Effects of Tea Seed Oil and Onion on Lipoprotein Metabolism in Hamsters

GUAN, Lei

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Thesis/ Assessment Committee

Professor CHEUNG Chi Keung Peter (Chair)

Professor CHUNG Hau Yin (Thesis Supervisor)

Professor CHEN Zhen Yu (Committee Member)

Professor LI Ke Ji (External Examiner)

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Abstract of dissertation entitled:

Effects of Tea Seed Oil and Onion on Lipoprotein Metabolism in Hamsters

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Cardiovascular disease (CVD) is a major health problem in developed countries and, with increasing prevalence in developing countries and Eastern Europe. Due to the increased incidence with advancing age, there is a need to develop primary preventive interventions to prolong the period of healthy life. Diet has a substantial influence on health and aging. The composition of the human diet plays an important role in the management of lipid and lipoprotein. In this respect, we have focused on the effects of two kinds of functional foods, tea seed oil and dietary onion on their hypocholesterolemic activities and underlying mechanisms in the present study.

Interest in tea seed oil (named tea oil) as a cooking oil is increasing. However, its effect on blood cholesterol is not known. This study was therefore conducted to compare the hypocholesterolemic activity of tea oil with grape seed, canola and corn oils. Fifty 8-week-old male hamsters were first fed a high fat diet (5% lard), and supplemented with 0.1% cholesterol for 2 weeks and then divided into five groups. Control group was continuously fed high fat high cholesterol diet, while the experimental groups were fed high fat, high cholesterol diet plus 10% tea oil, grape seed oil, canola oil and corn oil for 12 weeks. Results showed that plasma total cholesterol (TC), non-HDL-cholesterol (non-HDL-C) and triacylglycerols (TG) in hamsters fed a 0.1% cholesterol diet containing tea, grape, canola or corn oil was significantly reduced compared with those in lard-fed group. Tea oil decreased only non-HDL-C and had no or little effect on HDL-C concentration, while grape oil reduced both. Besides, tea oil-fed hamsters excreted less neutral but greater acidic

sterols compared with other three oils. Unlike grape oil, tea oil up-regulated sterol regulatory element binding protein (SREBP-2) and LDL receptor. Differences between tea oil and the tested vegetable oils could be attributable partially to >80% oleic acid in tea oil.

Clearly, there are many claims on health benefits of *Alliums*, however, most, with the exception of garlic, have not received any rigorous or even gentle scientific investigation. Thus, the present study was carried out to explore hypocholesterolemic effects of onion supplementation. After fed for 2 weeks of the high fat high cholesterol diet, thirty-six 8-week male hamsters were divided into four groups. Control group was continued fed with high fat high cholesterol diet, while the other two experimental groups were fed control diet plus 1% (1OP) and 5% (5OP) onion powder for 8 weeks. It was found that feeding high dose of onion powder diet significantly prevented the increase in serum TC, Non-HDL-C and the ratio of non-HDL-C/HDL respectively in hamsters fed a 0.1% cholesterol diet. In contrast, the ratio of HDL/TC in high dose group was significantly increased than that in the control. Low onion dose group tended to have the similar effects as high dose group but, statistically, no difference was observed between the control and low dose groups. Besides, both doses of onion powder diets could significantly countered the increase in serum TG levels. High dose of onion supplementation tended to increase output of fecal neutral and acidic sterols, resulting in reduction of cholesterol retained and absorption. High dose of onion powder diet could significantly up-regulate SREBP-2, LXRβ, and CYP7A1 protein expressions. The hypocholesterolemic activities of onion might due to the richness in alkyl and alkenyl sulfoxide compounds, anthocyanin, quercetin and cycloalliin, all of which have therapeutic effects.

In conclusion, diet plays an important role in reducing the risk of CVD. This has

led to the search for specific foods and food components that may help to improve the serum lipoprotein profile. In present study, tea seed oil and onion was proved to help favorably modify the plasma lipoprotein profile, serving as health supplementation. However, their potential mechanisms were not fully studied and need to be further explored.

摘要

當前，心血管疾病依然是全球關注的重大健康問題，持續升高的發病率不僅發生在發達國家，更蔓延至發展中國家。因此有必要建立一種預防干預手段來使人們的生活更加健康和長壽。膳食在健康生活及延緩衰老中有著至關重大的影響。其中，人類膳食中的各種成分在調節脂質代謝方面起著極其重要的作用。在本實驗中，我們將會對兩種健康食品，茶籽油及洋蔥粉在改善血脂的表現及其可能的機理進行進一步的研究。

近年來，茶籽油作為一種烹飪油得到了的持續上升關注度，然而，其在血脂的影響還未得到全面具體的研究。在本次實驗中，我們首次對茶籽油在降脂方面的作用進行了較為系統的探討，並同時將其與葡萄籽油、芥花籽油和玉米油的降脂作用比較。本次的實驗是選用 50 隻 8 週齡大的雄性倉鼠，適應 1 週後以高脂高膽固醇飲食 (含有 10%豬油及額外添加 0.1%膽固醇) 餵食 2 週。之後隨機分為五組：陽性對照組繼續餵食高脂高膽固醇飲食；實驗組則是餵食高膽固醇飲食之下，分別以 10% 茶籽油、10% 葡萄籽油、10% 芥花籽油及 10% 玉米油取代豬油部分，餵養 12 週。實驗結果顯示與食用豬油的對照組對比，食用茶籽油、葡萄籽油、芥花籽油和玉米油均可以顯著降低給與高脂高膽固醇膳食的成年雄性倉鼠的血總膽固醇，非高密度脂蛋白膽固醇和甘油三酯，其中，茶籽油僅僅降低的是非高密度脂蛋白膽固醇的水準，但對高密度脂蛋白膽固醇並沒有影響，然而，葡萄籽油對兩者都有降低作用。與其他三種食用油相比，食用茶籽油的倉鼠會排出更多的酸性固醇及較少的中性固醇。此外，與葡萄籽油不同，茶籽油可以向上調控固醇調節元件結合蛋白-2(SREBP-2)和低密度脂蛋白受體(LDLR)的蛋白表達水準。茶籽油與其他植物油對血脂調節表現的不同可能部分由於茶籽油中包含的超過 80% 的油酸所造成。

現今，有很多關於蔥屬植物在促進人類健康方面的報導，然而，除了大蒜，其他蔥屬植物並沒有任何系統綜合的研究。本次實驗的另一個實驗目的即對凍幹洋蔥粉在血液調節、肝臟脂質及膽固醇穩態的相關基因表現之影響進行探索。本試驗繼續採用成年雄性倉鼠作為高血脂動物模型對凍幹洋蔥粉的降血脂效果進行了評價。將 36 只成年雄性倉鼠經高脂高膽固醇飼料給與兩周後，

隨機分為三組 (n = 12)：陽性對照組僅需給與高脂高膽固醇飼料，試驗組則以高脂高膽固醇飼料低劑量組及高劑量組，均給與高脂高膽固醇飼料 (0.1% 膽固醇，5%豬油)。兩周後，陽性對照組繼續給與高脂高膽固醇飼料，其他兩組則在高脂高膽固醇量基礎上分別加入 1% (低劑量組)及 5% (高劑量組)凍幹洋蔥粉，實驗組飼料中的其他成分按比例減少。實驗結果顯示高劑量凍幹洋蔥粉可以顯著降低高脂高膽固醇飲食倉鼠血總膽固醇，非高密度脂蛋白膽固醇及非高密度脂蛋白與高密度脂蛋白膽固醇的比例。相反，高劑量組倉鼠血中高密度脂蛋白占總膽固醇的比例也明顯增加。低劑量組倉鼠的血脂變化趨勢與高密度組一致，但與陽性對照組相比，未有顯著性差異。但兩種劑量的凍幹洋蔥粉均已顯著降低。此外，高劑量進食洋蔥粉可以增加中性及酸性固醇的排出，進而減少膽固醇在體內的保留及吸收。同時，高劑量洋蔥粉還可顯著向上調控固醇調節元件結合蛋白-2(SREBP-2)，肝 X 受體 β(LXRβ)，膽固醇 7α 羥化酶 (CYP7A1)的蛋白表達水準。洋蔥中含有豐富的蔥屬組分如，烷基及烯丙基硫醚化合物，花青素，槲皮素，環蒜氨酸等等，這些成分均具有極佳的治療效果，因此可能均對洋蔥的降脂作用起到重要作用。

膳食可以對降低心血管疾病發生率起到至關重要的作用，這一觀念早已深入人心，由此引發近年來大量研究以探求可以改善血脂的特定食物及食物成分。在本研究中，我們對茶籽油及洋蔥粉進行系統研究，證實二者都可以調節倉鼠體內脂質及膽固醇的代謝機制進而改善血脂及改善高脂血症的情形。然而機理方面的研究還不完善，還需要更進一步的探討。

List of Abbreviations

1-PeCSO	*trans*-(+)-S-(propen-1-yl)-L-cysteine sulphoxide
ABC	ATP-binding cassette
ACAT 2	Acyl-coenzyme A:cholesterol acyltransferase 2;
ACSOs	Alk(en)yl cysteine sulfoxides;
ALA	Alpha-linolenic acid;
AMD	Age-related macular degen eration;
ANOVA	Analysis of variance;
Apo A-I	Apolipoprotein A-I;
Apo B	Apolipoprotein B;
ATP	Adenosine triphosphate;
CA	Cholic acid;
CDCA	Chenodeoxycholic acid;
CE	Cholesteryl esters;
CHD	Coronary heart disease;
CRs	Chylomicron remnants;
CVD	Cardiovascular disease;
CYP7A1	Cholesterol 7α-hydroxylase;
DCA	Deoxycholic acid;
DHA	Docosahexanoic acid;
EPA	Eicosapentanoic acid;
FXR	Farnesoid X receptor;
HCA	Toxic hyocholic acid;
HDCA	Hyodeoxycholic acid;

HDL	High density lipoprotein;
HMG-CoA	3-hydroxy-3methylglutaryl coenzyme A;
HMGR	HMG-CoA (3-hydroxy-3-methylglutaryl-CoA) reductase;
IDLs	Intermediate density lipoproteins;
LCA	Lithocholic acid;
LDL	Low density lipoprotein;
LDLR	Low density lipoprotein receptor;
LXR	Liver X receptor;
MCSO	(+)-S-methyl-L-cysteine sulphoxide;
MUFA	Monounsaturated fatty acids;
NPC1L1	Niemann-Pick C1-Like protein 1;
PrCSO	(+)-S-propyl-L-cysteine sulphoxide;
PUFA	Polyunsaturated fatty acid;
PVDF	Polyvinylidene difluoride;
RCT	Reverse cholesterol transport;
SD	standard deviation;
SFA	Saturated fatty acid;
SMCS	S-methylcysteine sulfoxide;
SREBP	Sterol regulatory element-binding protein;
SREBP-2	Sterol regulatory element binding protein 2;
TFA	*Trans*-fatty acids;
TG	Triglyceride;
VLDL	Very low density lipoprotein;

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Chapter 1

General Introduction of Atherosclerosis and Functional Food

1.1 Cholesterol and atherosclerosis

1.1.1 Atherosclerosis

Atherosclerosis is still the leading cause of morbidity and mortality in the Western world, with increasing prevalence in developing countries and Eastern Europe. One of the main causes of atherosclerosis is hypercholesterolemia, which is a hallmark of the so-called "Western lifestyle", contributing to the onset and development of these cardiovascular diseases (Yusuf *et al.*, 2004). These diseases are an inflammatory, degenerative affection of the arterial wall of the large and medium arteries. It is characterized by "a) the accumulation of lipids (both saturated and unsaturated fatty acids) and cholesterol in the subendothelial space, b) infiltration of inflammatory cells and fibrous elements into the intima, and c) the proliferation of smooth muscle cells in the media" (Lecerf *et al.*, 2009). The pathologic process underling a group of syndromes can be divided into three main categories: (1) coronary heart disease (CHD) (e.g., angina pectoris, myocardial infarction), (2) cerebrovascular disease (e.g., stroke, transient ischemic attacks), and (3) peripheral arterial disease (e.g., gangrene, intermittent claudication) (Yusuf *et al.*, 2004; Plat *et al.*, 2005). Indeed there is a linear relationship between the risk factor cholesterol and protection from atherosclerosis (Levine *et al.*, 1995). Hypertriglyceridemia is also a major risk factor for atherosclerosis (Assmann and Schulte, 1992).

1.1.2 Cholesterol

Cholesterol, the predominant sterol found in humans, is present in all animal tissues. It serves as a structural element in all cell membranes where it occupies the spaces between the polar headgroups of the phospholipid molecular bilayer, reducing its fluidity (Myant, 1981). Whilst the remainder is transported via the blood and acts as a precursor for other steroid based molecules such as sex hormones, steroid hormones, vitamin D or bile acids. The principle sites of cholesterol biosynthesis are

the liver and CNS. The major sterol found in mammals is the C-27 compound cholesterol (Tapiero, 2003). In animals the triterpenoid alcohol lanosterol is converted into cholesterol, a process requiring the loss of three methyl groups, reduction of the side-chain double bond and generation of a $\triangle^{5,6}$ double bond in place of the $\triangle^{8,9}$ double bond (Fig 1.1).

Major contributors to the amount of cholesterol in plasma include exogenous cholesterol absorbed through the gastrointestinal tract and endogenously synthesized cholesterol. As much as 60% of the cholesterol entering the body each day is derived from the diet. In addition, the amount of dietary cholesterol absorbed also influences endogenous cholesterol biosynthesis in the liver (Hui *et al.*, 2008).

Cholesterol homeostasis in mammals is maintained through the coordinate regulation of three major metabolic pathways in the liver. Endogenous biosynthetic pathway ensures the supply of cholesterol to cell, by converting acetate into cholesterol; and in exogenous pathway, the members of the low-density lipoprotein (LDL)-receptor family bind and internalize cholesterol-carrying particles from the blood; the third pathway involves the conversion of cholesterol into bile acids for catabolism (Turkey and Dietschy, 1988).

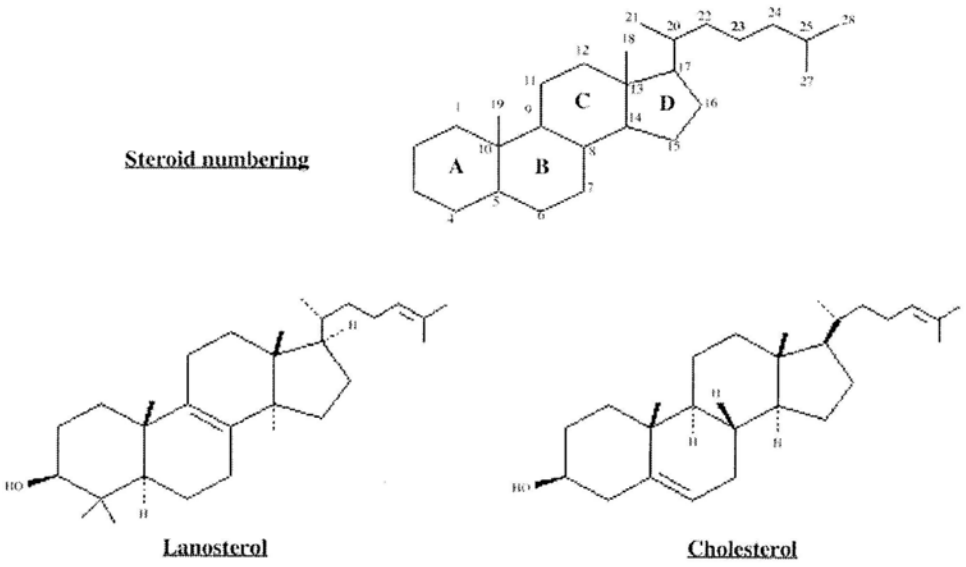

Figure 1.1 Chemical structure of cholesterol and lanosterol (Tapiero *et al.*, 2003)

1.2 Cholesterol in body

1.2.1 Absorption

Absorption is defined as "the transport of dietary fat and fat-soluble vitamins from the intestinal lumen to the plasma" (Hussain *et al.*, 2005). Absorption consists of three major steps. First, dietary fat is emulsified and hydrolyzed in the lumen of the intestine. Second, hydrolyzed products are taken up by enterocytes. Third, fat is re-synthesized in enterocytes and packaged into lipoproteins and secreted (Hussain *et al.*, 2005).

Lipid, from the oral cavity to the duodenum, undergo the process called early lipid digestion, producing crude emulsions including free cholesterol, triglycerides, free fatty acids, and phospholipids. Cholesterol absorption is achieved through passage across brush border membranes and into intestinal entercytes in the jejunum. In intestine, they are mixed with bile salt micelles, which are synthesized by liver to catalyze lipid emulsification into smaller droplets, which are more easily interacted with lipase enzymes (Young and Hui, 1999).

There are two major sources of the cholesterol in the intestinal tract available for absorption: the diet and the bile. The amount of cholesterol absorbed from the diet is a major contributor to the levels of cholesterol in circulation. Whereas dietary intake ranges from < 50 mg/day (pure vegetarians) to 750 mg/day, biliary cholesterol input is 3 to 10 times higher and ranges from 500 to 2400 mg/day (Mok *et al.*, 1979). About 30~60% of dietary cholesterol is absorbed through intestinal enterocytes, while the rest can be lost from the body as the fractions of bile salts and intestinal cholesterol which are not absorbed and excreted through feces (Ostland *et al.*, 1999).

1.2.2 Metabolism

Cholesterol metabolism is mainly regulated in the small intestine and the liver. This, in turn, affects the distribution of cholesterol between the liver and the blood. Low uptake of cholesterol or bile acids from the small intestine results in low levels of cholesterol in the hepatocytes, which leads to a compensatory hepatic up-regulation of LDL receptors, and consequently, achieves a normal cholesterol level in the body.

Liver plays a central role in maintaining cholesterol homeostasis by balancing multiple pathways including *de novo* cholesterol and bile acid synthesis, dietary

cholesterol uptake, biliary cholesterol excretion, lipoprotein synthesis, and reverse cholesterol transport (RCT) (Li and Chiang, 2009). Cholesterol is converted into bile acids by pathways that involve 17 different enzymes which are mostly expressed in the liver, and the immediate products of these pathways are referred to as primary bile acids. In fact, the structures of these bile acids vary widely among different vertebrate species. For instance, in human and rats, both cholic acid (CA) and chenodeoxycholic acid (CDCA) are the primary bile acids, whereas in mice, both cholic acid and β-muricholic acid predominate (Hylemon and Harder, 1998).

1.2.2.1 Hepatic bile acid synthesis

Approximately 500mg of cholesterol is converted into bile acids each day in adult human liver. Newly synthesized bile acids are secreted into the bile and delivered to the lumen of the small intestine where they serve as detergents for the absorption of dietary lipids, cholesterol, and fat-soluble vitamins. The solubilized nutrients are incorporated into lipoproteins, which are delivered to the liver and metabolized (Russell, 2003).

In humans, bile acid pool consists of primary bile acids (cholic acid [CA], and chenodeoxycholic acid [CDCA]) and secondary bile acids (deoxycholic acid [DCA], and lithocholic acid [LCA]) (Russell and Setchell, 1992). Primary bile acids are synthesized from cholesterol exclusively in the liver through two general pathways, namely, the classic and the alternative pathways (Chiang, 2002). The classic pathway is also known as the neutral pathway for most of its intermediates are neutral sterol. Cholesterol 7-α-hydroxylase (CYP7A1) catalyzed the first and rate-limiting step in the classic pathway to convert cholesterol into 7α-hydroxycholesterol (Myant and Mitropoulos, 1977). The alternative pathway, also called the acidic pathway, was originally revealed by the identification of several acidic intermediates which are not present in the classic pathway. The alternative pathway mainly produces CDCA (Axelson and Sjövall, 1990; Axelson *et al.*, 1988). In humans, the classic pathway is thought to be the major bile acid biosynthesis pathway in normal physiologic status.

Secondary bile acids are derived from primary bile acids in the intestine, catalyzed by bacterial enzymes which are located in the endoplasmic reticulum, mitochondria, cytosol, and peroxisomes (Chiang, 2002).

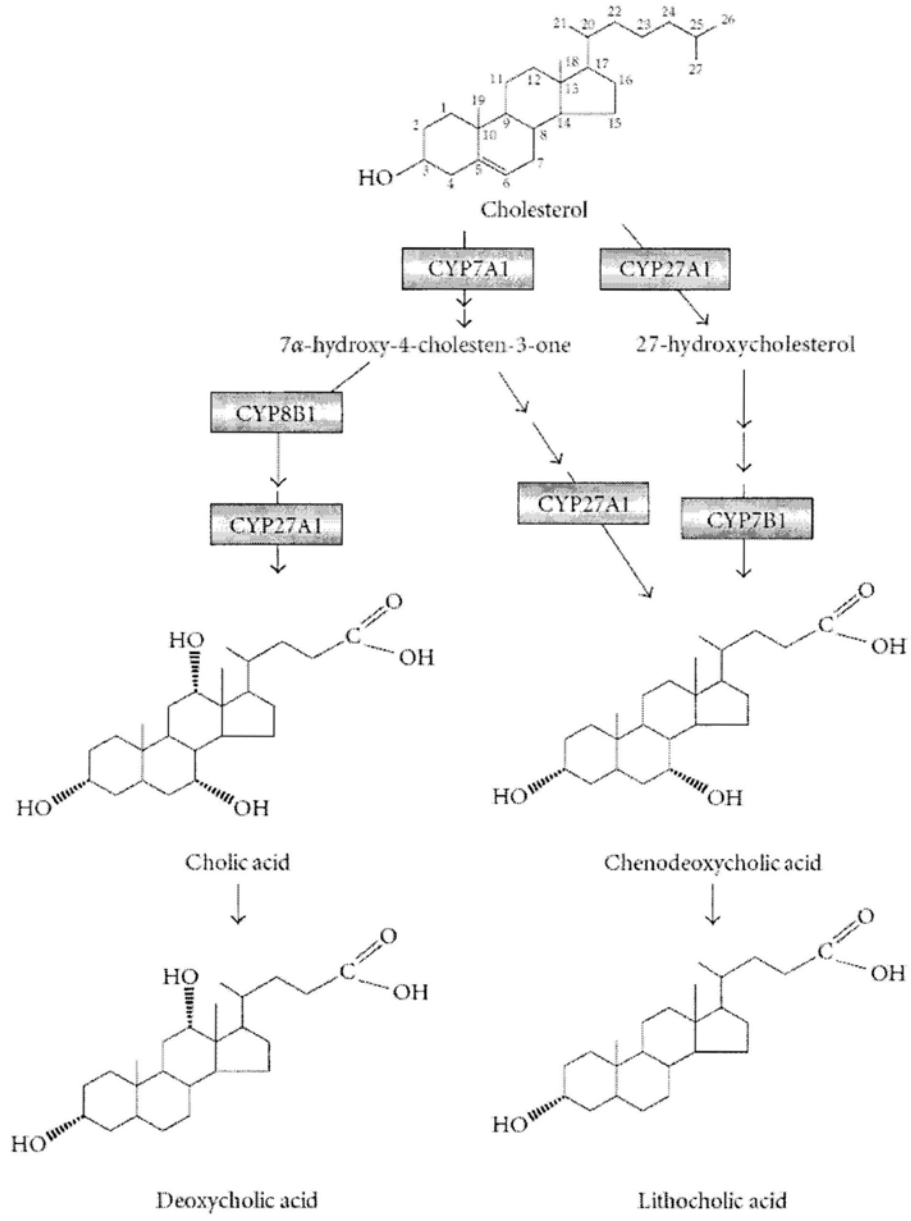

Figure 1.2 Bile acid synthesis. Bile acids are synthesized from cholesterol in the liver through two pathways: the classic pathway and the alternative pathway. In human liver, bile acid synthesis mainly produces two primary bile acids, CA, and CDCA. Key regulatory enzymes in both pathways are indicated. CYP7A1 catalyzes the first the rate-limiting step in the classic pathway to convert cholesterol into 7α-hydroxycholesterol, while CYP27A1 initiates the alternative pathway by converting cholesterol into 27-hydroxycholesterol, which is then 7α-hydroxylated by oxysterol 7α-hydroxylase (CYP7B1). CYP8B1 regulates the CA synthesis in the classic pathway. In the intestine, primary bile acid CA and CDCA are dehydroxylated at the 7α-position by the bacterial enzymes to produce the secondary bile acids, DCA, and LCA, respectively (Li and Chiang, 2009).

