



Long-Term Induction by Duck Egg Yolk Resulting in Increased Total Cholesterol, Low Density Lipoprotein, High Density Lipoprotein and Atherogenic Index in Experimental Animals

Muhammad Hanif Al As'ad Budiyo¹, Dimas Agus Cholili¹,
Diva Berliana Adhyaksa¹, Dwina Permatasari¹, Fathiyatul Mudzkiro¹,
Anindya Amanda Damayanti¹, and Dwi Nur Ahsani²(✉)

¹ Faculty of Medicine, Universitas Islam Indonesia, Yogyakarta, Indonesia

² Department of Histology, Faculty of Medicine, Universitas Islam Indonesia, Yogyakarta, Indonesia

dwi.nurahsani@uii.ac.id

Abstract. Induction by high-fat diet (HFD) is frequently performed for research on hypercholesterolemia in experimental animals. In general, HFD is obtained by combining various components, but only few studies have investigated the potential of each component to induce hypercholesterolemia in experimental animals. This study aimed to identify the potential of a number of HFD components found on a day-to-day basis in society for induction of hypercholesterolemia in animal models. The research involved a posttest only group design conducted on 30 *Rattus norvegicus* rats for 28 days. In addition to receiving standard ad-libitum feed, the rats were given appropriate treatment according to the group, each at a dose of 1ml/100gBW for P1 = distilled water, P2 = chicken egg yolk, P3 = quail egg yolk, P4 = duck egg yolk, P5 = liquid butter, and P6 = liquid margarine. The lipid profile (total cholesterol, LDL, HDL, triglycerides) was examined at the end of the study. The obtained blood serum was examined using a spectrophotometry to yield the lipid profile data. The atherogenic index (AI) was measured by dividing LDL by HDL. The data were analyzed using a one-way ANOVA. This study showed that induction by duck egg yolk for 28 days resulted in the highest levels of total cholesterol, LDL, HDL, and AI (P4; total cholesterol = 85.00 ± 22.86 , LDL = 23.20 ± 9.05 , HDL = 37.16 ± 12.21 , AI = 0.63 ± 0.12). The induction by liquid margarine was able to result in the highest increase in the triglyceride level compared to the healthy group and other induction of hypercholesterolemia, but there was no difference in the levels of total cholesterol ($p = 0.362$), LDL ($p = 0.112$), HDL ($p = 0.631$), triglycerides ($p = 0.427$), and AI (0.094). This study shows that duck egg yolk has the potential to be used in induction of hypercholesterolemia in experimental animals..

Keywords: Hypercholesterolemia · Lipid Profile · Egg Yolk · Margarine · Butter

1 Introduction

Hypercholesterolemia is a condition that can lead to various diseases, including cardiovascular disease which is closely related to hypercholesterolemia. High cholesterol levels can also lead to the formation of plaque in blood vessels. Such plaque can progress to a process of atherosclerosis or induce thromboembolism [1, 1]. Both of these conditions can interfere with organ perfusion (resulting in decreased function), and can even result in death [[3]. A further study should be conducted to prevent or to treat hypercholesterolemic condition.

Animal models are often used to study pathophysiology, including hypercholesterolemia treatment. Various methods have been used to achieve hypercholesterolemia in experimental animals. One of these methods is the administration of a long-term/chronic high-fat diet (HFD) [4, 5]. HFD administration is easy to do, making it popular to use for studies of hypercholesterolemic conditions in experimental animals in Indonesia. Some of the HFD frequently used to induce hypercholesterolemia come from a combination of diets with various components [6]. Such diet combination is also capable of inducing various organ abnormalities [7, 8]. The components of the diet combination that are often used in Indonesia include oils (pork oil, vegetable oil, coconut oil), various egg yolks (chicken, duck, quail), pure cholesterol, cholic acid, lamb fat, and beef brain [9–12].

