Research Article

Choosing hamsters but not rats as a model for studying plasma cholesterol-lowering activity of functional foods

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Rats and hamsters are commonly used rodents to test the efficacy of cholesterol-lowering functional foods. In general, a diet containing 1% cholesterol for rats whereas a diet containing 0.1% cholesterol for hamsters is used to induce the hypercholesterolemia. The present study was carried out to compare hamsters with rats as a hypercholesterolemia model. Golden Syrian hamsters and Sprague Dawley rats were randomly divided into four groups and fed one of the four diets containing 0 – 0.9% cholesterol. Results demonstrated that serum total cholesterol (TC) in hamsters was raised 73 – 81% higher than that in rats fed the same cholesterol diets. Unlike rats in which HDL-C accounted very little for serum TC, the lipoprotein profile in hamsters was closer to that in humans. We investigated interaction of higher cholesterol diets with 3-hydroxy-3-methylglutary-CoA (HMG-CoA) reductase, low-density lipoprotein receptor (LDL-R) and cholesterol-7α-hydroxylase (CYP7A1), sterol regulatory element binding protein-2 (SREBP-2), and liver X receptor (LXR-α). Results showed hamsters and rats metabolized cholesterol differently. In view that hamsters synthesize and excrete cholesterol and bile acids in a manner similar to that in humans, it is concluded that hamsters but not rats shall be chosen as a model to study efficacy of cholesterol-lowering functional foods.

Keywords: ACAT / CYP7A1 / Hamsters / LDL receptor / Rats

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1 Introduction

Interest in functional foods to treat hypercholesterolemia is growing [1]. Rats and hamsters are commonly used animal models to test the efficacy of these functional foods in lowering blood cholesterol levels. When the literatures are carefully searched, hypercholesterolemia (about 200 mg cholesterol /dl plasma) in rats is usually induced by feeding a 1% cholesterol diet [2, 3], whereas in hamsters it is achieved by feeding a 0.1% cholesterol diet [4, 5]. It remains poorly justified why a 1% cholesterol diet used for rats induce the same degree of hypercholesterolemia as a 0.1% cholesterol diet used for hamsters. It is apparent that addition of 1% cholesterol in diet used for rats is unreasonably high and requires validation, because diet in any mammals shall never contain cholesterol at such a high level.

Hypocholesterolemic activity of functional foods is usually mediated by their interaction with cholesterol metabolism. Cholesterol has acquired an abhorrent reputation for many years due to the strong correlation between plasma total cholesterol (TC) and the incidence of coronary heart disease (CHD). Elevated levels of plasma TC and low-density lipoprotein cholesterol (LDL-C) are the major risk factors, whereas high concentrations of plasma high-density lipoprotein cholesterol (HDL-C) and a low ratio of TC to

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Abbreviations: ACAT, acyl coenzyme A:cholesterol acyltransferase; **CYP7A1,** cholesterol 7α-hydroxylase; **HDL-C,** high-density lipoprotein cholesterol; **HMG-CoA-R,** 3-hydroxy-3-methylglutary-CoA reductase; **LDL-C,** low-density lipoprotein cholesterol; **LDL-R,** LDL receptor; **LXR-α**, liver X receptor; **SREBP-2,** sterol regulatory element binding protein-2; **TC,** total cholesterol; **TG,** triacylglycerols

HDL-C are protective against CHD [6]. Cholesterol homeostasis must be maintained to avoid over-accumulation of cholesterol to a harmful level in circulation. In this regard, two transcriptional factors, sterol regulatory element binding protein-2 (SREBP-2) and liver X receptor (LXR-α), act in a coordinated manner to govern cholesterol metabolism [7]. 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. In addition, intestinal acyl coenzyme A:cholesterol acyltransferase (ACAT), which catalyzes the conversion of cholesterol to cholesteryl ester, may also affect blood cholesterol level by regulating dietary cholesterol absorption.

The present study was to characterize the differences between rats and hamsters in interaction of dietary cholesterol with serum lipoprotein profile, gene expression of SREBP-2, LXR-α, HMG-CoA-R, LDL-R, and intestinal ACAT. The objective was to address a fundamental issue of which species, rats or hamsters, should be chosen as a model for studying the cholesterol-lowering activity of functional foods.