1.2.2.2 Neutral sterol excretion

Fecal excretion of total neutral sterols has been reported to range between 350~900 mg/day with a mean of 700 mg/day. Cholesterol accounts for about 20% of total daily neutral sterol output or 150 mg/day. The range of cholesterol excreted is between 75 to 200 mg/day. There are three primary sources of fecal cholesterol: 1) unabsorbed cholesterol from the diet, which accounts for 20%, 2) bile, which contribute the bulk of the cholesterol or 67%, and 3) and the intestinal epithelium or lining of the small intestine which constitute the remaining 13% of the cholesterol (Ferezou *et al.*, 1981).

1.2.2.2.1 Mechanism for intestinal cholesterol bacterial metabolism

Intestinal bacterial microflora is responsible for the cholesterol metabolism. The possible mechanism involves "an indirect pathway with 4-cholesten-3-one as the intermediate. This compound is formed by the oxidation of the 3 beta-hydroxyl group to a ketone and isomerization of the 5-6 double bonds to the 4-5 position. Coprostanone is formed by the reduction of the 4-5 double bonds. And, coprostanol is formed by the reduction of the 3-keto to a hydroxyl group" (Lichtenstein, 1990). The concentration of coprostanol is much higher than coprostanone (Eyssen and Parmentier, 1974).

It was suggested that the composition of the intestinal microflora was dependent on diet. Variation of dietary intakes influenced intestinal secretions and the substrates available for the bacterial metabolism (Aries *et al.*, 1969).

1.2.2.2.2 Compositions of fecal neutral sterols

The compositions of the cholesterol and its metabolites found in human feces are as follows: 1) cholesterol, which constitutes about 20% of the neutral sterol concentration; 2) coprostanol, accounting for 65% of the bulk; 3) coprostanone, making up 10%; 4) cholestanol and cholestanone (5 alpha compounds); and 5) epicoprostanol (3 alpha hydroxyl compound), the latter three compounds contributes about 5% of the neutral sterols in feces, collectively (Raddy *et al.*, 1977).

1.3 Cholesterol regulation in the body

Cholesterol homeostasis of the whole body requires precise regulation of processes that control *de novo* cholesterol genesis, cholesterol absorption and cholesterol excretion. An imbalance of these processes may lead to elevated plasma cholesterol levels, cholesterol accumulation in various tissues, and therefore increased risk of cardiovascular diseases (CVD) (Calpe-Beerdiel *et al.*, 2009).

1.3.1 Lipoproteins, cholesterol homeostasis, and cardiac health

Intestinal lipoprotein assembly is essential for the absorption of dietary fat and fat-soluble vitamins. While in circulation, cholesterol, being a lipid, requires a transport vesicle to shield it from the aqueous nature of plasma, in such case, cholesterol circulates as a component of lipoproteins. Lipoprotein particles are not only heterogenous in composition, which composed of the stored forms of fatty acids and cholesterol (triglycerides and cholesteryl esters, respectively), and amphipathic phospholipids, but also heterogeneous in size, shape, function, and perhaps most importantly, their contribution to vascular disease (Tyler *et al.*, 2009; Daniels *et al.*, 2009). The concentration of lipoprotein in humans is typically in the range 100-300 mg/dL (2.5-7.5 mmol/L).

Cholesterol usually has undergone a maturation process, beginning with the hepatic or intestinal synthesis of very low density lipoprotein (VLDL), before it reaches a particle of the HDL or LDL sub-fraction. There are six major sub-fractions of lipoproteins, including chylomicrons, chylomicron remnants (CRs), intermediate density lipoproteins (IDLs), VLDLs, LDLs and high density lipoproteins (HDLs) (Daniels *et al.*, 2009).

1.3.1.1 Chylomicron

Once inside enterocytes, dietary cholesterol is packaged into chylomicrons, and secreted by enterocytes into the lacteals of the intestine, and put into circulation from lymph via the thoracic duct. This process is initiated by the esterification of large amounts of free cholesterol by the cholesteryl transferase protein and the synthesis of triglycerides from free fatty acids by mono- and di-acylglycerol acyltransferase (Purdy and Field, 1984; Hui and Howles, 2005). Chylomicron is rich in triglyceride. While the digestion and packaging of dietary lipids into chylomicrons take about one

hour, the half-life of lipids in chylomicrons is only 4.5 minutes (Grundy and Mok, 1976).

1.3.1.2 Very low density lipoprotein (VLDL)

The assembly of VLDL begins inside the rough endoplasmic reticulum of hepatocytes, at the site of apoB-100 translation. Just like chylomicrons, VLDLs distribute free fatty acids to muscle and adipose tissues. When remodeled VLDL particles lose triglycerides, they become IDLs that either are removed by the liver or are subjected to further lipase activity and develop into LDL (Daniels *et al.*, 2009).

1.3.1.3 Low density lipoprotein (LDL)

LDL, the classic antagonists of circulatory system, does not always reach its most appropriate destination, but rather accumulates in the connective tissue in the intimal sub-layer of artery walls causing atherosclerosis. Thus, the quantity of circulating LDL is a well-known risk factor for heart disease, and is the primary focus of most lipid lowering therapies (Mourão and Bracamonte, 1984; National Cholesterol Education Program, 2001).

1.3.1.4 High density lipoproteins (HDL)

In humans, HDL particles extract cholesterol from tissues, including atherosclerotic plaques, and deliver them back to the liver to promote vascular heath (Mourão and Bracamonte, 1984). The levels of HDL are very well known measurement of cardiac health due to their strong inverse relationship with coronary heart disease (Wilson *et al.*, 1988; Stamler *et al.*, 2000). The principle HDL pathway, termed reverse cholesterol transport (RCT) contributes major effects to lipid homeostasis.

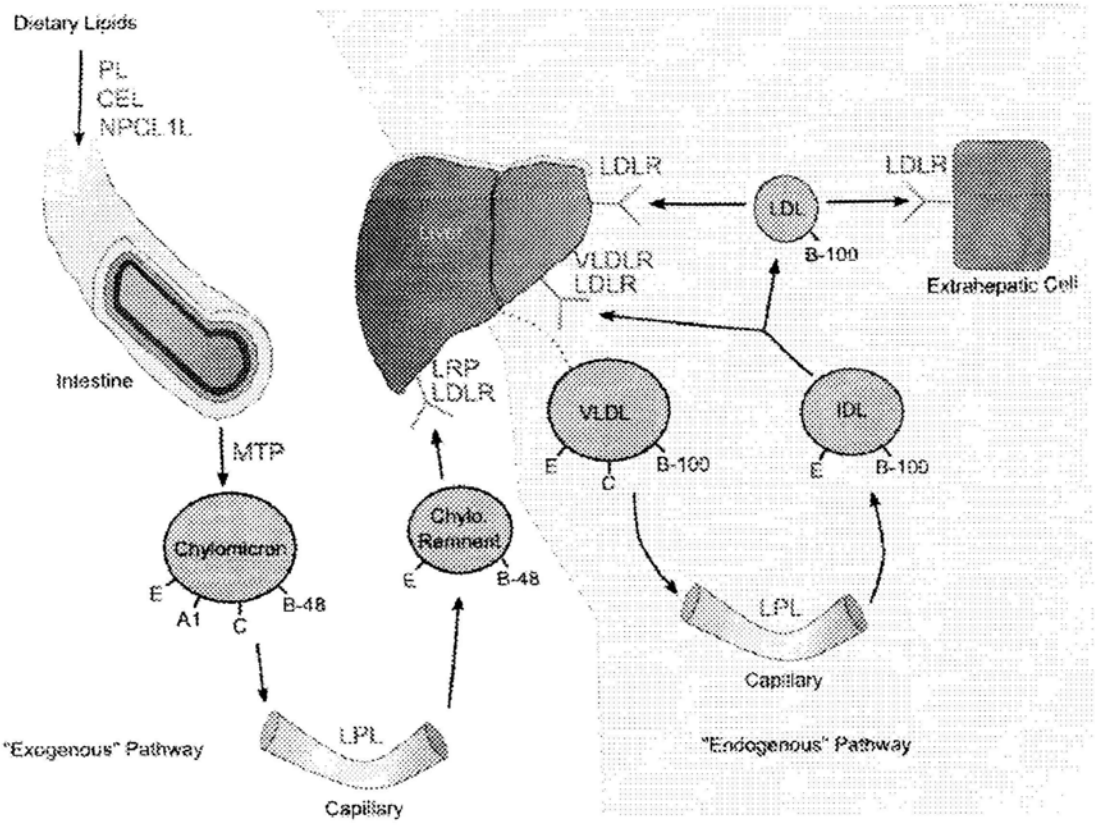

Figure 1.3 Transport of exogenous cholesterol and *de novo* cholesterol requires a diversity of lipoprotein and proteins (Daniels *et al.*, 2009).

1.3.1.5 Lipoprotein ratio: Physiological significance and clinical usefulness in cardiovascular prevention

Although atherogenesis is a multifactorial process, abnormality in lipoprotein metabolism is one of the key factors, "representing around 50% of the population-attributable risk of developing cardiovascular disease" (Yusuf *et al.*, 2004). In an attempt to optimize the predictive capacity of the lipid profile, several lipoprotein ratios or "atherogenic indices" were defined. These ratios can provide information to better mirror the metabolic and clinical interactions between lipid fractions as described in the following paragraphs.

1.3.1.5.1 Total cholesterol/HDL cholesterol and LDL/HDL cholesterol ratios

The total/high-density lipoprotein (HDL) cholesterol ratio, also known as the atherogenic or Castelli index, was regarded as important components and indicators of vascular risk, as well as the LDL/HDL, its discriminatory power and predictive value are greater than the isolated parameters (Millán *et al.*, 2009).

Like the total/HDL cholesterol ratio, LDL/HDL cholesterol ratio may have more predictive power if triglyceridemia is taken into account (Pinto and Ros, 2000). Their similarity can be explained by the fact that approximately two-thirds of the plasma cholesterol is found in LDL cholesterol, and consequently, total and LDL cholesterols are closely related (Millán *et al.*, 2009).

In this respect, an increase in total cholesterol, especially, in LDL cholesterol and decrease in HDL cholesterol result in enhanced levels of total cholesterol/HDL cholesterol and LDL/HDL cholesterol ratios, predicting a greater cardiovascular risk. The risk is remarkably higher when hypertriglyceridemia is present (Manninen *et al.*, 1992).

1.3.1.5.2 Logarithmic transformation of the triglyceride/HDL cholesterol molar concentration ratio

Atherogenic plasma index shows a positive correlation with the HDL esterification rate (FER_{HDL}) and an inverse correlation with LDL size (Dobiásová and Frohlich, 2001). Simultaneous use of triglycerides and HDL cholesterol in this ratio reflects the complex interactions of lipoprotein metabolism overall and can be useful for predicting plasma atherogenicity (Dobiásová, 2004).

1.3.1.5.3 Non-HDL cholesterol/HDL cholesterol ratio

Non-HDL cholesterol, which is the total cholesterol minus the HDL cholesterol, is a measure of the cholesterol in LDL, IDL, and VLDL particles. The non-HDL cholesterol/HDL ratio is a lineal combination of total/HDL cholesterol (Millán *et al.*, 2009).

1.3.1.5.4 LDL cholesterol/ Apolipoprotein B (ApoB) ratio

Apolipoprotein B (ApoB) is present not only in LDL, but also found in other atherogenic lipoprotein fractions such as IDL and VLDL, rather than HDL. However, the LDL cholesterol/ApoB concentration ratio provides approximate information on LDL particle size (Millán *et al.*, 2009).

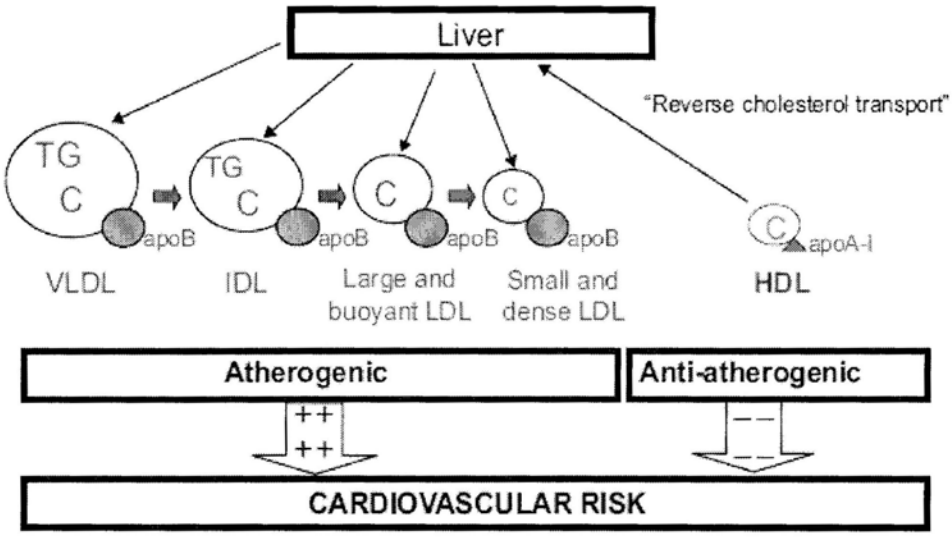

Figure 1.4 Atherogenic and anti-atherogenic lipoproteins. This diagram shows that there is one single apolipoprotein B (apoB) molecule in VLDL, IDL, and LDL. Therefore, apoB represents the total number of potentially atherogenic particles. Whereas, Apolipoprotein A-I (apo A-I) is the principal protein component in HDL and is responsible for starting RCT. The balance between apoB and apoA-I is indicative of cardiovascular risk: the greater the ratio, the greater the risk (Millán *et al.*, 2009).

Abbreviations: TG, triglycerides; C, cholesterol; + +, increased risk; - -, reduced risk.

1.3.2 Regulation of bile acid synthesis and oxysterols in cholesterol homeostasis

1.3.2.1 Bile acid feedback regulation of bile acid synthesis

Bile acid, once synthesized and excreted from the liver, are transported across the canalicular membrane of the hepatocytes into the bile and stored in the gall bladder. After each meal, bile acids are released into the intestinal tract for digestion of fats, efficiently reabsorbed in the ileum, and transported back to the liver via portal circulation for re-excretion into the bile. This process is referred to as enterohepatic circulation of the bile (Chiang *et al.*, 1998). However, primary bile acids, CA and CDCA, escaping reabsorption are converted to their secondary bile acids, DCA and LCA, respectively, a portion of which is also absorbed by 7α-dehydrolase in the bacterial flora. Back to the liver, amphipathic bile acids are efficiently 6-hydroxylated by the cytochrome P450 (CYP)3A4 enzyme into the less toxic hyocholic (HCA) and hyodeoxycholic acids (HDCA) (Trottier *et al.*, 2006). About 95% of the bile acids are reabsorbed after reaching the small intestine in humans, and only 5% are lost in feces, which are replenished by *de novo* bile acid synthesis (Bahar and Stolz, 1999).

1.3.2.2 Cholesterol homeostasis in the liver

Cholesterol is important for synthesis of bile acids, biological membranes, and steroid hormones, and its homeostasis needs to be maintained in body. Cholesterol directly affects two enzymes which are critical in its own removal. It activates acyl-CoA: cholesterol acyl transferase (ACAT), the enzyme required to synthesize cholesterol esters, which are the major storage form of cholesterol, and cholesterol also activates cholesterol 7α-hydroxylase (CYP7A1), the initial and rate-limiting enzyme of bile acid synthesis, the leading pathway of cholesterol elimination (Jackson *et al.*, 1997). Liver is vital in maintaining bile acid and cholesterol homeostasis. Interruption of the enterohepatic circulation of bile acids leads to an increase in bile acid synthesis and a reduction of plasma LDL cholesterol level (Cohen, 1999; Einarsson and Angelin, 1991). Elevated input of cholesterol and decreased output of bile acids may cause hypercholesterolemia, atherosclerosis, cholestasis, and cholelithiasis in humans (Hofmann, 1999; Jansen *et al.*, 2001).

1.3.2.3 Oxysterol regulation of cholesterol homeostasis

Many hydroxylated derivatives of cholesterol, known as the oxysterols, share with cholesterol as the potent regulators of cholesterol synthesis and lipid metabolism. Oxysterols are the intermediates of the cholesterol and bile acid synthesis pathways by either enzymatic or non-enzymatic oxidation (Kandutsch and Chen, 1974; Schroepfer *et al.*, 2000). In atherosclerotic plaque, 27-hydroxycholesterol, 7-ketocholesterol, and 7β-, 7α-hydroxycholesterol are the most abundant oxysterols. They cause foam cell formation from macrophages and lead to atherosclerosis in humans (Brown and Jessup, 1999). Some oxysterols in peripheral tissues are excreted to circulation, transported to the liver, and converted to bile acids. This feedback process to down-regulate cholesterol synthesis has been suggested as a defense against atherosclerosis analogous to RCT in humans (Bjorkhem *et al.*, 1994; Bjorkhem *et al.*, 1999).

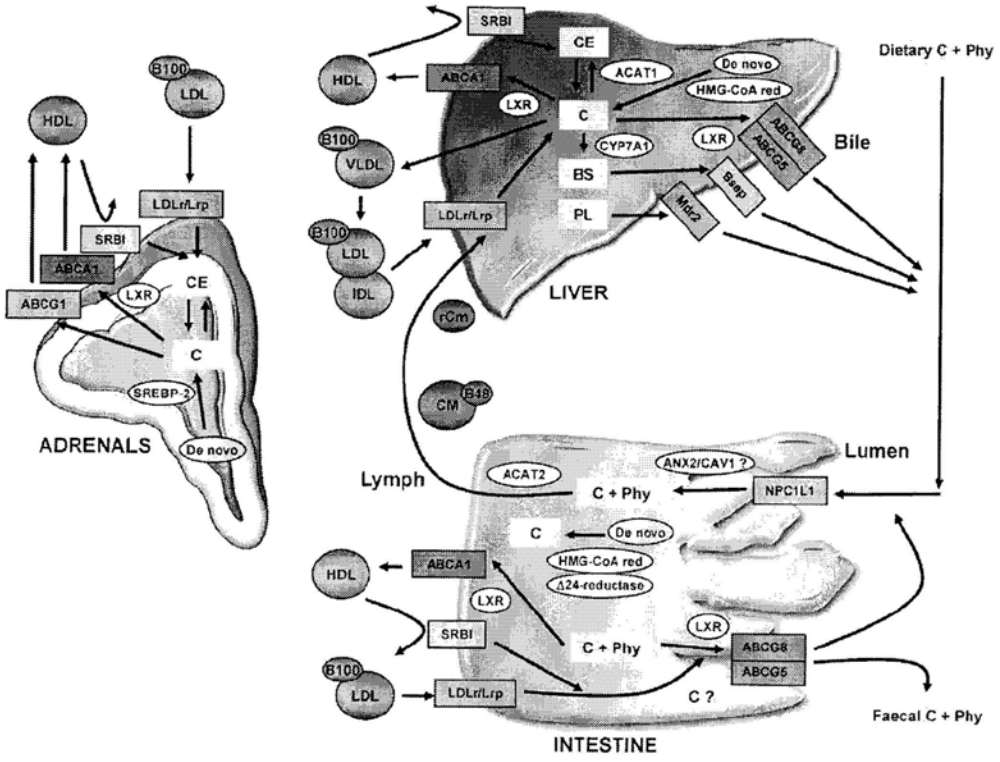

Figure 1.5 Schematic overview of the major routes of cholesterol in the human body (Calpe-Beerdiel *et al.*, 2009).

1.3.3 Molecular regulation of cholesterol in body

1.3.3.1 Transporters in liver

1.3.3.1.1 Liver X receptors (LXRs)

Liver X receptors (LXRs) have emerged as key regulators of cholesterol and lipid metabolism (Barish and Evans, 2004; Li and Glass, 2004; Tontonoz and Mangelsdorf, 2003). There are two LXRs, α and β, sharing considerable sequence homology at about 77%, and appearing to respond to the same endogenous ligands (Willy *et al.*, 1995; Tontonoz and Mangelsdorf, 2003). LXRβ is ubiquitously expressed, whereas LXRα is highly expressed in liver, intestine, adipose tissue, and macrophages. Both LXRα and LXRβ are activated by physiological concentrations of oxysterols, such as 22(R)-hydroxycholesterol, 24(S)-hydroxycholesterol, 27-hydroxycholesterol, and 24(S), 25-epoxycholesterol (Xu *et al.*, 2001; Janowski *et al.*, 1996; Lehmann *et al.*, 1997).

LXRs bind to their target DNA sequences in heterodimeric complexes with the retinoid X receptor (RXR). LXR/RXR is a so-called permissive heterodimer, in that it can be activated by ligands for either LXR or RXR (Willy *et al.*, 1995). The first LXR target to be identified was CYP7A1, the enzyme that catalyzes the rate-limiting step in bile acid synthesis. Considerable evidences implicate members of the Adenosine triphosphate (ATP) binding cassette (ABC) transporter family is involved in these LXR effects. Initial studies postulated that ABCA1 may be the key target responsible for LXR inhibition of cholesterol absorption; however, recent studies suggested that ABCG5/8 may play a more prominent role in this effect (Graf *et al.*, 2002; Yu *et al.*, 2002).

Lack of both LXR isoforms, which are act as global regulator of cholesterol homeostasis, increases aortic foam cell formation as a precursor for atherosclerosis (Schuster *et al.*, 2002). In the liver, LXRs regulate lipid metabolism, but, LXRα is more important for cholesterol metabolism and LXRβ plays a role in triglyceride metabolism (Michael *et al.*, 2005).

1.3.3.1.2 Cholesterol-regulated control of sterol regulatory element-binding protein (SREBPs)

The intracellular and membrane levels of fatty acids and cholesterol are under constant surveillance coordinated with *de novo* lipid biosynthesis controlled by ER-

bound SREBPs (Bengoechea-Alonso and Ericsson, 2007; Eberlé *et al.*, 2004). At physiological level, SREBPs play an important role in regulation of expression of the LDL receptor, which enables the hepatocytes to remove LDL-cholesterol from the blood. In cholesterol-depleted cells, the SREBPs are transported to the Golgi complex where they are processed by two proteases to release a soluble fragment that enters the nucleus where it activates transcription of the genes encoding HMG-CoA reductase and all the other enzymes of cholesterol biosynthesis as well as the LDL receptor (Horton *et al.*, 2002). While high cholesterol intake prevents SREBPs maturation and cut of both cholesterol and LDL receptor synthesis, resulting in high blood cholesterol and imminent danger of atherosclerotic plaque formation (Weber *et al.*, 2004).

