Coconut Oil and Cholesterol as Challenge Agents to Induce Hyperlipidemia and Atherosclerosis in Hamster Animal Model

SYARIFAH-NORATIQAH SB¹, FAIRUS S², ZULFARINA MS¹, 'ATIQAH A¹, QODRIYAH HMS¹, NAINA-MOHAMED I¹

¹Department of Pharmacology, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaacob Latif, Bandar Tun Razak, 56000 Cheras, Kuala Lumpur, Malaysia.
²Metabolics Unit, Malaysian Palm Oil Board (MPOB), 6, Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia.

ABSTRAK


Kata kunci: diet, Mesocricetus auratus, penyakit kardiovaskular

Address for correspondence and reprint requests: Isa Naina-Mohamed. Department of Pharmacology, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaacob Latif, Bandar Tun Razak, 56000 Cheras, Kuala Lumpur, Malaysia. Tel: +603-9145 9568 E-mail: isanaina@ppukm.ukm.edu.my
ABSTRACT

Hyperlipidemia is a condition of high lipid levels in the plasma and often linked with the deposition of lipid droplets in the aorta which initiate the progression of atherosclerosis. Atherosclerosis is a common cardiovascular disorder initiated by the formation of foams cells in the vascular wall which leads to turbulent blood flow, injury to the endothelial layer and subsequent vascular thrombosis. Since the early 1980’s, Golden-Syrian hamsters have been widely used as an animal model in the research of hyperlipidemia and atherosclerosis. The use of hamsters in the hyperlipidemic and atherosclerotic model is due to their lipoprotein profile that is closer to human setting, sensitive to high-fat high-cholesterol (HFHC) diet and a suitable rodent model. Atherosclerosis can be induced in hamsters through dietary challenge with HFHC diet. Over the decades, coconut oil (CNO) was commonly used as the source of fat in the diet design of high saturated fatty acids (SFA) composition. In this review, we summarized published literature with designs involving CNO plus cholesterol-induced hyperlipidemia, atherosclerosis or both. The factors that may influence the ability of CNO and cholesterol combination to induce hyperlipidemia such as the period of dietary intervention, hamster strains and the dietary amount were evaluated and summarized.

Keywords: diet, cardiovascular disease, Mesocricetus auratus

INTRODUCTION

In any new pharmaceutical treatment, initial studies on animal model to evaluate the toxicity and effectiveness of drugs are crucial before embarking on human study. Animal model study is still the best choice for preclinical or initial stage biomedical research since it is impossible and unethical to conduct experiments on humans with insufficient data on safety and efficacy. In addition, animals are easy to manage through their diet and environmental factors, have a shorter lifespan, similar with human in their anatomical basis and physiological functions as well as being suitable for drug safety study (Leong et al. 2015). Choosing the suitable types of animals for a particular disease model is another important consideration as different types of animals may be induced or spontaneously develop disease(s) which represent the pathology that happens in human.

Lipid, also known as ‘fats’ in the body are commonly divided into cholesterol and triglycerides (TG). In general, the elevation of both cholesterol and TG in the bloodstream often indicates hyperlipidemia, a condition that initiates the development of atherosclerosis (hardening of the arteries) (Ginghina et al. 2011; Jorgensen et al. 2013). Atherosclerosis is well known as a significant risk factor for cardiovascular disease (CVD), the
number one death-causing disease globally (Ghosh & Ghosh 2012; Rohilla et al. 2012). The consumption of an unhealthy diet containing high cholesterol and fats often leads to the elevation of these blood lipids. This review does not aim to discuss the pathogenesis of hyperlipidemia in detail and neither to discuss on human studies or in vitro models. A good animal model is needed to be translated into clinical studies. We narrowed down our discussion on only animal models of hyperlipidemia which requires appraisals and careful evaluation. We specifically focussed on hamsters as the animal model of choice in the study of hyperlipidemia and atherosclerosis because the lipoprotein profile is closer to human lipoprotein distribution (Dalboge et al. 2015; Shankar et al. 2015). CNO was chosen as the fat source due to the high amount of saturated fatty acid (SFA) found in this tropical oil and is known to raise low density lipoprotein-cholesterol (LDL-c) as well as stimulate the formation of atherosclerotic lesions (Denke & Grundy 1992; Temme et al. 1996). Together with CNO, dietary cholesterol is usually added into the diet in a specific range to raise the TC and TG levels in the serum blood (Zha et al. 2009).

This review aims to give an overview on what has been found so far on inducing atherosclerosis and hyperlipidemia in hamster animal models utilising cholesterol and CNO diet. This review is subdivided into three sections. The first part of this review gives a brief overview of both hyperlipidemia and atherosclerosis as well as the risk factors. The second section highlights the reasons of hamster as the animal model of choice in the study of hyperlipidemia and atherosclerosis. In the third section, we reviewed high-fat high-cholesterol (HFHC) diet as the challenge agent to induce hyperlipidemic state. We focused our review on the CNO as the primary fat source in the diet design and discussed few other contributing factors which caused the inconsistency development of the hyperlipidemic state.