2 Materials and methods

2.1 Diets

Four diets were prepared. The control diet (0% cholesterol added into diet) was prepared by mixing the following ingredients per kg diet (g): corn starch, 508; casein 242; sucrose, 119; lard, 50; AIN-76 mineral mix, 40; AIN-76 A vitamin mix, 20; gelatin, 20; DL-methionine, 1. The three experimental diets were prepared by adding 0.3, 0.6, and 0.9% cholesterol into the control diet.

2.2 Animals

Forty Sprague Dawley (SD) rats (300 – 340 g; age = 10 wk) and forty Golden Syrian hamsters (*Mesocricetus auratus*, 120 – 130 g, age = 10 wk) were randomly divided into four groups (n = 10 each) and fed one of the four diets *ad libitum* for a period of six weeks. Food intake was measured daily and body weight was recorded twice a week. The total fecal output was pooled from each animal. At the end of six weeks, all animals were fasted overnight and killed under CO_2 anaesthesia. Blood was collected and serum was obtained. Liver, heart, kidney, adipose tissues (perirenal and epididymal pads) were removed, washed in saline, and weighed. For measurement of intestinal ACAT, the first 10 cm of duodenum was discarded, and the next 30 cm of the small intestine was kept. All tissue samples were flash frozen in liquid nitrogen and stored at -80°C until analyses. The experimental protocol was approved by the Animal Experimentation Ethics Committee, The Chinese University of Hong Kong.

2.3 Serum lipids

Serum TC and total triacylglycerols (TG) were determined using enzymatic kits from Infinity (Waltham, MA, USA) and Stanbio Laboratories (Boerne, TX, USA), respectively. 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, Boerne, TX, USA). LDL-C was calculated as the difference between TC and HDL-C.

2.4 Western blotting analyses of liver SREBP-2, LDL-R, HMG-CoA-R, LXR-α and CYP7A1

Total liver proteins were extracted according to the method described by Vaziri *et al*. [8] with some modifications. In brief, the liver was homogenized in buffer A which contained 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 $12\,000 \times g$ for 15 min at 4°C and the supernatant was retained (total protein). A portion of total protein was centrifuged at $126\,000 \times g$ for 60 min at 4°C. The pellet (membrane protein) was re-suspended in buffer A. Protein concentration of two fractions was determined using a protein concentration assay kit obtained from BioRad (Hercules, CA, USA).

LDL-R, CYP7A1, and HMG-CoA-R were quantified as we previously described [9]. The membrane protein was size-fractionated on a 7% SDS-PAGE gel. The proteins bound in the gel were transferred to a Hybond-P PVDF membrane (Amersham Pharmacia Biosciences, Uppsala, Sweden), which was immediately blocked with 5% nonfat milk in 1 × TBS containing 0.1% Tween-20 and then incubated overnight at 4°C with anti-LDL-R (Santa Cruz Biotechnology, Inc., CA, USA), anti-HMG-CoA-R (Upstate, Lake Placid, NY, USA) or anti-CYP7A1 antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The membrane was further incubated with diluted horseradish peroxidase-linked rabbit anti-goat IgG (Zymed Laboratories, San Francisco, CA, USA) or donkey anti-rabbit IgG (Santa Cruz Biotechnology) followed by detection with ECL enhanced chemiluminescence agent (Amersham Life Science) and subjected to autoradiography on Super*RX* medical X-ray film (Fuji, Tokyo, Japan). Quantification was made using the computer software Photoshop® (Adobe Systems Inc, CA, USA). To quantify SREBP-2, equal amounts of the membrane protein and the total protein aliquots were

mixed and run on a 7% SDS-PAGE gel. The primary antibody used was anti-SREBP-2 antibody (Santa Cruz Biotechnology). Data on abundance of SREBP-2, LDL-R, HMG-CoA-R, LXR-α, and CYP7A1 were normalized with β-tubulin.