SREBP-activated genes predominantly belong to lipid metabolism pathways, viz, cholesterogenesis, fatty acid synthesis, lipogenesis, triglyceride and phospholipid synthesis, but also glucose metabolism. It has been discovered that SREBPs-1a and -1c actions favor fatty acid synthesis, whereas SREBP-2 action favors cholesterol synthesis (Guan *et al.*, 1997; Guan *et al.*, 1998).

1.3.3.1.3 Cholesterol 7á-hydroxylase (CYP7A1)

The main bile acid biosynthetic pathway is initiated by cholesterol 7α-hydroxylase (CYP7A1), a cytochrome P450 isozyme of the CYP7A family, by catalyzing the hydroxylation of cholesterol at the 7α position. Transcriptional activity of CYP7A1 controls the efficacy of the cholesterol catabolic pathway, and is crucial to hepatic cholesterol homeostasis. Meanwhile, transcription of CYP7A1 is also inhibited by bile acid feedback and is stimulated by dietary factors such as cholesterol (Russell and Setchell, 1992; Chiang, 1998). Farnesoid X receptor (FXR), one of the many important transcriptional regulators, acting as a bile acid sensor, suppress CYP7A1 activity when hepatic concentrations of bile salts are high (Wang *et al.*, 1999).

1.3.3.1.4 Hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase

The endoplasmic reticulum-bound enzyme, 3-hydroxy-3methylglutaryl coenzyme A (HMG-CoA) reductase, is generally regarded as catalyzing the rate-limiting step in the synthesis of cholesterol (Bucher *et al.*, 1960). Impairment in the synthesis of cholesterol, resulting in very low serum and tissue cholesterol level,

occurs in patients with the Smith-Lemli-Opitz syndrome, characterized with congenital heart disease, delayed myelinization, jaundice, etc (Irons *et al.*, 1994). On the other hand, increased serum cholesterol levels predispose people to atherosclerotic vascular disease.

Although HMG-CoA reductase is found in nearly all tissues, only liver expresses the highest levels of this enzyme, and the most importantly, its feedback regulation by cholesterol also occurs mainly in liver (Spady and Dietschy, 1983). Decreased hepatic HMG-CoA reductase activity in response to an increase in cholesterol reaching the liver *via* chylomicron remnants provides an effective means to maintain desirable cholesterol levels (Kita *et al.*, 1982). Thus, a high expression level of hepatic HMG-CoA reductase can serve to buffer excess dietary cholesterol. Interestingly, the Sprague-Dawley rat has a high level of hepatic cholesterol biosynthesis and is known to be rather resistant to dietary cholesterol; in contrast, rabbits and hamsters exhibit low levels and are very sensitive to dietary cholesterol (Spady and Dietschy, 1983).

1.3.3.1.5 LDL receptors (LDLR)

The existence of the postulated LDL receptor was formally demonstrated in 1974 (Goldstein, and Brown, 1974). It was revealed that the receptor-bound LDL remained on the surface of normal cells for less than 10 minutes on average, within this time most of the surface-bound LDL particles entered the cells (Goldstein *et al.*, 1976). Meanwhile, the cholesteryl esters of LDL were hydrolyzed, generating unesterified cholesterol which remained within the cells (Brown[a] *et al.*, 1975).

Lysosome is the only cellular organelle in which LDL could have been degraded completely and rapidly. This LDL-derived cholesterol within the lysosome proved to be the agent responsible for suppressing HMG-CoA reductase activity by blocking SREBP pathway, by which LDL also suppresses transcription of the LDL receptor gene. These activities allow cells to adjust the number of LDL receptors to provide sufficient cholesterol for metabolic needs without causing cholesterol over accumulation (Brown and Goldstein, 1999). The LDL-derived cholesterol also activates ACAT so that excess cholesterol can be stored as cholesteryl ester in the cytoplasm (Brown[b] *et al.*, 1975).

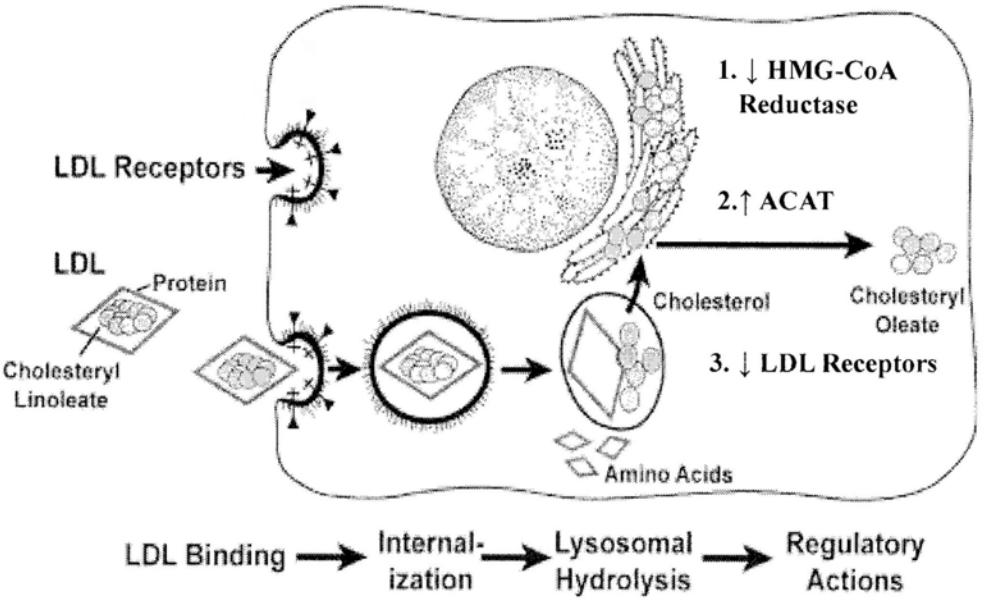

Figure 1.6 Sequential steps in the LDL receptor pathway of mammalian cells (Brown and Goldstein, 1979)

1.3.3.2 Transporters for intestinal cholesterol absorption

1.3.3.2.1 Niemann-Pick C1-Like protein 1 (NPC1L1)

Recently, NPC1L1, considered as the target of the cholesterol absorption inhibitor ezetimibe, was reported as intestinal cholesterol transporter and was shown to be responsible for the majority of both cholesterol and structurally related phytosterol uptake into enterocytes (Altmann *et al.*, 2004; Davis *et al.*, 2004). When assessing the NPC1L1 mRNA expression in human tissues, Altmann *et al.* (2004) found the highest levels of it expressed not only in small intestine, gall bladder, and stomach, but also in other tissues, such as heart, lung, and colon. But among the rodents including mice, hamsters, and rats, NPC1L1 is primarily found in the small intestine. In intestine, NPC1L1, as the putative transporter of cholesterol, is expressed on the brush border membranes of the absorptive jejunal enterocytes paralleling that of maximum cholesterol absorption. Naturally occurring mutations in human NPC1L1 are associated with reduced cholesterol absorption and circulating levels of LDL (Altmann *et al.*, 2004; Cohen *et al.*, 2006).

NPC1L1 gene expression is downregulated by LXR activators in the intestine suggesting a role for LXR nuclear factors in the regulation of NPC1L1 expression (Duval *et al.*, 2006). More recently, in a SREBP-2-dependant mechanism, the modulation of human NPC1L1 expression and promoter activity by cholesterol was demonstrated (Alrefai *et al.*, 2007).

1.3.3.2.2 Adenosine triphosphate (ATP) binding cassette (ABC) half-transporters: ABCG5 and ABCG8

Two ATP-binding cassette (ABC) half transporters ABCG5 and ABCG8 are predominantly and coordinately expressed in the liver and intestine, while their expressions are highest in the intestine with higher level in duodenum and jejunum than that in the ileum, and ABCG5/8 heterodimers limit sterol absorption by pumping sterols from the intestinal epithelium into the lumen (Lee *et al.*, 2001; Calpe-Berdiel *et al.*, 2009). It was found that in the ABCG5-/- and ABCG8-/- mice, an increased absorption of dietary sterols and impaired biliary sterol excretion were observed compared with their wild-type littermates, with 30-fold higher level of plasma sitosterol (Yu *et al.*, 2002). The expression of ABCG5/8 seems to be controlled by dietary cholesterol via LXRs. Dietary cholesterol causes an increase in the expression of the ABCG5 and ABCG8 transporters in mice which is controlled

by the activation of LXRs (Berge *et al.*, 2000; Repa *et al.*, 2002).

The function of ABCG5/8 is required to export phytosterols into the bile and intestinal lumen, respectively. Mutations of these leads to the excessive accumulation of plant sterols, a disorder called sitosterolaemia, in which phytosterols accumulate in tendons and arteries (Durrington, 2007).

1.3.3.2.3 Acyl-coenzyme A:cholesterol acyltransferase 2 (ACAT 2)

In mammals, excess cellular cholesterol is stored as cholesteryl esters. The conversion of cholesterol to cholesteryl esters (CE) is catalyzed by the action of enzyme acyl-coenzyme A (CoA): cholesterol acyltransferase 2 (ACAT 2). CE is then released into lymph associated with chylomicrons. Usually, CE is present only in low levels, mainly as cytoplasmic lipid droplets in most cell types. In plasma, CE is part of the neutral lipid vehicle present in the intestinal chylomicrons and in the hepatic VLDL. In steroidogenic tissues such as adrenals, CE serves as the cholesterol reservoir for producing steroid hormones. In the disease atherosclerosis, chronic accumulation of CE in macrophages causes these cells to appear foamy, thus, CE level is a hallmark of early stages in atherosclerosis. For reasons described above, the inhibition of intracellular esterification of cholesterol as a means to prevent the arterial CE accumulation in atherosclerosis has been a potential preventive and therapeutic strategy for many years. Therefore, ACAT 2 has been considered as a drug target for treatment against atherosclerosis and other human diseases (Chang *et al.*, 2009).

The expression of the ACAT 2 protein is limited to only two cell types, the small intestinal enterocytes and hepatocytes (Lee *et al.*, 2000). ACAT 2 in the enterocytes has been hypothesized to play a crucial role in the absorption of cholesterol into the body by esterification of newly absorbed dietary cholesterol to incorporate CE product into the core of chylomicron particles, which are subsequently secreted into the circulation (Klein and Rudel, 1983; Dawson and Rudel, 1999). It has been demonstrated that cholesterol and phytosterols are transported into the enterocytes through NPC1L1 (Davis *et al.*, 2004). However, once in the enterocytes, about 50% of dietary cholesterol is absorbed into the circulation, while less than 5% of the dietary phytosterols are absorbed. Recent discovery showed that once in the intestine, ACAT 2 acts as "gate keeper", preferentially esterifies cholesterol instead of phytosterols to limit the absorption of phytosterol (Temel *et al.*, 2003). The function

of ACAT 2 in the hepatocyte is to provide CE for incorporation into VLDL, as well as for formation of cytoplasmic lipid droplets as a mean for storage in response to hepatic cholesterol excess (Rudel *et al.*, 2005).

1.3.3.2.4 Microsomal triacylglycerol transfer protein (MTP)

MTP, a heterodimeric lipid transfer protein, is present in the lumen of the endoplasmic reticulum of both liver and intestine and is involved in the assembly of very low-density lipoprotein (VLDL) and chylomicrons (Wetterau and Zilversmit, 1986; Gordon and Jamil, 2000). MTP is crucial for the first step of lipoprotein assembly, since it transfers lipids and binds to apoB (Hussain *et al.*, 2003).

1.4 Functional foods

1.4.1 History and definition of functional foods

That foods might provide the therapeutic benefits is clearly not a new concept. The tenet, "*Let food be thy medicine and medicine be thy food*" was embraced about 2500 years ago by Hippocrates, the father of medicine. However, this "food as medicine" philosophy fell into relative obscurity in the 19th century with the advent of modern drug therapy. Until the 1900s, the important role of diet in disease prevention and health promotion was brought to the foreground once again (Hasler, 2002). "Food has been thought as the primary vehicle to transport us along the road to optimal health and wellness" (Hasler, 2000).

Functional foods have been defined as foods that, by virtue of the presence of physiologically-active component, provide a health benefit beyond basic nutrition (International Life Sciences Institute, 1999). Another term often used interchangeably with functional foods, although it is less favored by consumers, is "nutraceuticals", a term coined in 1991 by the Foundation of Innovation in Medicine to refer to nearly any bioactive component that delivers a health benefit. According to the Food and Nutrition Board of the Institute of Medicine, a functional food is "any food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains" (Wildman, 2001).

Currently, dozens of physiologically-active functional food components, from both plants and animals (known as phytochemicals and zoochemicals, respectively) that potentially could reduce risk for a variety of chronic diseases, including cancer,

heart disease, osteoporosis, arthritis and age-related macular degeneration (Hasler, 1998).

1.4.2 Functional foods in China

China is the home of traditional Chinese medicine. There is an ancient saying that both food and medicine originate from the same source, which is also the foundation of the functional foods today (Yang, 2008). Over the past decade in China, functional foods, also called healthy foods, played an important role in the health of the Chinese and have brought great economic benefits to the society.

1.4.3 Individual functional foods

1.4.3.1 Antioxidants

1.4.3.1.1 Polyphenols and flavonoids

The term polyphenol includes simple phenols and flavonoids, which are found in fruits, vegetables, nuts and their products, and possess important antioxidant activities. The famous phenomenon so called "French Paradox", which was the observation of residents in certain locations in France who were avid drinkers of red wine had less heart disease than other Western populations even though they consumed more fat in their diet, confirmed the presence of high concentrations of polyphenolics in red grape skins (Constant, 1997).

The largest and best studied polyphenols are flavonoids, which include proanthocyanidins, quercetin, and epicatechin, found mainly in chocolate, tea, and wine (Charles, 2003). Both epidemiologic and animal studies have demonstrated the inverse relationship between the consumptions of these flavonoids and certain kinds of diseases, such as cancers and CHD.

1.4.3.1.2 Lycopene

Tomatoes and tomato products are the most significant dietary source of lycopene, a non-provitamin A carotenoid that is also a potent antioxidant (Clinton, 1998). A comprehensive review of epidemiologic studies found an inverse association between tomato intake or plasma lycopene concentration and the risk of cancer (Giovannucci, 1999).

1.4.3.1.3 Lutein

Recently, another carotenoid that has received much attention for its role in disease risk reduction is lutein, the main pigment in the macula of the eye. High intake of lutein may reduce the risk of age-related macular degeneration (AMD) or cataracts (Mares-Perlman *et al.*, 2002; Seddon *et al.*, 1994). Good sources of lutein include green leafy vegetables such as spinach, kale and cooked cabbage.

1.4.3.2 Fatty acids

Dietary fatty acids and cholesterol interact to modulate plasma and intracellular cholesterol homeostasis. The relationship between type of dietary fat, cardiovascular disease risk, and lipid/lipoprotein profiles have been studied since the early 1900s (Finking and Hanke, 1997). It is well known now that the fatty acid profile of the dietary fat is a major determinant of the concentration of the circulating LDL cholesterol (Ross, 1993). In humans, dietary fat saturation plays a considerable role in modulating plasma cholesterol concentration and determining the risk for CHD (Temme *et al.*, 1996). Saturated fatty acids (SFA) and *trans*-fatty acids (TFA) contribute to higher LDL-C concentrations than that by monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) (Lichtenstein *et al.*, 1999).

1.4.3.2.1 Effects of saturated fatty acids on lipids and lipoproteins

In fact, SFAs are recognized to have the greatest negative effect on LDL cholesterol concentration (Hu *et al.*, 2001). Above a certain threshold, SFAs cause LDL cholesterol to increase, especially if the unsaturated fatty acid (linoleic acid) intake is low, but they also produce an increase in HDL cholesterol (Jones *et al.*, 1998; Fernandez *et al.*, 1989). However, the effect of the SFAs cannot be isolated from that of the unsaturated fatty acids. The LDL-C/HDL-C ratio is more favorably influenced by replacing SFAs with unsaturated fatty acids than by reducing SFAs alone (Fernandez *et al.*, 1992).

However, SFAs must be considered individually according to their chain length, because lauric (C12:0), myristic (C14:0), palmitic (C16:0), and stearic (C18:0) acids all have different effects. For instance, stearic acid is not a hypercholesterolemic agent (Rumsey *et al.*, 1995). Myristic acid is the most hypercholesterolemic but only when intakes are very high (Spady *et al.*, 1993). Furthermore, stearic acid is supposedly less thrombogenic than palmitic, myristic, and lauric acids (Daumerie *et*

al., 1992).

Cholesterol-raising SFAs (12:0, 14:0, 16:0) decrease LDL receptor activity, protein, and mRNA abundance and suppress that of ACAT, resulting in a greater proportion of cholesterol remaining in the regulatory pool (Fernandez *et al.*, 2005).

1.4.3.2.2 Effects of unsaturated fatty acids on lipids and lipoproteins

1.4.3.2.2.1 Monounsaturated fatty acids (MUFAs)

The effects of monounsaturated fatty acids, which are mostly refer to oleic acid, have long been considered as neutral and thus devoid of interest. This neutral effect of oleate seems to result from a concomitant increase of plasma HDL cholesterol levels and a modest reduction of plasma LDL cholesterol level, consequently increase the ratio of HDL/LDL, which is inversely related to CHD (Poli *et al.*, 2008).

The Seven Countries Study indicated that there may be an inverse relationship between intake of MUFAs and coronary mortality. Besides, the American prospective studies of healthcare professionals and nurses have shown "an estimated reduction of 19% in the risk of CHD when the intake in MUFAs was increased by 5% (as a percentage of the total energy)" (Hu *et al.*, 1997).

1.4.3.2.2.2 n-6 polyunsaturated fatty acids

PUFAs of n-6 series occur widely in food, essentially as linoleic acid, and in particular in some seed oils, such as corn, sunflower, and soybean oils. The data on n-6 PUFAs are contradictory. While it is clear that linoleic acid, unlike MUFAs, induce a LDL cholesterol reduction in the absence of a significant effect on HDL cholesterol, or at most, a small decrease, and lower postprandial lipemia. The ability of linoleic acid to affect LDL cholesterol might arise from an increased expression of LDL receptors in the liver (Fernandez and West, 2005). However, it has been suggested that high intakes of n-6 PUFAs may have harmful effect, especially on the inflammatory process (Hu *et al.*, 1997).

1.4.3.2.2.3 n-3 polyunsaturated fatty acids

There are three important n-3 PUFAs: alpha-linolenic acid (ALA) and long-chain PUFAs eicosapentanoic acid (EPA) and docosahexanoic acid (DHA). The n-3 fatty acids are found in nuts and vegetables as ALA, and in marine products as EPA

and DHA. The effects of n-3 fatty acids are on VLDL metabolism via reduction of VLDL secretion and triglyceride transport, and an increased VLDL clearance resulting from increased VLDL catabolism (Poli *et al.*, 2008).

PUFAs may induce the LXRα protein, mRNA, and gene transcription in the liver, then the LXRα-regulatory pathway facilitates the elimination of excess cholesterol by stimulating CYP7A1, thereby resulting in the conversion of cholesterol to bile acids. Besides, PUFAs increase LDL receptor expression by the elimination of cholesterol from the liver via increased bile acid synthesis (Fernandez and West, 2005).

Some researchers proposed that the intake of both n-3 and n-6 fatty acids were not assessed in absolute terms, but should be evaluated as a ratio between these two fatty acid classes. However, the role of the ratio between n-6 and n-3 in the control of hypercholesterolemia has not been convincingly demonstrated (Poli *et al.*, 2008).

1.4.3.3 Phytosterols

Phytosterols are also known as plant sterols. Campesterol, β-sitosterol and stigmasterol are the most common and abundant phytosterols in nature, comparable in chemical structure to cholesterol but contain one or two methyl or ethyl groups in the molecule's side-chain. Saturation with hydrogen cause the phytosterols form plant stanols, such as campestanol and sitostanol (Ling and Jones, 1995). Phytosterols have long been reported to lower plasma LDL cholesterol levels by reducing the absorption of dietary and endogenously derived cholesterol in the intestine, while not significantly altering HDL-cholesterol or triglycerides (Miettinen *et al.*, 1995; Moghadasian and Frohlich, 1999).

Human body cannot synthesize phytosterols, thus, these components are obtained only from the diet. Phytosterols are found in all foods of plant origin, usually occurs in vegetable oils, nuts, cereals, beans, and seeds, their dietary intake varies from 150~350 mg/day, or even higher in vegetarians in Western diets. For incorporation into foods, phytosterols are often esterified with fatty acids to improve their fat solubility. One gram of phytosterols is equivalent to about 1.6 gram of esterified phytosterols (Kerckhoffs *et al.*, 2002).

Despite their relatively similar dietary intake, phytosterols present in human plasma in a concentration, from 500 (campesterol) to 20000 times (stigmasterol) less than that of cholesterol. These large differences in plasma concentration may due to

several mechanisms: (1) Much of the cholesterol in plasma is derived from endogenous synthesis, mainly in the liver, while plant sterols are not synthesized in the human body and are exclusively obtained from the diet, (2) Phytosterols are absorbed far less efficiently by the small intestine than is cholesterol, In particular, cholesterol absorption is usually 30~80%, while the absorption of phytosterols vary between 0.1~5% depending on the molecular structure of the specific plant sterol (Heinemann *et al.*, 1993), and (3) phytosterols are probably not metabolized to bile acids, and excreted much faster from the liver into bile compared with cholesterol (Thompson *and* Grundy, 2005; von Bergmann *et al.*, 2005). However, phytosterols have a greater affinity for micelles than cholesterol because of their greater hydrophobicity. Therefore, they can easily displace intestinal cholesterol from micelles, decreasing intestinal cholesterol absorption (Plat and Mensink, 2001). This reduction leads to the compensatory increase in endogenous cholesterol synthesis and LDL receptor expression However, the net overall effect is that circulating LDL cholesterol concentrations are lowered by 9~14% after consumption of phytosterols (Miettinen and Gylling, 1999; Plat and Mensink, 2002). Phytosterols reduce plasma cholesterol even if there is no cholesterol in the diet, because the uptake of both endogenous cholesterol from the bile and exogenous cholesterol from the diet are inhibited (Ikeda and Sugano, 1998). The same effect on plasma cholesterol is achieved whether the phytosterols are consumed only in the morning or if the intake is spread throughout the day (Plat *et al.*, 2000).

Therefore, the U.S. FDA authorized the claim that "*foods containing at least 0.65 g per serving of plant sterol/stanol esters (daily total intake ≥1.3 g), as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease.*" (FDA Talk Paper, 2000) In 2003, the FDA further stated that the lowest effective daily intake of free sterols is 0.8 g/d (FDA Letter, 2003).

