SYSTEMATIC REVIEW

# Induction of Hypercholesterolemia in Rodents Based on Different Dietary Requirements: A Systematic Review

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**ABSTRACT**

**Introduction:** Cardiovascular diseases (CVDs) are the most common cause of death around the world. CVDs claimed the lives of 17.9 million individuals worldwide in 2019, accounting for 32% of all deaths and 38% of premature deaths before the age of 70. One of the common risk factors of CVDs is hypercholesterolemia. Therefore, research on hypercholesterolemia using animal models is important to understand cholesterol metabolism and regulation, as well as to find the best medications and treatments for this condition. This study is critical because it is related to the leading cause for CVDs. **Methods:** The systematic review was conducted according to the updated PRISMA guidelines. Online searches for articles published between 2018 and 2022 were conducted using PubMed, Science Direct, and Hindawi. **Results:** A total of 587 articles were initially identified for analysis. After screening, 22 articles were selected for the final report. Result showed that a diet with a mixture of other substances such as cholesterol, cholic acid, and oil will be able to increase LDL cholesterol in rodents thus creating a hypercholesterolemic animal model. A study using 2% of cholesterol and 0.4% of cholic acid increase the serum level of TC (3.41±0.41) mmol/L, LDL (2.24±0.42) mmol/L of positive control group compared to normal group TC (1.14±0.15) mmol/L, LDL (0.30±0.07) mmol/L. **Conclusion:** A diet composition with 2% cholesterol and 0.4% cholic acid mixed with other compounds such as peanut oil and coconut oil is the most effective and low-cost diet to promote high cholesterol conditions in rodents.

**Keywords:** Hypercholesterolemic diet; Dietary model; Rodents; Total cholesterol; Low-density lipoprotein

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## INTRODUCTION

Cardiovascular diseases (CVDs) are the most common cause of death around the world. CVDs claimed the lives of 17.9 million individuals worldwide in 2019, accounting for 32% of all deaths. CVDs were responsible for 38% of the 17 million premature deaths (before the age of 70) caused by NCDs in 2019 [1]. Most cardiovascular illnesses can be avoided by addressing behavioural risk factors such as cigarette use, poor diet, obesity, physical inactivity, and alcohol abuse [2]–[3].

The term hypercholesterolemia refers to a high level of cholesterol in the blood with normal plasma triglycerides as a result of an increase in cholesterol and apoB-rich lipoproteins, also known as low-density lipoproteins (LDL)[4]. Hypercholesterolemia is diagnosed if the blood cholesterol level is above 240 mg/dL. The normal cholesterol range in humans is 125 to 200mg/dL. Hypercholesterolemia is the leading cause of atherosclerosis and related diseases such as coronary heart disease, ischemic cerebrovascular disease, and peripheral vascular diseases [5]. Lipids are a group of fats and fatlike substances that are important constituents of cells and sources of energy. A lipid profile measures the level of specific lipids in the blood. The particles measured with a lipid profile are classified by their density into high-density lipoproteins (HDL), low-density lipoproteins (LDL), and very-low-density lipoproteins (VLDL).

A high level of cholesterol in the blood can be caused by eating too many foods high in saturated and trans unsaturated fats, or by having an inherent susceptibility[6]. The excess cholesterol may form plaques on the inside walls of blood vessels. Plaques can constrict or block blood channel openings, causing atherosclerosis, heart disease, and stroke [7].

Hypercholesterolemia has been investigated extensively in animal models as one of the key risk factors for CVD, particularly in the search for innovative treatment methods. There is, however, no agreement on which hypercholesterolemia induction technique to utilize. Various animal species have been used

as animal models for cholesterol study including rodents [8]–[10], rabbits [11]–[12], Guinea pig [13]–[14] and opossum [15]. Among these, rodents are widely utilized in the development of hypercholesterolemia-induced animal models of human diseases [16]. In study of pharmaceutical effectiveness, it is important to make sure a successful induction of the disease model. However, there was no consensus on the best dietary feeding to induce hypercholesterolemia in animal model. Many studies used method of mixing the high fat ingredient such as corn starch, soybean oil, milkfat or even a high level of cholesterol in their study but the induction model is not considered the best since it also interferes with physical state of the model. In addition, reviews on the dietary model to induce hypercholesterolemia in rodents are limited. Therefore, the goal of this study was to analyse experimental research that used rodents to induce hypercholesterolemia by diet. .