2.5 Real time PCR analyses of mRNA for liver SREBP-2, LDL-R, HMG-CoA-R, LXR-α and CYP7A1

mRNA levels for liver SREBP-2, LDL-R, HMG-CoA-R, LXR-α, and CYP7A1 were quantified as previously described (9). In brief, total liver mRNA was extracted and isolated using Tizol® Reagent (Invitrogen, Carslbad, CA, USA). Briefly, after addition of Trizol, the liver sample was homogenized on ice and then centrifuged at $4000 \times g$ for 25 min at 4°C. The supernatant was retained and incubated at room temperature for 5 min. RNA was precipitated by addition of chloroform and the aqueous layer was collected and added into isopropyl alcohol. The RNA pellet formed after centrifugation was re-suspended with 75% v/v ethanol, followed by air drying. After dissolution in DEPC-treated water solution, the samples were stored at −80°C.

Total RNA was converted to complementary DNA (cDNA) using High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA, USA). Reverse transcription was carried out in a thermocycler (Gene Amp® PCR system 9700, Applied Biosystems), with program set as initiation for 10 min at 25°C, followed by incubation at 50°C for 90 min and at 85°C for additional 5 min. The cDNA synthesized was stored at −20°C.

Real-time PCR analysis was carried out on a Fast Real-time PCR System 7500 (Applied Biosystems). Primers and TaqMan® probes used for real-time PCR for these genes in hamsters were: GAPDH, F: GAACATCATCCCTGCATCCA, R: CCAGTG AGCTTCCCGTTCA, TaqMan probe: CTTGCCCACAGCCTTGGCAGC (10); CYP7A1: F: GGTAGTGTGCTGTTGTATATGGGTTA, R: ACAGCCCAGGTATGG AATCAAC, TaqMan probe: CACCTGCTTTCCTTCTCC; HMG-CoA R: F: CGAAGGGTTTGCAGTGATAAAGGA, R: GCCATAGTCACATGAAGCTTCTGT A, TaqMan probe: ACGTGCGAATCTGCT; LDL-R: F: GCCGGGACTGGTCAG ATG, R: ACAGCCACCATTGTTGTCCA, TaqMan probe: GCACTCATTGGTCCT GCAGTCCTT (10); SREBP-2: F: GGACTTGGTCATGGGAACAGATG, R: TGTAATCAATGGCCTTCCTCAGAAC, TaqMan probe: CCAAGATGCACAA ATC. The expressions of target genes were normalized with that of GAPDH, a housekeeping gene and being used as an internal control. Assay-on-Demand Gene Expres b sion Products (Applied Biosystems) were used as primers and probes for all the genes in rats.

Real-time PCR was performed using a TaqMan Fast Universal PCR Master Mix (Applied Biosystems). The reaction mixture was subject to thermal cycling under the following conditions: heating up to 95°C in 20 s, followed by 40 cycles at 95°C for 3 s and 60°C for 30 s. Data were analyzed using the Sequence Detection Software version 1.3.1.21 (Applied Biosystems). Gene expressions were calculated according to the comparative Threshold cycle (C_T) method (Applied Biosystems). Levels of gene expressions in the treatment groups were presented as a ratio of treatment to the control group.

2.6 Measurement of intestinal ACAT activity

Intestinal ACAT activity was measured according to the method previously described [9]. Briefly, the intestinal microsome was prepared and cholesterol in 45% w/v 2-hydroxypropyl β-cyclodextrin aqueous solution was added followed by incubation in a 37°C water bath for 5 min. The reaction was initiated by adding an assay reagent of 0.52 nmol of [^{14}C] oleoyl-Coenzyme A (0.03 μCi), 7.48 nmol of non-radioactive oleoyl-Coenzyme A and 10 nmol of fatty acid-free bovine serum albumin. After 20 min, the reaction was stopped by adding 4.8 mL of chloroform: methanol mixture (2:1, v/v) and 1 mL saline. After addition of 10 μg [^{3}H] cholesterol oleate (0.002 μCi), the mixture was centrifuged at $800 \times gg$ for 10 min at 4°C and the lower organic layer was collected and transferred to a new tube and evaporated under a gentle nitrogen stream until dryness. Cholesteryl oleate (10 μg) in 50 μL of chloroform was then added and the tube was vortexed thoroughly. The re-suspension was then spotted on a thin-layer silica gel plate (Merck, NJ, USA) and developed in a solvent mixture of hexane/ethyl acetate/acetic acid (80:20:1, v/v) for 45 min. The band corresponding to cholesterol oleate was cut off and transferred into a scintillation vial. 10 mL of OptiPhase HiSafe 2 scintillation fluid (PerkinElmer) was added into the vial and incubated with agitation overnight. Radioactivity was then measured in a LS 6500 scintillation counter (Beckman) and the data were calculated on the basis of [^{3}H] recovery.