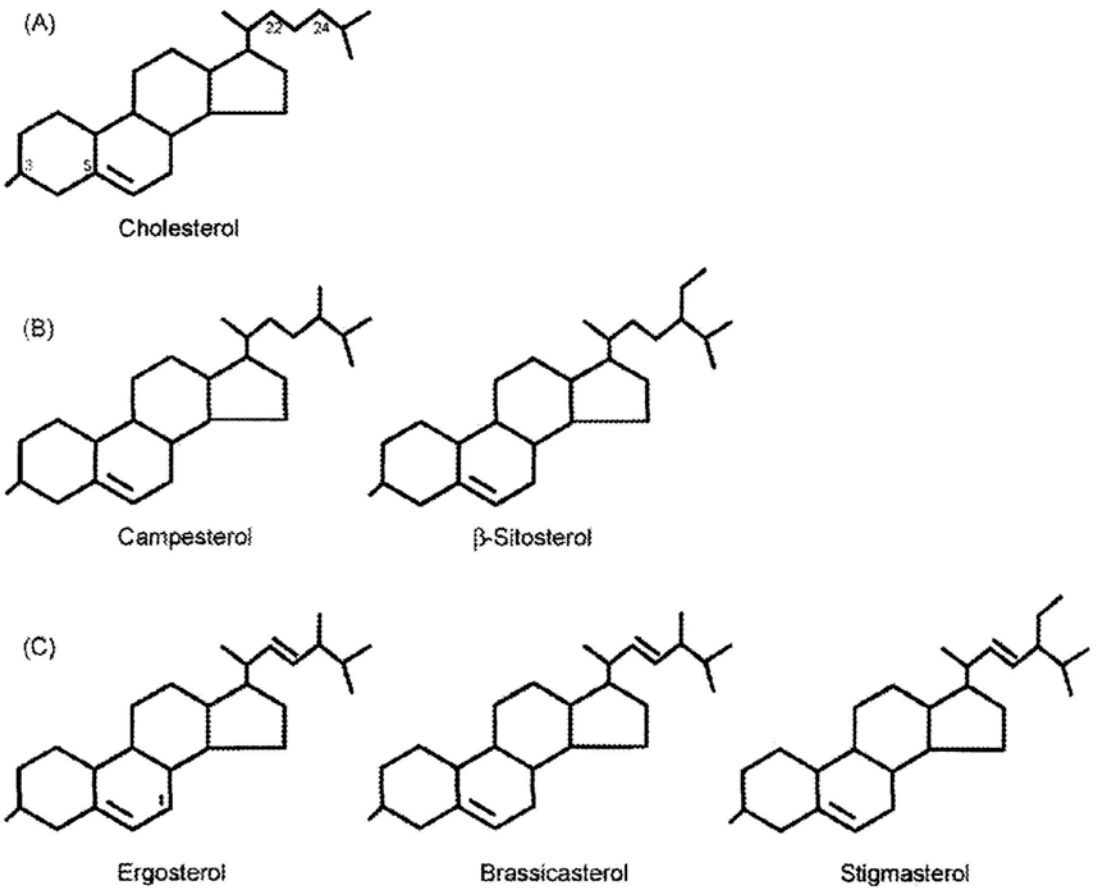

Figure 1.7 Chemical structures of cholesterol and the main phytosterols. (Calpe-Berdiel *et al.*, 2009)

1.4.3.4 Soy protein and isoflavones

In 1999, the U.S. Food and Drug Administration (FDA) proved a health claim that "*25 g of soy protein a day, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease*" (FDA and HHS, 1999; FDA Talk Paper, 1999).

The component in soybean and its soy protein-containing products (e.g., soy milk, soy flour, tofu, miso) that may attribute to the hypocholesterolemic effects in blood has been suggested to be soybean isoflavones, which are phytoestrogens or plant estrogens, possessing estrogenic properties because of their similarities in chemical structures to the estrogenic compounds (Charles, 2003). Both genistein and daidzein are predominant among soybean isoflavones.

The blood lipid lowering effects of soybean isoflavones could be explained by their weak estrogenic activity through binding to estrogen receptors (Kurzer and Xu, 1997; Tikkanen and Adlercreutz, 2000). However, the effects of isoflavones on plasma lipids and lipoproteins are controversial. Not all studies have reported a hypocholesterolemic effect of isoflavones. Based on the inconsistent findings, it remains uncertain whether isoflavones are responsible for the potential hypocholesterolemic effects in soybean. In some studies, plasma lipoprotein concentrations were unaffected by consumption of isolated soybean isoflavones (without soybean protein) showing that both soybean protein and isoflavones may be needed to exert cholesterol lowering effect (Kerckhoffs *et al.*, 2002).

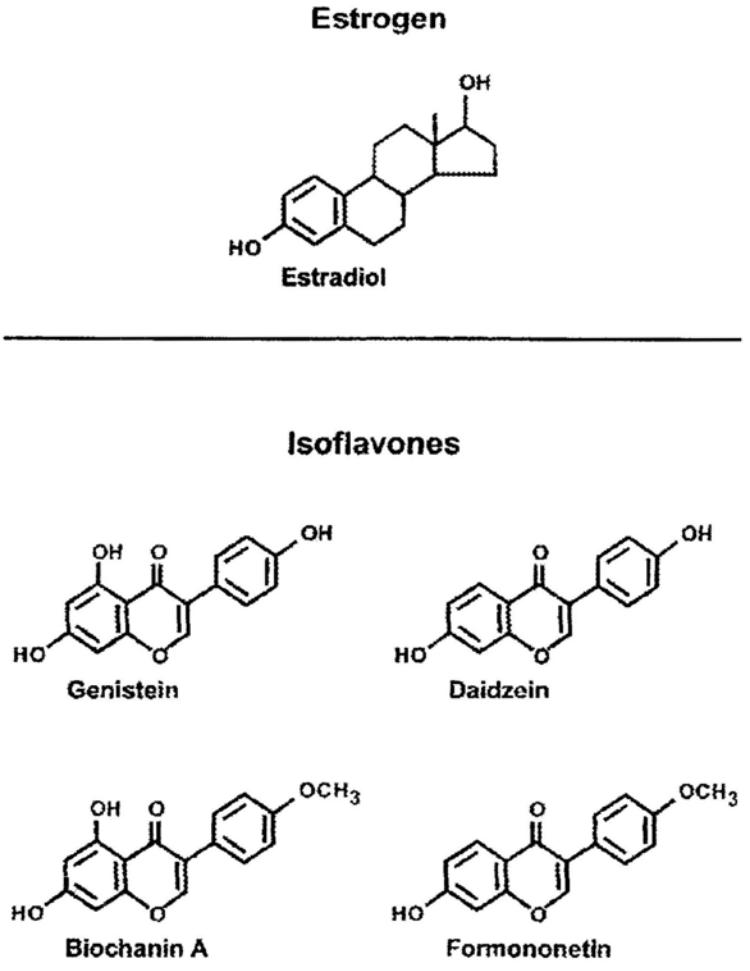

Figure 1.8 Chemical structures of isoflavones. The isoflavones, which are structurally related to the estrogen, estradiol, include genistein and daidzein and their methyl ether derivatives biochanin A and formononetin, respectively (Kerckhoffs *et al.*, 2002).

1.4.3.5 Oat products and β-glucan

Dietary fibers include a variety of plant substances, mainly nonstarch polysaccharides and lignins, which are resistant to digestion by digestive enzymes. They can be classified into two groups based on water solubility. Most soluble fibers were reported to exert a specific effect on LDL cholesterol to further lower total cholesterol (Anderson *et al.*, 1990). The mechanisms for hypocholesterolemic effects involved that soluble fibers may increase the binding of bile acids in the intestinal lumen, leading to a decreased enterohepatic circulation of bile acids and a subsequent increase in the hepatic conversion of cholesterol to bile acids (Glore *et al.*, 1994; Bell *et al.*, 1999). Besides, soluble fibers may also increase viscosity of the food mass in the small intestine and form a thick unstirred water layer adjacent to the mucosa to reduce the absorption of nutrients and bile acids (Lund *et al.*, 1989; Beer *et al.*, 1995). Furthermore, soluble fibers may reduce the rate of glucose absorption, resulting in a lower glycemic response and lower insulin concentration, which may cause a reduced hepatic cholesterol synthesis (Anderson *et al.*, 1990; Bell *et al.*, 1999).

Oats is a rich source of soluble fiber. In 1997, the U.S. FDA approved a health claim of food products that "*a diet high in soluble fiber from whole oats (oat bran, oatmeal, and oat flour) and low in saturated fat and cholesterol may reduce the risk of heart disease.*" The FDA recommended that at least 3 g/d of β-glucan from oats should be consumed to achieve a clinically relevant reduction in serum total cholesterol concentration (FDA and HHS, 1996; FDA Talk Paper, 1997).

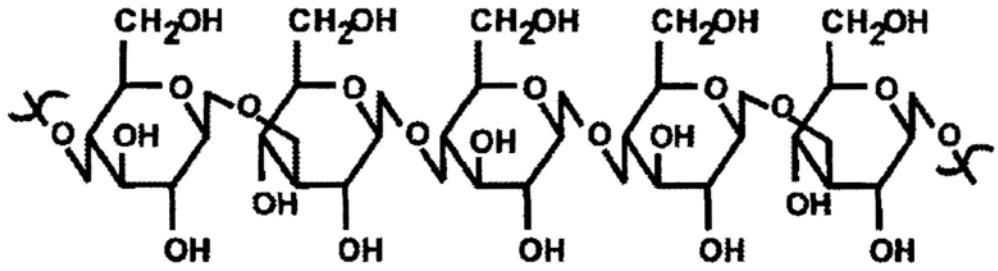

Figure 1.9 Chemical structure of β-glucan from oats. Oat β-glucan is often referred to as mixed linkage β-glucan (Kerckhoffs *et al.*, 2002).

1.4.3.6 Fish oils

Dietary fish oils, appearing as n-3 polyunsaturated fatty acids, are mainly found in cold water fish, such as salmon, tuna, mackerel, sardines and herring, The two major n-3 fatty acids are EPA (20:5) and DHA (22:6). Many clinical studies have reported the beneficial effects of n-3 fatty acids in certain chronic conditions such as cancer, rheumatoid arthritis, psoriasis, Crohn's disease, cognitive dysfunction and cardiovascular disease (Rice, 1999).

The 2000 American Heart Association Dietary Guidelines recommend that "*two servings of fatty fish per week for a healthy heart*" (Krauss *et al.*, 2000). Besides, FDA also authorized a qualified health claim stating that "*Consumption of omega-3 fatty acids may reduce the risk of coronary heart disease*" (FDA, 2002). The biological effects of fish oils include inhibition of hepatic synthesis and secretion of triacylglycerol and VLDL cholesterol with reduced postprandial lipemia, increased circulating HDL, inhibition of platelet aggregation, and prevention of cardiac arrhythmias (Connor, 2001).

However, certain safety concerns with regards to the high level intake of n-3 fatty acids include increased bleeding times and risk for hemorrhagic stroke, the formation of biologically active oxidation products from the oxidation of n-3 fatty acids, and increased levels of LDL cholesterol. Therefore, FDA concluded that "*use of n-3 fatty acids supplements is safe, provided daily intake of EPA and DHA do not exceed 2 g/d*" (FDA, 2002).

1.4.3.7 Garlic (*Allium sativum Linn*)

Garlic has been used for thousands of years for a wide variety of medicinal purposes, garlic has been described as stimulant, carminative, antirheumatic and alternative, garlic oil is said to be a powerful antiseptic. Clove of garlic was known as a home remedy on account of its prophylactic and curative properties in the East and also used in Norway and Middle Europe as a folk medicine against diabetes (Augusti, 1996). Epidemiologic studies in the past twenty years have revealed an inverse relationship between garlic consumption and the incidence of certain types of cancer, including stomach, colon, and laryngeal cancers (Buiatti *et al.*, 1991; Steinmetz *et al.*, 1994; Zheng *et al.*, 1992). Besides, garlic has been shown to have a modest blood pressure-lowering effect in clinical studies (Silagy and Neil, 1994). In fact, the best-documented clinical effect of garlic focus on its cardiovascular

protection, this has been only evaluated extensively in recent years.

The presences of numerous physiologically active organosulfur components, such as allicin and allylic sulfides, are likely attributed to the effects (Block, 1992). Allicin, dially disulfide oxide (C_3H_5-S-S(O)-C_3H_5), which causes the characteristic garlic odor, is believed to be the active lipid-lowering compound in garlic. In the process of crushing, cutting, or chewing the garlic clove, the enzyme alliinase comes into contact with the odorless alliin (SACS), which is then converted into allicin (Kerckhoffs *et al.*, 2002). Allicin is an inhibitor of HMG-CoA synthetases, and diallyl disulfide is an inhibitor of HMG-CoA reductase (Focke *et al.*, 1990, Kumar *et al.*, 1991). The precursor alliins have the antioxidant and hypolipidemic activities (Sheela and Augusti, 1995).

However, excessive intake of *allium* species may interfere with hemoglobin production and may lead to lysis of red blood cells. Prolong feeding of high levels of raw garlic to rats has resulted in anemia, weight loss and failure to grow, and even more, abdominal hemorrhage and death (Augusti, 1996).

1.4.3.8 Onion (*Allium cepa Linn.*)

The bulb onion is thought to have originated in central Asia and is used as flavoring additive to food or as salad (Augusti, 1996). Onions are grown in every part of the world where plants are farmed and exhibit a great diversity in form including color, shape, dry matter content and pungency (Griffiths *et al.*, 2002).

Onions are rich in two chemical groups that have perceived health benefits to man. These are the flavonoids and the alk(en)yl cysteine sulfoxides (ACSOs). The two flavonoids subgroups are found in onion are anthocyanins, imparting a red or purple color to some varieties, and flavanols, such as quercetin and its derivatives responsible for the yellow flesh and brown skins of many other varieties (Leighton *et al.*, 1992). The flavor of onion mainly due to sulfur-containing compounds, formed by the cleavage of three-S-alk(en)yl-L-cysteine sulfoxides (ACSOs) by alliin alkyl-sulfenate –lyase (alliinase).

Onion is traditionally used to treat fever, dropsy, chronic bronchitis, colic, scurvy, and etc. onion oil (about 0.005% of bulbs) contains a heart stimulant, increase pulse volume and frequency of systolic pressure and coronary flow. It promotes bile production and reduces blood sugar (Augusti, 1996). Observed lipid lowering effects of allium reveals that S-methylcysteine sulfoxide (SMCS) present in onion and

cabbage are active hypocholesterolemic agents. Other health effects of onions were also demonstrated by large numbers of research, such as anticarcinogenic properties, antiasthmatic activity, antibiotic activities, antioxidant properties, and antidiabetic effects (Augusti, 1996).

1.4.3.9 Probiotics and prebiotics

Probiotics can be defined as "*viable microbial food supplements which benefit the host by improving the intestinal microbial form*" (Dunne *et al.*, 2001). Scientific evidences for the therapeutic and/or preventive use of these functional ingredients for various health concerns include cancer, intestinal tract function, immune function, allergy, stomach health, urogenital health, cholesterol lowering and hypertension (Sanders, 1999).

More recently, research efforts have focused on prebiotics, i.e. "*nondigestible food ingredients that beneficially after the host by selectively stimulating the growth and/or activity of one or a limited number of beneficial bacteria in the colon, thus improving host health*" (Gibson and Roberfroid, 1995).

"Symbiotics" is still a new concepts, which is mixture of probiotics and prebiotics that "*beneficially affect the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract, by selectively stimulating the growth and/or by activating the metabolism of one or a limited number of health-promoting bacteria, and thus improving host welfare*" (Gibson and Roberfroid, 1995).

1.4.3.10 Eggs as a functional food

Eggs have not traditionally been regarded as a functional food because of their link with adverse effects on blood cholesterol. Evidence from both the Nurses' Health Study and the Health Professional Follow-Up Study showed that consumption of up to one egg per day did not have a substantial overall impact on the risk of CHD and stroke (Hu *et al.*, 1999). Furthermore, another epidemiologic study also demonstrated that eating more than four eggs per week may result in a significant lower mean plasma cholesterol level than those eat one or fewer eggs per week (Song and Kerver, 2000).

In fact, eggs are excellent, inexpensive and low calorie source of high quality protein and several important nutrients, including riboflavin (15% RDA), selenium

(17% RDA) and vitamin K (31% RDA) (United States Department of Agriculture Human Nutrition Information Service, 1989). Besides, one egg contains about 2000 mg choline, which is reported to have beneficial effects on cognitive function, particularly during early brain development (Zeisel, 1992; Blusztajn, 1998).

1.5 Summary

The composition of the diet is important, since there is good evidence that a vegetarian diet (rich in antioxidants), the Mediterranean diet (high in olive oil with MUFAs), and the Okinawan diet (high in fruits and vegetables plus n-3 fatty acids in fish) are beneficial to reduce the risk of developing age-associated diseases. This has led to the research for specific foods and food components that may help to improve the plasma lipoprotein profiles.

However, it is not only important to pay attention on the health effects of functional food ingredients to the lipoprotein profiles, but also need to concern their adverse effects, and its mechanism under various conditions, such as in combination with cholesterol-lowering diets or drugs, and in different populations (Kerckhoffs *et al.*, 2002).

In the present study, we have focus on the effects of two kinds of functional foods, tea seed oil and dietary onion, which draw more and more attention but have not been fully studied, on their hypocholesterolemic activities and underlying mechanisms.

Chapter 2

Comparison of Hypocholesterolemic Activity of Tea Seed Oil with Commonly Used Vegetable Oils in Male Golden Syrian Hamsters

2.1 Introduction

Tea leaves (*Camellia sinensis*) are popularly used to brew different beverages worldwide whereas tea seed has been utilized to produce cooking oil in some Asian countries. Extensive research has linked a wide range of health benefits to drinking tea including lowering the risk of certain cancers and heart disease as well as weight loss and protection against Alzheimers (Stangl *et al.*, 2006; Chen *et al.*, 2008). However, benefit associated with the tea seed oil (known as tea oil) remains relatively unexplored except for the two recent reports showing that tea oil possessed the antioxidant activity (Fazel *et al.*, 2009) and anti-lipogenic activity (Kim *et al.*, 2008). Tea oil has been utilized as cooking oil for more than 1000 years in China, particularly in Southern China such as Hunan province where more than 50% of the cooking oil is tea oil (Shanan and Ying 1982; Xia *et al.*, 1993). Tea oil is chemically stable because it is rich in oleic acid (18:1n-9).

Accumulating evidence has demonstrated that grape seed and wine polyphenols are able to scavenge the free-radicals, lower plasma cholesterol level, and reduce the risk of cardiovascular disease and cancer (Castilla *et al.*, 2006). Grape seed oil (called grape oil) has been long used for salad dressings, marinades, deep frying, flavored oils, baking, massage oil, sunburn repair lotion, and hand creams in all over the world. Chemically, grape oil is known for abundance in both linoleic acid (18:2n-6) and oleic acid. However, nutritional properties of grape oil remains unknown compared with other vegetable oils.

Blood total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) correlate directly with risk of heart diseases, whereas high-density lipoprotein cholesterol (HDL-C) correlates inversely with risk. Dietary fatty acids affect plasma cholesterol level largely mediated by their interaction with two transcriptional factors, sterol regulatory element binding protein-2 (SREBP-2) and liver X receptor (LXR-α)

(Elerle *et al.*, 2004; Kastelein, 2007; Rotheblat *et al.*, 2002). SREBP-2 governs the transcription of LDL receptor (LDL-R) and 3-hydroxy-3-methylglutary-CoA reductase (HMG-CoA-R). LDL-R is responsible for the removal of LDL-C from the circulation whereas HMG-CoA-R is a key enzyme in cholesterol synthesis in the liver. On the other hand, LXR-α regulates the transcription of CYP7A1 encoding cholesterol 7α-hydroxylase, which is a rate-limiting enzyme in conversion of cholesterol to bile acids in the liver and is responsible for elimination of excessive cholesterol in the bile fluid. No study to date has characterized the interaction of tea and grape oil with these transcriptional factors, receptor and enzymes that involve in cholesterol metabolism.

2.2 Objective

Despite extensive research on vegetable oils, little is known on how tea and grape oils affect blood cholesterol and interact with the genes and proteins described above, involved in cholesterol metabolism *in vivo*. The present study was therefore undertaken to (i) use hamsters as a model to examine the relative hypocholesterolemic activity of tea oil compared with grape oil, canola oil and corn oil with lard being a reference animal fat; and (ii) characterize the interaction of dietary tea oil with SREBP-2, LXR, HMGR, LDLR and CYP7A1.

2.3 Materials and Methods

2.3.1 Animals and diets

Five diets were prepared as previously described by Lam *et al.* (2008). The basal diet was formulated by mixing the following ingredients per kg diet (g): corn starch, 500; casein 200; sucrose, 100; AIN-76 mineral mix, 40; AIN-76 A vitamin mix, 20; gelatin, 20; DL-methionine, 1; cholesterol, 1. The five experimental diets were prepared by adding 10% lard, 10% tea oil, 10% grape oil, 10% canola oil, and 10% corn oil, respectively, into the basal diet (Table 2.1).

Male Golden Syrian hamsters (n = 50, 5 wk, 100~120g) were obtained from the Laboratory Animal Services Centre, The Chinese University of Hong Kong. Experiments were approved and conducted in accordance with the guidelines set by the Animal Experimental Ethical Committee, The Chinese University of Hong Kong. Hamsters were housed (2 hamsters per cage) in wire-bottomed cages in an animal room at 25°C with 12:12-h light-dark cycles. In brief, hamsters were allowed free access to a standard cereal-based chow diet (PicoLab® Rodent Diet 20-Lab Diet, Australia) for a 2-week acclimation period. Afterwards, hamsters were weighed, ear-punched, and randomly divided into five groups (n=10 each) maintained on one of the five diets for a period of 12 weeks. Body weight was recorded once a week, and total fecal output was collected, and food consumption was recorded every 2 days. Blood was collected from the retro-orbital sinus into a heparinized capillary tube under light anesthetization, using a mixture of ketamine, xylazine and saline (v/v/v, 4:1:5) after a 16-hour food deprivation at week 0, 4, 8 and 12. At the end of 12 weeks, all the hamsters were sacrificed after overnight fasting. Blood was collected via the abdominal aorta. The liver, heart, kidney, testis, perirenal fat and epididymal fat were also removed, rinsed with ice-cold saline, weighted, flash frozen in liquid nitrogen and stored at –80°C until analysis.

2.3.2 Determination of fatty acid composition and phytosterol content in oils

The methyl ester derivatives of fatty acids by boron trifluoride in methanol (14%, Sigma, MO, USA) were analyzed with GC. After saponification and extraction of the unsaponifiable matter with cyclohexane, the cyclohexane phase was evaporated to dryness under nitrogen, and the TMS-ester derivatives of phytosterols by a

commercial TMS-reagent (dry pyridine-hexamethyldisilazane- trichlorosilane, 9: 3: 1, v/v/v, Sil-A regent, Sigma, MO, USA) were dissolved in hexane and analyzed with GC.

2.3.3 Plasma lipid and lipoprotein determinations

Plasma TC and total triacyglycerol (TG) levels were determined using enzymatic kits from Infinity (Waltham, MA, USA) and Stanbio Laboratories (Boerne, TX, USA), respectively. The concentration of HDL-C was measured after precipitation of LDL and very low-density lipoprotein (VLDL) with phosphotungstic acid and magnesium chloride, using a commercial kit (Stanbio Laboratories). Non-HDL-C was calculated from the difference between TC and HDL-C.