HYPERLIPIDEMIA AND Atherosclerosis

Based on the European Cardiovascular Disease Statistics report, CVD is the leading cause of death in both Europe and the European Union with 3.9 million and 1.8 million of deaths each year respectively (Elizabeth et al. 2017) and 15.6 million death worldwide (Townsend et al. 2015). Hyperlipidemia, one of the risk factors for CVD is defined as elevations of fasting total cholesterol (TC) or TG concentration, or both (Nelson 2013). The elevation of TC and TG are respectively known as hypercholesterolemia and hypertriglyceridemia, which are the subcategories of hyperlipidemia. This lipid disorder is also known as hyperlipoproteinemia. Lipoproteins are lipids that attached to proteins and carried in the blood (Niroska et al. 2014). Family history, unhealthy diets intake, obesity, chronic diseases (diabetes mellitus, renal failure, nephritic syndrome and hypothyroidism), smoking and
alcoholism are the critical factors that play a role in hyperlipidemia incidence (Hassan 2013). These factors would further classify hyperlipidemia into two categories which are primary hyperlipidemia and secondary hyperlipidemia. Primary hyperlipidemia which is also known as familial hyperlipidemia usually takes place as a result of genetic problems. Secondary hyperlipidemia which is also called acquired hyperlipidemia arises as a result of other underlining disorder due to factors such as diet, alcohol intake and diseases that lead to alterations in plasma lipid and lipoprotein metabolism (Goldstein et al. 1973; Hassan 2013; Nirosha et al. 2014).

Atherosclerosis is a condition in which the building up of lesions which consists of an accumulation of cells, lipid and matrix components including minerals, inside the arteries. These histological criteria are associated with structural disorganization, repair, and thickening of the intima, as well as deformity of the arterial wall (Stary et al. 1995). Hyperlipidemia is often linked to the development of atherosclerosis. The development of atherosclerosis from dietary fat may be mediated through the deposition of lipids and lipoprotein from plasma into the arterial wall, due to the high concentration of cholesterol (Goodnight et al. 1982). Consumption of a diet rich in fat and cholesterol leads to the increase of LDL-c levels, also known as ‘bad cholesterol’ in plasma and triggers the process of atherosclerosis. The rise of LDL-c and very low-density lipoprotein (VLDL) level will cause these lipoproteins to infiltrate the artery wall beyond the capacity for elimination and retained in the extracellular matrix. LDL-c undergoes oxidative modification and turns into oxidized LDL (oxLDL) in the intima, leading to release of proinflammatory mediators that can activate endothelial cells. Activated endothelial cells express leukocyte adhesion molecules, for example, vascular cell adhesion molecule-1 (VCAM-1) will cause the cells carrying counter receptors for VCAM-1 such as monocytes and lymphocytes to adhere to these sites (Hansson et al. 2006). Figure 1 illustrates the link between hyperlipidemia and atherosclerosis which leads to the development of CVD.

THE GOLDEN-SYRIAN HAMSTER AS HYPERLIPIDEMIA AND ATHEROSCLEROSIS ANIMAL MODEL

The best animal model that can closely mimic the human disease should be carefully selected for translational research. In order to extrapolate animal data into human data, the mechanisms of the drug action should be first understood before selecting the animal model. Thus, the drug’s effect on the human can be predicted based on the results obtained through the animal study (Food Drug Administration 2015). The Golden or Syrian hamsters, also known as the Golden-Syrian hamsters (Mesocricetus auratus) originated from Syria and live in deep tunnels that provide them with cooler temperature and higher humidity environment
(Miedel & Hankenson 2015). Over the years, numerous studies reported on the usage of hamster as an animal model to study a variety of diseases such as carcinogenesis, metabolic, cardiovascular as well as infectious diseases (Homburger 1969; Miedel & Hankenson 2015; Warner et al. 2017). Since early 1980’s, Golden-Syrian hamsters have been widely used as an animal model in the research of hyperlipidemia and atherosclerosis (Dalboge et al. 2015; Dillard et al. 2010). The reasons of hamsters preferable in this research are as follows;

1. **Resembling the Human Setting.**
   Numerous studies have suggested that hamsters have an atherogenic lipoprotein profile which makes them a suitable model for the study of lipoprotein metabolism. Hamster does resemble the human setting since the main circulating lipoprotein in the hamster is non-high-density lipoprotein cholesterol (nHDL-c) and possess similarity in human plasma lipid distribution, synthesis, and excretion (Dalboge et al. 2015; Shankar et al. 2015). Unlike rodent models such as mice and rats, hamsters can develop aortic lesions and atherogenic lipoprotein profile that are similarly observed in human subjects [nHDL-c > HDL-c] when they are fed with HFHC diet (Dillard et al. 2010). Hamsters possess the activity of cholesteryl ester transfer protein (CETP) (Rémillard et al. 2001; Tsutsumi et al. 2001) and the production of the apolipoprotein (apo) B from the liver with a density close to that of a human having VLDL (Taghibiglou et al. 2000). The CETP in the hamster is similar to human regarding activity and inhibitor interactions (Wang et al. 2017). Hamster also responds to the lipid-
lowering agent, fenofibrate in a way similar to human (Guo et al. 2001). The combination of all these factors makes hamsters optimal for hyperlipidemia and atherogenic animal model studies.

2. **Sensitive to high-fat high-cholesterol (HCFC) diet.** Numerous studies have reported on the sensitivity of hamster to high-fat (HF) and high-cholesterol (HC) diet to develop hyperlipidemia and atherosclerosis. Hamster is reported to develop the hyperlipidemic condition as early as seven days (Singh et al. 2013) to two weeks of feeding (Ioriya et al. 2002; Takenaga et al. 2000) with HFHC diet. Additionally, researchers have also reported the ability of this model to develop the fatty streak in the aorta as early as 10 weeks (Asami et al. 1999; Huang et al. 2015; Vinson et al. 2001; Vinson et al. 2002) to 12 weeks (Auger et al. 2005; Kowala et al. 1995a; Vide et al. 2015) of feeding with HFHC diet. The percentage of fat and cholesterol added to the diet, as well as the diet duration, is important in the design of a disease model. HFHC diet as the challenge agent is being further elaborated in the third section of this review.