Although effective, the HFD combination is relatively more expensive than a single diet. Few studies have used a single diet to achieve hypercholesterolemia in experimental animals. This study aims to examine the potential of various diets/ingredients with high fat content commonly used in society (egg yolks, butter, and margarine) as hypercholesterol-inducing agents in experimental animals. Using the same dose of a variety of modalities to induce hypercholesterolemic conditions (1ml/100gBW), the potential of each agent can be identified. Induction of hypercholesterolemia in this study was performed chronically by observing changes in the lipid profile, such as total cholesterol, LDL, HDL and triglycerides (triacylglycerol). In addition, the researchers measured the atherogenic index (AI) of each type of induction given.

2 Method

2.1 Experimental Animal Handling

This study has obtained approval from the Ethics Committee of the Faculty of Medicine of Universitas Gadjah Mada with the ethics approval number KE/FK/1164/EC/2020. This was an experimental study with a posttest only with control group design. The research was conducted at the Laboratory of the Faculty of Medicine of Universitas Islam Indonesia Yogyakarta. A total of 30 male *Rattus norvegicus* aged 2–4 months and weighing 200–400 g were used in this study. Induction of hypercholesterolemia was done by administering an ad-libitum AD-2 Comfeed diet and a high-fat diet (based on the treatment group) for 28 days. There were 6 treatments (each consisting of 5 rats) in this study, including P1 = distilled water (normal group), P2 = chicken egg yolk, P3 = quail egg yolk, P4 = duck egg yolk, P5 = liquid butter, P6 = liquid margarine. A high-fat diet (butter, margarine, egg yolk) or distilled water was given at a dose of 1ml/100gBB, and all of the rats received ad libitum drink.

2.2 Induction of Hypercholesterolemia

All of the induction modalities used in this study were obtained from Pakem Traditional Market. All types of egg yolk (quail, chicken and duck) used in this study were carefully separated from the white and placed in different containers. Butter and margarine were put in a beaker glass and then heated on a Bunsen burner until they melted. The melted butter and margarine were then cooled at a room temperature. After cooling, the butter and margarine were administered to the experimental animals. Induction of hypercholesterolemia was done using NGT once a day at the same hour for 28 days.

2.3 Examination of Lipid Profile

Examination of the total cholesterol was done using the rat blood serum. Blood was collected through retro-orbital sampling at the end of the study (day 28). Prior to such a procedure, the rats were anesthetized using intraperitoneal injection of Zoletil (a dose of 0.01 ml/100grBW). The blood was then made into serum through centrifugation at 3000 rpm for 10 min. The lipid profile of the rat blood serum was determined using the Microlab 300 spectrophotometry. The lipid profile data consisted of total cholesterol, LDL, HDL, and triglycerides presented in mg/dL unit. The lipid profile examination was performed at LPPT UGM Yogyakarta.

2.4 Statistical Analysis

The atherogenic index data were obtained from LDL data divided by HDL (LDL/HDL). All of the research data were tested in the one-way ANOVA test. The data were declared significant if $p < 0.005$, with 95% CI.

3 Result and Discussion

This study shows that the variety of modalities to induce hypercholesterolemia produces different lipid profiles. Induction by duck egg yolk for 28 days resulted in the highest levels of total cholesterol, LDL HDL, and atherogenic index (AI). Conversely, induction using quail egg yolk yielded the lowest total cholesterol, LDL, and HDL levels. In contrast to the three lipid profile parameters, induction by liquid margarine was able to produce the highest increase in triglyceride levels compared to those in the healthy group and other hypercholesterolemia induction. However, there was no significant difference in the total cholesterol, LDL, HDL, TG, and AI among the treatment groups (Table 1.).

The high levels of total cholesterol, LDL, and AI in induction by duck egg yolk indicate that this modality has the potential use as single induction to establish hypercholesterolemia in experimental animals. Research on cholesterol induction using duck egg yolk can be found in a study by Bogorani et al. [13]. Using induction by two preparations of duck egg yolk for 30 days (made into pellets and fresh preparations), the experimental animals experienced a significant increase in the cholesterol, LDL, TG, and AI levels. Different from the research by Bogorani et al. which showed significant results, this current study found insignificant results. This is possible due to differences

Table 1. Lipid profile and atherogenic index after induction of hypercholesterolemia by a variety of modalities in experimental animal.