2.3.4 Determination of organ cholesterol

Cholesterol in organs was determined using a method described as previously described (Chan *et al.* 1999). The liver (100 mg) and heart (300 mg) were used to determine the cholesterol level. In brief, the tissue sample and 1 mg stigmastanol, as an internal standard, were homogenized in 15 mL chloroform-methanol (2:1, v/v) and 3 mL saline. The chloroform-methanol phase was removed and dried down under a nitrogen steam. After 1 hour mild hydrolysis with 5 mL of 1 N NaOH in 90% ethanol at 90 °C, 1 mL of water and 6 mL of cyclohexane were added for extraction of cholesterol. The cyclohexane phase was evaporated to dryness under nitrogen, and cholesterol was converted to its TMS-ether derivative. The TMS-ether derivative was dissolved in hexane for GC analysis.

2.3.5 Determination of fecal neutral and acidic sterols

Neutral and acidic sterols in the feces of the hamsters were determined as described previously with slight modifications (Chan *et al.* 1999). 300 mg dried fecal samples were mildly hydrolyzed with 1N NaOH in 90% ethanol at 90°C for 1 hour. Then, total neutral sterols were extracted with cyclohexane, and converted into their trimethylsilyl derivatives. The acidic sterol-containing lower aqueous phase were saponified and converted into their trimethylsilyl derivatives. The two trimethylsilyl derivatives were separately analyzed with GC.

2.3.6 Western blotting analysis of liver SREBP-2, LDL-R, HMGR, LXR and CYP7A1

Liver protein was extracted according to the method described previously by Vaziri and Liang with some modification (Vaziri and Liang, 1996). In brief, the liver was homogenized in a homogenizing buffer containing 20 mM Tris-HCL (pH 7.5), 2 mM $MgCL_2$, 0.2 M sucrose and Complete® protease inhibitor cocktail (Roche, Mannheim, Germany). The extract was centrifuged at 13000 g for 15 min at 4°C and the supernatant was collected (total protein). The total protein was centrifuged at 126,000 g for 60 min at 4°C. The pellet was re-suspended in the same homogenizing buffer.

The pellet protein was separated by electrophoresis in a 7% SDS-PAGE gel and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA) using a semi-dry transfer system. Membranes were then blocked in 5% nonfat milk Tris-buffered saline with Tween-20 for 1 hour and overnight at 4°C in the same solution containing 1:600 anti-LDL receptor antibody (Santa Cruz Biotechnology, Inc., California, USA), 1:500 anti-HMG-CoA reductase (Upstate USA Inc., Lake Placid, NY, USA), 1:200 anti-CYP7A1 (Santa Cruz Biotechnology, Inc., California, USA), 1:400 anti-LXR antibody, or anti-SREBP-2 antibody (Santa Cruz Biotechnology). The membrane was then incubated for one hour at 4°C in diluted (1:3000) horseradish peroxidase-linked goat anti-rabbit IgG (Santa Cruz Biotechnology, Inc. California, USA), donkey anti-rabbit IgG (Santa Cruz Biotechnology, Inc. California, USA) or goat anti-mouse IgG (Calbiochem, EMD Chemicals, Inc., San Diego, CA, USA). Then, membranes were developed with ECL enhanced chemiluminescence agent (Santa Cruz Biotechnology, Inc., California, USA) and subjected to autoradiography on SuperRX medical X-ray film (Fuji, Tokyo, Japan). Densitometry was quantified using the BioRad Quantity one® software (BioRad Laboratories, Hercules, USA). Data on abundance of LDLR, HMG-R, CYP7A1, LXR and SREBP-2 were normalized with β-actin (Santa Cruz Biotechnology, Inc., California, USA).

2.3.7 Statistics

Data are expressed as mean ± standard deviation (SD). Treatment effects were statistically analyzed among groups using one-way analysis of variance (ANOVA)

and post hoc LSD test on SigmaStat Advisory Statistical Software (SigmaStat version 16.0, SPSS Inc., Chicago, IL, USA). P-value less than 0.05 are regarded statistically significant. Finally, regression analysis was performed and Pearson's correlation coefficients were determined to evaluate the relationship between LXR protein abundance and plasma and hepatic lipid levels.

2.4 Results

2.4.1 Fatty acid composition

Fatty acid composition is different amount the five diets (Table 2.1). Lard has the greatest while canola oil has the least amount of palmitic acid (16:0) among the five diets. In contrast, tea oil has the greatest amount of oleic acid (18:1n-9) while the grape oil has the least. Regarding linoleic acid (18:2n-6), corn oil has the greatest while tea oil has the least. However, tea oil has the least α-linolenic acid (18:3n-3) whereas canola oil has the most.

Table 2.1 Composition of High Fat Diets plus 0.1% Cholesterol

Main Nutrients	g/kg
Corn starch	500
Casein	200
Sucrose	100
Fat[1]	100
Cholesterol[2]	1
Vitamin mixture[3]	20
Mineral mixture[4]	40
Gelatin	20
Methionine	1

1. The experimental diets contained 100 g fat / kg with type varying among diets.
2. The experimental diets contained 1 g cholesterol / kg.
3. Vitamins provided in the diet: Thiamin HCl: 12 mg/kg; Riboflavin: 12 mg/kg; Pyridoxine HCl: 14 mg/kg; Niacin: 60 mg/kg; Calcium Pantothenate: 32 mg/kg; Folic Acid: 4 mg/kg; Biotin: 0.4 mg/kg; Vitamin B12 (0.1% in mannitol): 20 mg/kg; Vitamin A Palmitate (500,000 IU/g): 16 mg/kg; Vitamin E, DL-alpha tocopheryl acetate (500 IU/g): 200 mg/kg; Vitamin D3, cholecalciferol (400,000 IU/g in sucrose): 5 mg/kg; Vitamin K, MSB complex: 3 mg/kg.
4. Minerals provided in the diet: Calcium Phosphate, dibasic: 2.0%; Sodium Chloride: 0.3%; Potassium Citrate, monohydrate: 0.9%; Potassium Sulfate: 0.2%; Magnesium Oxide: 960 mg/kg; Manganous Carbonate: 140 mg/kg; Ferric Citrate: 240 mg/kg; Zinc Carbonate: 64 mg/kg; Cupric Carbonate: 12 mg/kg; Potassium Iodate: 0.4 mg/kg; Sodium Selenite, pentahydrate: 0.4 mg/kg; Chromium Potassium Sulfate, dodecahydrate: 22 mg/kg.

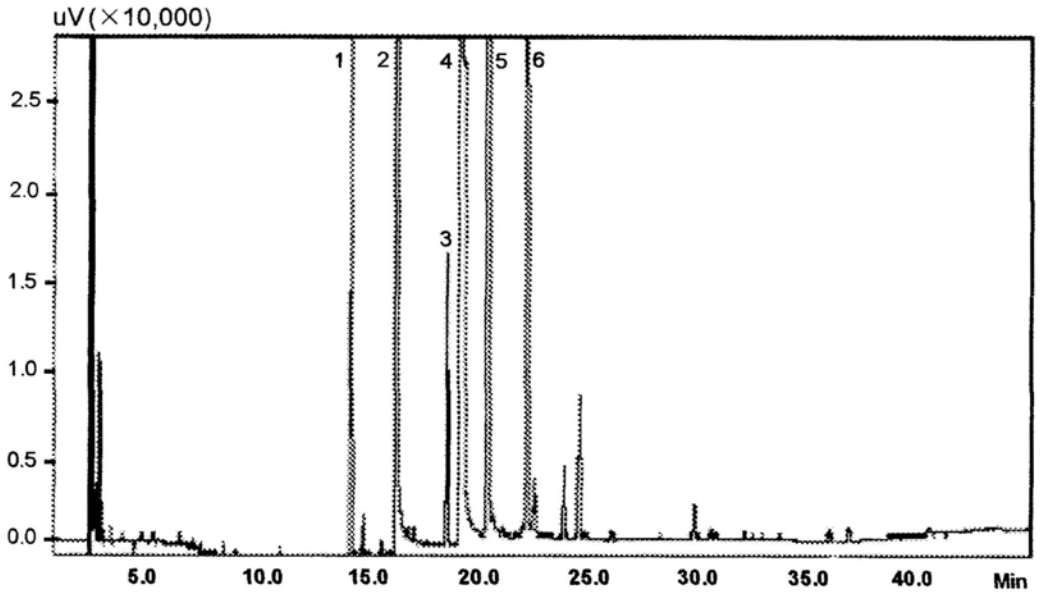

Figure 2.1 Gas liquid chromatogram of fatty acids in five oils. Identification of peaks: **1.** 16:0; **2.** 17:0 (internal standard); **3.** 18:0; **4.** 18:1*n-9*; **5.** 18:2*n-6* and **6.** 18:3*n-3*.

Table 2.2 Fatty acid composition (g/100g oil) and phytosterol content (mg/100g oil) in five oils

	Lard	Tea oil	Grape Oil	Canola oil	Corn oil
14:0	1.95	0	0	0	0
16:0	25.54	7.60	8.24	4.00	11.64
18:0	14.41	2.09	3.64	1.97	1.98
18:1*n-9*	43.16	77.36	28.57	55.60	29.08
18:2*n-6*	10.69	7.51	49.54	20.41	50.22
18:3*n-3*	0.51	0.11	2.50	9.34	0.94
M:S	1.08	7.98	2.41	9.32	2.14
P:S	0.27	0.79	4.38	4.99	3.76
Campesterol	0	22.82	29.66	187.43	120.51
Stigmasterol	0	10.31	20.95	ND	43.66
β-sitosterol	0	32.37	118.15	254.36	425.57
Total	0	65.50	168.76	441.78	589.74

M:S, monounsaturated : saturated fatty acids

P:S, polyunsaturated : saturated fatty acids

ND: no detected

2.4.2 Food intake, body and organ weights

No difference was seen in mean food consumption among the five groups (Table 2.3). It was found that dietary fat had no effect on the final body weight of the five groups. Liver, kidney, heart and epididymal fat pad weights were not significantly different among the five group except that corn oil-fed hamsters had significant lower relative liver weight than the lard-fed group, and the hearts in canola oil-fed and corn oil-fed groups weighed lesser than other the three groups.

Table 2.3 Change in food consumption, body weight and relative organ weights in male Golden Syrian hamsters fed one of the five diets containing 0.1% cholesterol and 10% lard, tea oil, grape oil, canola oil, or corn oil for 12 weeks

	Lard	Tea oil	Grape oil	Canola oil	Corn oil
Food intake	9.5 ± 0.9	9.2 ± 0.6	9.4 ± 0.5	9.1 ± 0.5	9.3 ± 0.6
Body Weight (g)					
Initial	112.5 ± 9.2	112.0 ± 6.7	113.0 ± 5.4	112.5 ± 10.3	113.0 ± 8.6
Final	130.0 ± 15.3	127.5 ± 7.2	130.5 ± 7.6	132.5 ± 14.6	134.0 ± 15.6
Relative Organ Weight (% Body Weight)					
Liver	4.01 ± 0.25[a]	3.79 ± 0.19[a]	3.99 ± 0.20[a]	3.84 ± 0.24[a]	3.76 ± 0.22[b]
Kidney	0.82 ± 0.04	0.80 ± 0.02	0.78 ± 0.06	0.79 ± 0.04	0.80 ± 0.04
Heart	0.35 ± 0.03[a]	0.34 ± 0.02[a]	0.35 ± 0.02[a]	0.33 ± 0.01[b]	0.33 ± 0.02[b]
Testis	2.92 ± 0.20	2.92 ± 0.20	2.94 ± 0.12	2.97 ± 0.22	2.92 ± 0.20
Epididymal fat pad	0.95 ± 0.13	0.91 ± 0.17	0.88 ± 0.12	0.93 ± 0.27	0.88 ± 0.21
Perirenal fat pad	1.64 ± 0.23	1.61 ± 0.21	1.68 ± 0.23	1.71 ± 0.37	1.69 ± 0.26

Data were expressed as mean ± SD; n=10 each group; Means at the same row with different superscript (a, b, c) differ significantly at $p < 0.05$.

2.4.3 Plasma lipoprotein concentrations

Plasma TC increased in all five groups compared with the baseline values (Table 2.4). Compared with that of lard group, plasma TC in the four vegetable oil groups was significantly reduced at the end of week 12. No significant difference in plasma TC was observed among the four vegetable oil groups at the end of week 12 (Table 2.4), though grape oil and canola oil groups appeared to have lower plasma TC than tea oil and corn oil groups. Similarly, non-HDL-C was markedly reduced in hamsters fed tea oil, grape seed oil, canola oil and corn oil compared with that of hamsters maintained on the lard diet at the end of experiment. Plasma HDL-C increased in all five groups compared with the baseline values throughout the experiment. However, grape oil and corn oil groups had HDL-C significantly lower than the lard-fed hamsters at the end of experiment. Interestingly, only tea oil and canola oil could decrease significantly the ratio of non-HDL-C to HDL-C at the end of experiment. Plasma TG concentrations in all five groups also increased substantially compared with their baseline values throughout the entire experiment. However, the four vegetable oils could equally be effective to suppress the elevation in plasma TG level (Table 2.4).

Liver cholesterol accumulation varied in the five groups. Liver cholesterol was greatest in tea oil group followed by canola oil group, grape oil group, lard group and corn oil group in a decreasing order (Table 2.4).

Table 2.4 Change in plasma and organ lipid concentrations in male Golden Syrian hamsters fed one of the five diets containing 0.1% cholesterol and 10% of lard, tea oil, grape oil, canola oil, or corn oil, respectively, for 12 weeks.

	Lard	Tea oil	Grape oil	Canola oil	Corn oil
Total Cholesterol					
Initial	121±17	120±11	120±19	121±13	120±12
4th Week	170±30^{a}	148±43^{ab}	146±24^{ab}	144±30^{ab}	124±20^{b}
8th Week	217±15^{a}	185±19^{bc}	196±29^{ab}	175±33^{bc}	170±18^{c}
12th Week	234±31^{a}	202±33^{b}	183±29^{b}	184±18^{b}	198±30^{b}
HDL-Cholesterol					
Initial	60±5	57±6	58±8	59±5	59±4
4th Week	84±10^{a}	79±11^{a}	70±4^{b}	75±13^{ab}	54±3^{c}
8th Week	82±11^{a}	73±12^{ab}	72±8^{b}	73±9^{ab}	66±8^{b}
12th Week	101±9^{a}	99±13^{a}	86±11^{b}	96±13^{ab}	90±12^{b}
Non-HDL-Cholesterol					
Initial	61±13	61±7	62±16	61±11	61±10
4th Week	86±28	78±32	77±22	69±19	70±20
8th Week	136±16^{a}	112±17^{bc}	124±27^{ab}	102±26^{c}	104±18^{c}

12th Week	133±29[a]	103±30[b]	97±26[b]	88±22[b]	108±30[b]
Non-HDL-C / HDL-C					
Initial	1.01±0.18	1.05±0.11	1.07±0.29	1.04±0.19	1.03±0.15
4th Week	1.03±0.35	1.01±0.39	1.10±0.29	0.92±0.15	1.29±0.37
8th Week	1.70±0.37[a]	1.57±0.40[ab]	1.74±0.42[ab]	1.39±0.25[b]	1.62±0.36[ab]
12th Week	1.32±0.30[a]	1.01±0.30[b]	1.13±0.31[ab]	0.94±0.32[b]	1.20±0.33[ab]
Triglyceride (mg/dL)					
Initial	96±32	90±29	114±43	106±33	109±30
4th Week	201±46	196±78	190±69	159±99	167±84
8th Week	241±79[a]	156±56[b]	130±34[b]	141±51[b]	134±56[b]
12th Week	269±94[a]	185±47[b]	185±38[b]	170±45[b]	175±69[b]
Organ Cholesterol (mg/g)					
Liver	72±11[bc]	90±12[a]	74±20[bc]	81±11[ab]	67±13[c]
Heart	3.3±0.2[bc]	3.4±0.1[ab]	3.5±0.2[a]	3.2±0.1[c]	3.3±0.2[abc]

Non-HDL-C = [TC] - [HDL-C];

Data were expressed as mean ± SD; n=10 each group; Means at the same row with different superscript (a, b, c) differ significantly at $p < 0.05$.

2.4.4 Fecal output of neutral and acidic sterols

Concentration of neutral sterols in the feces of the hamsters is shown in Table 2.5. Total fecal neutral sterol content in hamsters fed canola oil and corn oil was significantly greater than that in hamsters fed the lard, tea and grape oil. Compared with the lard group, tea oil increased the excretion of total fecal bile acids although the difference was not statistically significant. Hamsters fed the lard and tea oil, however, had greater excretion of total fecal bile acids than the other three groups.

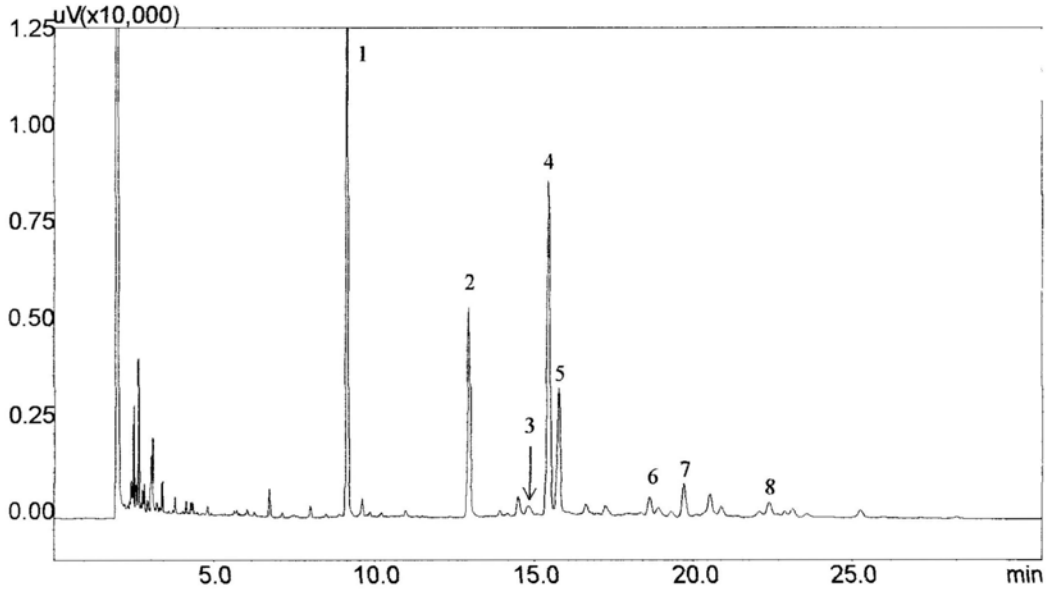

Figure 2.2 Gas liquid chromatogram of neutral sterols in feces. Identification of peaks: **1.** 5α-cholestane (internal standard); **2.** coprostanol; **3.** coprostanone; **4.** cholesterol; **5.** dihydrocholesterol; **6.** campersterol; **7.** stigmasterol and **8.** β-sitosterol

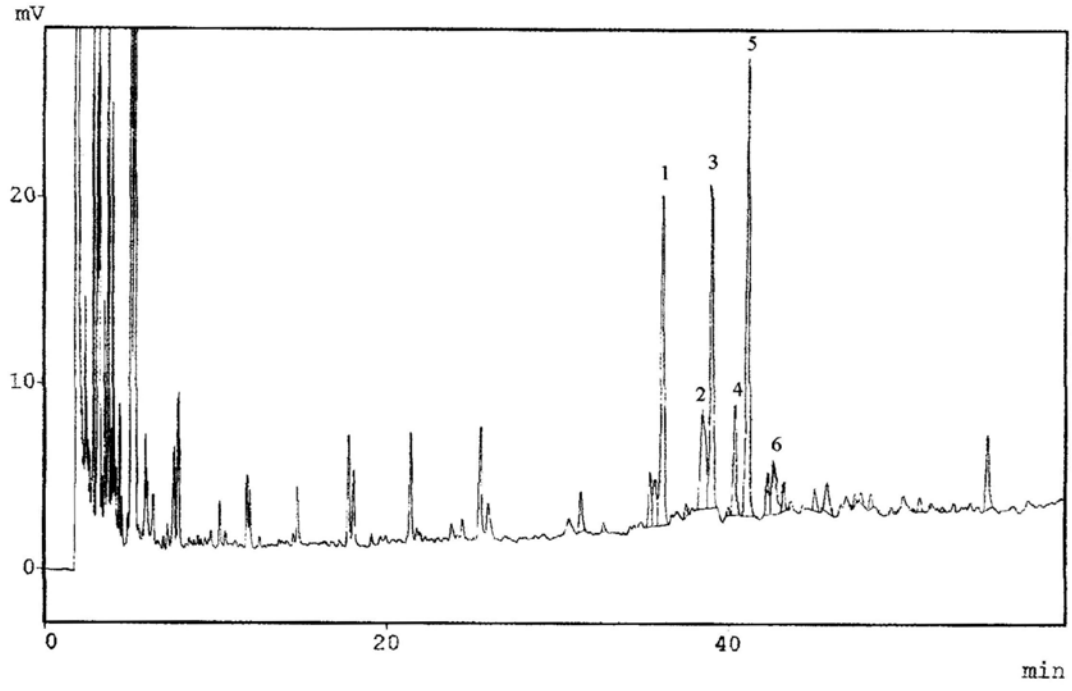

Figure 2.3 Gas liquid chromatogram of acidic sterols in feces. Identification of peaks: **1.** lithocholic acid; **2.** deoxycholic acid; **3.** chenodeoxcholic acid; **4.** cholic acid; **5.** hyodeoxycholic acid (internal standard) and **6.** Ursodeoxycholic acid.

2.4.5 Cholesterol balance

Total intake of cholesterol was compared with the excretion of neutral and acidic sterols (Table 2.5). Cholesterol retention was calculated according to the difference between the intake and excretion of both neutral and acidic sterols. It was found that cholesterol retention was lesser in the canola and corn oil groups than that in the other three groups. When the cholesterol retention was expressed as a percentage of cholesterol intakes per hamster, lard group had the greatest apparent cholesterol absorption followed by grape oil group, tea oil group, corn oil group and canola oil group in a decreasing order (Table 2.5).

Table 2.5 Cholesterol balance in hamsters fed the diets containing 0.1% cholesterol and 10% lard, tea oil, grape oil, canola oil and corm oil, respectively, for 12 weeks.