## MATERIALS AND METHODS

### Eligibility criteria

The analysis focused on published research that fed rodents a high cholesterol diet to induce hypercholesterolemia. The animals must be rodents and have complete information on diet composition, induction periods, name of rodents species, age, and last result of research. Only data from the control and diet intervention groups were used for analysis in studies that included drug manipulation. The data that are not related to the hypercholesterolemia induction is excluded. Data that does not have lipid serum test including LDL, TC, and non-HDL cholesterol is excluded since these is the crucial parameters to determine the successful induction. Data from experimental studies with successful induction of hypercholesterolemia through diet in rodents were included. Articles that were part of reviews, meta-analyses, book chapter, citations, patents, incomplete clinical trials, abstracts, and locked articles are excluded.

### Database Search and Extraction

Searched keywords include "Hypercholesterolemic diet AND Rodents in PubMed, Hindawi, and Science Direct. Truncation was used to capture plural keywords and synonyms. Through the search from PubMed, 201 articles were retrieved. A total of 365 and 21 articles were retrieved on Science Direct and Hindawi respectively. Through filter and advanced search on each website, only articles from 2018 to 2022 were included in this review to ensure current and updated information. All 587 articles were extracted from the website and are classified in their database.

### Screening for inclusion

The process of selecting articles for inclusion is shown in Fig. 1. Articles were first identified, and the duplicate articles were removed. Articles were then screened for eligibility using the information provided in the title and abstract. Articles that lack of key point of high cholesterol diet or hypercholesterolemia in their title or abstract were excluded in this study. The key point mentioned including 'high', 'cholesterol', 'cholesterol induction' or 'diet-induce'. Full texts of articles that passed the eligibility test were then screened to confirm that they had met the requirements for inclusion in the analysis.

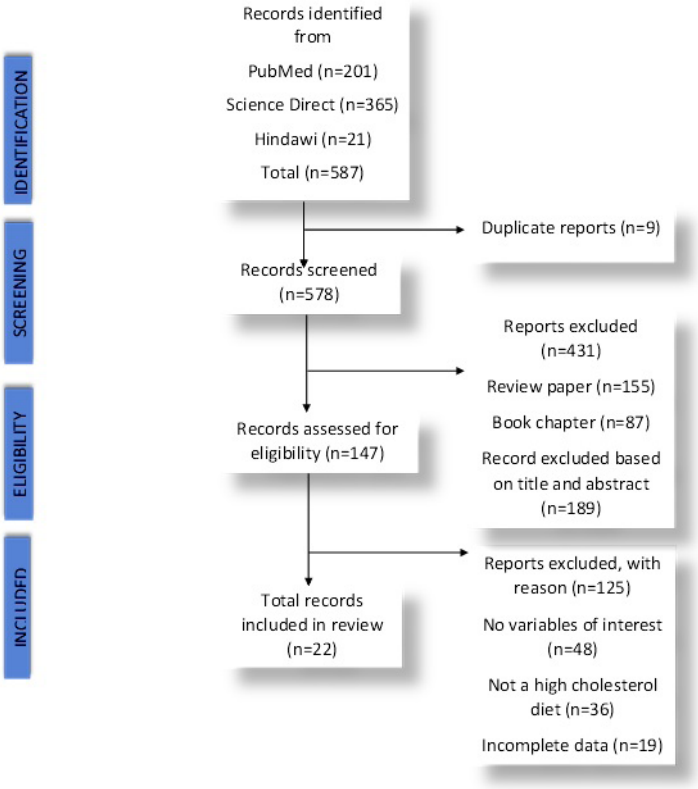


**Fig. 1 :** Figure 1: Flow chart showing the process of selecting articles for inclusion in the analysis based on Preferred Reporting Items for Systematic Review (PRISMA) guidelines.

## RESULTS AND DISCUSSION

The initial search yielded 587 articles. After the removal of duplicates (n=9), 578 articles were screened based on the types of articles. 155 review papers and 87 book chapters were excluded. 189 records were excluded after screening based on the title and abstract. Of the remaining, 147 articles reviewed in full text, a further 125 were excluded as they did not meet the inclusion criteria. A study that does not have complete information on the species of rodents or the induction methods was excluded.

