REVIEW ARTICLE

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

SYARIFAH-NORATIQAH SB[1], FAIRUS S[2], ZULFARINA MS[1], 'ATIQAH A[1], QODRIYAH HMS[1], NAINA-MOHAMED I[1]

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

ABSTRAK

Hiperlipiedmia adalah keadaan paras lipid tinggi dalam plasma dan sering dikaitkan dengan pemendapan titisan lipid dalam aorta yang menyebabkan kejadian aterosklerosis. Aterosklerosis adalah penyakit kardiovaskular yang bermula dengan pembentukan sel-sel busa di dalam dinding vascular, seterusnya menyebabkan aliran darah bergelora, kecederaan pada lapisan endotelial dan trombosis pada pembuluh darah. Sejak awal 1980-an, hamster Golden-Syrian telah digunakan secara meluas sebagai model haiwan dalam penyelidikan hiperlipiedmia dan aterosklerosis. Penggunaan hamster sebagai model hiperlipiedmia dan aterosklerosis adalah kerana profil lipoprotein mereka yang lebih dekat dengan penetapan manusia, sensitif terhadap diet tinggi-lemak tinggi-kolesterol (TLTK) dan model roden yang sesuai. Aterosklerosis boleh dicetus ke atas hamster melalui cabaran diet TLTK. Selama beberapa dekad, minyak kelapa (MK) biasa digunakan sebagai sumber lemak dalam reka bentuk diet berkomposisi asid lemak tepu tinggi (ALT). Dalam ulasan ini, kami merumuskan literatur yang diterbitkan dengan reka bentuk yang melibatkan MK- dan kolesterol-cetusan hiperlipidmia, aterosklerosis atau kedua-duanya. Faktor-faktor yang mungkin mempengaruhi kebolehan campuran MK dan kolesterol untuk menggalakkan hiperlipiedmia seperti tempoh diet, jenis hamster dan jumlah pemakanan akan dinilai dan diringkaskan.

Kata kunci: diet, Mesocricetus auratus, penyakit kardiovascular

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 details 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 (Nirosha 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
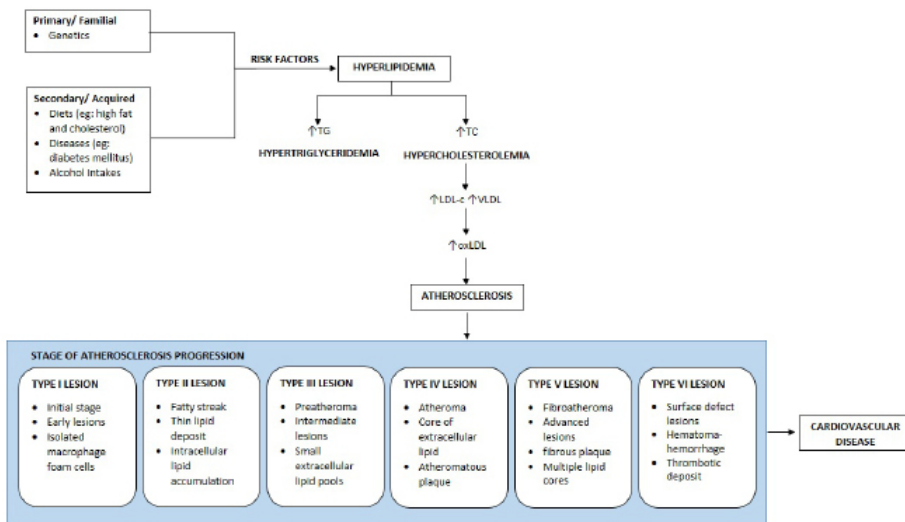
Figure 1: Summary of the disease development of hyperlipidemia and atherosclerosis as well as different stages of aortic lesion progression which leads to cardiovascular disease.

(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

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

CHO (%)	CNO/HCNO (%)	Strain	Study Duration	Disease		References
Cholesterol <0.1%						
				Hyperlipidemia	Atherosclerosis	
0.03	5	F1B	14 weeks	Yes	Yes*	(Ausman et al. 2005)
0.05	10	F1B	8 weeks	Yes	Yes*	
0.05	10	F1B	3 weeks	Yes	Yes*	(Kowala et al. 1994)
0.05	10	F1B	3 weeks	Yes	Yes*	(Kowala et al. 1995b)
0.05	10	F1B	4 weeks	Yes	-	(Wilson et al. 1998b)
0.05	10	F1B	4 weeks	Yes*	-	(Smith et al. 2001a)
0.05	10	F1B	8 weeks	Yes*	Yes*	(Kowala et al. 1991)
0.05	10	F1B	8 weeks	Yes	Yes*	(Wilson et al.1998a)
0.05	10	F1B	10 weeks	Yes	Yes*	(Kowala et al. 1993)
0.05	10	F1B	10 weeks	Yes	Yes*	(Rong et al. 1997)
0.05	10	F1B	10 weeks	Yes	Yes*	(Nicolosi et al. 1998)
0.05	10	SLC	10 weeks	Yes*	Yes*	(Asami et al. 1999)
0.05	10	F1B	11 weeks	Yes*	Yes*	(Pitman et al. 1998)
			12 weeks	Yes*	Yes*	
0.05	10	F1B	12 weeks	Yes	Yes*	(Wilson et al. 1999)
0.05	10	F1B	12 weeks	Yes*	Yes*	(Kowala et al. 1995a)
0.05	10	F1B	12 weeks	Yes*	Yes*	(Robins et al. 1995)
0.05	10	Not stated	12 weeks	Yes*	Yes*	(Pitha et al. 2010)
0.05	10	F1B	10 weeks	Yes*	Yes*	(Otto et al. 1995)
			12.5 weeks	Yes*	Yes*	
0.06	10	Sasco Lab	2 weeks	Yes*	-	(Krause et al. 1992)
0.07	20	Sasco Lab	4 weeks	Yes*	-	(Bertolotti & Spady 2001)