3. **Suitable rodent model.** Hamster and rabbit are widely used in the research of atherosclerosis. Unlike rabbit, the hamster is thought to be a more suitable model through their eating habit since rabbit is a total vegetarian. Atherosclerosis is developed in the rabbit model through abnormal diet with HC supplementation and the lipid metabolism which is far different when compared to human (Russell & Proctor 2006). When it comes to the best suitable model; factors such as being small in size, less expensive, large enough to allow for physiological experiments, as well as mimicking closer to the human disease process, will be prioritized. While rats is well known in the study of hypertension (Kumeshini et al. 2016), hamster is said to be an ideal model in the study of lipid metabolism and atherosclerosis. Compared to other rodents such as rats and mice, hamster is closer to human in terms of lipid utilization, sensitive to dietary cholesterol and can develop aortic lesion (Pereira et al. 2016; Zhao et al. 2017). Their sizes which are bigger than mice would make them easier to handle but smaller than rats, thus requiring less volume of treatment. On the other hand, rats and mice are resistant to HC diet (Chen et al. 2014) and do not have endogenous CETP genes (Wang et al. 2017). The CETP activity in mice and rats are very low to not detected (Tsutsumi et al. 2001).

**DIET-INDUCED HYPERLIPIDEMIA AND ATHEROSCLEROSIS IN SYRIAN HAMSTER MODEL**

When it comes to choosing the agent to induce disease and mimics human hyperlipidemia and atherosclerotic
plaque formation, HC and HFHC diet have been used successfully in hamster animal model. However, limitations arise with the inconsistent induction of hyperlipidemia as well as the development of early-stage atherosclerosis (Dillard et al. 2010). Many factors affect the disease progression in hamster animal model such as hamster strain (Dorfman et al. 2003; Trautwein et al. 1993), gender different (Robins et al. 1995; Wilson et al. 1998a), length of dietary intervention (Faia et al. 2002), amount of dietary cholesterol (Mcateer et al. 2007), as well as amount and type of dietary fat (Dillard et al. 2013; Dorfman et al. 2005; Lecker et al. 2010). It is also important to note the small factors that affect the plasma lipoprotein response of the hamster such as photoperiod (Smith et al. 2001a), blood fasting lipid (Mcateer et al. 2007; Weingand & Daggy 1991) and also cholesterol condition (oxidized or non-oxidized) during diet preparation (Ng et al. 2008). In this section, we highlight the literature on HFHC diets as challenge agent to induce hyperlipidemia as well as atherosclerosis in Syrian hamster animal model.

THE ROLE OF HIGH FAT HIGH CHOLESTEROL (HFHC) DIET IN THE DEVELOPMENT OF HYPERLIPIDEMIA AND ATHEROSCLEROSIS IN HAMSTER ANIMAL MODEL

HF and cholesterol diet affect the level of plasma lipoprotein and thus plays a vital role in the development of atherosclerosis and CVD. Diet rich in saturated fat and cholesterol is responsible for increasing the TG and TC level in plasma. HFHC diet was previously reported to induce hyperlipidemia and early stage atherosclerosis in hamster animal model (Agbor et al. 2012; Romain et al. 2012). This diet is formulated by adding a certain amount of cholesterol and fat in normal chow hamster food, in a specific time of dietary intervention. To ensure the constant development of the disease, considerable attention must be paid towards the diet preparation, and the method of preparation should be standardized. During diet preparations, cholesterol is usually dissolved in the heated fat before mixed thoroughly with the normal chow (Bhatia et al. 2003). The common sources of fat used are from tropical oils or dairy fats. Tropical oils are natural vegetable oils produced in tropical regions, for example, CNO and palm oil (PO) (Elson & Alfin-Slater 1992). Wilson and co-workers demonstrated the atherogenic effects of hamster-fed CNO and different PO preparations. He concluded that CNO was found to be more atherogenic compared to the PO (Wilson et al. 2005).

CNO had received much attention over the past decade as the source of fats to induce hyperlipidemia and atherosclerosis since it is rich in SFA (Jayachandran et al. 2015; Mangiapane et al. 1999; Spady & Dietschy 1985). SFA is known to raise LDL-c and stimulate the formation of atherosclerotic lesions. High amount of lauric acid is found in CNO, and studies demonstrated that lauric
acid could raise serum TC in human subjects (Denke & Grundy 1992; Temme et al. 1996). Apart from CNO, researchers also used hydrogenated coconut oil (HCNO) in the diet formulation to induce hyperlipidemia and atherosclerosis in hamster animal model (Alexaki et al. 2004; Cherng & Shih 2005; Delaney et al. 2003; Krause et al. 1992; Mangiapane et al. 1999; Mitchell et al. 2005; Robins et al. 1995; Romain et al. 2014; Romain et al. 2012; Vide et al. 2015). HCNO are more abundant in SFA compared to CNO and reported to be more atherogenic (Alexaki et al. 2004; Mangiapane et al. 1999).