The study that specifically uses a high-fat diet was also excluded because the study does not meet the criteria of a hypercholesterolemic diet. The high-fat diet was mostly used by researchers to induce obesity and diabetes but not cholesterol. These studies focused on other parameters related to their induction and lack of result related to cholesterol studies such as LDL, TC, and non-HDL cholesterol makes it reasonable to exclude them from this study.

After the initial 587 articles, only 22 studies that completely fit the set inclusion criteria were included. These studies have complete data on the species of rodent use, age, initial weight, diet composition, and diet duration [9], [17]–[36]. Out of the 22 studies, 8 studies use Wistar rats as a model, 9 used Sprague Dawley rats as a hypercholesterolemia model. One study used C5-7BL/6 mice, two use albino rats (Rattus rattus), another one-use Swiss albino mice and the last one use Golden Syrian hamster.

Most studies which are 82% induce hypercholesterolemia in male rodents compared to female. Female rodents are only used in four studies. All studies analyse blood lipid profiles including total cholesterol, HDL, LDL, and triglycerides to evaluate the change and efficiency of the induction. 21 out of 22 studies include cholesterol such as in their diet composition as a dietary method of induction of hypercholesterolemia in rodents. One study used 11.25% saturated fat, and 3.75% maize oil but was also able to induce hypercholesterolemia. Mixing cholesterol into a normal basal diet increases the non-HDL cholesterol including total cholesterol, LDL, triglycerides, and VLDL effectively. When not using cholesterol or using only a small amount of it, oil such as maize oil, peanut oil, and coconut oil is a good substitute to increase total cholesterol and triglycerides. The range of duration used in the induction study is between 3 to 24 weeks based on the parameters of the studies. The duration needed for the study is much shorter when the researchers only need lipid profile, liver function test and kidney function test. It takes longer duration when the researchers trying to test for more complex study such as the genetic or neurology. 10 out of 22 studies include cholic acid in their diet composition. Cholic acid causes an increase in intestinal cholesterol absorption and biliary cholesterol output [37], as well as a lithogenic phase change in bile, all of which appear to contribute to cholesterol supersaturation and fast crystallization. Cholic acid inhibits the rate-limiting enzyme in BS biosynthesis (cholesterol 7-hydroxylase) [38], disrupting the catabolic conversion of cholesterol to BS and likely leading to excessive cholesterol secretion rates, promoting cholesterol supersaturation even more.

Concerning the age of the rodents, 13 studies use rodents that are 8 weeks old and more, another 9 studies use 3-6 weeks old rodents. When diet duration is observed, studies use 3-23 weeks depending on the parameters that were investigated. Notably, most of the studies use both cholesterol and cholic acid to induce hypercholesterolemia. Only one study does not use either cholesterol or cholic acid and uses 11.25% saturated fat + 3.75% maize oil instead. All studies were able to induce hypercholesterolemia despite using different diet compositions. A complete analysis of all studies was summarized in Table I.

There is considerable evidence that rodents fed with a high cholesterol diet will be able to induce hypercholesterolemia but there were so much research using different diet compositions for different types of study. Therefore, this analysis aims to give evidence on how to induce hypercholesterolemia in rodents successfully and to aid other future researchers in choosing the right diet composition, age, and even species of rodents to use for their study. Throughout the analysis, there are two types of induction phases. There is research that carries out the induction simultaneously with the treatment. These induction methods mostly use to investigate significant change in drug treatment. There are some research that induces hypercholesterolemia in animal models first and then move to the treatment phase. These methods are use in disease prevention study. Since this study only use the comparison between normal and positive control group (induction), any method of treatment will not interfere with the data analysis for this study.

Most research uses adult rodents in their study rather than younger or older. For rats, 8 weeks is the most suitable age for research because, by this age, all the vital systems of the rats have matured [39]. Researchers employed male rats as the standard because they felt that hormone fluctuations during the female cycle caused female rats to behave differently to the same stimuli, rendering them unpredictable and resulting in inconsistent results during repeated trials. Many researchers also felt that female rats exhibited more variable behaviours among themselves, even when they were at the same stage of their cycles, but male rats exhibited more uniform activity [40]. Studying the male member of a species was the most effective approach to learning about the species' most basic functions and habits.