Mean \pm Standar Deviation (mg/dL)							p-value
	P1 (n=5)	P2 (n=5)	P3 (n=5)	P4 (n=5)	P5 (n=5)	P6 (n=5)	
Total cholesterol	73.54 \pm 14.15	71.42 \pm 12.60	65.44 \pm 3.95	85.00 \pm 22.86	71.30 \pm 6.57	75.40 \pm 12.44	0.362
LDL	16.20 \pm 2.28	19.20 \pm 4.41	14.80 \pm 2.38	23.20 \pm 9.05	17.16 \pm 2.58	20.08 \pm 4.13	0.112
HDL	33.80 \pm 6.06	35.04 \pm 4.07	30.24 \pm 2.16	37.16 \pm 12.21	31.90 \pm 3.38	33.36 \pm 5.54	0.631
TG	80.00 \pm 28.52	74.46 \pm 13.43	81.46 \pm 23.36	84.30 \pm 30.87	106.34 \pm 54.63	126.24 \pm 79.19	0.427
AI	0.49 \pm 0.75	0.54 \pm 0.72	0.49 \pm 0.05	0.63 \pm 0.12	0.54 \pm 0.09	0.60 \pm 0.10	0.094

Note: P1= distilled water (normal group), P2= chicken egg yolk, P3=quail egg yolk, P4=duck egg yolk, P5= liquid butter, P6=liquid margarine. The atherogenic index data were obtained from LDL data divided by HDL (LDL/HDL)

in the research doses and standard feed given. The dose of 1 ml/100gBW for duck egg yolk induction given in this study referred to the research by Harsa et al. which used a combination of duck egg yolk and lard [12]. In contrast to Harsa et al., single induction by duck egg yolk did not result in decreased HDL levels. In addition, the increase in triglyceride levels in the group induced by duck egg yolk was not much different compared to the healthy group. This shows that the oil component in the study by Harsa et al. was able to reduce HDL levels and increase triglyceride levels in experimental animals. The same finding also appears in a study by sa'Adah et al., which used a combination of duck egg yolk and used cooking oil [14]. Such combination (duck egg yolk and used cooking oil) was able to increase the total cholesterol, LDL, and AI index and reduce HDL. In line with such finding, this current study shows that induction by liquid butter and margarine resulted in increased triglyceride levels and affected HDL levels. Liquid butter increased triglyceride levels and reduced HDL levels, while induction by liquid margarine only increased triglyceride levels. This is similar to the research by Franczyk-Żarów et al. and de Angelis-Pereira et al. which showed that margarine administration was able to significantly increase TG levels in experimental animals. Meanwhile, liquid butter was able to lower HDL levels better than liquid margarine [15], [16].

Atherogenic index is a potential marker for metabolic-related diseases. Research shows that AI has a strong positive correlation with obesity [17]. Furthermore, AI is also closely associated with the occurrence of cardiovascular diseases, metabolic syndrome, hypertension, and diabetes mellitus [18]. It even becomes a strong, independent predictor of coronary heart disease and other risk factors [19]. AI is closely linked to the hardening of blood vessels. It can even be used to determine the occurrence of early subclinical atherosclerotic lesions [20]. Increased AI index after induction by duck egg yolk in this

study shows its potential to produce cardiovascular animal models by administering higher doses and longer durations of induction.

The potential of single induction to establish hypercholesterolemia in experimental animals is lower than that of the combination. Therefore, a large number of studies have used a combination of various dietary modalities to achieve hypercholesterolemia. The diet combination with soybeans, cholesterol, and fiber can increase LDL levels and decrease HDL without causing impaired liver function. This indicates that the induction model is suitable for hypercholesterolemia research in experimental animals without causing developmental disorders [21]. A high-fat diet combined with partial carotid artery ligation is able to create a marked atherosclerotic condition in experimental animals [22].

Egg yolks have a higher fat content than the rest of the egg. Therefore, this part of egg is often used for induction of hypercholesterolemia. The inability of quail egg yolks to increase total cholesterol and LDL levels in this study was caused by their low total fat content. Research shows that the cholesterol content of quail eggs is lower than that of duck and turkey eggs [23]. Other studies have similar findings with the highest to lowest total fat content found in eggs of ostrich, duck, turkey, goose, and quail, respectively. The content of triglycerides, free fatty acids (FFA), diglycerides (DG), monoglycerides and phosphatidylinositol in quail eggs is lower than in duck eggs [24].

