

MODELING OF MICE AS TEST ANIMALS FOR A PRECLINICAL STUDY OF HYPOLIPIDEMIC AGENTS

Setiyo Budi Santoso^{1,2}✉, Widarika Santi Hapsari^{1,2}, Renny Setyowati¹

¹Department of Pharmacy, Universitas Muhammadiyah Magelang, Magelang 56172, Indonesia

²Consortium for Pharmacology and Clinical Pharmacy Studies, Universitas Muhammadiyah Magelang, Magelang 56172, Indonesia

✉ sb@unimma.ac.id

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ABSTRACT

Animal models suitable for preclinical research are necessary for the discovery of hypolipidemic agents. Various publications have presented alternative dyslipidemia animal models, but identifying a feasible and stable method would serve as a solid reference for researchers. This investigation aimed to establish a sustained dyslipidemia induction that persists after several days of intervention with a hypolipidemic agent. Six groups of mice, each consisting of five primary test animals and one reserve test animal, were used. After a seven-day acclimatization period, we induced each group for 14 days using three different methods: (1) 5% body weight of quail egg yolks (5% QEY), (2) 10% body weight of used cooking oil (10% UCOs), and (3) a combination of 5% QEY and 10% UCOs. Once all mice reached their peak lipid levels, we evaluated lipid performance through a seven-day intervention with simvastatin (0.026 mg/20-gram body weight) in one of the paired groups. A 14-day combined induction of 5% QEY and 10% UCOs resulted in a 39% elevation in mouse lipids compared to baseline levels. Our findings offer an alternative to traditional dyslipidemia models. However, the development of an animal model for dyslipidemia still poses challenges. Therefore, the identification of novel biomarkers capable of targeting dyslipidemia in humans is crucial.

Keywords: Quail egg yolks; Used cooking oil; Simvastatin

1. INTRODUCTION

Dyslipidemia are the cause of death and morbidity in developed countries. The use of animal models has helped to expand our understanding by introducing novel ways aimed at improving the discovery of new hypolipidemic agents (Zaragoza et al., 2011). The suitable animal models must be picked to achieve this purpose (Cignarella, 2009; Hickman et al., 2017).

In order to modeling dyslipidemic mice, many studies used high-fat diets ranging from 20 to 60% of total calories. Plant-derived oils (maize, safflower, or olive oil) or animal fats can be used to make the fat component (beef tallow, lard) (Wong et al., 2016). Various national studies gave well-known inductions such as butter (Mutia et al., 2018; Octavia et al., 2015), used cooking oil (Azhari et al., 2017; Puspasari et al., 2016; Tandraini et al., 2020), quail eggs (Fauziah et al., 2018; Pangestuti, 2019), and propylthiouracil (Oktavia et al., 2018; Priatna, 2015; Sulastri et al., 2020). They provide a variety of induction days, with an average of 14 days (Kardela et al., 2019; Runtal et al., 2019), the shortest accelerating was 6 days (Mutia et al., 2018), and others maximizing up to 3 weeks until a month (Azhari et al., 2017; Puspasari et al., 2016; Tandraini et al., 2020).

Several previous studies have reported the utilization of quail egg yolks (QEY) and used cooking oil (UCO) in high-fat diets to induce dyslipidemia in animal models (Datu et al., 2022; Wiyati et al., 2020). This approach allows researchers to investigate the effects of various interventions on lipid metabolism and potentially develop therapeutic strategies for dyslipidemia

in humans (Emmanuel et al., 2017; Reda et al., 2020). The incorporation of quail egg yolks and used cooking oil in dyslipidemia modeling can be attributed to their high fat content, which plays a significant role in the development of dyslipidemia in animal models (Datu et al., 2022; Emmanuel et al., 2017; Reda et al., 2020; Wiyati et al., 2020).

In addition to the statins' activity in decreasing plasma low-density lipoprotein cholesterol for the treatment of dyslipidemia in humans, the responses of these parameters to simvastatin therapy in our mice model were studied (Yin et al., 2012). It is commonly used as a positive control substance in a variety of hypolipidemic drug development studies (Azhari et al., 2017; Octavia et al., 2015; Sulastri et al., 2020). The empirical finding that statins are more effective in lowering cholesterol from an external source, such as a high-fat diet, supports the basic mechanism (Pecoraro et al., 2014).