Throughout the study, five types of diet can be observed. The first diet contains pure cholesterol. Feeding pure cholesterol to rodents will increase the blood's total cholesterol and lead to successful induction. The second was the mixture of cholesterol

**Table I : Summary of the hypercholesterolemic diet-fed studies in rodents**

| Reference | Species | Gender | Age | Initial body weight (g) | Diet composition | Diet duration | Parameters observed |
|---|---|---|---|---|---|---|---|
| [5] | Wistar rats | male | 21 days | 60–65 | 11.25% saturated fat, 3.75% maize oil | 30 days | TC, TG ~ increase, HDL, LDL ~ decrease, ALT ~ increase, AST ~ decrease |
| [13] | Wistar mice | male | 60 days | 28–30 | 2% cholesterol, 0.2% cholic acid | 6 weeks | TC, LDL, AI, TG ~ increase, HDL, SOD ~ decrease, bodyweight ~ increase 9.03% |
| [14] | Wistar rats | male | 8 weeks | 270–324 | 2% cholesterol, 0.25% cholic acid | 8 weeks | TC, LDL, TG ~ significantly increase, HDL ~ decrease bodyweight ~ no change compared to control |
| [15] | Wistar rats | female | 8 weeks | 180 – 220 | 1.51% cholesterol, 15% fats | 3 weeks | TC, LDL, TG ~ significantly increase, HDL ~ no change, |
| [16] | Wistar rats | male | 5-6 weeks | 90 – 110 | 1.25% cholesterol | 4 weeks | TC ~ increase 74%, glucose ~ increase 36%, TG ~ increase 70%, LDL ~ increase 114%, VLDL ~ increase 100% 114%, HDL ~ decrease by 63% |
| [17] | Wistar rats | male | 8 weeks | 190 – 210 | 1% cholesterol, 10% egg yolk powder | 50 days | TC, LDL, TG, VLDL ~ significantly increase, HDL ~ no change |
| [18] | Wistar rats | male | 8 weeks | 150 – 154 | 2% cholesterol | 28 days | TC, LDL, TG, ALT, AST, ALP, AI, bodyweight ~ significantly increase, HDL ~ decrease |
| [19] | Wistar rats | female | 6 weeks | 130-150 | 10% cholesterol, | 8 weeks | body weight gain ~ increase by 173.71%, TC, TG, AI, CRI ~ significantly increase |
| [20] | Sprague-Dawley rats | male | 6 weeks | 130-150 | 2% cholesterol, 0.4% cholic acid | 10 weeks | TC, LDL ALT, AST, ALP, creatinine, urea, ~ significantly increase, HDL, TG ~ no change |
| [21] | Sprague-Dawley rats | male | 4 weeks | 100-150 | 1% cholesterol | 30 days | TC, LDL, ALT, AST ~ significantly increase<br>SOD, CAT ~ significantly decrease |
| [22] | Sprague-Dawley rats | male | 8 weeks | 160-180 | 1% cholic acid, 2% pure cholesterol, and 5.5% edible peanut oil | 38 days | TC, LDL, HDL ~ increase, TG ~ no significant change, AI ~ increase, Decrease in SOD (-26.86%) and CAT (-20.39%), increase in MDA (43.46%). |
| [23] | Sprague-Dawley rats | male | 8 weeks | 180-250 | 1% cholesterol, 0.05% cholic acid and 5% lard | 4 weeks | TC, LDL, TG, Glucose, bodyweight ~ increase, HDL ~ no significant change ApoA-I, ApoB ~ increase |
| [24] | Sprague-Dawley rats | male | 6 weeks | 160-180 | 2% cholesterol and 0.5% sodium cholate | 8 weeks | TC, TG ~ significantly increase, LDL ~ increase by 29.4%, body weight ~ increase by 31.7%, |
| [25] | Sprague-Dawley rats | male | 3-4 weeks | 50 | 2% cholesterol | 18-23 weeks | TC ~ increase by 30 mg/dL LDL ~ increase, HDL ~ no change |
| [26] | Sprague-Dawley rats | male | 6 weeks | 170-190 | 2% cholesterol | 24 weeks | TC, LDL ~ increase, HDL, TG ~ no change, bone ~ microstructural defect |
| [27] | Sprague-Dawley rats | male | 5 weeks | 140-160 | 1.25% cholesterol | 11 weeks | TC, TC, AST, ALT, MDA ~ increase |
| [28] | Sprague-Dawleyrats | male | 4 weeks | 150-200 | 1.5% cholesterol, 0.37% cholic acid | 8 weeks | TC, TC, AST, ALT, MDA ~ increase |
| [29] | C57BL/6 mice | female | 8-12 weeks | 25-30 | 0.7% cholesterol, cholic acid 0.1% | 60 days | TC, LDL ~ increase glycemia, urea, creatinine, AST, ALT ~ increase, bodyweight ~ increase |
| [30] | Swiss albino mice | male | 12 weeks | 45 | 20% fat, 1.5% cholesterol | 8 weeks | cholesterol levels ~ increase Glucose ~ increase, Bodyweight ~ increase, |