	Lard	Tea oil	Grape oil	Canola oil	Corn oil
Cholesterol intake (mg/day)	10.02 ± 1.19	9.23 ± 0.47	9.76 ± 0.37	9.39 ± 0.50	9.63 ± 0.23
Fecal total neutral sterol output (mg/day)	2.22 ± 0.67^{b}	1.78 ± 0.60^{b}	3.00 ± 0.49^{b}	6.00 ± 2.00^{a}	6.13 ± 1.86^{a}
Fecal total acidic sterol output (mg/day)	3.56 ± 1.12^{a}	4.90 ± 1.55^{a}	1.79 ± 0.53^{b}	1.84 ± 0.71^{b}	1.31 ± 0.68^{b}
Net Cholesterol equivalent retained (mg/day)	5.45 ± 1.46^{a}	3.99 ± 1.60^{a}	4.87 ± 1.11^{a}	2.15 ± 0.48^{b}	2.64± 0.90^{b}
Apparent cholesterol absorption (% intake)	53.39 ± 2.13^{a}	41.14 ± 1.39^{b}	48.57 ± 2.82^{ab}	22.09 ± 1.22^{d}	27.61± 1.31^{c}

Data were expressed as mean ± SD; n=10 each group; Apparent cholesterol absorption = [(cholesterol intake – excretion of neutral and acidic sterols)]/cholesterol intake; **Means** at the same row with different superscript (a, b, **c) differ** significantly at $p < 0.05$

2.4.6 Western blot of CYP7A1, HMGR, LDLR, LXR, and SREBP-2

No difference in hepatic CYP7A1, and HMG-CoA reductase was seen among the five groups (Figure 2.6, 2.7). However, LDL receptor appeared to be greater in tea oil group than that in the lard and canola oil groups (Figure 2.8). Except for the tea oil group, all the vegetable oil groups had LXR significantly greater than the lard group (Figure 2.5). Likewise, all the four vegetable oil diets could up-regulate SREBP-2 protein, however, only tea and canola oil groups statistically had greater SREBP-2 compared with control group (Figure 2.4).

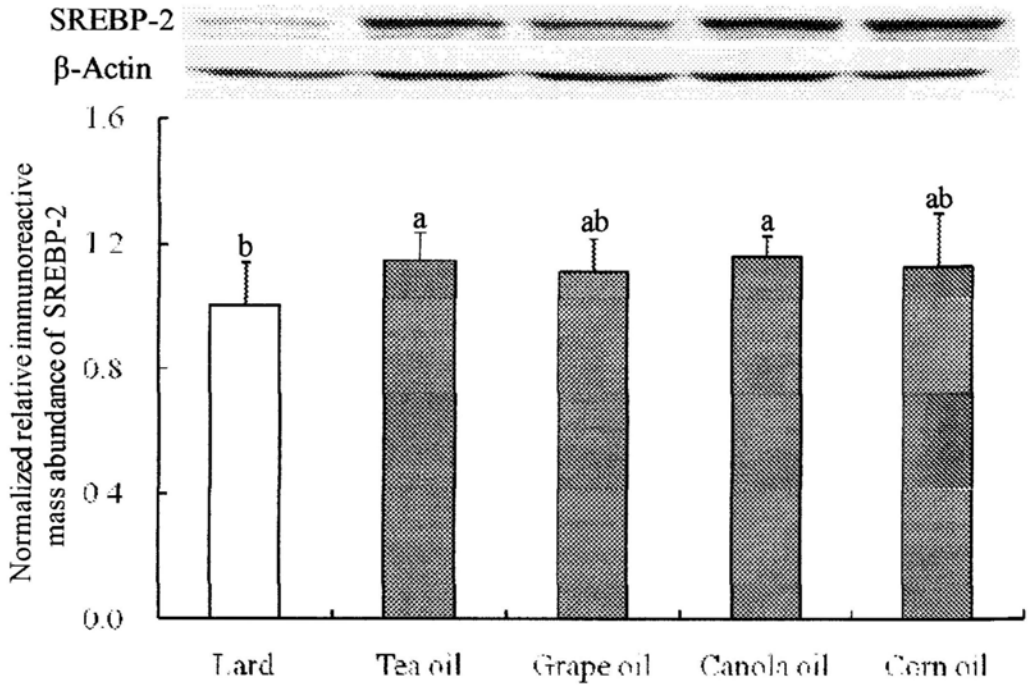

Figure 2.4 Effect of tea oil, grape oil, canola oil and corn oil compared with lard on the relative immunoreactive mass of sterol regulatory element binding protein (SREBP-2) as determined by Western blot analysis. Data were normalized with β-actin so that value of the control group was regarded as 1.0. Values were expressed as mean ± SD, n=10.

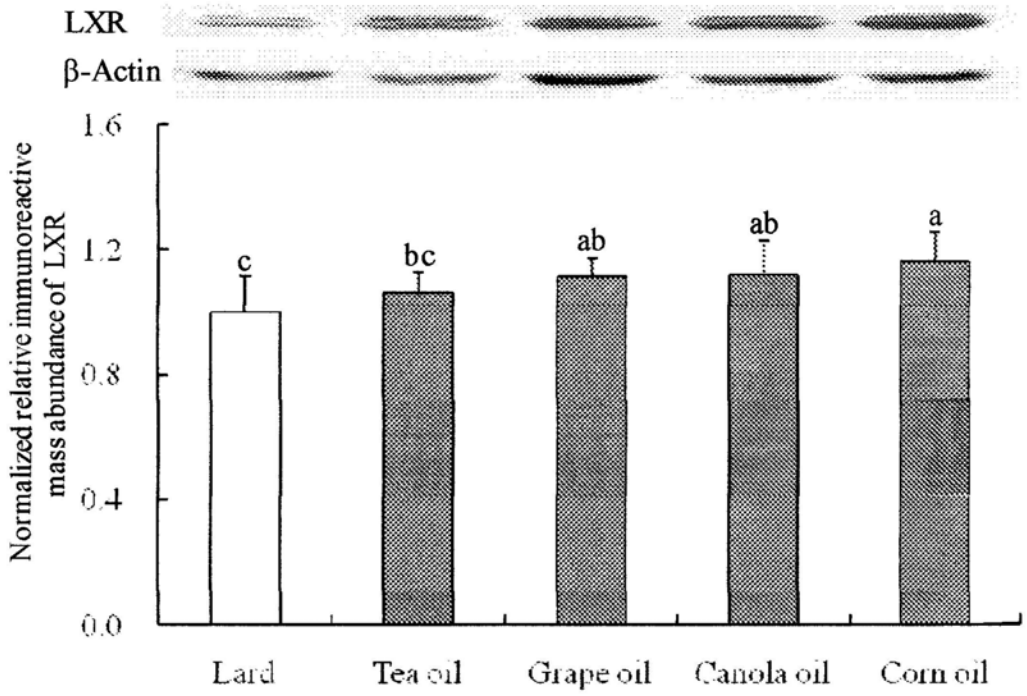

Figure 2.5 Effect of tea oil, grape oil, canola oil and corn oil compared with lard on the relative immunoreactive mass of liver X receptor (LXR) as determined by Western blot analysis. Data were normalized with β-actin so that value of the control group was regarded as 1.0. Values were expressed as mean ± SD, n=10.

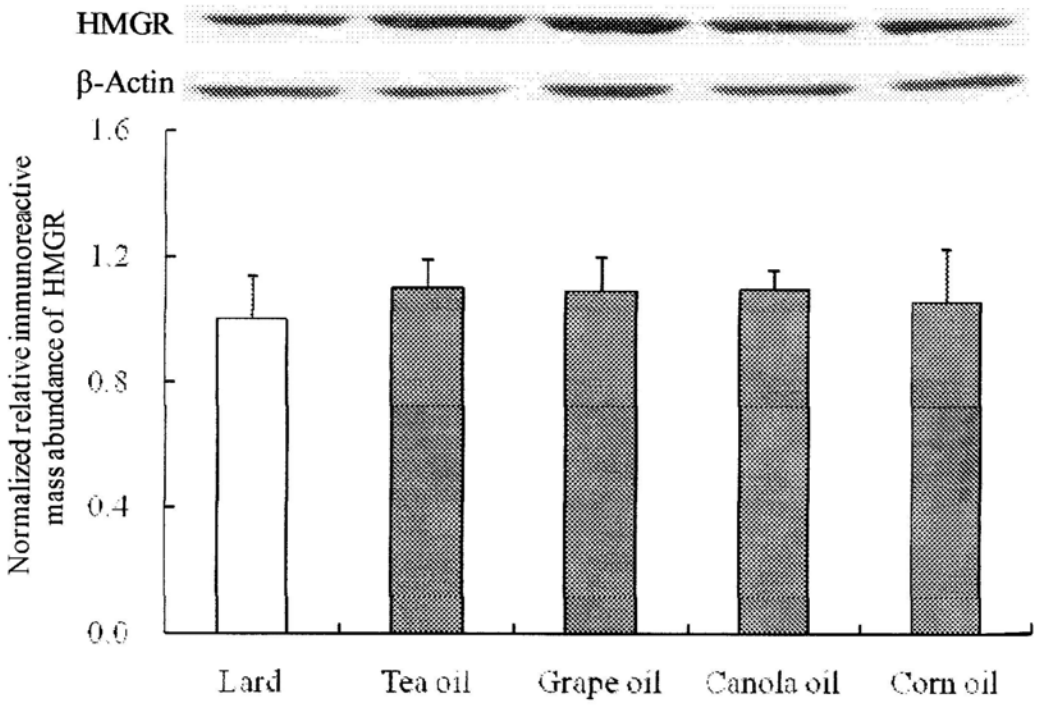

Figure 2.6 Effect of tea oil, grape oil, canola oil and corn oil compared with lard on the relative immunoreactive mass of 3-hydroxy-3-methylglutary-CoA reductase (HMGR) as determined by Western blot analysis. Data were normalized with β-actin so that value of the control group was regarded as 1.0. Values were expressed as mean ± SD, n=10.

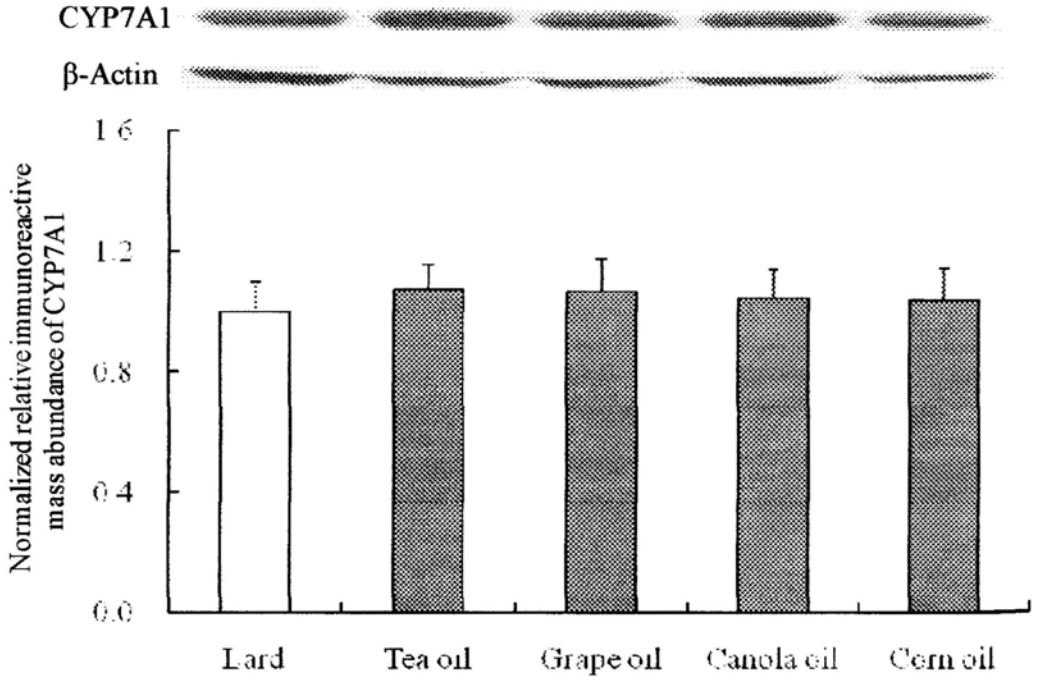

Figure 2.7 Effect of tea oil, grape oil, canola oil and corn oil compared with lard on the relative immunoreactive mass of cholesterol 7α-hydroxylase (CYP7A1) as determined by Western blot analysis. Data were normalized with β-actin so that value of the control group was regarded as 1.0. Values were expressed as mean ± SD, n=10.

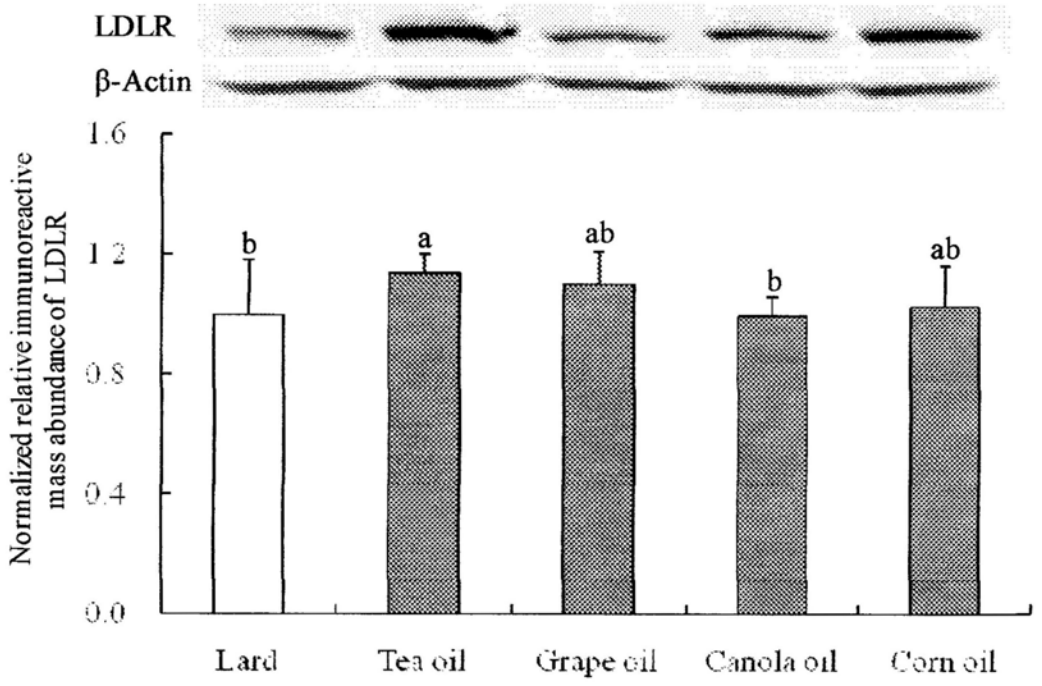

Figure 2.8 Effect of tea oil, grape oil, canola oil and corn oil compared with lard on the relative immunoreactive mass of Low density lipoprotein receptor (LDLR) as determined by Western blot analysis. Data were normalized with β-actin so that value of the control group was regarded as 1.0. Values were expressed as mean ± SD, n=10.

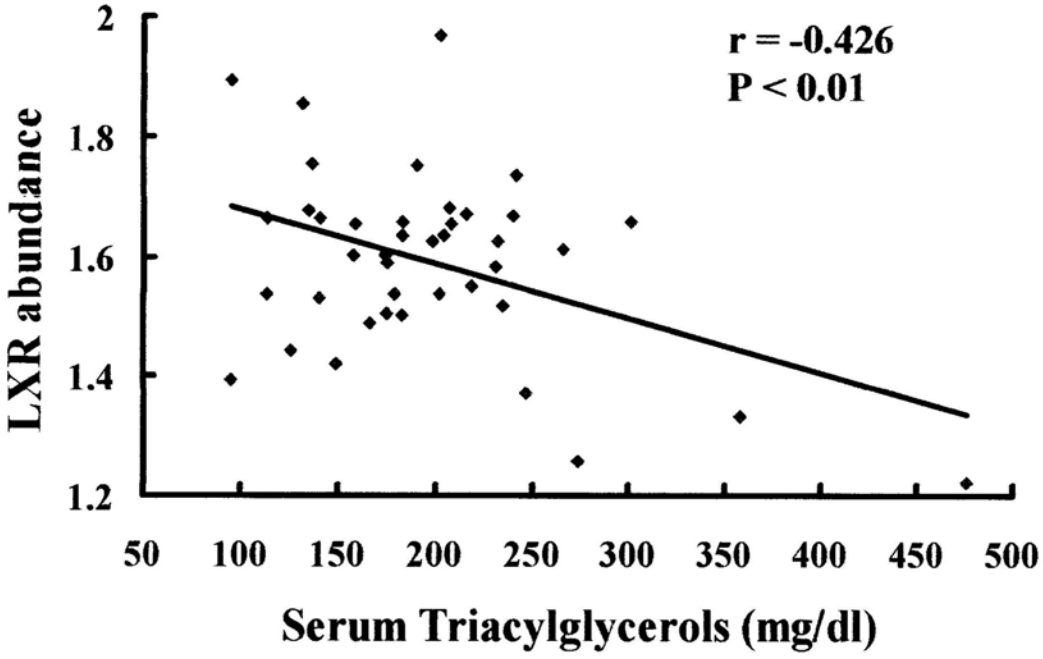

Figure 2.9 Correlation scatter plot of hepatic liver X receptor (LXR) protein and serum triacylglycerols. Data were obtained from all individual animals (n=50). The Pearson's correlation coefficients (r values) and level of significance are noted.

2.5 Discussion

The present report was the first of its kind to examine the relative hypocholesterolemic activity of tea oil, compared with that of grape oil, canola oil and corn oil with lard being as a control. The results clearly demonstrated that tea oil and grape oil were equally effective as canola oil and corn oil in lowering blood cholesterol in hamsters. However, tea oil and grape oil affected differently the lipoproteins with the former decreasing only non-HDL-C and having no or little effect on HDL-C concentration, while the latter decreasing both non-HDL-C and LDL-C concentrations. In this regard, grape oil behaved like corn oil while tea oil acted like canola oil. The varying effect of tea oil and grape oil on the ratio of non-HDL-C to HDL-C can be explained by their respective fatty acid composition. It is known that oleic acid has a lesser LDL-C lowering effect but possesses a greater HDL-raising effect compared with linoleic acid (Trautwein *et al.*, 1999; Kris-Etherton and Yu, 1997; Katan *et al.*, 1994). In fact, tea oil and canola oil are rich in oleic acid while grape oil and corn oil are rich in linoleic acid. Therefore, a smaller ratio of non-HDL-C/HDL-C in tea oil group and canola oil group was expected compared with that in grape oil and corn oil-fed hamsters (Table 2.2).

Polyunsaturated-to-saturated fatty acids (P:S) ratio in diet is a key determinant of plasma cholesterol levels. It is proved that the higher the ratio is, the lower the plasma TC is (Scott and Margo, 1990; McNamara, 1992). To be expected, plasma TC levels among the five groups were inversely correlated with their corresponding P:S values (Table 2.4). Dietary P: S ratio may be also associated with plasma HDL-C concentration. Ehnholm *et al.* (1982) reported that the plasma HDL-C concentration decreased as the ratio of the P: S increased in the diet. In addition, in contrast to the n-6 polyunsaturated fatty acid-enriched vegetable oils that lower HDL-C concentration, n-3 polyunsaturated fatty acid-rich oils did not or mildly decrease HDL-C concentration (Conner, 2000). The present study found that TC and HDL-C concentrations were affected by not only P: S but also n-3 : n-6 fatty acids. This was reflected by the observation that tea oil with the lowest P: S ratio and the lowest

percentage of n-6 fatty acid had the highest HDL-C concentration among the four unsaturated cooking oils, although the difference was not statistically warranted.

Four vegetable oils were equally effective in reducing plasma TG level (Table 2.4). Elevated plasma TG has emerged as a significant and independent risk factor for major coronary events (Paul, 2000). In the present study, excessive cholesterol and lard intake induced a marked increase in plasma TG concentration; however, feeding four vegetable oils could significantly attenuate the hypertriacylglycerolaemic response induced by cholesterol and lard feeding. It is known that dietary polyunsaturated fatty acids suppress transcription of a group of hepatic genes encoding glycolytic and lipogenic enzymes, thus leading to reduction in tissue and plasma TG (Teran-Garcia *et al.*, 2007). Therefore, reduced plasma TG levels were expected in the four vegetable oil groups because these vegetable oils are rich in monounsaturated or polyunsaturated fatty acids. In addition, LXR has emerged as a key regulator of cholesterol and lipid metabolism (Barish and Evans, 2004; Li and Glass, 2004; Tontonoz and Mangelsdorf, 2003). The present study found a striking negative correlation between protein expression of LXR and plasma TG levels (Figure 2.6). It was further affirmed that hamsters fed the four unsaturated vegetable cooking oil diets showed a marked up-regulation of LXR protein abundance compared with the lard-fed group, although the difference between the tea oil and lard groups was not statistically significant.

Tea oil could up-regulate while grape oil did not significantly increase expression of SREBP-2 and LDL receptor (Figure 2.1, Figure 2.5). In both humans and hamsters, most non-HDL-C is taken up by the LDL receptor-mediated pathway (Chen *et al.*, 1996). LDL-receptor expression is a key determinant of plasma cholesterol levels. It is evident that saturated fatty acids reduce clearance of LDL-C by suppression of LDL receptor, whereas unsaturated fatty acids relatively activate the activity of LDL receptor and decrease the LDL-C concentration (Spady and Dietschy, 1989; Elke *et al.*, 1997). In response to changes in plasma cholesterol levels, the liver maintains cholesterol homeostasis by regulating internalization of LDL particles and *de novo* cholesterol synthesis (Cheema *et al.*, 1997). Under normal conditions, as much as

80% of the circulating LDL particles are cleared by the liver mainly through an LDL receptor-mediated process (Dietschy, 1995). At any level of dietary cholesterol, receptor-dependant LDL uptake by the liver is always higher in animals fed unsaturated fatty acids than in animals fed saturated fatty acids (Horton *et al.*, 1993). In the present study, the hepatic cholesterol accumulation was significantly elevated in the liver of tea oil group and, to a lesser degree, in canola oil group. These changes are in accordance with previous studies indicating that monounsaturated fatty acid-rich diet may increase hepatic cholesteryl esters (Elke *et al.*, 1997; Spady *et al.*, 1993). Tea oil, rich in monounsaturated fatty acid, decreased LDL-C concentration accompanied with a higher level of LDL receptor compared with the other groups, suggesting higher rates of LDL clearance, thereby resulting higher level of hepatic cholesterol concentration in the present study (Table 2.4).

Tea oil affected differently the excretion of neutral and acidic sterols compared with other three vegetable oils (Table 2.5). The major finding was that tea oil had greater excretion of bile acids (acidic sterols) and lesser excretion of neutral sterols compared with the other three vegetable oils. This could be attributable to high oleic acid content in tea oil. It has been shown that monounsaturated fatty acid (olive oil) and linoleic acid (corn oil) affected the bile composition with the former having relative lesser neutral sterols and greater acidic sterols in the bile compared with the latter in rats (Bravo *et al.* 1998). When total intake of cholesterol was compared with the excretion of neutral and acidic sterols, cholesterol retention was calculated according to the difference between the intake and excretion of both neutral and acidic sterols (Table 2.5). It was found that cholesterol retention was less in four groups fed the vegetable oils compared with that in the lard-fed hamsters. The present result was in agreement with that of Bravo *et al.*, who found that mono-or n-6 polyunsaturated fatty acids reduced the absorption of cholesterol compared with dietary saturated fatty acids. The present study suggests that cholesterol-lowering activity associated with these four vegetable oils be mediated by their inhibitory effect on cholesterol absorptions.