There is a need for appropriate animal models in preclinical research to discover hypolipidemic agents. Numerous studies have presented various models for dyslipidemia testing in animals. However, it is crucial to identify a feasible and stable method that can serve as a reliable reference for researchers. In our study, we established a sustained induction of dyslipidemia using QEY and UCO, which persisted even after several days of intervention with a hypolipidemic agent. These findings provide valuable insights for further investigations into dyslipidemia and its potential therapeutic approaches.

2. METHODS

2.1. Ethics

The study protocol set was approved by Medical and Health Research Ethics Committee (MHREC) Faculty of Medicine, Public Health and Nursing Universitas Gadjah Mada – Dr. Sardjito General Hospital with reference number: KE/FK/0328/EC/2022.

2.2. Animals

BALB/C mice bred by Gadjah Mada University's Pharmacology Laboratory were placed and handled in accordance with an approved protocol. We used male heterozygous BALB/C mice (2-3 months old) in this study.

2.3. Materials

All mice were fed the standard feed on a daily basis. We prepared boiled quail egg yolk (from the traditional market) and cooking oil (Bimoli by Salim Ivomas Pratama), which we used for induction five times. Drugs for intervention containing 10 mg simvastatin (Hexpharm Jaya). A cholesterol meter (Easy Touch) is used to measure lipids.

2.4. Study Design

All mice were divided into six groups, each with five main test animals and one reserve test animal. After a seven days acclimatization period, they were treated for 14 days in the induction phase, followed by 7 days in the intervention phase.

2.4.1. Induction Period

During the modeling induction, we fed quail egg yolks -5% body weight (QEY-5%) to groups A and D, used cooking oil 10% body weight (UCOs-10%) to groups B and E, and a combination of (QEY-5%) and (UCOs-10%) to groups C and F. After taking baseline lipid levels on day 0, we took periodic measures on the 5th and 11st days, and concluded on the 14th. Throughout the experiment, all mice were fed daily.

2.4.2. Intervention Period

We investigated the cholesterol performance with simvastatin intervention (0.026 mg/20-gram body weight) for 7 days after all mice attained the greatest level on day 14. The stability of lipid levels was measured on the 15th, 16th, and 18th days, and concluded on the 21st. Throughout the experiment, all mice were fed daily. In line with the research objective, a comprehensive

evaluation was conducted on the entire treatment group utilizing the simvastatin intervention. Each group was managed and observed according to the same schedule.

3. RESULTS AND DISCUSSION

3.1. Performance of 5% QEY Induction

After 14 days of 5% QEY induction, both the intervention (A) and control (D) groups had an increase in lipid to 149 mg/dl. However, after seven days, cholesterol levels in the simvastatin intervention group (A) maintained at 123 mg/dL, whereas the control group (D) rise up to 155 mg/Dl (Figure 1).