2.7 Determination of hepatic cholesterol

Total hepatic cholesterol was analyzed as we previously described [11]. In brief, total lipids were extracted from 300 mg liver sample with addition of 1 mg of stigmastanol as an internal standard, using 15 mL chloroform/methanol (2:1, v/v) and 3 mL saline. After the mild hydrolysis with 5 mL NaOH in 90% ethanol at 90°C, esterified cholesterol was converted to free cholesterol. Total cholesterol was then extracted with 1 mL of water and 6 mL of cyclohexane. After centrifugation, the extract was evaporated to dryness under a gentle stream of nitrogen. Cholesterol was converted to its trimethyl-silyl (TMS)-ether derivative by a commercial TMS reagent (dry pyridine/hexamethyldisilazane/trichlorosilane, 9:3:1 v/v/v, Sil-A reagent; Sigma, St. Louis, MO, USA). Analysis of the cholesterol TMS-ether

derivative in hexane was performed in a fused silica capillary column (SAC^{TM}-5, 30 m × 0.25 mm, id; Supelco, Bellefonte, PA, USA) in a Shimadzu GC-14B GLC equipped with a flame-ionization detector (Shimadzu, Tokyo, Japan).

2.8 Determination of fecal neutral and acidic sterols

All fecal outputs were pooled, mixed, and ground in a coffee grinder. Individual fecal neutral and acidic sterols were quantified as previously described [11]. In brief, 300 mg fecal sample was taken and stigmasterol (0.5 mg in 1 mL of chloroform) as an internal standard for neutral sterols was added. The sample was saponified using 9 mL of 1 mol/L NaOH in 90% ethanol containing 0.5 mg hyodeoxycholic acid in 2 mL of 1N NaOH as an internal standard for acidic sterols (Sigma, St. Louis, MO, USA). The total neutral sterols were extracted using 8 mL of cyclohexane. After the cyclohexane extraction, 1 mL of 10 mol/L NaOH was added to the remaining aqueous layer and heated at 120°C for 3 h. After cooling down, 1 mL of distilled water and 3 mL of 3N HCl were added followed by extraction with 7 mL of diethyl ether twice. The diethyl ether layers were then pooled followed by adding 2 mL of methanol, 2 mL of dimethoxypropane and 40 μL of concentrated HCl (12 mol/L). After standing overnight at room temperature, the solvents were dried down and the acidic sterols were similarly converted to their TMS-ether derivatives at 60°C for GLC analysis.

2.9 Statistics

The data were expressed as means ± standard deviation (SD). The statistical significance of differences between rats and hamsters was assessed by one-way analysis of variance (ANOVA) followed by post hoc LSD test on SigmaStat Advisory Statistical Software (SigmaStat version 14.0, SPSS Inc., Chicago, IL, USA). The significance across all treatments and treatment trend were assessed using Bonferroni's method. Significance was defined as *p*-value less than 0.05.

3 Results

3.1 Effect of dietary cholesterol in diet on blood cholesterol

Serum TC and LDL-C in both hamsters and rats were elevated in a dose-dependent manner when cholesterol in diet increased from 0 to 0.9% (Fig. 1). However, serum TC in hamsters was elevated 73 – 81% higher than that for rats fed the same diets. To be specific, serum TC increased up to 354 mg/dl in hamsters, whereas it increased only up to 205 mg/dl in rats fed the same 0.9% cholesterol diet (Fig. 1). In the other words, hamsters were hyper-responsive whereas rats were relatively hypo-responsive to dietary cholesterol.