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

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

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; Cherng & 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

				0.1%≤Cholesterol≤1.0%		
CHO (%)	CNO/ HCNO (%)	Strain	Study Duration	Disease		References
				Hyperlipidemia	Atherosclerosis	
0.1	10	CR	2 weeks	Yes	Yes	(Wilson et al. 2006b)
0.1	10	CR	6 weeks	Yes*	Yes	(Dorfman et al. 2005)
0.1	20	Taiwan Nat. Sc. Council	8 weeks	Yes*	-	(Cherng & Shih 2005)
0.1	5	F1B	10 weeks	Yes	Yes	(Rong et al. 1997)
0.1	10	F1B	10 weeks	Yes	Yes	(Wilson et al. 2005)
0.1	10	F1B	12 weeks	Yes	Yes	(Wilson et al. 2006a)
0.1	10	F1B	12 weeks	Yes	Yes	(Wilson et al. 2007)
0.1	10	F1B	12 weeks	Yes	-	(Lecker et al. 2010)
0.1	5	F1B	14 weeks	Yes	Yes	(Wilson et al. 1998b)
0.1	10	F1B CR	20 weeks	Yes*	No	(Dorfman et al. 2003)
0.5	10	F1B CR	20 weeks	Yes*	No	
1.0	10	F1B CR	20 weeks	Yes*	Yes*	
0.1	10	F1B	15 months	Yes	Yes*	(Wilson et al. 2003)
0.12	10	SLC	24 days	Yes*	-	(Ioriya et al. 2002)
0.12	20	CR	30 days	Yes*	-	(Spady & Dietschy 1985)
0.12	10	CR	4 weeks	Yes	-	(Bensch et al. 1999)
0.12	10	VR lab	5 weeks	Yes*	-	(Chen et al. 2014)
0.12	20	CR	12 weeks	Yes*	Yes*	(Mitchell et al. 2005)
0.15	20	F1B	9 weeks	Yes*	Yes*	(Delaney et al. 2003)
0.15	20	F1B	10 weeks	Yes	Yes	(Alexaki et al. 2004)
0.15	10	F1B	12 weeks	Yes	No	(Dillard et al. 2013)
0.2	10	Sasco Lab	2 weeks	Yes*	-	(Krause et al. 1992)
0.2	12	Taiwan Nat. Sc. Council	6 weeks	Yes*		(Lin et al. 2012)
0.2	12	Taiwan Nat. Sc. Council	6 weeks	Yes*	-	(Lin et al. 2013)
0.2	10	Not stated	44 days	Yes*	-	(Ahmed 2009)
0.2	15	F1B CR	9 weeks	Yes / Yes	Yes / Yes	(Smith et al. 2001b)
0.2	10	CR	10 weeks	Yes	Yes	(Foxall et al. 1992)
0.2	10	CR	10 weeks	Yes	Yes	(Vinson et al. 2001)

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*	
0.3	10	SLC	40 weeks	Yes*	Yes*	(Mawatari et al. 2004)
0.3	10	F1B	15 months	Yes	Yes	(Kowala, Rose, et al. 1995)
0.5	10	Taiwan Nat. Sc. Council	10 weeks	Yes*	-	(Huang et al. 2015)
0.5	10	KBT	12 weeks	Yes*	Yes*	(Uehara et al. 2002)
0.5	15	F1B DSNI	4 week/ 12months	Yes	Yes	(Mcateer et al. 2007)
1.0	10	CR	2 weeks	Yes*	-	(Takenaga et al. 2000)
1.0	15	CSIR-CDRI	35 days	Yes*	Yes*	(Singh et al. 2013)
1.0	10	CSIR-CDRI	5 weeks	Yes*	Yes	(Singh et al. 2015)
1.0	22	F1B	49 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.

2001b; Wilson et al. 2003; Wilson et al. 2005; Wilson et al. 2006a; Wilson et al. 2007; Wilson et al. 1998b) followed by CR strain (Agbor et al. 2012; Bensch et al. 1999; Choi et al. 2008; Choi et al. 2009; Dorfman et al. 2003; Dorfman et al. 2005; Foxall et al. 1992; Jang et al. 2008; Mitchell et al. 2005; Smith

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.

Table 3: Effects of high fat high cholesterol (Cholesterol > 1.0%)-enriched diets on development of hyperlipidemia and atherosclerosis

CHO (%)	CNO/ HCNO (%)	Strain	Study Duration	Disease		References
				Hyperlipidemia	Atherosclerosis	
3.0	15	DSNI	12 weeks	Yes	Yes*	(Mangiapane et al. 1999)
3.0	15	CDRI	90 days	Yes*	-	(Singh et al. 2011)
5.0	15	F1B DSNI	4 week/ 12 months	Yes	Yes	(Mcateer et al. 2007)

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.

2002; Vide et al. 2015; Zha et al. 2009).

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

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.

ACKNOWLEDGEMENT

REFERENCES

Agbor, G.A., Vinson, J.A., Sortino, J. Johnson, R. 2012. Antioxidant and anti-atherogenic activities of three piper species on atherogenic diet fed hamsters. *Exp Toxicol Pathol* **64**(4): 387-91.