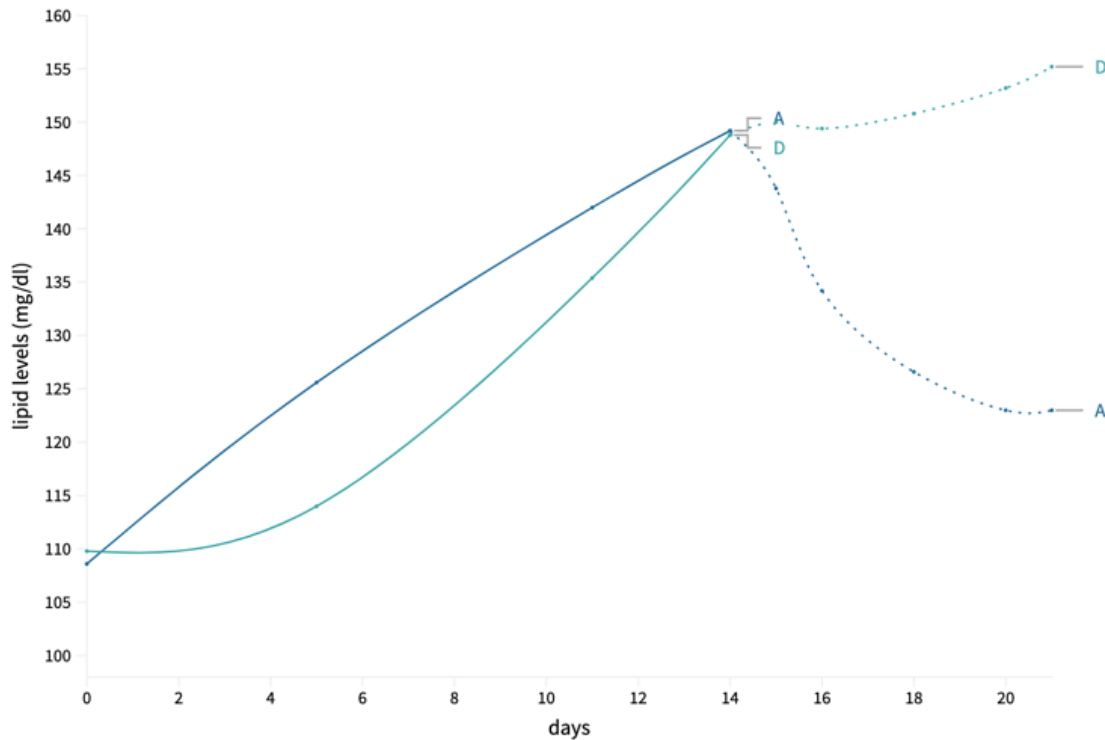


Figure 1. The 5% QEY induction performance is shown by intervention group (A) and control group (D). The solid line represents the model's induction phase, while the dashed line represents the intervention phase.

3.2. Performance of 10% UCOs Induction

After 14 days of 10% UCOs induction, the lipid increased in both the intervention (B) and control (E) groups, reaching 147 mg/dL and 143 mg/dL, respectively. However, after seven days, cholesterol levels in the simvastatin (B) intervention group declined to 112 mg/dL, whereas the control group (E) climbed to 146 mg/dL (Figure 2).

3.3. Performance of 5% QEY and 10% UCO Combination

After 14 days of combination of QEY-5% and UCOs-10% induction, both the intervention (C) and control (F) groups had elevated lipids at 156 mg/dL and 149 mg/dL, respectively. However, cholesterol levels in the simvastatin (C) intervention group maintained at 141 mg/dL after seven days, whereas the control group (F) increased to 159 mg/dL (Figure 3).

3.4. All induction alternatives' performance is compared.

Group C had the largest lipid rise (54%) after 14 days of induction, followed by group B (44%) and then group A (37 percent). Group C had the greatest lipid performance stability after simvastatin testing (39 percent rise from baseline), followed by group A (13 percent), and group B (13 percent) (10 percent) (Figure 4).