In summary, we found that like canola and corn oil, tea and grape seed oils were

able to lower plasma cholesterol in hamsters fed a 0.1% cholesterol diet. However, tea oil decreased only non-HDL-C and had no or little effect on HDL-C concentration, while grape oil reduced both non-HDL-C and LDL-C concentrations. Unlike grape oil, tea oil could up-regulate SREBP-2 and LDL receptor. Hamsters fed the tea oil diet excreted less neutral but greater acidic sterols compared with other three vegetable oils. The present study suggests that cholesterol-lowering activity associated with these four vegetable oils be mediated by their inhibitory effect on cholesterol absorptions. This could be explained, at least in part, tea oil is rich in oleic acid, a monounsaturated fatty acid.

Chapter 3

Hypocholesterolemic Effects of Dietary Onion Powders on Male Golden Syrian Hamsters

3.1 Introduction

Over the centuries, species in the genus *Allium* have acquired reputations in the folklore as powerful prophylactic and therapeutic medicinal agents in many cultures. Onion (*Allium cepa Linn*) and garlic (*Allium sativum Linn*) are two important *Allium* species which have been studied for their therapeutic uses as antibiotic, antidiabetic, antioxidant, antiatherogenic, anticancer, and fibrinolytic effect (Augusti, 1996). Onion is traditionally used to treat fever, dropsy, chronic bronchitis, colic, scurvy, *etc*. Onion oil (about 0.005% of bulbs) contains a heart stimulant, increase pulse volume and frequency of systolic pressure and coronary flow. It promotes bile production and reduces blood sugar (Augusti, 1996).

Onions are grown in different parts of the world where they are farmed and exhibit a great diversity in form including color, shape, dry matter content and pungency (Brewster, 1994). World production of onion has increased by at least 25% over the past decade, which makes it the second most important horticultural crop after tomatoes (Food and Agriculture Organization of the United Nations Production Yearbook, 1999). Onions are highly versatile and are used as an ingredient in many dishes and are accepted by almost all traditions and cultures. China and India are the major producers in Asia, and contributes over half of the world's production (Griffiths *et al.*, 2002).

The chemical contents present in onion are shown in Table 3.1. Onion contains small amount of vanadium (680-750 μg/100g) and carotene (630-890 μg/100g). All types of free amino acids (8.2-10 mg/100g) are also found in onion, in particular, arginine and glutamic acid which are in abundance. Fatty acids, palmitic, oleic and linoleic acids, account for 75% of the fats in onion. Ratio of total unsaturated and

saturated fatty acids is 1: 9. Sulfur containing compounds are important in *Allium* species, but the concentrations of L-cysteine, L-cystine and L-methionine are relatively low, suggesting their rapid metabolism (Augusti, 1996).

The main groups of compounds rich in onions that have perceived health benefits to man are flavonoids and the alk(en)yl-L-cysteine sulfoxides (ACSOs) and their breakdown products found in onion oil viz. alkyl thiosulfinate, disulfides and polysulfides (Kumari and Augusti, 2006) (Fig 3.1). Two flavonoids subgroups found in onion are (1) anthocyanins, imparting a red or purple color to some varieties, and (2) flavanols, such as quercetin and its derivatives responsible for the yellow flesh and brown skins of many other varieties (Leighoton *et al.*, 1992). Onions contain about tenfold higher levels of quercetin than other vegetables and along with tea, they are the major source of quercetin in the human diet (Hollman and Arts, 2000). There are three ACSOs, namely, (+)-S-methyl-L-cysteine sulphoxide (MCSO, methiin), (+)-S-propyl-L-cysteine sulphoxide (PrCSO, propiin) and *trans*-(+)-S-(propen-1-yl)-L-cysteine sulphoxide (1-PeCSO, isoalliin) (Table 3.2). Quercetin is known to possess antioxidant properties, suppress the oxidative modification of LDL-C, and have the ability to lower plasma lipid in animal studies (Chu *et al.*, 2000; Leake, 2001; Bok *et al.*, 2002). The active compounds in onion including alkyl and alkenyl sulfoxides and breakdown products, have been described to lower plasma total and LDL cholesterol, increase HDL cholesterol, protect LDL cholesterol from oxidation and improve the rheological properties of the blood (Steiner *et al.*, 1996; Augusti *et al.*, 1975; Vatsala and Singh,1980). Observed lipid lowering effects of allium reveals that S-methylcysteine sulfoxide (SMCS) present in onion and cabbage are active hypocholesterolemic agents.

3.2 Objective

While epidemiological studies have shown a correlation between diets rich in onion and a reduced risk of mortality from CHD (Sogani and Katoch, 1981; Hertog *et al.*, 1993), few clinical and animal researches have explore the benefits of onion consumption and dose responses, and the potential mechanism of its hypocholesterolemic effects. Besides, when onions are consumed, many active compounds present may form complex metabolites which cannot always be stimulated in an *in vitro* system, until now, little attention has been paid to the efforts of the consumption of whole onion, therefore, the present study was undertaken to examine the effects of onion powder supplemented in the diets at two levels on the indices of cardiovascular health using the male Golden Syrian hamsters as a model.

Table 3.1 Chemical contents in onion (*A.cepa*) (Augusti *et al.*, 1996)

Content	Onion *Chemical contents (g/100g wet wt)*
Moisture (%)	87-93
Proteins	0.9-1.5
Fat	0.2-0.4
Carbohydrate	5.2-10.5
Ash	0.7
Energy (cal)	23-38
	Bulk element (mg/100g wet wt)
Ca	190-540
P	200-430
K	80-110
Na	31-50
Mg	81-150
Al	0.5-1
Ba	0.1-1
Fe	1.8-2.6
	Sulfur, Cholorine and trace elements (mg/100mg, wet wt)
Sr	0.8-7
B	0.6-1
Cu	0.05-0.64
Zn	1.5-2.8
Mn	0.5-1
Cr	< 0.5
Sr	51
Cl	36
	Vitamins (mg/100mg wet wt)
Thiamin	0.3
Riboflavin	0.05
Nicotinic acid	0.2
Vitamin C	10
Vitamin B_6	0.1
Pantothenic acid	0.14
	(μg/100g wet wt)
Folic acid	16
Biotin	0.9
Retinol	25

Table 3.2 Volatile sulfur compounds in onion (*A. cepa*) (Augusti *et al.*, 1996)

Compounds	Onion
L-Cystein	+
L-Cystine	+
L-Methionine	+
S-Methyl L-cysteine	+
S-Prophyl L-cysteine	+
S-(2-propenyl) L-cysteine	+
S-(carboxyehyl) L-cysteine	+
S-(carboxy propyl) L-cysteine	+
trans S-(1-propenyl) L-cysteine	+
S-(carboxy isopropyl) L-cysteine	+
S-(carboxymethyl) L-cysteine	+
L-Methionine sulfoxide	+
S-(2-propenyl)cysteine sulfoxide	Abundant (2%)
S-Methyl cysteine sulfoxide	+
S-prophylcysteine sulfoxide	+
S-allyl cysteine sulfoxide	−

(+) means "present"; and (−) means "not present"

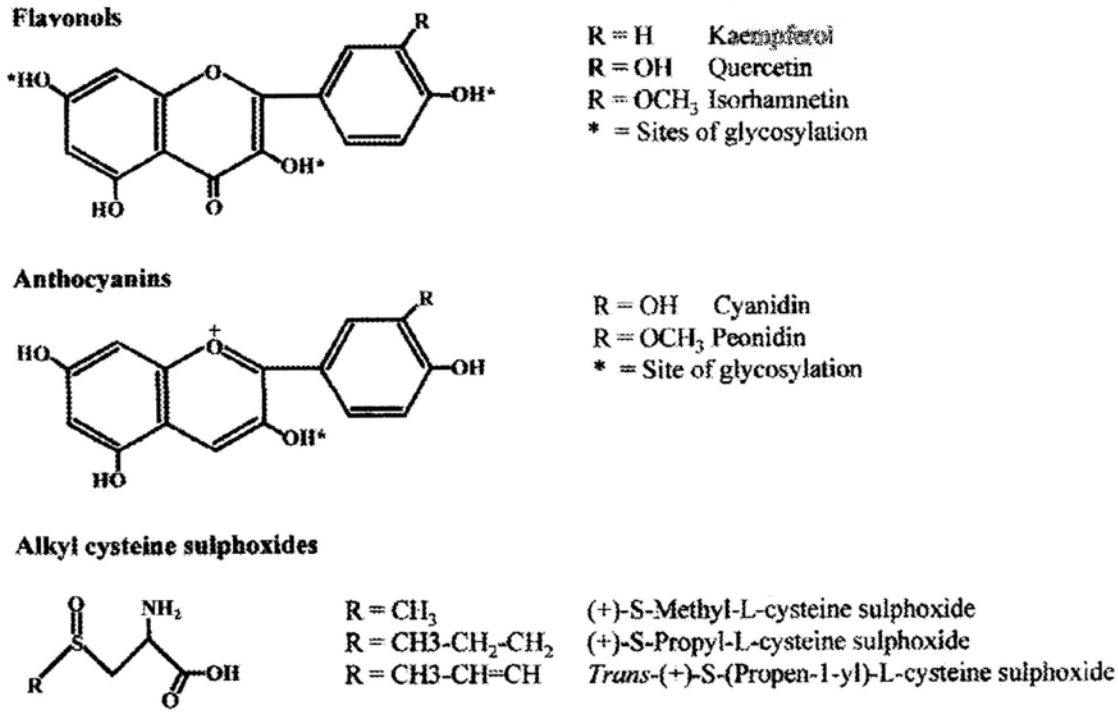

Figure 3.1 Beneficial health compounds in onion (Griffiths *et al.*, 2002)

3.3 Methodology

3.3.1 Diet

The control diet was prepared by mixing the following ingredients in proportion (g/kg diet): cornstarch, 508; casein, 242; lard, 50, sucrose, 119; mineral mix AIN-76, 40; vitamin mix AIN-76A, 20; DL-methionine, 1; cholesterol, 1. The two experimental diets were prepared by adding 1 and 5% onion powder w/w into the control diet, respectively. The powdered diets were mixed with a gelatin solution (20g/L) in a ratio of 200 g diet per liter of solution (Table 3.3). Once the gelatin has set, the diets were cut into pieces of approximately 10 g cubes and stored frozen at -20 °C.

3.3.2 Animal

Golden Syrian male hamsters (n = 36, 100~120 g) were housed in an animal room at 25 °C with 12:12-h light-dark cycles. Experiments were approved and conducted in accordance with the guidelines set by the Animal Experimental Ethical Committee, The Chinese University of Hong Kong. The animals were given free access to a standard cereal-based chow diet (PicoLab® Rodent Diet20-Lab Diet, Australia) and water for a 2-week acclimation period. The health status of the hamsters was monitored daily. All animals were allowed free access to a 0.1% cholesterol diet (control diet) and tap water for two weeks. Then they were weighed, ear-punched, and randomly divided into three groups (n=12 each) to fed control diet and one of the two experimental diets, which were similar to the control diet except that they were supplemented with 1% OP (1OP) and 5% OP (5OP) by weight, respectively, for a period of 8 weeks.

The procedure of the animal handling was as previously described in chapter 2 (2.1).

3.3.3 Study design

The study design and the measurement of experimental parameters were as previously described in chapter 2 (2.1, 2.3, 2.4, 2.5, and 2.6).

3.3.4 Statistics

The statistical analysis was as same as previously described in the chapter 2 (2.7).

Table 3.3 Composition (g) of the control and two experimental diets supplemented with 1% OP (1OP) and 5% OP (5OP)

Main Nutrients	Control	1OP	5OP
Corn starch	508	508	508
Casein	242	242	242
Lard	50	50	50
Sucrose	119	119	119
Mineral mixture AIN-76	40	40	40
Vitamin mixture AIN-76A	20	20	20
DL-Methionine	1	1	1
Cholesterol	1	1	1
OP	0	10	50
Gelatin	20	20	20

3.4 Results

3.4.1 Body weight, food intake, food efficiency, and relative organ weight

The data on body weight, food intake, food efficiency and relative organ weight are shown in Table 3.4. All animals offered onion powder diet remained in good health throughout the experimental period. No signs of toxicity were observed and onion consumption had no effect on body weight among the control, 1OP and 5OP groups.

Food intake was expressed as the diet each hamster consumed each day, whereas the food efficiency ratio was defined as the body weight gained by each hamster that consumed every 100 g of diet. Food intake and food efficiency did not differ among the experimental groups.

No difference in relative organ weight was seen among the groups, although hamsters fed OP with both doses had smaller epididymal and peripheral pad than control group.

Table 3.4 Body weight, food intake, food efficiency, and relative organ weight in hamsters fed the control, and two experimental diets supplemented with 1OP and 5OP

	Control	1OP	5OP
Body weight (g)			
Initial	127.1±6.6	124.2±10.2	124.6±12.1
Final	138.0±11.8	131.0±8.4	132.2±10.3
Food Intake (g/day/hamster)	9.44±0.34	9.89±0.48	9.32±0.35
Food Efficiency (g/100g diet)	7.32±1.40	7.72±1.27	8.07±1.70
Relative organ weight (g/100g body weight)			
Liver	4.06±0.15	4.06±0.29	4.04±0.16
Heart	0.35±0.03	0.35±0.02	0.36±0.03
Kidney	0.80±0.06	0.80±0.02	0.78±0.09
Epididymal fat	1.52±0.27	1.45±0.22	1.46±0.33
Peripheral fat	0.93±0.15	0.88±0.14	0.87±0.16

Data were expressed as mean ± SD; n=12 each group; means at the same raw with different superscripts (a, b, c) differ significantly at $p<0.05$.

3.4.2 Plasma and organ cholesterol

The plasma lipoprotein profiles in hamsters fed control and two experimental diets are shown in Table 3.5. There was no difference in plasma lipoprotein profiles among the three groups at week 0. Feeding of 5% onion powder diet significantly prevented the increase in plasma TC levels by 11.2% and 20.3% at week 4 and week 8, respectively. Non-HDL-C and the ratio of non-HDL-C/HDL in 5OP groups were also significantly decreased by 23.4% and 18.8%, respectively. In contrast, the ratio of HDL/TC in 5OP groups was significantly increased than that in the control. 1OP group tended to have similar effects as 5OP group but, statistically, no difference was observed between the control and 1OP groups.

Besides, both doses of onion powder diets could significantly countered the increase in plasma TG levels by 23.9 and 33.5% in 1OP and 5OP groups, respectively, compared with that in the control.

However, no significant differences were found in hepatic and heart cholesterol content among the three groups.

Table 3.5 Change in plasma and organ lipid concentrations in hamsters fed the control diet, and two experimental diets supplemented with 1OP and 5OP

	Control	1OP	5OP
TC			
Initial	207.40±36.41	207.19±48.92	207.34±47.98
4th week	216.4±24.9[a]	199.4±38.5[ab]	192.2±20.6[b]
8th week	215.1±39.6[a]	204.7±46.8[ab]	171.4±25.0[b]
HDL			
Initial	80.63±14.31	84.73±10.19	83.86±12.14
4th week	102.83±13.91	112.41±19.97	102.45±17.09
8th week	96.48±7.99[a]	102.83±10.13[a]	84.31±7.38[b]
non-HDL			
Initial	126.76±29.92	122.46±41.02	123.49±42.12
4th week	118.00±7.68	97.71±41.07	94.10±15.03
8th week	107.36±27.78[a]	99.45±26.36[ab]	82.29±13.35[b]
HDL/TC			
Initial	0.39±0.06	0.42±0.07	0.42±0.07
4th week	0.46±0.04	0.50±0.11	0.51±0.06
8th week	0.46±0.08[b]	0.49±0.09[ab]	0.51±0.03[a]
non-HDL/HDL			
Initial	1.60±0.35	1.43±0.38	1.48±0.47
4th week	1.17±0.22	0.99±0.54	0.97±0.21
8th week	1.17±0.35[a]	0.95±0.23[ab]	0.95±0.11[b]
TG			
Initial	155.4±39.7	157.6±45.3	147.3±73.2
4th week	211.9±61.7	172.9±50.5	167.1±43.9
8th week	218.3±92.0[a]	166.3±73.6[b]	145.4±36.0[b]
Organ cholesterol			
Liver	80.18±10.50	88.47±11.33	77.60±13.28
Heart	3.72±0.35	3.72±0.26	3.64±0.24

Non-HDL-C= [TC]-[HDL-C];

Data were expressed as mean ± SD; n=12 each group; means at the same raw with different superscripts (a, b, c) differ significantly at $p<0.05$.

3.4.3 Fecal excretion

Concentration of individual neutral and acidic sterols in the feces of the hamsters at week 8 is shown in Table 3.6. In general, the control group excreted lesser amount of neutral sterols than the other two experimental groups. Similar observation was found in individual acidic sterols except for chenodeoxycholic acid, cholic acid and ursodeoxycholic acid.

Total intake of cholesterol was compared with its excretion in neutral and acidic sterols (Table 3.7). Net cholesterol equivalent retained was calculated by difference between intake and excretion of both neutral and acidic sterols. It was found that the net cholesterol retention was the highest in the control group, followed by 1OP and 5OP in a decreasing order. The apparent cholesterol absorption was calculated in an equation [(cholesterol intake – excretion of neural and acidic sterols)/cholesterol intake]. It was shown that onion powder diet could decrease cholesterol absorption.

Table 3.6 Changes in fecal output of individual neutral and acidic sterols in hamsters fed the control, and two experimental diets supplemented with 1OP and 5OP

	Control	1OP	5OP
Neutral sterols			
Coprostanol	0.59±0.14	0.70±0.01	0.86±0.43
Coprostanone	0.03±0.01	0.05±0.01	0.05±0.01
Cholesterol	0.79±0.19	0.81±0.14	0.84±0.15
Dihydrocholesterol	0.35±0.06	0.38±0.02	0.40±0.03
Campesterol	ND	0.06±0.01	0.10±0.04
Stigmasterol	ND	0.11±0.01	0.15±0.01
β- sitosterol	ND	0.03±0.01	0.05±0.02
Total	1.76±0.38^{b}	2.14±0.15^{ab}	2.45±0.34^{a}
Acidic sterols			
Lithocholic acid	1.95±1.25	3.14±0.74	3.21±0.93
Deoxycholic acid	0.52±0.11	0.38±0.05	0.44±0.22
Chenodeoxycholic acid + Cholic acid	1.14±0.89	0.68±0.22	0.51±0.11
Ursodeoxycholic acid	0.26±0.05	0.28±0.06	0.63±0.09
Total	3.88±0.19	4.49±0.97	4.79±1.12

Non-HDL-C= [TC]-[HDL-C];

Data were expressed as mean ± SD; n=12 each group; means at the same raw with different superscripts (a, b, c) differ significantly at $p<0.05$.

Table 3.7 Cholesterol balance in hamsters fed the control diet, and two experimental diets supplemented with 1OP and 5OP

	Control	1OP	5OP
Food Intake (g/d/hamster)	12.19±0.79	11.56±0.56	11.19±0.97
Neutral Sterol (mg/d/hamster)	1.69±0.50^{b}	1.94±0.16^{ab}	2.15±0.31^{a}
Acetic Sterol (mg/d/hamster)	3.88±0.19	4.49±0.97	4.79±1.12
Total Sterol output (mg/d/hamster)	5.88±0.76	6.53±0.11	6.44±0.69
Cholesterol Retained (mg/d/hamster)	6.84±1.46	5.03±0.31	5.17±0.09
Apparent cholesterol absorption (% intake)	0.54±0.09	0.42±0.01	0.45±0.03

Non-HDL-C= [TC]-[HDL-C];

Data were expressed as mean ± SD; n=12 each group; means at the same raw with different superscripts (a, b, c) differ significantly at $p<0.05$.

3.4.4 Western Blot results of SREBP-2, HMGR, LXRα/β, CYP7A1 and LDLR

Results of Western blot analysis of SREBP-2, HMGR, LXRα/β, CYP7A1 and LDLR are shown in Fig 3.2, 3.3, 3.4, 3.5, 3.6, 3.7. 5% onion powder diet could significantly up-regulate SREBP-2, LXRβ, and CYP7A1 protein expression. 1OP group had the similar effects but, statistically, no difference was observed between control and 1OP group. Onion powder diet also slightly increases the HMGR, LXRα and LDLR protein mass.

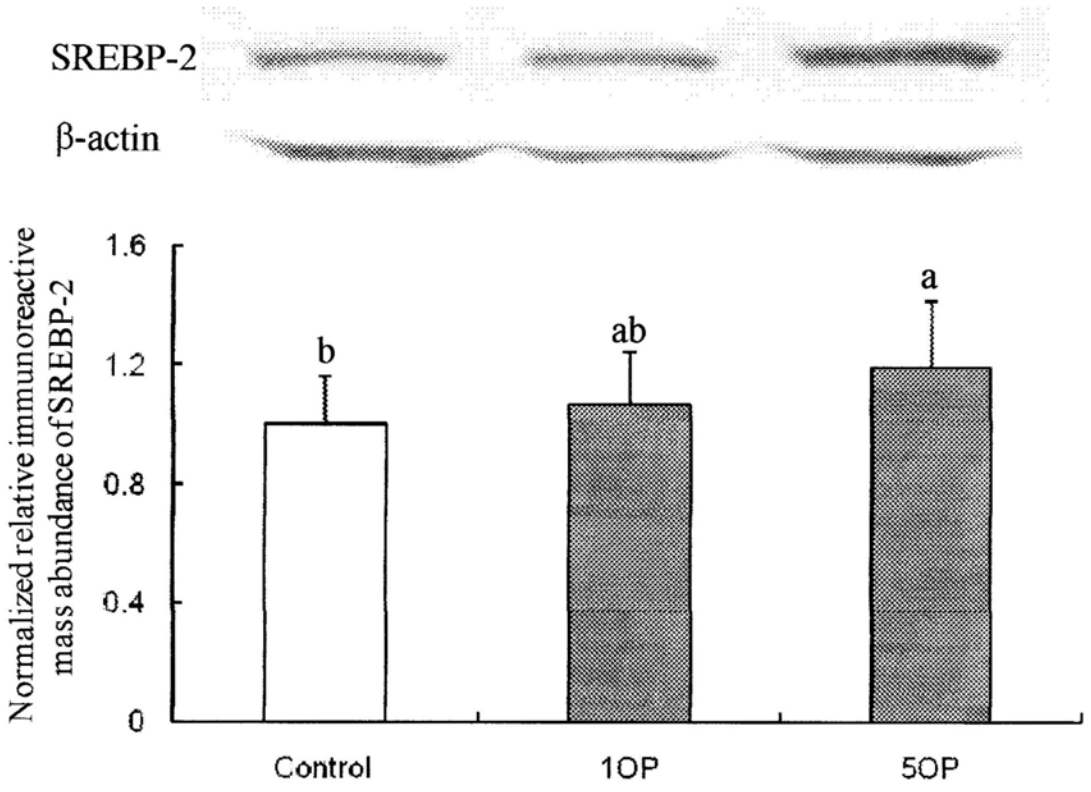

Figure 3.2 Effects of OP on the relative immunoreactive mass of hepatic SREBP-2 as determined by Western blot analysis. Data were normalized with β-actin so that value of the control group was regarded as 1.0. Values were expressed as mean ± SD, n=12.