Ahmed, A.A. 2009. Beneficial effects of combined administration of sodium molybdate with atorvastatin in hyperlipidemic hamsters. *Drug Discov Ther* **3**(2): 62-70.

Ahn, Y.S., Smith, D., Osada, J., Li, Z., Schaefer, E.J. Ordovas, J.M. 1994. Dietary fat saturation affects apolipoprotein gene expression and high density lipoprotein size distribution in golden syrian hamsters. *J Nutr* **124**(11): 2147-55.

Alexaki, A., Wilson, T.A., Atallah, M.T., Handelman, G. Nicolosi, R.J. 2004. Hamsters fed diets high in saturated fat have increased cholesterol accumulation and cytokine production in the aortic arch compared with cholesterol-fed hamsters with moderately elevated plasma non-hdl cholesterol concentrations. *J Nutr* **134**(2): 410-5.

Asami, Y., Yamagishi, I., Akiyoshi, K., Tomoike, H., Tsuchida, K. Higuchi, S. 1999. Inhibitory effect of ts-962 on the formation of early atherosclerotic lesions in high fat-fed hyperlipidemic hamsters. *Atherosclerosis* **146**(2): 237-42.

Auger, C., Rouanet, J.M., Vanderlinde, R., Bornet, A., Decorde, K., Lequeux, N., Cristol, J.P. Teissedre, P.L. 2005. Polyphenols-enriched chardonnay white wine and sparkling pinot noir red wine identically prevent early atherosclerosis in hamsters. *J Agric Food Chem* **53**(25): 9823-9.

Ausman, L.M., Rong, N. Nicolosi, R.J. 2005. Hypocholesterolemic effect of physically refined rice bran oil: studies of cholesterol metabolism and early atherosclerosis in hypercholesterolemic hamsters. *J Nutr Biochem* **16**(9): 521-9.

Bensch, W.R., Gadski, R.A., Bean, J.S., Beavers, L.S., Schmidt, R.J., Perry, D.N., Murphy, A.T., Mcclure, D.B., Eacho, P.I., Breau, A.P., Archer, R.A. Kauffman, R.F. 1999. Effects of ly295427, a low-density lipoprotein (LDL) receptor up-regulator, on ldl receptor gene transcription and cholesterol metabolism in normal and hypercholesterolemic hamsters. *J Pharmacol Exp Ther* **289**(1): 85-92.

Bertolotti, M., Spady, D.K. 2001. Inhibition of intestinal cholesterol absorption by surfomer (alpha-olefin maleic acid) affects hepatic cholesterol synthesis and low density lipoprotein transport in hamsters fed a fat-enriched diet. *Dig Liver Dis* **33**(2): 145-50.

Bhatia, G., Rizvi, F., Saxena, R., Puri, A., Khanna, A.K., Chander, R., Wulff, E.M. Rastogi, A.K. 2003. In vivo model for dyslipidemia with diabetes mellitus in hamster. *Indian J Exp Biol* **41**(12): 1456-9.

Chen, C.L., Pan, T.M. 2012. Red mold dioscorea: a potentially safe traditional function food for the treatment of hyperlipidemia. *Food Chem* **134**(2): 1074-80.

Chen, W., Fan, S., Xie, X., Xue, N., Jin, X. Wang, L.

2014. Novel PPAR pan agonist, ZBH ameliorates hyperlipidemia and insulin resistance in high fat diet induced hyperlipidemic hamster. *PLoS One* **9**(4): e96056.

Chen, Y.Y., Lee, P.C., Wu, Y.L. Liu, L.Y. 2015. In vivo effects of free form astaxanthin powder on anti-oxidation and lipid metabolism with high-cholesterol diet. *PLoS One* **10**(8): e0134733.

Cherng, J.Y. Shih, M.F. 2005. Preventing dyslipidemia by chlorella pyrenoidosa in rats and hamsters after chronic high fat diet treatment. *Life Sci* **76**(26): 3001-13.

Choi, M.S., Jung, U.J., Kim, H.J., Do, G.M., Jeon, S.M., Kim, M.J. Lee, M.K. 2008. Du-zhong (Eucommia ulmoides Oliver) leaf extract mediates hypolipidemic action in hamsters fed a high-fat diet. *Am J Chin Med* **36**(1): 81-93.

Choi, M.S., Lee, M.K., Jung, U.J., Kim, H.J., Do, G.M., Park, Y.B. Jeon, S.M. 2009. Metabolic response of soy pinitol on lipid-lowering, antioxidant and hepatoprotective action in hamsters fed-high fat and high cholesterol diet. *Mol Nutr Food Res* **53**(6): 751-9.

Dalboge, L.S., Pedersen, P.J., Hansen, G., Fabricius, K., Hansen, H.B., Jelsing, J., Vrang, N. 2015. A hamster model of diet-induced obesity for preclinical evaluation of anti-obesity, anti-diabetic and lipid modulating agents. *PLoS One* **10**(8): e0135634.

de Oliveira, F.G., Rossi, C.L., De Oliveira, M.G., Saad, M.J. Velloso, L.A. 2000. Effect of vitamin e supplementation on antibody levels against malondialdehyde modified ldl in hyperlipidemic hamsters. *Cardiovasc Res* **47**(3): 567-73.

Delaney, B., Nicolosi, R.J., Wilson, T.A., Carlson, T., Frazer, S., Zheng, G.H., Hess, R., Ostergren, K., Haworth, J. Knutson, N. 2003. Beta-glucan fractions from barley and oats are similarly antiatherogenic in hypercholesterolemic syrian golden hamsters. *J Nutr* **133**(2): 468-75.