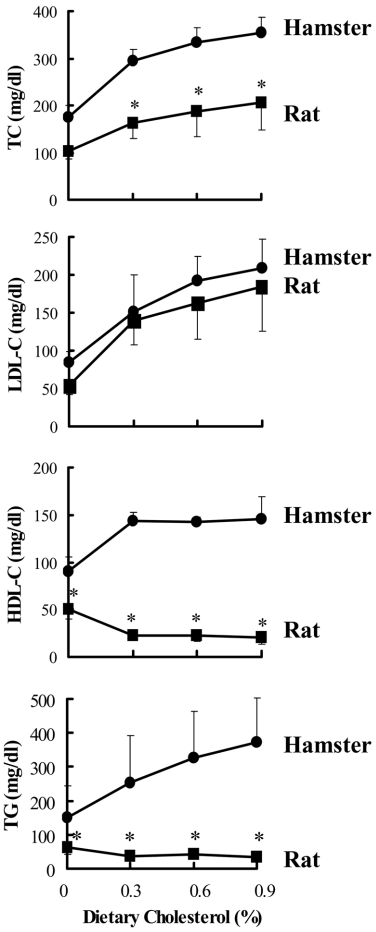

Figure 1. Serum TC, LDL-C, HDL-C, and triacylglycerols (TG) in rats and hamsters fed one of the four diets containing 0, 0.3, 0.6, and 0.9% cholesterol, respectively, for six weeks. Data are expressed as means ± SEM for ten rats or hamsters. *Means for the same diet groups differ significantly between rats and hamsters at $p < 0.05$.

Serum HDL-C in hamsters responded differently from that in rats to the increasing dietary cholesterol. When dietary cholesterol increased from 0 to 0.3%, HDL-C in hamsters was elevated from 91 to 144 mg/dl, and no further elevation was seen thereafter. In contrast, HDL-C level in rats was lowered when dietary cholesterol increased from 0 to 0.3% and thereafter, no further decrease in HDL-C level was seen when dietary cholesterol increased from 0.3 to 0.9% (Fig. 1).

Serum TG levels were raised from 150 to 375 mg/dl in a dose-dependent manner as dietary cholesterol increased from 0 to 0.9% in hamsters. In contrast, serum TG level was decreased from 64 to 36 mg/dl and thereafter no further change was seen when cholesterol in diet increased from 0 to 0.9% in rats (Fig. 1).

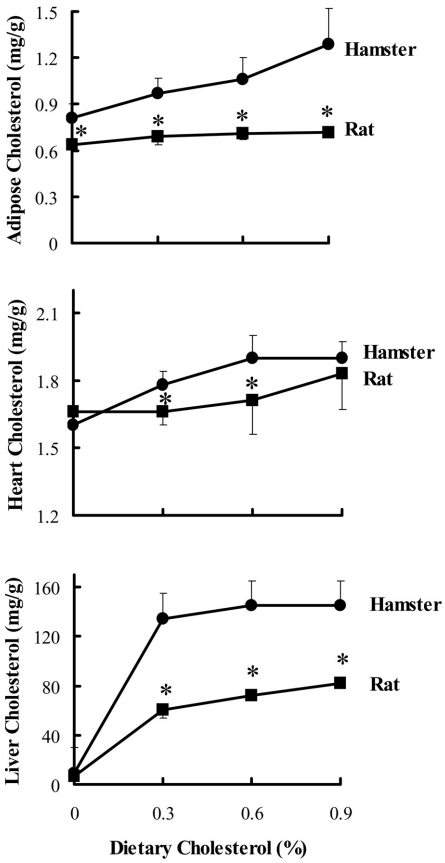

Figure 2. Tissue cholesterol levels in rats and hamsters fed one of the four diets containing 0, 0.3, 0.6 and 0.9% cholesterol, respectively, for six weeks. Data are expressed as means ± SD for ten rats or hamsters. *Means for the same diet groups differ significantly between rats and hamsters at $p < 0.05$.

3.2 Effect of dietary cholesterol on tissue cholesterol

Hepatic cholesterol in both hamsters and rats increased significantly when dietary cholesterol was increased from 0 to 0.9% in diet (Fig. 2). In general, cholesterol in the liver increased sharply when dietary cholesterol increased from 0 to 0.3% and thereafter no further increase was seen with dietary cholesterol being increased from 0.3 to 0.9% in both species. However, hepatic cholesterol concentration was much greater in hamsters than that for rats in response to the same amount of cholesterol in diets (Fig. 2).

A dose-dependent increase in adipose tissue cholesterol concentration was seen in hamsters with the increase in cholesterol in diets (Fig. 2). In contrast, adipose tissue cholesterol in rats remained unchanged when dietary cholesterol increased from 0 to 0.9%. Cholesterol concentration in heart of hamsters was greater in general than that in rats in response to the increase in amounts of cholesterol in diets (Fig. 2).