The similarity of HFHC in mimicking western diet serve had received much attention as the challenge agent of hyperlipidemia and early-stage atherosclerosis. Together with SFA, cholesterol is added to the diet to further raise the TC and nHDL-c in the hamster model. Several studies were carried out using HC diet as the challenge agent of the disease without the addition of HF agent. These studies have demonstrated the positive outcomes of disease by using cholesterol with significant results (Chen & Pan 2012; Chen et al. 2015; Lee et al. 2013; Patel et al. 2017; Wang et al. 2015). However, the amount of cholesterol used as the inducer of the disease as well as the duration of diet intervention varied from one study to another. The fundamental problem with much of the literature regarding the HC as well as HFHC diet as the challenge agent in hyperlipidemia and atherosclerosis is the inconsistent development of the disease. It is plausible that some limitations such as; method design, hamster strain, dietary intervention, amount of cholesterol as well as amount and type of fat used in the study could have influenced the results obtained. In the next section, we discuss the literature from the 1990’s on diet-induced hyperlipidemia and atherosclerosis, with CNO as the primary source of fat. Our primary focus is to explore the most exceptional amount of cholesterol and period of dietary intervention based on the hamster strain to develop the target disease. This section is divided further into three subsections based on the percentage of cholesterol added into the diet preparation.

1. Cholesterol <0.1%

Table 1 lists the data on the effects of HFHC (<0.1%) -enriched diets in development of hyperlipidemia and atherosclerosis. In this table, we put together literature that used cholesterol less than 0.1% in the diet preparation to induce diseases. Based on the listed literature, the range of cholesterol use was 0.03-0.07%, with 0.05% (Asami et al. 1999; Ausman et al. 2005; Kowala et al. 1994; Kowala et al. 1993; Kowala et al. 1991; Kowala et al. 1995a; Kowala et al. 1995b; Nicolosi et al. 1998; Otto et al. 1995; Pitha et al. 2010; Pitman et al. 1998; Robins et al. 1995; Rong et al. 1997; Smith, et al. 2001a; Wilson et al. 1998a; Wilson et al. 1999; Wilson et al. 1998b) being the frequently used dose of cholesterol in diet preparations. Even though the amount of cholesterol can be considered small in this group, the effects of the
Based on the listed literature in this group, all of the diets were designed with 10% supplemental fat except Bertolotti and colleagues (Bertolotti & Spady 2001) who added 20% of supplemental fat in their diet. Next, we moved on the hamster strain. In this group of cholesterol, F1B is the most common strain preferred (Ausman et al. 2005; Kowala et al. 1994; Kowala et al. 1993; Kowala et al. 1991; Kowala et al. 1995a; Kowala et al. 1995b; Nicolosi et al. 1998; Otto et al. 1995; Pitman et al. 1998; Robins et al. 1995; Rong et al. 1997; Smith et al. 2001a; Wilson et al. 1998a; Wilson et al. 1999; Wilson et al. 1998b) followed by Sasco Laboratory (Bertolotti & Spady 2001; Krause et al. 1992), with no literature used CR strain. Those reports suggest

cholesterol was significant enough to promote disease in the animal model. Based on the listed literature in this group, all of the diets were designed with 10% supplemental fat except Bertolotti and colleagues (Bertolotti & Spady 2001) who added 20% of supplemental fat in their diet. Next, we moved on the hamster strain. In this group of cholesterol, F1B is the most common strain preferred (Ausman et al. 2005; Kowala et al. 1994; Kowala et al. 1993; Kowala et al. 1991; Kowala et al. 1995a; Kowala et al. 1995b; Nicolosi et al. 1998; Otto et al. 1995; Pitman et al. 1998; Robins et al. 1995; Rong et al. 1997; Smith et al. 2001a; Wilson et al. 1998a; Wilson et al. 1999; Wilson et al. 1998b) followed by Sasco Laboratory (Bertolotti & Spady 2001; Krause et al. 1992), with no literature used CR strain. Those reports suggest

Table 1: Effects of high-fat high-cholesterol (<0.1%) -enriched diets on development of hyperlipidemia and atherosclerosis