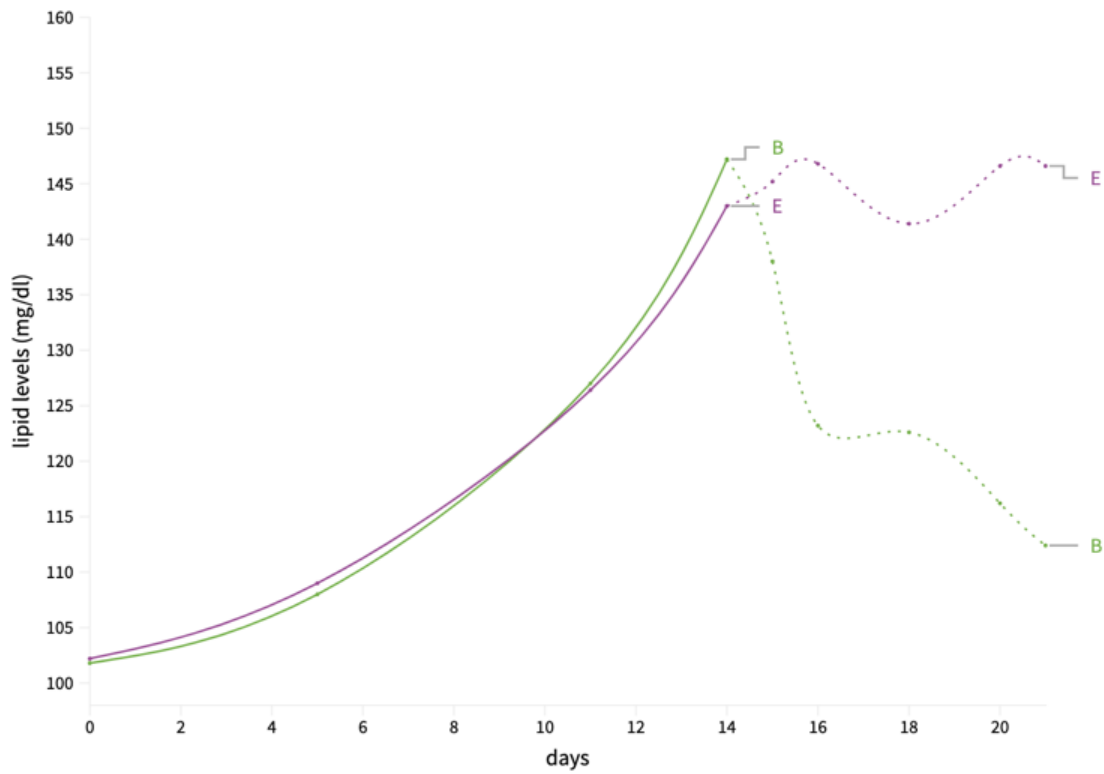


Figure 2. The 10% UCOs induction performance is showed by intervention group (B) and control group (E). The solid line represents the model's induction phase, while the dashed line represents the intervention phase.