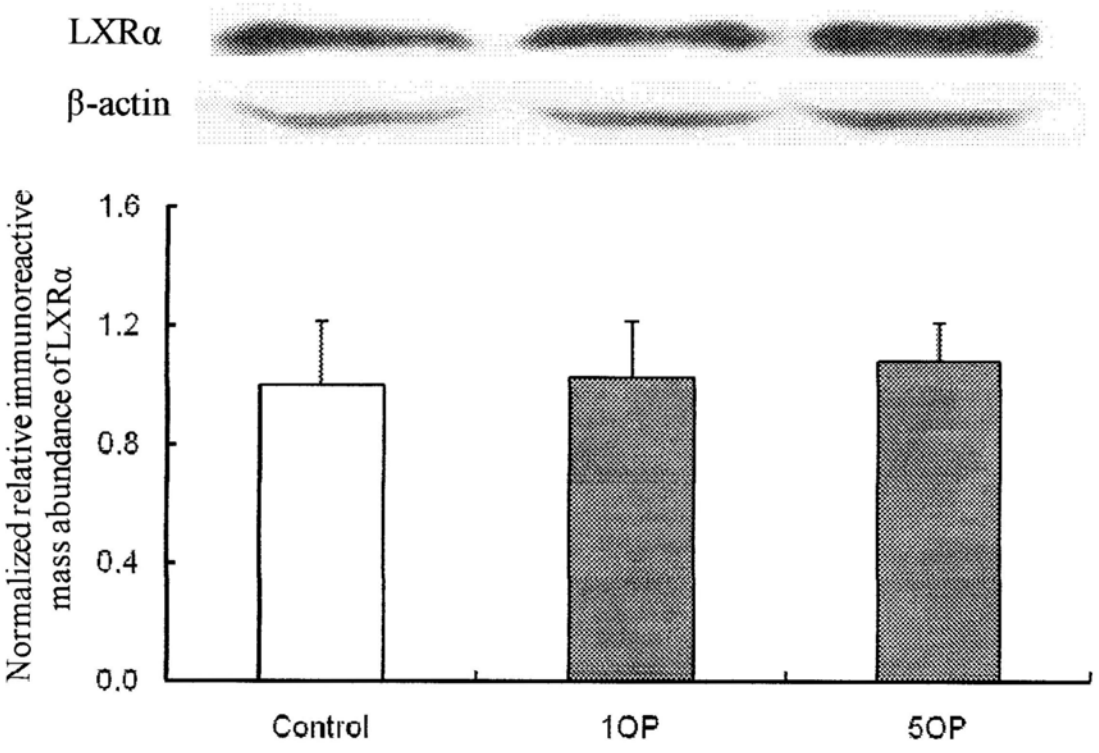

Figure 3.3 Effects of OP on the relative immunoreactive mass of hepatic LXRα as determined by Western blot analysis. Data were normalized with β-actin so that value of the control group was regarded as 1.0. Values were expressed as mean ± SD, n=12.

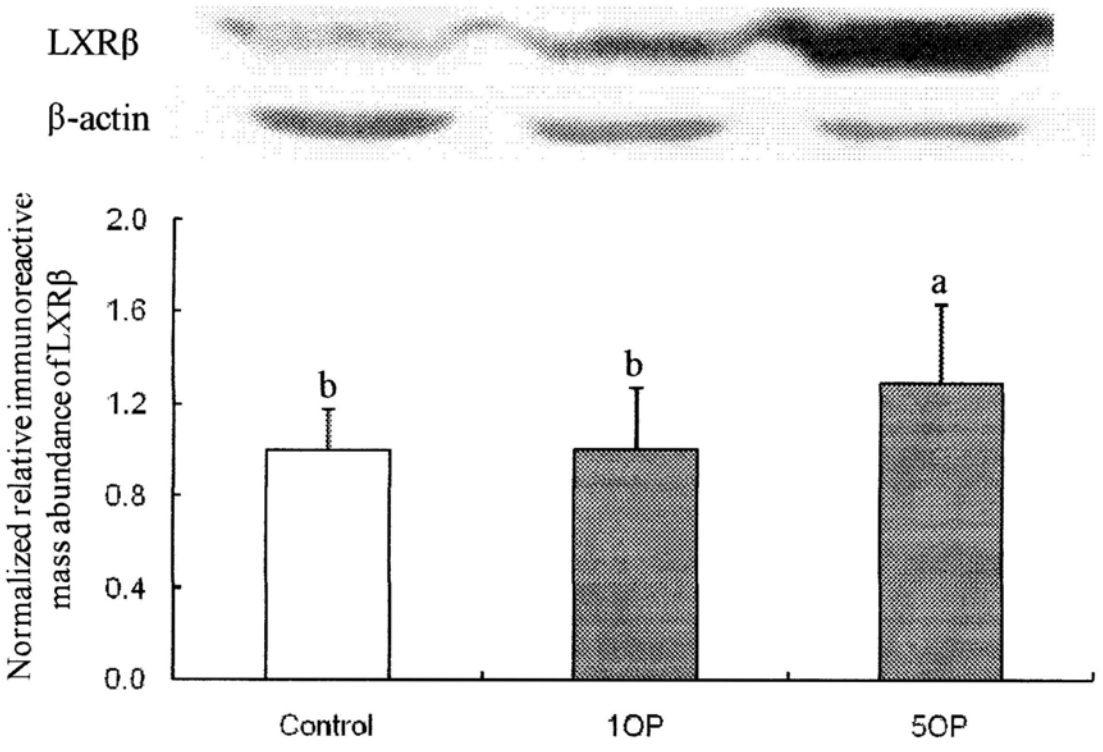

Figure 3.4 Effects of OP on the relative immunoreactive mass of hepatic LXRβ as determined by Western blot analysis. Data were normalized with β-actin so that value of the control group was regarded as 1.0. Values were expressed as mean ± SD, n=12.

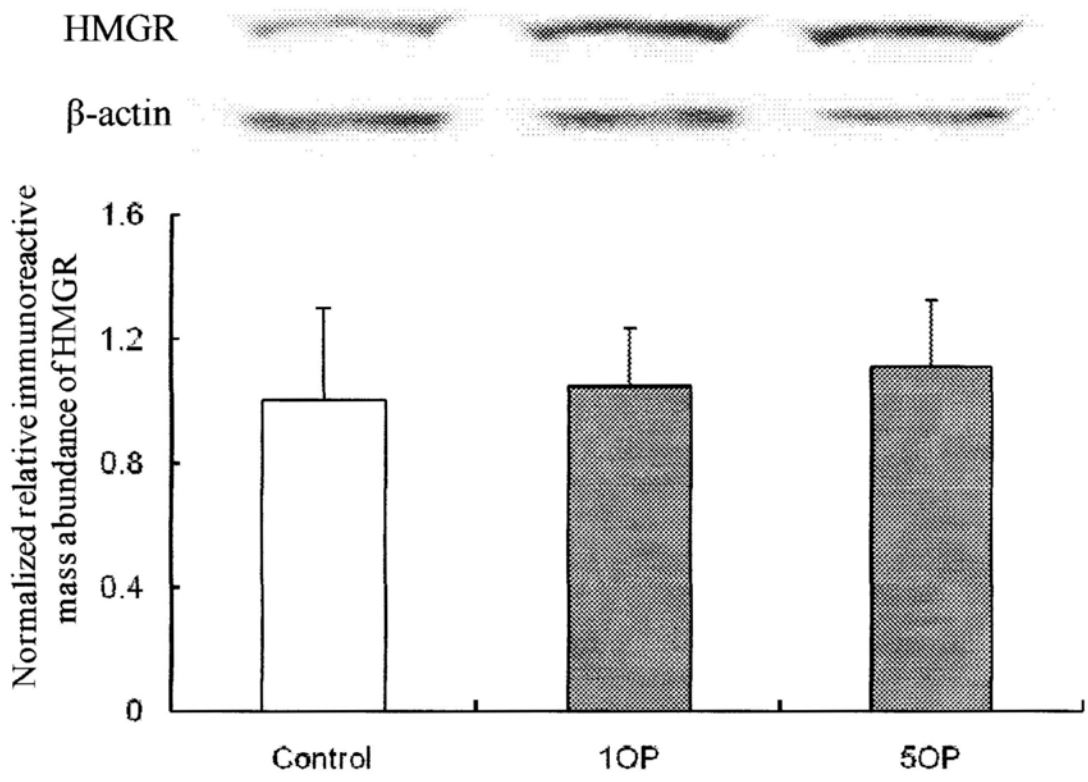

Figure 3.5 Effects of OP on the relative immunoreactive mass of hepatic HMGR as determined by Western blot analysis. Data were normalized with β-actin so that value of the control group was regarded as 1.0. Values were expressed as mean ± SD, n=12.

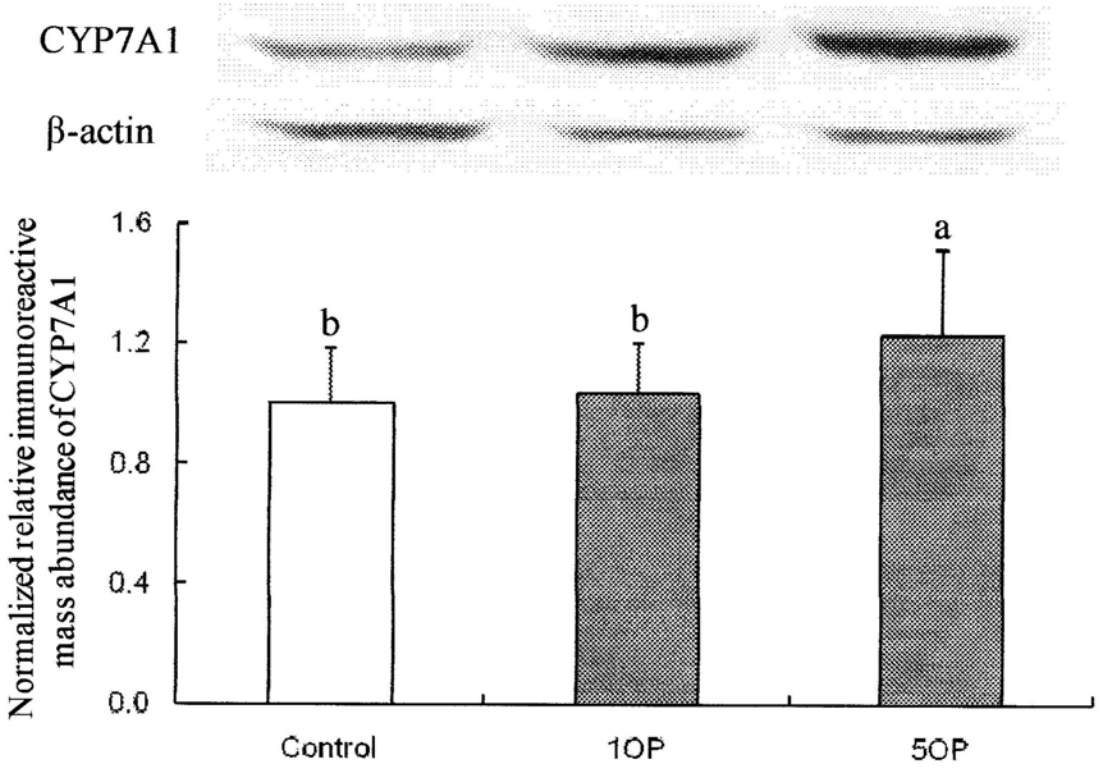

Figure 3.6 Effects of OP on the relative immunoreactive mass of hepatic CYP7A1 as determined by Western blot analysis. Data were normalized with β-actin so that value of the control group was regarded as 1.0. Values were expressed as mean ± SD, n=12.

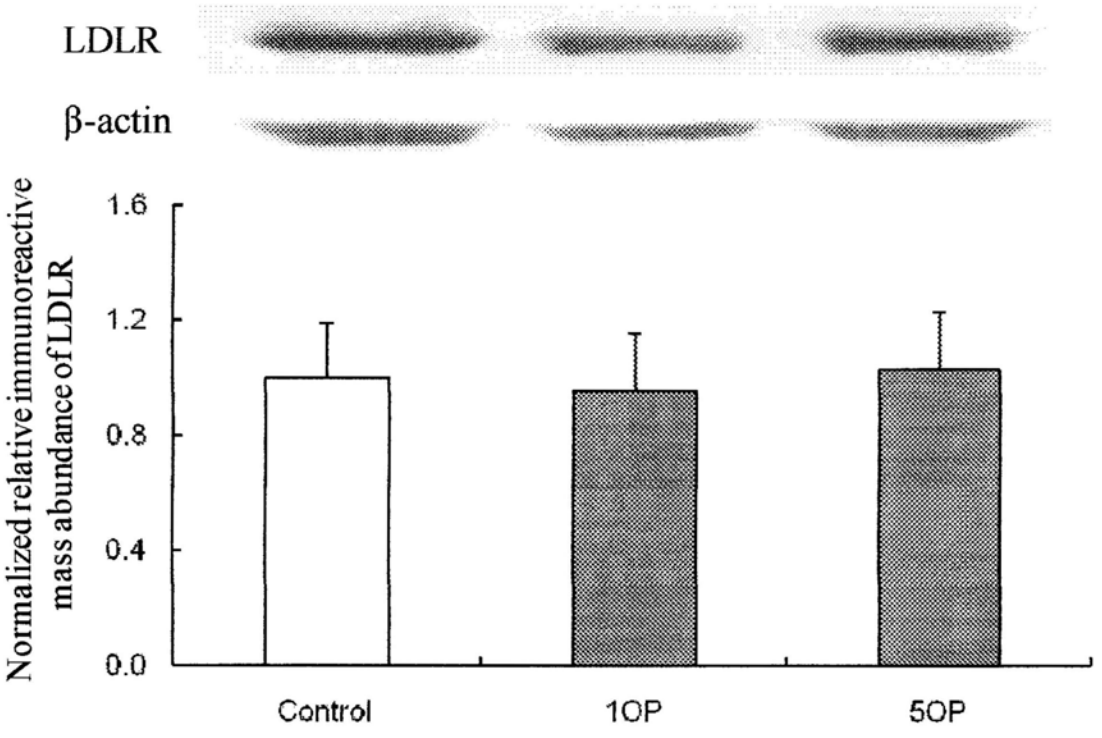

Figure 3.7 Effects of OP on the relative immunoreactive mass of hepatic LDLR as determined by Western blot analysis. Data were normalized with β-actin so that value of the control group was regarded as 1.0. Values were expressed as mean ± SD, n=12.

3.5 Discussion

Allium spices, garlic and onion have been reported to possess health beneficial hypolipidemic, hypocholesterolemic and hydrocholagoguic influence (Srinivasan *et al.*, 2004). The present report was the first one to demonstrate the underlying mechanism of the hypocholesterolemic effect of dietary onion using the male Golden Syrian hamsters which are often used animal model for cholesterol study.

The current study demonstrated that supplemented with dietary onion powder in the diet could favorably modify the plasma lipid profile. High dose of onion powder (5%) could significantly reduce the plasma total TC, non-HDL-C, and TG, subsequently decrease the ratio of non-HDL-C to HDL-C statistically and increase in the ratio of HDL/TC. The ratio of LDL-C to HDL-C is commonly calculated to assess the risk of CHD. In the present study, since LDL-C accounts for most of the cholesterol in the hamsters' non-HDL-C, non-HDL-C was therefore calculated instead of LDL-C. The decreased ratio of non-HDL-C to HDL-C in the onion-fed hamsters indicated that the onion powder supplemented in the diet would be beneficial in prevention of CHD.

It is noteworthy to note that there was a dose-dependent reduction in circulating TG concentration, with the low and high levels of dietary onions by 23.9 and 33.5%, respectively, compared with the control. It is believed that in the liver, LXRs regulate lipid metabolism, but, LXRα is more important in cholesterol metabolism, and LXRβ plays a role in triglyceride metabolism (Michael *et al.*, 2005). In the present study, the increase in the protein abundance of LXRβ after onion feeding could explain the decrease in plasma TG in the onion-fed hamsters. Besides, the mechanisms underlining the hypotriacylglyceridaemic effect of onion might be partly attributed to a decreased rate of *de novo* fatty acid synthesis in the adipose tissue which is the main site of fat metabolism (O'Hea and Leveille, 1969). In the present study, both epididymal fat and peripheral fat were decreased in both onion-fed groups. Furthermore, over-production of ApoB100- containing lipoproteins was reported to lead to atherosclerosis and CHD. The plasma TG lowering effect was postulated

partially to the decreases in intestinal microsomal TG transfer protein (MTP) mRNA and apolipoprotein B100 (ApoB100) secretion (Lin *et al.*, 2002; Han *et al.*, 2002).

The increases in the excretion of bile acids and neutral sterols might be one of the mechanisms for lowering cholesterol content. SMCS contains a cysteine moiety, which has been reported to raise the level of CYP7A1 activity, a key enzyme in the synthesis of bile acids from cholesterol, by conferring its antagonist effect to the Farnesoid X receptor (FXR) which inhibits the expression of CYP7A1, similar to gugulipid (Stephan *et al.*, 1987). This is in accordance with the observations in the present study showing that the increased level of CYP7A1 protein abundance was associated with the higher amount of bile acids excretion in the onion-fed hamsters.

It is interesting to note that the major phytosterols, i.e. β-sitosterol, campesterol, and stigmasterol, were detected in the feces of the two onion-fed groups, and the amount of the three kinds of phytosterols determined in the feces among the three groups was in order of 5OP > 1OP > control. This could partially explain the relatively lower level of TC in the onion-fed hamster body because phytosterols competed with cholesterol for absorption, resulting in the decrease in the cholesterol absorption but increase in the cholesterol and neutral sterols excretion.

The response seen in the protein abundance of SREBP-2 after onion powder feeding is noteworthy. Similar to the pattern observed for the abundance in HMGR protein, SREBP-2 protein abundance was the highest in hamsters fed with 5% onion diet relative to the control. SREBP-2, as an activated transcriptional factor, is regulated at the transcriptional level by both the sterol depletion and the proteolytic cleavage cascade at the posttranslational level (Brown and Goldstein, 1997). The modest rise in HMGR protein abundance may be a result of an increase in the SREBP-2 transcription in the present study. In fact, cholesterol depletion could up-regulate the cholesterol biosynthesis pathway (Sato *et al.*, 1996). Correlation between HMGR mRNA and protein abundance, and resistance to dietary cholesterol induced hypercholesterolemia among animal species were reported by Ness and Gertz (2004). Animals with the highest HMGR mRNA and protein abundance such as rat, were the most resistant to dietary cholesterol relative to animals with lower abundance such as

hamster. This could explain the cause-and-effect relationship between the low HMGR protein abundance and the high non-HDL-C level in response to the control fed, i.e. lard, compared with onion- fed hamsters.

Biosynthesis of cholesterol is regulated by HMGR, whereas its biodegradation to bile acids in hepatocytes is carried out by cholesterol 7β-hydroxylase and sterol 27-hydroxylase (Dietschy *et al.*, 1993). Vidyashankar *et al.* (2009) reported that hepatic HMGR activity was diminished by lithogenic diet, prepared by supplemented 0.5% cholesterol and 0.25% bile salts to the AIN-76 basal diet, due to negative feedback inhibition. Feeding onion powder diet (2%) could restore this altered activity significantly. The possible explanation for this could be that endogenous cholesterol synthesized by *de novo* process might be preferred over the exogenous cholesterol for conversion into bile acids and for further excretion in bile. Vidyashankar *et al.* (2009) also documented both CYP7A1 and sterol 27-hydroxylase activities were inhibited by feeding lithogenic diet to the experimental animals, while onion diet could counteract the decrease in the enzyme activity significantly. Therefore, in the present study, reduced levels of cholesterol in both plasma and liver might be the combined effects of modestly increased in cholesterol synthesis and the significantly enhanced conversion of cholesterol to bile acid in the onion-fed hamsters.

In conclusion, the *Allium cepa* are rich in alkyl and alkenyl sulfoxide compounds, anthocyanin, quercetin and cycloalliin. All of them have therapeutic properties. This study was the first to show the relationship of the genes associated with the protein expression of cholesterol-regulation with the plasma cholesterol modification, and both fecal neutral sterol and bile acid excretions. Although the exact mechanisms involved in the therapeutic effects of *Allium cepa* to hypercholesterolemia remained unknown, its protection against cardiovascular diseases through the decrease in plasma cholesterol, increase in the excretion of both bile acids and neutral sterols, and decrease in triglyceride by activation of SREBP-2, CYP7A1, and LXRβ at translational level were observed in this investigation.

Chapter 4
Overall Conclusion

In the past twenty years, consumers began to regard their diets no longer as a means to satisfy hunger or provide essential nutrients for the maintenance and / or repair of body tissues, this changing concept of food brought into new area of the foods and nutritional sciences known as functional foods. The interest in functional foods has resulted in a number of new foods in the marketplace designed to address specific health concerns, particularly as regards chronic diseases, such as aging, cancer, diabetes mellitus, and cardiovascular diseases. In the present study, two kinds of functional foods were chosen to investigate their cholesterol modifying effects.

While tea leaves are used worldwide, tea seed oil is only used in some Asian countries. Extensive research has shown the health benefits of tea drinking. However, benefit associated with the consumption of tea seed oil remains unclear. We have shown that like grape, canola and corn oils, tea seed oils were able to dramatically lower plasma cholesterol in hamsters fed a 0.1% cholesterol diet. The hypocholesterolemic activity of tea oil was characterized by decreasing only non-HDL-C and having no effect on HDL-C concentration. Most importantly, tea oil could up-regulate SREBP-2 and LDL receptor. It was further demonstrated that hamsters fed the tea oil diet excreted less neutral but greater acidic sterols compared with other three vegetable oils, leading to decreased cholesterol absorption. These results suggest that the tea seed oil could be alternative healthy oil for human consumption.

In the mean time, onion had been used as medical agents for many centuries in the folklore, along with other members of Allium family acquired reputation in many cultures. However, the work on the health benefits of onion is less advanced, the underlying mechanisms were still remaining unclear, and therefore onion became another investigated subject in the present study. It was found that onion could

significantly reduce plasma TC and non-HDL-cholesterol in hamsters fed a 0.1% cholesterol diet. Besides, onion powder diets could remarkably counter the increase in plasma TG levels compared with that in the control. Onion could increase the output of fecal neutral sterols and acidic sterols, resulting in lesser amount of cholesterol retained and cholesterol absorption. It is noteworthy that onion powder diet could up-regulate SREBP-2, LXRβ, and CYP7A1 protein. The positive results from present study as well as the fact that onions are eaten by almost all consumers and ethic groups make it a potential functional food.

In conclusion, diet plays an important role in reducing the risk of CVD. This has led to the search for specific foods and food components that may help to improve the plasma lipoprotein profile. Extensive research is currently directed toward increasing our understanding of "functional foods". It is also important to monitor not only the effects of a functional food ingredient on the lipoprotein profile but also on other aspects of health effects and its mechanism under various conditions, such as in combination with cholesterol-lowering diets or drugs, and in different population groups. Attention should be paid not only to beneficial but also to potentially adverse health effects.

It is must realized that there are no good or bad "foods", only good or bad dietary pattern. Diet is only one aspect of a comprehensive lifestyle approach toward good health, once combined with regular exercise, tobacco avoidance, stress reduction, maintenance of healthy body weight and other positive health practices, functional foods can become part of an effective strategy to maximize health and reduce risk of diseases.

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