<table>
<thead>
<tr>
<th>Cholesterol (%)</th>
<th>CNO/HCNO (%)</th>
<th>Strain</th>
<th>Study Duration</th>
<th>Hyperlipidemia</th>
<th>Atherosclerosis</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.03</td>
<td>5</td>
<td>F1B</td>
<td>14 weeks</td>
<td>Yes</td>
<td>Yes*</td>
<td>(Ausman et al. 2005)</td>
</tr>
<tr>
<td>0.05</td>
<td>10</td>
<td>F1B</td>
<td>8 weeks</td>
<td>Yes</td>
<td>Yes*</td>
<td>(Kowala et al. 1994)</td>
</tr>
<tr>
<td>0.05</td>
<td>10</td>
<td>F1B</td>
<td>3 weeks</td>
<td>Yes</td>
<td>Yes*</td>
<td>(Kowala et al. 1995b)</td>
</tr>
<tr>
<td>0.05</td>
<td>10</td>
<td>F1B</td>
<td>4 weeks</td>
<td>Yes</td>
<td>-</td>
<td>(Wilson et al. 1998b)</td>
</tr>
<tr>
<td>0.05</td>
<td>10</td>
<td>F1B</td>
<td>4 weeks</td>
<td>Yes*</td>
<td>-</td>
<td>(Smith et al. 2001a)</td>
</tr>
<tr>
<td>0.05</td>
<td>10</td>
<td>F1B</td>
<td>8 weeks</td>
<td>Yes*</td>
<td>Yes*</td>
<td>(Kowala et al. 1991)</td>
</tr>
<tr>
<td>0.05</td>
<td>10</td>
<td>F1B</td>
<td>8 weeks</td>
<td>Yes</td>
<td>Yes*</td>
<td>(Wilson et al. 1998a)</td>
</tr>
<tr>
<td>0.05</td>
<td>10</td>
<td>F1B</td>
<td>10 weeks</td>
<td>Yes</td>
<td>Yes*</td>
<td>(Kowala et al. 1993)</td>
</tr>
<tr>
<td>0.05</td>
<td>10</td>
<td>F1B</td>
<td>10 weeks</td>
<td>Yes</td>
<td>Yes*</td>
<td>(Rong et al. 1997)</td>
</tr>
<tr>
<td>0.05</td>
<td>10</td>
<td>F1B</td>
<td>10 weeks</td>
<td>Yes</td>
<td>Yes*</td>
<td>(Nicolosi et al. 1998)</td>
</tr>
<tr>
<td>0.05</td>
<td>10</td>
<td>SLC</td>
<td>10 weeks</td>
<td>Yes*</td>
<td>Yes*</td>
<td>(Asami et al. 1999)</td>
</tr>
<tr>
<td>0.05</td>
<td>10</td>
<td>F1B</td>
<td>11 weeks</td>
<td>Yes*</td>
<td>Yes*</td>
<td>(Pitman et al. 1998)</td>
</tr>
<tr>
<td>0.05</td>
<td>10</td>
<td>F1B</td>
<td>12 weeks</td>
<td>Yes*</td>
<td>Yes*</td>
<td>(Wilson et al. 1999)</td>
</tr>
<tr>
<td>0.05</td>
<td>10</td>
<td>F1B</td>
<td>12 weeks</td>
<td>Yes</td>
<td>Yes*</td>
<td>(Kowala et al. 1995a)</td>
</tr>
<tr>
<td>0.05</td>
<td>10</td>
<td>F1B</td>
<td>12 weeks</td>
<td>Yes*</td>
<td>Yes*</td>
<td>(Robins et al. 1995)</td>
</tr>
<tr>
<td>0.05</td>
<td>Not stated</td>
<td>12 weeks</td>
<td>Yes*</td>
<td>Yes*</td>
<td>(Pitha et al. 2010)</td>
<td></td>
</tr>
<tr>
<td>0.05</td>
<td>10</td>
<td>F1B</td>
<td>10 weeks</td>
<td>Yes*</td>
<td>Yes*</td>
<td>(Otto et al. 1995)</td>
</tr>
<tr>
<td>0.06</td>
<td>10</td>
<td>Sasco Lab</td>
<td>2 weeks</td>
<td>Yes*</td>
<td>-</td>
<td>(Krause et al. 1992)</td>
</tr>
<tr>
<td>0.07</td>
<td>20</td>
<td>Sasco Lab</td>
<td>4 weeks</td>
<td>Yes*</td>
<td>-</td>
<td>(Bertolotti &amp; Spady 2001)</td>
</tr>
</tbody>
</table>

The symbol ‘Yes*’ indicates disease successfully develops through diet with significant outcomes. The symbol ‘Yes’ indicates disease successfully develops through diet but in the absence of normal control/baseline group to compare the significant outcomes. The symbol ‘No’ indicates disease does not successfully develop through diet. The symbol ‘-’ indicates disease not being evaluated in the study. CHO=cholesterol; CNO=coconut oil; HCNO=hydrogenated coconut oil.
that F1B strain of Syrian hamster are sensitive to HFHC diet even with a small amount of dietary cholesterol. Furthermore, studies conducted on the strain comparison also proved the sensitivity of F1B strain on HFHC diet compared to other hamster strain (Dorfman et al. 2003; Mcateer et al. 2007; Smith et al. 2001b; Srivastava 2011). Despite the same amount of cholesterol and similar hamster strain, the time in which hyperlipidemia and atherosclerosis begin to develop varies among studies. This inconsistent period of hyperlipidemia and atherosclerosis development may be due to the differences in the study design. In the model of F1B hamster strain with 0.05% supplemental cholesterol; hyperlipidemia significantly developed as early as four weeks of dietary intervention (Smith et al. 2001a) and significantly developed early-stage atherosclerosis at eight weeks of dietary intervention (Kowala et al. 1991). However, there are prevention studies that reported significant development of both hyperlipidemia and early stage atherosclerosis later from ten weeks up to twelve weeks of dietary intervention (Asami et al. 1999; Kowala et al. 1995a; Otto et al. 1995; Pitman et al. 1998; Robins et al. 1995). Other studies with 0.05% supplemental cholesterol as illustrated in Table 1 (Ausman et al. 2005; Kowala et al. 1994; Kowala et al. 1993; Kowala et al. 1995b; Nicolosi et al. 1998; Rong et al. 1997; Wilson et al. 1998a; Wilson et al. 1999; Wilson et al. 1998b) had no time frame for disease development. Throughout the literature, hamster strain from Sasco laboratories breeder was also sensitive to HFHC diet and reported to develop hyperlipidemia significantly as early as two to four weeks with 0.06-0.07% cholesterol (Bertolotti & Spady 2001; Krause et al. 1992). The limitation lies in the fact that not much study was conducted using this hamster strain with a small amount of dietary cholesterol.