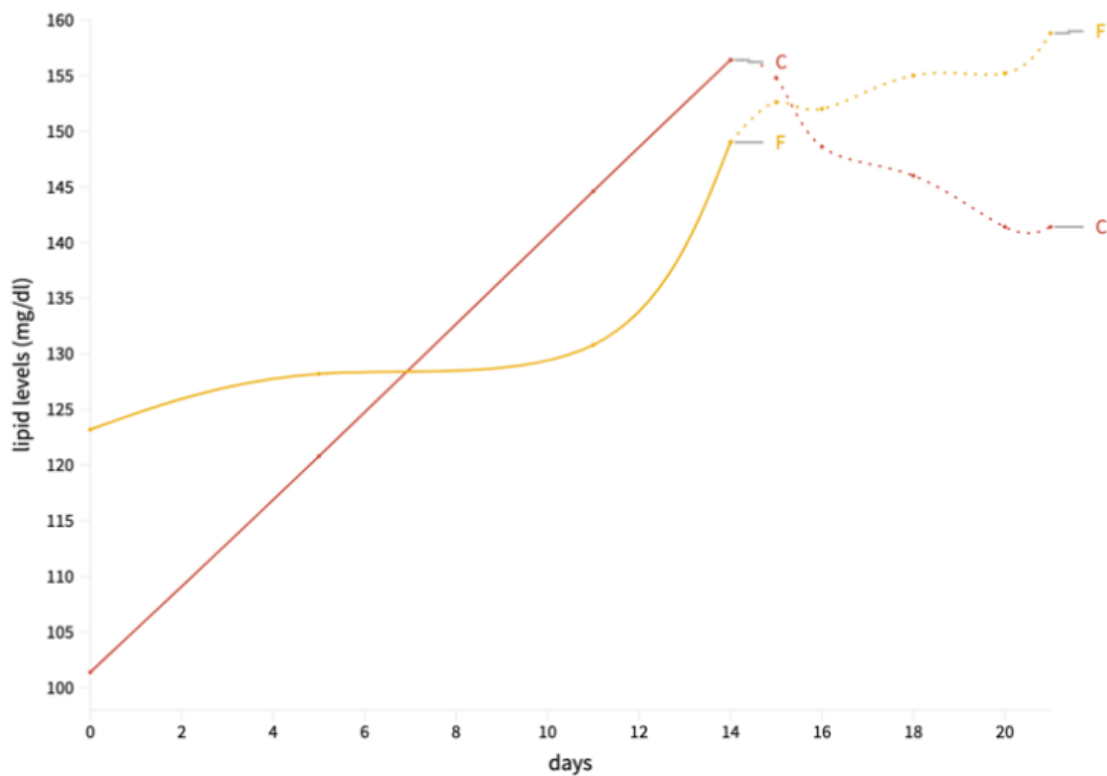


Figure 3. The Combination of 5% QEY and 10% UCOs induction performance is showed by intervention group (C) and control group (F). The solid line represents the model's induction phase, while the dashed line represents the intervention phase.

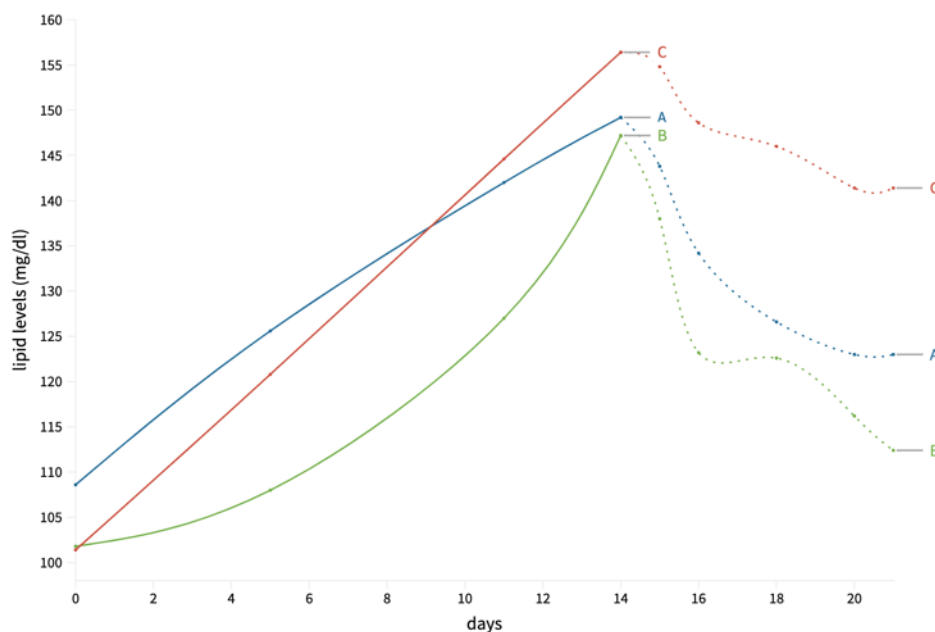


Figure 4. All induction alternatives' performance is compared; the 5% QEY (A), the 10% UCOs (B), and the combination of 5% QEY and 10% UCOs (C). The solid line represents the model's induction phase, while the dashed line represents the intervention phase.

Quail egg yolks are recognized for their high cholesterol content, which may pose challenges for individuals with hyperlipidemia (Datu et al., 2022; Khalifa & Noseer, 2019; Wiyati et al., 2020). However, this study discovered that the low total fat content of quail egg yolks prevented an increase in total cholesterol and LDL levels. Conversely, used cooking oil is a known source of trans fats, which can raise LDL cholesterol levels and lower HDL cholesterol levels (Khalifa & Noseer, 2019; Reda et al., 2020). The total cholesterol concentration, HDL, and LDL in quail egg yolks were significantly higher than those in chicken egg yolks (Ukachukwu et al., 2017). Nevertheless, the limited impact of quail egg yolks on total cholesterol and LDL levels can be attributed to their low total fat content (Budiyanto et al., 2023). Additionally, the total cholesterol concentration, HDL, and LDL in quail egg yolks were significantly higher compared to chicken egg yolks (Reda et al., 2020). Overall, the composition of quail egg yolks and used cooking oil can be manipulated to induce different dyslipidemia profiles in animal models, while the choice of oil source in quail diets can influence both egg quality and quail health (Datu et al., 2022; Khalifa & Noseer, 2019; Reda et al., 2020; Wiyati et al., 2020).