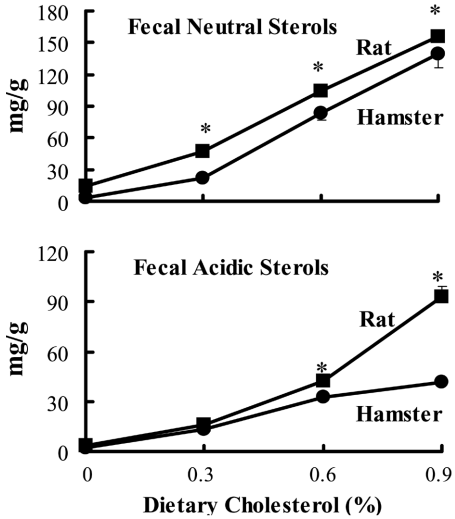

Figure 3. Fecal output of neutral and acidic sterols (mg/g feces) in rats and hamsters fed one of the four diets containing 0, 0.3, 0.6, and 0.9% cholesterol, respectively. Data are expressed as means ± SD for ten rats or hamsters. *Means for the same diet groups differ significantly between rats and hamsters at $p < 0.05$.

3.3 Analyses of total fecal neutral and acidic sterols

Total fecal neutral sterols represent the unabsorbed cholesterol from either diet or bile fluid. The gas chromatographic analysis showed that both hamsters and rats had a sharp increase in total fecal neutral sterol output when dietary cholesterol was increased from 0 to 0.9%, although total fecal neutral sterol output in rats was in general greater than that in hamsters (Fig. 3).

Total fecal acidic sterols were excreted in a dose-dependent manner with the increasing dietary cholesterol (Fig. 3). However, rats had greater excretion of acidic sterols than hamsters. To be more specific, total acidic sterol in rats fed a 0.9% cholesterol diet had three folds of acidic sterol excretion as that in hamsters fed the same diet.

3.4 Immunoblot and mRNA analyses of SREBP-2, LDL-R and HMG-CoA-R

The western blot analyses demonstrated that SREBP-2 was decreased in response to the increasing cholesterol intake in hamsters (p for trend < 0.05, Fig. 4). The change in SERBP-2 mRNA expression pattern in hamsters was consistent with that of the western blotting (p for trend < 0.01). In contrast, addition of cholesterol (0.3 – 0.9%) into diets had no significant effect on SREBP-2 in rats, however, it decreased the SERBP-2 mRNA expression in a dose-dependent manner (p for trend < 0.05).

The immunoblot analysis showed that addition of cholesterol in diet decreased sharply HMG-CoA-R in a dose-