Denke, M.A. Grundy, S. 1992. Comparison of effects of lauric acid and palmitic acid on plasma lipids and lipoproteins. *Am J Clin Nutr* **56**(5): 895-8.

Dillard, A., Matthan, N.R. Lichtenstein, A.H. 2010. Use of hamster as a model to study diet-induced atherosclerosis. *Nutr Metab (Lond)* **7**: 89.

Dillard, A., Matthan, N.R., Spartano, N.L., Butkowski, A.E. Lichtenstein, A.H. 2013. Background diet and fat type alters plasma lipoprotein response but not aortic cholesterol accumulation in f1b golden syrian hamsters. *Lipids* **48**(12): 1177-84.

Dorfman, S.E., Smith, D.E., Osgood, D.P. Lichtenstein, A.H. 2003. Study of diet-induced changes in lipoprotein metabolism in two strains of Golden-Syrian hamsters. *J Nutr* **133**(12): 4183-8.

Dorfman, S.E., Wang, S., Vega-Lopez, S., Jauhiainen, M. Lichtenstein, A.H. 2005. Dietary fatty acids and cholesterol differentially modulate hdl cholesterol metabolism in golden-syrian hamsters. *J Nutr* **135**(3): 492-8.

Elson, C.E. 1992. Tropical oils: nutritional and scientific issues. *Crit Rev Food Sci Nutr* **31**(1-2): 79-102.

Faia, K.L., Davis, W.P., Marone, A.J. Foxall, T.L. 2002. Matrix metalloproteinases and tissue inhibitors of metalloproteinases in hamster aortic atherosclerosis: correlation with in-situ zymography. *Atherosclerosis* **160**(2): 325-37.

Food Drug Administration. 2015. *Product Development under the Animal Rule: Guidance for Industry.* Maryland: FDA.

Foxall, T.L., Shwaery, G.T., Stucchi, A.F., Nicolosi, R.J. Wong, S.S. 1992. Dose-related effects of doxazosin on plasma lipids and aortic fatty streak formation in the hypercholesterolemic hamster model. *Am J Pathol* **140**(6): 1357-63.

Ghosh, R.K. Ghosh, S.M. 2012. Current status of cetp inhibitors in the treatment of hyperlipidemia: an update. *Curr Clin Pharmacol* **7**(2): 102-10.

Ginghina, C., Bejan, I. Ceck, C.D. 2011. Modern risk stratification in coronary heart disease. *J Med Life* **4**(4): 377-86.

Goldstein, J.L., Schrott, H.G., Hazzard, W.R., Bierman, E.L. Motulsky, A.G. 1973. Hyperlipidemia in coronary heart disease. Ii. Genetic analysis of lipid levels in 176 families and delineation of a new inherited disorder, combined hyperlipidemia. *J Clin Invest* **52**(7): 1544-68.

Goodnight, S.H., Harris, W.S., Connor, W.E. Illingworth, D.R. 1982. Polyunsaturated fatty acids, hyperlipidemia, and thrombosis. *Arteriosclerosis* **2**(2): 87-113.

Guo, Q., Wang, P.R., Milot, D.P., Ippolito, M.C., Hernandez, M., Burton, C.A., Wright, S.D. Chao, Y.S. 2001. Regulation of lipid metabolism and gene expression by fenofibrate in hamsters. *Biochim Biophys Acta* **1533**(3): 220-32.

Hansson, G.K., Robertson, A.K., Söderberg-Nauclér, C. 2006. Inflammation and atherosclerosis. *Annu Rev Pathol* **1**(1): 297-329.

Hassan, B.A.R. 2013. Overview on Hyperlipidemia. *J Chromat Sep Tech* **4**(3): e113.

Homburger, F. 1969. Chemical carcinogenesis in the syrian golden hamster. A review. *Cancer* **23**(2): 313-38.

Huang, T.W., Chang, C.L., Kao, E.S. Lin, J.H. 2015. Effect of hibiscus sabdariffa extract on high fat diet-induced obesity and liver damage in hamsters. *Food Nutr Res* **59**: 29018.

Ioriya, K., Kino, K., Sato, Y.F. Ohashi, N. 2002. Effect of smp-500, a novel acyl-coa:cholesterol acyltransferase inhibitor, on serum cholesterol level and ldl cholesterol clearance in hamsters with induced hyperlipidemia. *Pharmacology* **66**(2): 107-14.

Jang, E.M., Choi, M.S., Jung, U.J., Kim, M.J., Kim,

H.J., Jeon, S.M., Shin, S.K., Seong, C.N. Lee, M.K. 2008. Beneficial effects of curcumin on hyperlipidemia and insulin resistance in high-fat-fed hamsters. *Metabolism* **57**(11): 1576-83.

Jayachandran, M., Chandrasekaran, B., Namasivayam, N. 2015. Effect of geraniol, a plant derived monoterpene on lipids and lipid metabolizing enzymes in experimental hyperlipidemic hamsters. *Mol Cell Biochem* **398**(1-2): 39-53.

Jayachandran, M., Chandrasekaran, B. Namasivayam, N. 2015. Geraniol attenuates oxidative stress by nrf2 activation in diet-induced experimental atherosclerosis. *J Basic Clin Physiol Pharmacol* **26**(4): 335-46.