Mice are the most often utilized animals in biomedical research (Hickman et al., 2017). The majority of genes in mice serve the same functions as genes in humans (The Jackson Laboratory, 2022). They have a reproductive cycle, a short lifespan, and a wealth of information on their anatomy, genetics, biology, and physiology (Hickman et al., 2017). That is, we grow in the same way from egg and sperm and have the same organs (heart, brain, lungs, kidneys, etc.) as well as circulatory, reproductive, digestive, hormonal, and neurological systems (The Jackson Laboratory, 2022). In addition to their small size, which makes them easy to care for, we can turn mice into most suitable models based on our research (Hickman et al., 2017). BALB/C male mice were used in our investigation since they are one of proper animal for developing hyperlipidemia and are widely accessible (Fernandes et al., 2016; Hickman et al., 2017; Madariaga et al., 2015; Putra et al., 2022).

The distribution of the eight primary lipid fractions in mice and dyslipidemic humans is noteworthy (cholesteryl ester, triglyceride, diacylglycerol, free fatty acid, phosphatidylcholine, phosphatidylethanolamine, lysophosphatidylcholine, and free cholesterol) (Yin et al., 2012). Further, mice are often resistant to dyslipidemia due to total cholesterol levels of less than 100

mg/dl (Hewing & Fisher, 2012), which are mostly regulated by HDL cholesterol (Cignarella, 2009; Hewing & Fisher, 2012). Because HDL particles make up the majority of plasma cholesterol in mice, it should be noted that plasma cholesterol metabolism in mice differs significantly from that in humans (Cignarella, 2009).

Dietary and genetic modification lead to significant increases in non-HDL plasma lipoproteins (Hewing & Fisher, 2012). Despite the fact that diet modification is the most often utilized method for developing dyslipidemic animals. Mice fed a cholesterol-containing diet looked more like dyslipidemic humans than mice fed a chow diet (Yin et al., 2012). A high fat diet in mice increases all fat components, one of which is total fat pads, which cause lipid metabolic difficulties when circulating in the blood, causing around 70% of released free fatty acids to be re-esterified (lipogenesis) to form triglycerides (Wong et al., 2016).

According to the latest expert assessments, mice lacking low density lipoprotein receptors (LDLR^{-/-} mice) and mice with insufficient apolipoprotein E (apoE^{-/-} mice) are the best simulators for human dyslipidemia (Yin et al., 2012; Zaragoza et al., 2011). Knocking down apoE slows lipoprotein clearance and elevates their levels. On a low-fat diet, ApoE^{-/-} mice spontaneously raise total plasma cholesterol levels by 300–400 mg/dl. A high-fat/high-cholesterol diet boosts plasma cholesterol levels (mostly VLDL and chylomicrons) to far above 1000 mg/dl by 14-16 weeks (Hewing & Fisher, 2012).

The investigation of modeling animal remains challenging. Novel biomarkers capable of establishing the concept of a novel target in humans are critically needed (Hewing & Fisher, 2012). In addition to investigating drug effects on dyslipidemia, the model may be used to uncover dyslipidemia susceptibility-modifying genes using candidate-gene and gene-mapping approaches, decode molecular pathways and cell types implicated in dyslipidemia, and assess potential therapeutics that limit lesion development (Zaragoza et al., 2011).

4. CONCLUSION

A 14-day combined induction of 5% QEY and 10% UCOs was reported to elevate mice lipids by 39% than baseline levels. Our findings undoubtedly provide an alternative to traditional dyslipidemia models. Indeed, the investigation of modeling animal remains challenging. Novel biomarkers capable of establishing the concept of a novel target in humans are critically needed.

5. ACKNOWLEDGMENT

The findings of the study are the research output of revitalizing the institutional vision for the field of pharmacology at the University of Muhammadiyah Magelang.

6. CONFLICT OF INTEREST

All authors declared that there was no conflict of interest.

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