2. 0.1% ≤ Cholesterol ≤ 1.0%

Table 2 summarized the data on the effects of HFHC (0.1-1.0%) -enriched diets in development of hyperlipidemia and atherosclerosis. In this table, we grouped literature that used cholesterol in between the range of 0.1-1.0% in the diet preparation to induce diseases, and we termed it as a moderate amount of supplemental cholesterol. Based on the literature searches, most of the diet design would fall within this group. Similarly to the previous cholesterol group, many studies were designed with 10% of supplemental fat, except for a few studies conducted with 5% (Rong et al. 1997; Wilson et al. 1998b), 12% (Lin et al. 2013; Lin et al. 2012), 13% (Ahn et al. 1994), 15% (Mcateer et al. 2007; Singh et al. 2013; Smith et al. 2001b), 20% (Alexaki et al. 2004; Cherg & Shih 2005; Delaney et al. 2003; Mitchell et al. 2005; Spady & Dietschy 1985) and 22% (Faia et al. 2002) of supplemental fat. The most common use of hamster strain in this group was F1B (Ahn et al. 1994; Alexaki et al. 2004; Delaney et al. 2003; Dillard et al. 2013; Dorfman et al. 2003; Faia et al. 2002; Kowala et al. 1995a; Lecker et al. 2010; Mcateer et al. 2007; Rong et al. 1997; Smith et al.
### Table 2: Effects of high fat high cholesterol (0.1%≤Cholesterol≤1.0%)-enriched diets on development of hyperlipidemia and atherosclerosis

<table>
<thead>
<tr>
<th>CHO (%)</th>
<th>CNO/HCNO (%)</th>
<th>Strain</th>
<th>Study Duration</th>
<th>Disease</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>10 CR</td>
<td>2 weeks</td>
<td>Yes</td>
<td>Yes</td>
<td>(Wilson et al. 2006b)</td>
</tr>
<tr>
<td>0.1</td>
<td>10 CR</td>
<td>6 weeks</td>
<td>Yes*</td>
<td>Yes</td>
<td>(Dorfman et al. 2005)</td>
</tr>
<tr>
<td>0.1</td>
<td>20 Taiwan Nat. Sc. Council</td>
<td>8 weeks</td>
<td>Yes*</td>
<td>-</td>
<td>(Cherng &amp; Shih 2005)</td>
</tr>
<tr>
<td>0.1</td>
<td>5 F1B</td>
<td>10 weeks</td>
<td>Yes</td>
<td>Yes</td>
<td>(Rong et al. 1997)</td>
</tr>
<tr>
<td>0.1</td>
<td>10 F1B</td>
<td>10 weeks</td>
<td>Yes</td>
<td>Yes</td>
<td>(Wilson et al. 2005)</td>
</tr>
<tr>
<td>0.1</td>
<td>10 F1B</td>
<td>12 weeks</td>
<td>Yes</td>
<td>Yes</td>
<td>(Wilson et al. 2006a)</td>
</tr>
<tr>
<td>0.1</td>
<td>10 F1B</td>
<td>12 weeks</td>
<td>Yes</td>
<td>Yes*</td>
<td>(Wilson et al. 2007)</td>
</tr>
<tr>
<td>0.1</td>
<td>5 F1B</td>
<td>12 weeks</td>
<td>Yes</td>
<td>-</td>
<td>(Lecker et al. 2010)</td>
</tr>
<tr>
<td>0.1</td>
<td>10 F1B</td>
<td>14 weeks</td>
<td>Yes</td>
<td>Yes</td>
<td>(Wilson et al. 1998b)</td>
</tr>
<tr>
<td>0.1</td>
<td>10 F1B CR</td>
<td>20 weeks</td>
<td>Yes*</td>
<td>No</td>
<td>(Dorfman et al. 2003)</td>
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<tr>
<td>0.5</td>
<td>10 F1B CR</td>
<td>20 weeks</td>
<td>Yes*</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>10 F1B CR</td>
<td>20 weeks</td>
<td>Yes*</td>
<td>Yes*</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>10 F1B</td>
<td>15 months</td>
<td>Yes</td>
<td>Yes*</td>
<td>(Wilson et al. 2003)</td>
</tr>
<tr>
<td>0.12</td>
<td>10 SLC</td>
<td>24 days</td>