Jorgensen, T., Capewell, S., Prescott, E., Allender, S., Sans, S., Zdrojewski, T., De Bacquer, D., de Sutter, J., Franco, O.H., Logstrup, S., Volpe, M., Malyutina, S., Marques-Vidal, P., Reiner, Z., Tell, G.S., Verschuren, W.M. Vanuzzo, D. 2013. Population-level changes to promote cardiovascular health. *Eur J Prev Cardiol* **20**(3): 409-21.

Kanashiro, A., Kabeya, L.M., Martinello, F., Turato, W.M., Paula, F.S., Polizello, A.C., Uyemura, S.A., Lucisano-Valim, Y.M. 2007. Effect of rutin on polymorphonuclear leukocytes oxidative metabolism in hypercholesterolemia golden syrian hamsters: evaluation by chemiluminescence and flow cytometry. *Pharmazie* **62**(4): 295-8.

Kim, H.J., Jeon, S.M., Lee, M.K., Cho, Y.Y., Kwon, E.Y., Lee, J.H. Choi, M.S. 2010. Comparison of hesperetin and its metabolites for cholesterol-lowering and antioxidative efficacy in hypercholesterolemic hamsters. *J Med Food* **13**(4): 808-14.

Kowala, M.C., Nunnari, J.J., Durham, S.K. Nicolosi, R.J. 1991. Doxazosin and cholestyramine similarly decrease fatty streak formation in the aortic arch of hyperlipidemic hamsters. *Atherosclerosis* **91**(1-2): 35-49.

Kowala, M.C., Mazzucco, C.E., Hartl, K.S., Seiler, S.M., Warr, G.A., Abid, S., Grove, R.I. 1993. Prostacyclin agonists reduce early atherosclerosis in hyperlipidemic hamsters. Octimibate and bmy 42393 suppress monocyte chemotaxis, macrophage cholesteryl ester accumulation, scavenger receptor activity, and tumor necrosis factor production. *Arterioscler Thromb* **13**(3): 435-44.

Kowala, M.C., Grove, R.I. Aberg, G. 1994. Inhibitors of angiotensin converting enzyme decrease early atherosclerosis in hyperlipidemic hamsters. Fosinopril reduces plasma cholesterol and captopril inhibits macrophage-foam cell accumulation independently of blood pressure and plasma lipids. *Atherosclerosis* **108**(1): 61-72.

Kowala, M.C., Recce, R., Beyer, S. Aberg, G. 1995a. Regression of early atherosclerosis in hyperlipidemic hamsters induced by fosinopril and captopril. *J Cardiovasc Pharmacol* **25**(2): 179-86.

Kowala, M.C., Rose, P.M., Stein, P.D., Goller, N., Recce, R., Beyer, S., Valentine, M., Barton, D., Durham, S.K. 1995b. Selective blockade of the endothelin subtype a receptor decreases early atherosclerosis in hamsters fed cholesterol. *Am J Pathol* **146**(4): 819-26.

Krause, B.R., Bousley, R.F., Kieft, K.A., Stanfield, R.L. 1992. Effect of the acat inhibitor ci-976 on plasma cholesterol concentrations and distribution in hamsters fed zero- and low-cholesterol diets. *Clin Biochem* **25**(5): 371-7.

Kumeshini, S., Nurshazana, A., Faizah, O., Kamsiah, J., Srijit, D., Zar Chi, T. 2016. Development of hypertensive animal model using ovariectomised rat fed with short-term 2% cholesterol diet. *Med & Health* **11**(2): 171-80.

Lecker, J.L., Matthan, N.R., Billheimer, J.T., Rader, D.J., Lichtenstein, A.H. 2010. Impact of dietary fat type within the context of altered cholesterol homeostasis on cholesterol and lipoprotein metabolism in the F1B hamster. *Metabolism* **59**(10): 1491-501.

Lee, C.L., Hung, Y.P., Hsu, Y.W., Pan, T.M. 2013. Monascin and ankaflavin have more anti-atherosclerosis effect and less side effect involving increasing creatinine phosphokinase activity than monacolin k under the same dosages. *J Agric Food Chem* **61**(1): 143-50.

Leong, X.F., Ng, C.Y., Jaarin, K. 2015. Animal models in cardiovascular research: hypertension and atherosclerosis. *Biomed Res Int* **2015**: 528757.

Lin, Y.L., Chou, C.H., Yang, D.J., Chen, J.W., Tzang, B.S. Chen, Y.C. 2012. Hypolipidemic and antioxidative effects of noni (morinda citrifolia l.) Juice on high- fat/cholesterol-dietary hamsters. *Plant Foods Hum Nutr* **67**(3): 294-302.

Lin, Y.L., Chang, Y.Y., Yang, D.J., Tzang, B.S. Chen, Y.C. 2013. Beneficial effects of noni (morinda citrifolia l.) Juice on livers of high-fat dietary hamsters. *Food Chem* **140**(1-2): 31-8.

Mangiapane, E.H., Mcateer, M.A., Benson, G.M., White, D.A. Salter, A.M. 1999. Modulation of the regression of atherosclerosis in the hamster by dietary lipids: comparison of coconut oil and olive oil. *Br J Nutr* **82**(5): 401-9.

Mawatari, K., Kakui, S., Harada, N., Ohnishi, T., Niwa, Y., Okada, K., Takahashi, A., Izumi, K. Nakaya, Y. 2004. Endothelin-1(1–31) levels are increased in atherosclerotic lesions of the thoracic aorta of hypercholesterolemic hamsters. *Atherosclerosis* **175**(2): 203-12.

Mcateer, M.A., Grimsditch, D.C., Vidgeon-Hart, M., Benson, G.M. Salter, A.M. 2007. Dietary cholesterol reduces lipoprotein lipase activity in

the atherosclerosis-susceptible Bio F1B hamster. *Br J Nutr* **89**(3): 341-50.