This study has a number of limitations, including the inability of the researchers to determine whether there have been morphological and functional abnormalities of the organs in this induction model. Also, the researchers did not examine how free radical changes occurred after induction. However, this study has been able to demonstrate the early potential of egg yolk to achieve hypercholesterolemia in experimental animal

4 Conclusion

Induction by duck egg yolk leads to increased levels of total cholesterol, LDL, HDL, and AI, while induction by liquid margarine raises the triglyceride levels. Duck egg yolk is potential as induction modality to trigger a hypercholesterolemic condition in experimental animals.

Acknowledgment. Some part of this research was funded through the research innovation grants for medical students by Universitas Islam Indonesia

Author's Contribution. MHAB contributed as the research drafter, conducted the research, processed the data, and drafted the initial manuscripts for publication. DAC, DBA, DP, FM, and AA conducted the research and processed the data. DNA contributed as the research drafter, interpreted the data, and prepared the final manuscript for publication

References

1. M. A. W. Broeders, G. J. Tangelder, D. W. Slaaf, R. S. Reneman, and M. G. A. Oude Egbrink, "Hypercholesterolemia enhances thromboembolism in arterioles but not venules: Complete reversal by L-arginine," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 22, no. 4, pp. 680–685, 2002.
2. N. A. Boynosky and L. Stokking, "Atherosclerosis associated with vasculopathic lesions in a golden retriever with hypercholesterolemia," *Canadian Veterinary Journal*, vol. 55, no. 5, pp. 484–488, 2014.
3. S. S. Gidding, K. Wilemon, and S. Chugh, "Familial Hypercholesterolemia Causes Sudden Death," *Journal of the American College of Cardiology*, vol. 74, no. 23, pp. 2951–2952, 2019.
4. G. A. Otunola, O. B. Oloyede, A. T. Oladiji, and A. A. Afolayan, "Effects of diet-induced hypercholesterolemia on the lipid profile and some enzyme activities in female Wistar rats," *African Journal of Biochemistry Research*, vol. 4, no. 6, pp. 149–154, 2010.
5. Z. J. Lin, B. Zhang, X. Q. Liu, and H. L. Yang, "Abdominal fat accumulation with hyperuricemia and hypercholesterolemia quail model induced by high fat diet," *Chinese Medical Sciences Journal*, vol. 24, no. 3, pp. 191–194, 2009.
6. H. M. Al-Muzafar and K. A. Amin, "Efficacy of functional foods mixture in improving hypercholesterolemia, inflammatory and endothelial dysfunction biomarkers-induced by high cholesterol diet," *Lipids in Health and Disease*, vol. 16, no. 1, 2017.
7. C. Csonka et al., "Isolated hypercholesterolemia leads to steatosis in the liver without affecting the pancreas," *Lipids in Health and Disease*, vol. 16, no. 1, pp. 1–14, 2017.
8. M. Ibrahim et al., "Baccaurea angulata fruit juice reduces atherosclerotic lesions in diet-induced Hypercholesterolemic rabbits," *Lipids in Health and Disease*, vol. 16, no. 1, 2017.
9. T. Heriansyah, "Pengaruh Berbagai Durasi Pemberian Diet Tinggi Lemak Terhadap Profil Lipid Tikus Putih (Rattus Novergicus Strain Wistar) Jantan," *Jurnal Kedokteran Syiah Kuala*, vol. 13, no. 3, pp. 144–150, 2013.
10. N. Fatimuzzahro and R. Chriestedy, "Efek Kopi Robusta terhadap Profil Lipid Darah Tikus yang Diinduksi Seduhan dan Berat Badan Diet Tinggi Lemak Effect of Robusta Coffee on Blood Lipid Profile Rat Induced High Fat Diet and Body Weight," *Jurnal Kedokteran Brawijaya*, vol. 30, no. 1, pp. 7–11, 2018.
11. P. M. Ulfa, Y. Alioes, and B. O. Putri, "Pengaruh Pemberian Ekstrak Biji Melinjo (Gnetum gnemon) Terhadap Kadar Trigliserida pada Tikus dengan Diet Tinggi Lemak," *Jurnal Kesehatan Andalas*, vol. 7, no. 2, p. 192, 2018.
12. I. M. S. Harsa, "Efek Pemberian Diet Tinggi Lemak Terhadap Profil Lemak Darah Tikus Putih (Rattus norvegicus)," *Universitas Wijaya Kusuma*, vol. 31, no. 1, pp. 21–28, 2014.
13. N. W. Bogoriani, A. A. B. Putra, and W. E. Heltyani, "the Effect of Intake Duck Egg Yolk on Body Weight, Lipids Profile and Atherosclerosis Diseases in Male Wistar Rats," *International Journal of Pharmaceutical Sciences and Research*, vol. 10, no. 2, pp. 926–932, 2019.
14. N. N. Sa'Adah, K. I. Purwani, A. P. D. Nurhayati, and N. M. Ashuri, "Analysis of lipid profile and atherogenic index in hyperlipidemic rat (Rattus norvegicus Berkenhout, 1769) that given the methanolic extract of Parijoto (Medinilla speciosa)," *AIP Conference Proceedings*, vol. 1854, 2017.
15. M. Franczyk-Żarów, E. Kuś, and R. B. Kostogrys, "Effect of conjugated linoleic acid and different type of dietary fat on serum lipid profile, liver enzymes activity and oxidative stress markers in Wistar rats," *Roczniki Panstwowego Zakladu Higieny*, vol. 70, no. 1, pp. 27–33, 2019.
16. M. C. de Angelis-Pereira, M. de F. P. Barcelos, J. de A. R. Pereira, R. C. Pereira, and R. V. de Souza, "Effect of different commercial fat sources on brain, liver and blood lipid profiles of rats in growth phase1," *Acta Cirurgica Brasileira*, vol. 32, no. 12, pp. 1013–1025, 2017.