<td>Yes*</td>
<td>-</td>
<td>(Ioriya et al. 2002)</td>
</tr>
<tr>
<td>0.12</td>
<td>20 CR</td>
<td>30 days</td>
<td>Yes*</td>
<td>-</td>
<td>(Spady &amp; Dietschy 1985)</td>
</tr>
<tr>
<td>0.12</td>
<td>10 CR</td>
<td>4 weeks</td>
<td>Yes</td>
<td>-</td>
<td>(Bensch et al. 1999)</td>
</tr>
<tr>
<td>0.12</td>
<td>10 VR lab</td>
<td>5 weeks</td>
<td>Yes*</td>
<td>-</td>
<td>(Chen et al. 2014)</td>
</tr>
<tr>
<td>0.12</td>
<td>20 CR</td>
<td>12 weeks</td>
<td>Yes*</td>
<td>Yes*</td>
<td>(Mitchell et al. 2005)</td>
</tr>
<tr>
<td>0.15</td>
<td>20 F1B</td>
<td>9 weeks</td>
<td>Yes*</td>
<td>Yes*</td>
<td>(Delaney et al. 2003)</td>
</tr>
<tr>
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<td>20 F1B</td>
<td>10 weeks</td>
<td>Yes</td>
<td>Yes</td>
<td>(Alexaki et al. 2004)</td>
</tr>
<tr>
<td>0.15</td>
<td>10 F1B</td>
<td>12 weeks</td>
<td>Yes</td>
<td>No</td>
<td>(Dillard et al. 2013)</td>
</tr>
<tr>
<td>0.2</td>
<td>10 Sasco Lab</td>
<td>2 weeks</td>
<td>Yes*</td>
<td>-</td>
<td>(Krause et al. 1992)</td>
</tr>
<tr>
<td>0.2</td>
<td>12 Taiwan Nat. Sc. Council</td>
<td>6 weeks</td>
<td>Yes*</td>
<td>-</td>
<td>(Lin et al. 2012)</td>
</tr>
<tr>
<td>0.2</td>
<td>12 Taiwan Nat. Sc. Council</td>
<td>6 weeks</td>
<td>Yes*</td>
<td>-</td>
<td>(Lin et al. 2013)</td>
</tr>
<tr>
<td>0.2</td>
<td>10 Not stated</td>
<td>44 days</td>
<td>Yes*</td>
<td>-</td>
<td>(Ahmed 2009)</td>
</tr>
<tr>
<td>0.2</td>
<td>15 F1B CR</td>
<td>9 weeks</td>
<td>Yes*</td>
<td>Yes</td>
<td>(Smith et al. 2001b)</td>
</tr>
<tr>
<td>0.2</td>
<td>10 CR</td>
<td>10 weeks</td>
<td>Yes</td>
<td>Yes</td>
<td>(Foxall et al. 1992)</td>
</tr>
<tr>
<td>0.2</td>
<td>10 CR</td>
<td>10 weeks</td>
<td>Yes</td>
<td>Yes</td>
<td>(Vinson et al. 2001)</td>
</tr>
</tbody>
</table>
0.2 10 CR 10 weeks Yes Yes (Vinson et al. 2002)
0.2 10 CR 10 weeks Yes - (Choi et al. 2008)
0.2 10 CR 10 weeks Yes - (Jang et al. 2008)
0.2 10 CR 10 weeks Yes - (Choi et al. 2009)
0.2 10 Not stated 12 weeks Yes* - (Kanashiro et al. 2007)
0.2 10 CR 12 weeks Yes* Yes* (Agbor et al. 2012)
0.2 10 Jackson Lab 12 weeks Yes - (Kim et al. 2010)
0.2 10 Janvier-Labs 12 weeks Yes* Yes* (Vide et al. 2015)
0.2 10 Not stated 13 weeks Yes* Yes* (Romain et al. 2012)
0.2 10 Janvier 13 weeks Yes* - (Romain et al. 2014)
0.2 13 F1B 18 weeks Yes* - (Ahn et al. 1994)
0.2 13 F1B 18 weeks Yes* - (Ahn et al. 1994)
0.25 10 Hyderabad Nat. Ins. Nutrition 12 weeks Yes* Yes* (Jayachandran et al. 2015)
0.25 10 Hyderabad Nat. Ins. Nutrition 12 weeks Yes* - (Jayachandran et al. 2015)
0.3 10 CR 12 weeks Yes* Yes* (Zha et al. 2009)
0.8 10 CR 12 weeks Yes* Yes* (Mawatari et al. 2004)
0.3 10 SLC 40 weeks Yes* Yes* (Kowala, Rose, et al. 1995)
0.3 10 F1B 15 months Yes Yes (Huang et al. 2015)
0.5 10 Taiwan Nat. Sc. Council 10 weeks Yes* - (Uehara et al. 2002)
0.5 10 KBT 12 weeks Yes* Yes* (Mcateer et al. 2007)
0.5 15 F1B DSNI 4 week/12months Yes Yes (Takenaga et al. 2000)
1.0 10 CR 2 weeks Yes* - (Singh et al. 2013)
1.0 15 CSIR-CDRI 35 days Yes* Yes* (Singh et al. 2015)
1.0 10 CSIR-CDRI 5 weeks Yes* Yes (Faia et al. 2002)