Miedel, E.L. Hankenson, F.C. 2015. Chapter 5 - Biology and Diseases of Hamsters. In *Laboratory Animal Medicine.* 3rd edition. Elsevier Inc.; 209-45.

Mitchell, P.L., Langille, M.A., Currie, D.L. Mcleod, R.S. 2005. Effect of conjugated linoleic acid isomers on lipoproteins and atherosclerosis in the syrian golden hamster. *Biochim Biophys Acta* **1734**(3): 269-76.

Nelson, R.H. 2013. Hyperlipidemia as a risk factor for cardiovascular disease. *Prim Care* **40**(1): 195-211.

Ng, C.H., Qiang Yao, X., Huang, Y. Chen, Z.Y. 2008. Oxidised cholesterol is more hypercholesterolaemic and atherogenic than non-oxidised cholesterol in hamsters. *Br J Nutr* **99**(4): 749-55.

Nicolosi, R.J., Wilson, T.A. Krause, B.R. 1998. The acat inhibitor, ci-1011 is effective in the prevention and regression of aortic fatty streak area in hamsters. *Atherosclerosis* **137**(1): 77-85.

Nirosha, K., Divya, M., Vamsi, S. Sadiq, M. 2014. A review on hyperlipidemia. *Int J Novel Trends Pharm Sci* **4**(5): 81-92.

Otto, J., Ordovas, J.M., Smith, D., van Dongen, D., Nicolosi, R.J. Schaefer, E.J. 1995. Lovastatin inhibits diet induced atherosclerosis in f1b golden syrian hamsters. *Atherosclerosis* **114**(1): 19-28.

Patel, V., Joharapurkar, A., Kshirsagar, S., Patel, H.M., Pandey, D., Patel, D., Shah, K., Bahekar, R., Shah, G.B. Jain, M.R. 2017. Central and peripheral glucagon reduces hyperlipidemia in rats and hamsters. *Drug Res (Stuttg)* **67**(6): 318-26.

Pereira, P.C., Pernomian, L., Côco, H., Gomes, M.S., Franco, J.J., Marchi, K.C., Hipólito, U.V., Uyemura, S. A., Tirapelli, C.R., de Oliveira, A.M. 2016. Auto-inhibitory regulation of angiotensin II functionality in hamster aorta during the early phases of dyslipidemia. *Eur J Pharmacol* **781**: 1-9.

Pitha, J., Bobkova, D., Kovar, J., Havlickova, J. Poledne, R. 2010. Antiatherogenic effect of simvastatin is not due to decrease of ldl cholesterol in ovariectomized golden syrian hamster. *Physiol Res* **59**(3): 401-6.

Pitman, W.A., Osgood, D.P., Smith, D., Schaefer, E.J. Ordovas, J.M. 1998. The effects of diet and lovastatin on regression of fatty streak lesions and on hepatic and intestinal mrna levels for the ldl receptor and hmg coa reductase in f1b hamsters. *Atherosclerosis* **138**(1): 43-52.

Rémillard, P., Shen, G., Milne, R. Maheux, P. 2001. Induction of cholesteryl ester transfer protein in adipose tissue and plasma of the fructose-fed hamster. *Life Sci* **69**(6): 677-87.

Robins, S.J., Fasulo, J.M., Patton, G.M., Schaefer, E.J., Smith, D.E. Ordovas, J.M. 1995. Gender differences in the development of hyperlipemia and atherosclerosis in hybrid hamsters. *Metabolism* **44**(10): 1326-31.

Rohilla, A., Dagar, N., Rohilla, S., Dahiya, A., Kushnoor, A. 2012. Hyperlipidemia- a Deadly Pathological Condition. *Int J Curr Pharm Res* **4**(2): 15-8. – edi dr google

Romain, C., Bresciani, L., Gaillet, S., Feillet-Coudray, C., Calani, L., Bonafos, B., Vide, J., Rugani, N., Ramos, J., Del Rio, D., Cristol, J.P. Rouanet, J.M. 2014. Moderate chronic administration of vineatrol-enriched red wines improves metabolic, oxidative, and inflammatory markers in hamsters fed a high-fat diet. *Mol Nutr Food Res* **58**(6): 1212-25.

Romain, C., Gaillet, S., Carillon, J., Vide, J., Ramos, J., Izard, J.C., Cristol, J.P. Rouanet, J.M. 2012. Vineatrol and cardiovascular disease: beneficial effects of a vine-shoot phenolic extract in a hamster atherosclerosis model. *J Agric Food Chem* **60**(44): 11029-36.

Rong, N., Ausman, L.M., Nicolosi, R.J. 1997. Oryzanol decreases cholesterol absorption and aortic fatty streaks in hamsters. *Lipids* **32**(3): 303-9.

Russell, J.C. Proctor, S.D. 2006. Small animal models of cardiovascular disease: tools for the study of the roles of metabolic syndrome, dyslipidemia, and atherosclerosis. *Cardiovasc Pathol* **15**(6): 318-30.

Shankar, K., Singh, S.K., Kumar, D., Varshney, S., Gupta, A., Rajan, S., Srivastava, A., Beg, M., Srivastava, A.K., Kanojiya, S., Mishra, D.K., Gaikwad, A.N. 2015. Cucumis melo ssp. Agrestis var. Agrestis ameliorates high fat diet induced dyslipidemia in syrian golden hamsters and inhibits adipogenesis in 3t3-l1 adipocytes. *Pharmacogn Mag* **11**(Suppl 4): S501-10.