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| [31] | albino rats (Rattus rattus) | female | 8 weeks | 90-110 | 3% cholesterol, 8% cocoa butter, 2% cholic acid, 1% thiouracil and 1% starch | 4 months | TC, LDL, TG ~ increase, bodyweight ~ increase CAT, SOD and GST activities decrease (7.81 ± 0.39, 4.92±0.03, 15.02±0.75, respectively) MDA increase (4.16±0.24) 51.84±1.67, respectively) |
| [32] | albino rats | male | 6-8 weeks | 100-150 | 2.5% cholesterol and cholic acid | 8 weeks | TC, TG, VLDL ~ increase, lipoprotein a, qRT-PCR |
| [35] | *Golden Syria*n hamsters | male | 5 weeks | 85 | 0.2% cholesterol, 10% coconut oil | 12 weeks | TC ~ +227%, TG ~ +192% non-HDL ~ +704% Aortic Fatty Streak Area (AFSA) ~ 6.2% covered with lipid deposit, body weight ~ +28% from control group |

TC: total cholesterol; TG: triglycerides; LDL: low density lipoprotein; HDL: high density lipoprotein; VLDL: very low-density lipoprotein; AST: aspartate aminotransferase; ALT: alanine transaminase; CAT: catalase; SOD: Superoxide dismutase; MDA: malondialdehyde; GST: Glutathione S-transferases; AI: atherogenic index; BS: bile salt

and cholic acid. Cholic acid was used to disrupt the cholesterol conversion to bile acid. The third was a mixture of cholesterol and another cholesterol-promoting compound such as coconut oil, fat, or egg yolk powder. This promoting compound was proven to increase LDL and non-HDL cholesterol effectively. The fourth diet consists of cholesterol, cholic acid, and a mixture of other compounds such as peanut oil, butter, or lard. The last diet observed was fat mixed with maize oil.

Considering diet composition of the induction, we can conclude that the mixture of cholesterol and cholic acid is the best way to induce hypercholesterolemia, but it is more effective to include another cholesterol promoting compound such as oil and fat. The serum level of LDL and non-HDL cholesterol were put into consideration when comparing the effective diet for induction. Hence, the most effective and low cost diet used is a combination of 2% of cholesterol and 0.4% of cholic acid [35]. In this study, serum level of TC (3.41±0.41) mmol/L, LDL (2.24±0.42) mmol/L of positive control group increase significantly compared to normal group TC (1.14±0.15) mmol/L, LDL (0.30±0.07) mmol/L.

## CONCLUSION

This article reviewed the diet composition to induce hypercholesterolemia in the rodent's model. The result shows that a diet with a mixture of other substances such as cholesterol, cholic acid, and oil will be able to increase cholesterol and non-HDL cholesterol in rodents thus creating a hypercholesterolemia animal model for the study. A diet composition with 2% of cholesterol and 0.4% of cholic acid and mixed with other compounds such as peanut oil and coconut oil is the best diet to promote high cholesterol in rodents.

## ABBREVIATIONS

CVD: cardiovascular disease; TC: total cholesterol; TG: triglycerides; LDL: low density lipoprotein; HDL: high density lipoprotein; VLDL: very low-density lipoprotein; AST: aspartate aminotransferase; ALT: alanine transaminase; CAT: catalase; SOD: Superoxide dismutase; MDA: malondialdehyde; GST: Glutathione S-transferases; AI: atherogenic index; BS: bile salt.

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