The table represents the effects of supplemental fat and cholesterol (<0.1%) in diet-induced hyperlipidemia and atherosclerosis. The symbol ‘Yes*’ indicates disease successfully develops through diet with significant outcomes. The symbol ‘Yes’ indicates disease successfully develops through diet but in the absence of normal control/baseline group to compare the significant outcomes. The symbol ‘No’ indicates disease does not successfully develop through diet. The symbol ‘-’ indicates disease not being evaluated in the study. CHO=cholesterol; CNO=coconut oil; HCNO=hydrogenated coconut oil.

et al. 2001b; Spady & Dietschy 1985; Takenaga et al. 2000; Vinson et al. 2002; Vinson et al. 2001; Wilson et al. 2006b). As discussed earlier, the F1B strain was well known to be sensitive towards the atherogenic diet. In this section, we aimed to focus on the CR strain Syrian hamster. CR strain from Charles River Laboratory was the second preferable strain after F1B. A study conducted by Dorfman and co-workers demonstrated the comparison of lipoprotein profile between these two strains. Both of the strains were challenged with HFHC diet, and they reported that plasma lipid level of CR was significantly lower but possessed higher aortic cholesterol ester (CE) when compared to F1B (Dorfman et al. 2003). It can be seen in Table 2 that the most common amount of dietary cholesterol fed to CR hamster strain was 0.2% (Agbor et al. 2012; Choi et al. 2008; Choi et al. 2009; Foxall et al. 1992; Jang et al. 2008; Vinson et al. 2001). However, a diet supplemented with 0.1% dietary cholesterol, was able to significantly develop hyperlipidemia in this hamster strain after six weeks of diet challenge (Dorfman et al. 2005). Studies were also conducted using 0.12% cholesterol supplemented into the diet in CR hamster strain (Bensch et al. 1999; Mitchell et al. 2005; Spady & Dietschy 1985). An amount of 0.12% cholesterol with 20% of fat can significantly develop hyperlipidemia after 30 days of dietary intervention (Spady & Dietschy 1985) and both hyperlipidemia and atherosclerosis after 12 weeks of dietary intervention (Mitchell et al. 2005). Studies demonstrated with 0.2% cholesterol for 10 weeks were also able to induce the disease, but limitation arises as no normal or baseline group was there for comparing the significant development (Choi et al. 2008; Choi et al. 2009; Foxall et al. 1992; Jang et al. 2008; Vinson et al. 2001). Another study conducted by Agbor and colleagues reported that CR strain was able to significantly develop both hyperlipidemia and atherosclerosis with 0.2% of cholesterol after 12 weeks of dietary intervention (Agbor et al. 2012). The increment of cholesterol up to 1.0% was able to significantly develop hyperlipidemia in a short period, which was two weeks (Takenaga et al. 2000). However, the major drawbacks of using this amount of dietary cholesterol was the period of dietary intervention. The prolonged dietary intervention reduced the lipoprotein profile which indicated hepatotoxicity in this animal model. Dorman and co-workers reported the decline of TC : HDL-C ratios indicated the toxic effects of 1.0% cholesterol after 20 weeks of dietary intervention (Dorfman et al. 2003). In addition, studies also reported a significant development of the disease for other hamster strain, with a range of cholesterol from 0.1-1.0% and the period of dietary intervention as early as two weeks up to 40 weeks (Ahmed 2009; Chen et al. 2014; Cherng & Shih 2005; Huang et al. 2015; Ioriya et al. 2002; Jayachandran et al. 2015, 2015; Kanashiro et al. 2007; Krause et al. 1992; Lin et al. 2013; Lin et al. 2012; Mawatari et al. 2004; Romain et al. 2014; Romain et al. 2012; Singh et al. 2013; Singh et al. 2015; Uehara et al.
3. Cholesterol >1.0%

The third part, as shown in Table 3 of the data, was on the effect of HFHC (>1.0%) -enriched diets in development of hyperlipidemia and atherosclerosis. This group of cholesterol was classified as large amount group of cholesterol. The amount of cholesterol which was more than 1.0% was of concern due to the hepatotoxic effects on hamster animal model (Dillard et al. 2010). Not many previous studies were found using this amount of cholesterol in the combination of CNO as the fat source. As illustrated in Table 3, the amount of dietary cholesterol supplemented into the diet were in the range of 3-5% together with the addition of 15% CNO (Mangiapane et al. 1999; Mcateer et al. 2007; Singh et al. 2011). On the other hand, there are also studies which are conducted with the dietary design of more than 1.0% cholesterol in the combination of another type of dietary fat such as butter (de Oliveira et al. 2000; Sima et al. 2001; Simionescu et al. 1996; Stancu et al. 2014) and lard (Zhao et al. 2014). Mangiapane and co-workers demonstrated a significant development of atherosclerosis within 12 weeks of dietary intervention with 3.0% of supplemental cholesterol (Mangiapane et al. 1999). In 2011, Singh and colleagues reported a significant development of hyperlipidemia in HFHC fed hamster compared to normal control and baseline result. However, the lipoprotein profile for TC, TG, and LDL-c started to decrease slightly after 30 days of diet challenge (Singh et al. 2011).

CONCLUSION

We believe that the present review would benefit researchers in designing the dietary challenge to induce hyperlipidemia and atherosclerosis with studies utilising Syrian hamster animal model. Hamster animal model is an optimal animal model to study

<table>
<thead>
<tr>
<th>CHO (%)</th>
<th>CNO/HCNO (%)</th>
<th>Strain</th>
<th>Study Duration</th>
<th>Disease</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0</td>
<td>15</td>
<td>DSNI</td>
<td>12 weeks</td>
<td>Yes</td>
<td>Yes*</td>
</tr>
<tr>
<td>3.0</td>
<td>15</td>
<td>CDRI</td>
<td>90 days</td>
<td>Yes*</td>
<td>-</td>
</tr>
<tr>
<td>5.0</td>
<td>15</td>
<td>F1B</td>
<td>4 week/12 months</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

The table represents the effects of supplemental fat and cholesterol (< 0.1%) in diet-induced hyperlipidemia and atherosclerosis. The symbol ‘Yes*’ indicates disease successfully develops through diet with significant outcomes. The symbol ‘Yes’ indicates disease successfully develops through diet but in the absence of normal control/baseline group to compare the significant outcomes. The symbol ‘No’ indicates disease does not successfully develop through diet. The symbol '-' indicates disease not being evaluated in the study. CHO=cholesterol; CNO=coconut oil; HCNO=hydrogenated coconut oil.
hyperlipidemic and atherosclerotic changes since the lipoprotein profile is closer to that of human and they are sensitive to HFHC diet. Based on the literature searched, the F1B strain is the most common used hamster strain followed by CR strain for this type of research. The amount of cholesterol and period of dietary intervention are crucial and should be carefully selected. Higher amounts of supplemental cholesterol (>1.0%) and/or more extended period of dietary intervention would result in toxic effects and cause reduction of lipoprotein profile after a certain period of dietary challenge. To develop both hyperlipidemia and atherosclerosis, recommended cholesterol concentration for F1B strain and CR strain are 0.05% and 0.2-0.5%, respectively together with the addition of 10-15% of dietary fat. The recommended period of dietary intervention is 12 weeks, since it is enough to promote the development of early-stage atherosclerosis in the form of foam cell accumulation and fatty streak formation.

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