Sima, A., Stancu, C., Constantinescu, E., Ologeanu, L. Simionescu, M. 2001. The hyperlipemic hamster-a model for testing the anti-atherogenic effect of amlodipine. *J Cell Mol Med* **5**(2): 153-62.

Simionescu, M., Popov, D., Sima, A., Hasu, M., Costache, G., Faitar, S., Vulpanovici, A., Stancu, C., Stern, D. Simionescu, M. 1996. Pathobiochemistry of combined diabetes and atherosclerosis studied on a novel animal model.The hyperlipemic-hyperglycemic hamster. *Am J Pathol* **148**(3): 997-1014.

Singh, V., Jain, M., Prakash, P., Misra, A., Khanna, V., Tiwari, R.L., Keshari, R.S., Singh, S., Dikshit, M. Barthwal, M.K. 2011. A time course study on prothrombotic parameters and their modulation by anti-platelet drugs in hyperlipidemic hamsters. *J Physiol Biochem* **67**(2): 205-16.

Singh, V., Jain, M., Misra, A., Khanna, V., Rana,

M., Prakash, P., Malasoni, R., Dwivedi, A.K., Dikshit, M. Barthwal, M.K. 2013. Curcuma oil ameliorates hyperlipidaemia and associated deleterious effects in golden syrian hamsters. *Br J Nutr* **110**(3): 437-46.

Singh, V., Rana, M., Jain, M., Singh, N., Naqvi, A., Malasoni, R., Dwivedi, A.K., Dikshit, M. Barthwal, M.K. 2015. Curcuma oil attenuates accelerated atherosclerosis and macrophage foam-cell formation by modulating genes involved in plaque stability, lipid homeostasis and inflammation. *Br J Nutr* **113**(1): 100-13.

Smith, D., Pedro-Botet, J., Cantuti-Castelvetri, I., Shukitt-Hale, B., Schaefer, E.J., Joseph, J. Ordovas, J.M. 2001a. Influence of photoperiod, laboratory caging and aging on plasma lipid response to an atherogenic diet among f1b hamsters. *Int J Neurosci* **106**(3-4): 185-94.

Smith, D., Ahn, Y.S., Pedro-Botet, J., Osada, J., Mata, P., Schaefer, E.J. Ordovas, J.M. 2001b. Dietary fat saturation distinctly affects apolipoprotein gene expression and high density lipoprotein size distribution in two strains of golden syrian hamsters. *Nutr Res* **21**(1): 215-28.

Spady, D.K. Dietschy, J.M. 1985. Dietary saturated triacylglycerols suppress hepatic low density lipoprotein receptor activity in the hamster. *Proc Natl Acad Sci U S A* **82**(13): 4526-30.

Srivastava, R.A. 2011. Evaluation of anti-atherosclerotic activities of PPAR-α, PPAR-γ, and LXR agonists in hyperlipidemic atherosclerosis-susceptible F(1)B hamsters. *Atherosclerosis* **214**(1): 86-93.

Stancu, C.S., Sanda, G.M., Deleanu, M. Sima, A.V. 2014. Probiotics determine hypolipidemic and antioxidant effects in hyperlipidemic hamsters. *Mol Nutr Food Res* **58**(3): 559-68.

Stary, H.C., Chandler, A.B., Dinsmore, R.E., Fuster, V., Glagov, S., Insull, W., Rosenfeld, M.E., Schwartz, C. J., Wagner, W.D. Wissler, R.W. 1995. A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the committee on vascular lesions of the council on arteriosclerosis, american heart association. *Circulation* **92**(5): 1355-74.

Taghibiglou, C., Rudy, D., Van Iderstine, S.C., Aiton, A., Cavallo, D., Cheung, R. Adeli, K. 2000. Intracellular mechanisms regulating apob-containing lipoprotein assembly and secretion in primary hamster hepatocytes. *J Lipid Res* **41**(4): 499-513.

Takenaga, T., Imada, K. Otomo, S. 2000. Hypolipidemic effect of taurine in golden syrian hamsters. *Adv Exp Med Biol* **483**: 187-92.

Temme, E.H., Mensink, R.P. Hornstra, G. 1996. Comparison of the effects of diets enriched in lauric, palmitic, or oleic acids on serum lipids and lipoproteins in healthy women and men.

Am J Clin Nutr **63**(6): 897-903.

Townsend, N., Nichols, M., Scarborough, P. Rayner, M. 2015. Cardiovascular disease in europe-epidemiological update 2015. *Eur Heart J* **36**(40): 2696-705.

Trautwein, E.A., Jingsheng, L. Hayes, K.C. 1993. Plasma lipoproteins, biliary lipids and bile acid profile differ in various strains of syrian hamsters *Mesocricetus auratus*. *Comp Biochem Physiol Comp Physiol* **104**(4): 829-35.

Tsutsumi, K., Hagi, A. Inoue, Y. 2001. The relationship between plasma high density lipoprotein cholesterol levels and cholesteryl ester transfer protein activity in six species of healthy experimental animals. *Biol Pharm Bull* **24**(5): 579-81.

Uehara, Y., Urata, H., Ideishi, M., Arakawa, K. Saku, K. 2002. Chymase inhibition suppresses high-cholesterol diet-induced lipid accumulation in the hamster aorta. *Cardiovasc Res* **55**(4): 870-6.

