. Yonezawa R, Wada T, Matsumoto N, Morita M, Sawakawa K, Ishii Y, et al. Central versus peripheral impact of estradiol on the impaired glucose metabolism in ovariectomized mice on a high-fat diet. Am J Physiol Endocrinol Metab 2012; 303(4):E445-56. https://doi.org/10.1152/ajpendo.00638.2011
12. Correia AL Jr, Aguila MB, Mandarim-de-Lacerda CA, Faria TS. Effects of high-fat diet on plasma lipids, adiposity, and inflammatory markers in ovariectomized C57BL/6 mice. Nutrition 2012;28(3):316-23. https://doi.org/10.1016/j.nut.2011.07.014
13. Safar ME, Czernichow S, Blacher J. Obesity, arterial stiffness, and cardiovascular risk. J Am Soc Nephrol 2006; 17(4 Suppl 2):S109-11. https://doi.org/10.1681/ASN.2005121321
14. Smith JB, Mangkoewidjojo. Pemeliharaan, Pembiakan, dan Penggunaan Hewan Percobaan di Daerah Tropis. Jakarta: UI Press, 1998.
15. Safar ME, Sebastian C, Jacques B. Obesity, arterial stiffness, and cardiovascular risk. J Am Soc Nephrol 2006; 17:S109-11.

https://doi.org/10.1681/ASN.2005121321
16. Reeves PG, Nielsen FH, Fahey GC Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr 1993; 123(11):1939-51. https://doi.org/10.1093/jn/123.11.1939
17. Yonezawa R, Wada T, Matsumoto N, Morita M, Sawakawa K, Ishii Y, *et al*. Central versus peripheral impact of estradiol on the impaired glucose metabolism in ovariectomized mice on a high-fat diet. Am J Physiol Endocrinol Metab 2012; 303:E445–56. http://doi.org/10.1152/ajpendo.00638.2011
18. Mendelsohn ME & Karas RH. The protective effects of estrogen on the cardiovascular system. N Eng J Med1999; 340:1801-11. http://doi.org/10.1056/NEJM199906103402306