17. X. Zhu et al., "Atherogenic index of plasma is a novel and better biomarker associated with obesity: A population-based cross-sectional study in China," *Lipids in Health and Disease*, vol. 17, no. 1, 2018.
18. R. L. Yang, Y. H. Shi, G. Hao, W. Li, and G. W. Le, "Increasing oxidative stress with progressive hyperlipidemia in human: Relation between malondialdehyde and atherogenic index," *Journal of Clinical Biochemistry and Nutrition*, vol. 43, no. 3, pp. 154–158, 2008.
19. G. Cai, G. Shi, S. Xue, and W. Lu, "The atherogenic index of plasma is a strong and independent predictor for coronary artery disease in the Chinese Han population," *Medicine (United States)*, vol. 96, no. 37, 2017.
20. G. Yildiz et al., "Evaluation of association between atherogenic index of plasma and intima-media thickness of the carotid artery for subclinic atherosclerosis in patients on maintenance hemodialysis," *Hemodialysis International*, vol. 17, no. 3, pp. 397–405, 2013.
21. S. Leite Matos et al., "Dietary Models for Inducing Hypercholesterolemia in Rats," *Brazilian Archives of Biology and Technology*, vol. 48, no. 2, pp. 203–209, 2005.
22. A. Ishii et al., "Swine model of carotid artery atherosclerosis: Experimental induction by surgical partial ligation and dietary hypercholesterolemia," *American Journal of Neuroradiology*, vol. 27, no. 9, pp. 1893–1899, 2006.
23. D. E. Turk and B. D. Barnett, "Cholesterol content of market eggs.," *Poultry science*, vol. 50, no. 5, pp. 1303–1306, 1971.
24. V. J. Sinanoglou, I. F. Strati, and S. Miniadis-Meimaroglou, "Lipid, fatty acid and carotenoid content of edible egg yolks from avian species: A comparative study," *Food Chemistry*, vol. 124, no. 3, pp. 971–977, 2011.

Open Access This chapter is licensed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits any noncommercial use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license and indicate if changes were made.

The images or other third party material in this chapter are included in the chapter's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the chapter's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder.