Vide, J., Virsolvy, A., Romain, C., Ramos, J., Jouy, N., Richard, S., Cristol, J.P., Gaillet, S. Rouanet, J.M. 2015. Dietary silicon-enriched spirulina improves early atherosclerosis markers in hamsters on a high-fat diet. *Nutrition* **31**(9): 1148-54.

Vinson, J.A., Mandarano, M.A., Shuta, D.L., Bagchi, M. Bagchi, D. 2002. Beneficial effects of a novel ih636 grape seed proanthocyanidin extract and a niacin-bound chromium in a hamster atherosclerosis model. *Mol Cell Biochem* **240**(1-2): 99-103.

Vinson, J.A., Teufel, K. Wu, N. 2001. Red wine, dealcoholized red wine, and especially grape juice, inhibit atherosclerosis in a hamster model. *Atherosclerosis* **156**(1): 67-72.

Wang, X., Guan, L., Zhao, Y., Lei, L., Liu, Y., Ma, K.Y., Wang, L., Man, S.W., Wang, J., Huang, Y. Chen, Z.Y. 2015. Plasma cholesterol-lowering activity of dietary dihydrocholesterol in hypercholesterolemia hamsters. *Atherosclerosis* **242**(1): 77-86.

Wang, Z., Niimi, M., Ding, Q., Liu, Z., Wang, L., Zhang, J., Xu, J. Fan, J. 2017. Comparative studies of three cholesteryl ester transfer proteins and their interactions with known inhibitors. *PLoS One* **12**(8): e0180772.

Warner, B.M., Safronetz, D. Kobinger, G.P. 2017. Syrian hamsters as a small animal model for emerging infectious diseases: advances in immunologic methods. *Adv Exp Med Biol* **972**: 87-101.

Weingand, K.W. Daggy, B.P. 1991. Effects of dietary cholesterol and fasting on hamster plasma lipoprotein lipids. *Eur J Clin Chem Clin Biochem* **29**(7): 425-8.

Wilkins, E., Wilson, L., Wickramasinghe, K., Bhatnagar, P., Leal, J., Luengo-Fernandez, R., Burns, R., Rayner, M., Townsend, N. 2017.

European Cardiovascular Disease Statistics 2017. European Heart Network: Brussels.

Wilson, T.A., Meservey, C.M. Nicolosi, R.J. 1998a. Soy lecithin reduces plasma lipoprotein cholesterol and early atherogenesis in hypercholesterolemic monkeys and hamsters: beyond linoleate. *Atherosclerosis* **140**(1): 147-53.

Wilson, T.A., Romano, C., Liang, J. Nicolosi, R.J. 1998b. The hypocholesterolemic and antiatherogenic effects of cholazol h, a chemically functionalized insoluble fiber with bile acid sequestrant properties in hamsters. *Metabolism* **47**(8): 959-64.

Wilson, T.A., Nicolosi, R.J., Lawton, C.W. Babiak, J. 1999. Gender differences in response to a hypercholesterolemic diet in hamsters: effects on plasma lipoprotein cholesterol concentrations and early aortic atherosclerosis. *Atherosclerosis* **146**(1): 83-91.

Wilson, T.A., Foxall, T.L. Nicolosi, R.J. 2003. Doxazosin, an alpha-1 antagonist, prevents further progression of the advanced atherosclerotic lesion in hypercholesterolemic hamsters.*Metabolism* **52**(10): 1240-5.

Wilson, T.A., Nicolosi, R.J., Kotyla, T., Sundram, K. Kritchevsky, D. 2005. Different palm oil preparations reduce plasma cholesterol concentrations and aortic cholesterol accumulation compared to coconut oil in hypercholesterolemic hamsters. *J Nutr Biochem* **16**(10): 633-40.

Wilson, T.A., Nicolosi, R.J., Saati, A., Kotyla, T. Kritchevsky, D. 2006a. Conjugated linoleic acid isomers reduce blood cholesterol levels but not aortic cholesterol accumulation in hypercholesterolemic hamsters. *Lipids* **41**(1): 41-8.

Wilson, T.A., Kritchevsky, D., Kotyla, T. Nicolosi, R.J. 2006b. Structured triglycerides containing caprylic (8:0) and oleic (18:1) fatty acids reduce blood cholesterol concentrations and aortic cholesterol accumulation in hamsters. *Biochim Biophys Acta* **1761**(3): 345-9.

Wilson, T.A., Nicolosi, R.J., Woolfrey, B. Kritchevsky, D. 2007. Rice bran oil and oryzanol reduce plasma lipid and lipoprotein cholesterol concentrations and aortic cholesterol ester accumulation to a greater extent than ferulic acid in hypercholesterolemic hamsters. *J Nutr Biochem* **18**(2): 105-12.

Zha, W., A, J., Wang, G., Yan, B., Gu, S., Zhu, X., Hao, H., Huang, Q., Sun, J., Zhang, Y., Cao, B. Ren, H. 2009. Metabonomic characterization of early atherosclerosis in hamsters with induced cholesterol. *Biomarkers* **14**(6): 372-80.

Zhao, M.J., Wang, S.S., Jiang, Y., Wang, Y., Shen, H., Xu, P., Xiang, H. Xiao, H. 2017. Hypolipidemic Effect of Xh601 on Hamsters of Hyperlipidemia and Its Potential Mechanism. *Lipids Health Dis* **16**(1): 85.

Zhao, Y., Shu, P., Zhang, Y., Lin, L., Zhou, H., Xu, Z., Suo, D., Xie, A. Jin, X. 2014. Effect of centella asiatica on oxidative stress and lipid metabolism in hyperlipidemic animal models. *Oxid Med Cell Longev* **2014**: 7.