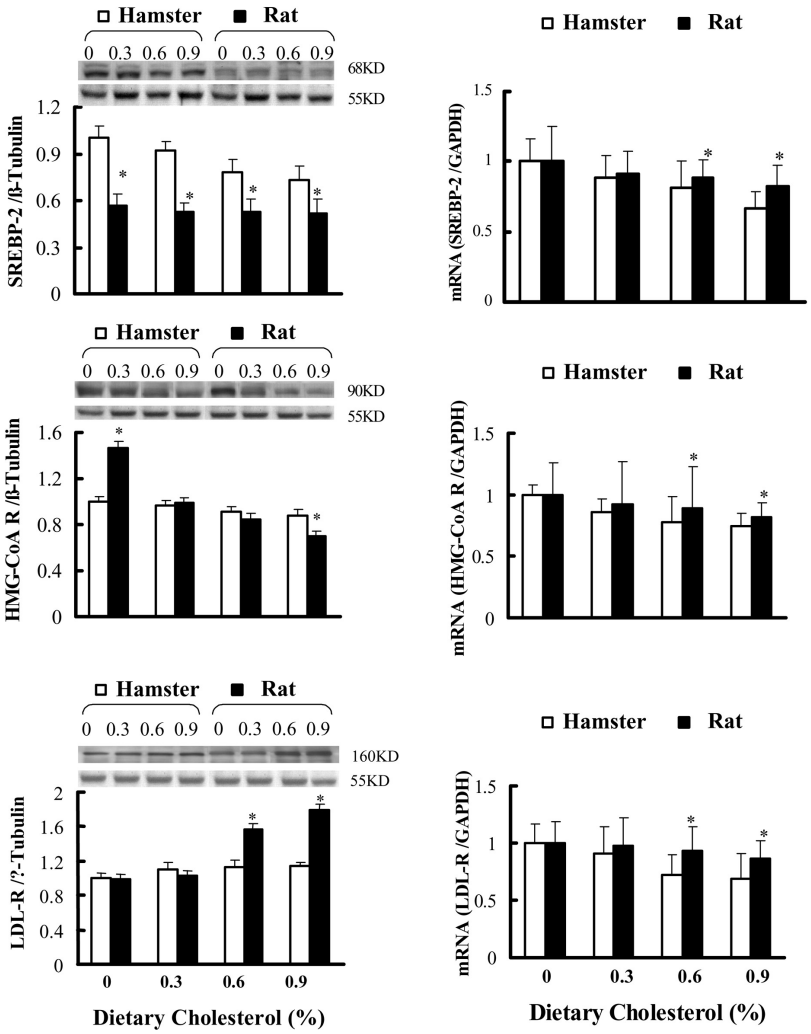

Figure 4. Western blot, real-time PCR and dose-dependent analyses of each target protein with the increasing cholesterol in diet. Rats and hamsters fed the same diets containing 0, 0.3, 0.6, and 0.9% cholesterol, respectively. Data are expressed as means ± SD for ten rats or hamsters. LEFT (Western): Liver sterol regulatory element binding protein 2 (SREBP-2): p_{trend} for hamsters < 0.05, p_{trend} for rats > 0.05; Low-density lipoprotein receptor (LDL-R): p_{trend} for hamsters > 0.05, p_{trend} for rats < 0.05; HMG-CoA-R: p_{trend}> for hamsters < 0.01, p_{trend} for rats < 0.05. RIGHT (Rear Time-PCR): SREBP-2: p_{trend} for hamsters < 0.01, p_{trend} for rats < 0.05; LDL-R: p_{trend} for hamsters <0.05, p_{trend} for rats < 0.05; HMG-CoA-R: p_{trend} for hamsters < 0.05, p_{trend} for rats < 0.01. *Means for the same diet groups differ significantly between rats and hamsters at $p < 0.05$.

dependent manner in rats (p for trend < 0.04) and so was HMG-CoA-R in hamsters (p for trend < 0.01, Fig. 4). In both rats and hamsters, mRNA HMG-CoA-R expression was inhibited in a dose-dependent manner by the increasing dietary cholesterol (p for trend for hamster <0.03, p for trend for rats < 0.01, Fig. 4).

Hepatic LDL-R in rats was sharply increased in a dose-dependent manner (p for trend < 0.05), but it was not seen in hamsters when cholesterol in diet was increased from 0 to 0.9% (Fig. 4). In contrast, LDL-R mRNA expression in both rats and hamsters was down-regulated in a dose-dependent manner as dietary cholesterol was increased from 0 to 0.9% (p for trend < 0.05).

3.5 Immunoblot analyses of CYP7A1 and LXR-α

Results demonstrated that dietary cholesterol (0.3 – 0.9%) had no effect on LXR-α in hamsters, although the protein level of LXR-α was greater in hamsters than that in rats (Fig. 5). In contrast, rats showed a dose-dependent increase in LXR-α with the increasing dietary cholesterol (p for trend < 0.05, Fig. 5). RT-PCR analysis found LXR-α mRNA in both hamsters and rats was increased in response to the increasing amounts of cholesterol in diet (p for trend < 0.01).

Dietary cholesterol at levels of 0.3 – 0.9% had no effect on CYP7A1 in hamsters whereas it increased CYP7A1 in a dose-dependent manner in rats (p for trend < 0.01, Fig. 5). CYP7A1 mRNA expression was significantly increased in rats (p for trend < 0.01) but it was slightly increased in hamsters fed the diets containing 0.3 – 0.9% cholesterol (p for trend < 0.05, Fig. 5).

3.6 Intestinal ACAT activity

Rats had a very low ACAT activity compared with hamsters maintained on the same diets (Fig. 6). The intestinal ACAT activity in hamsters was almost twenty folds of that in rats.

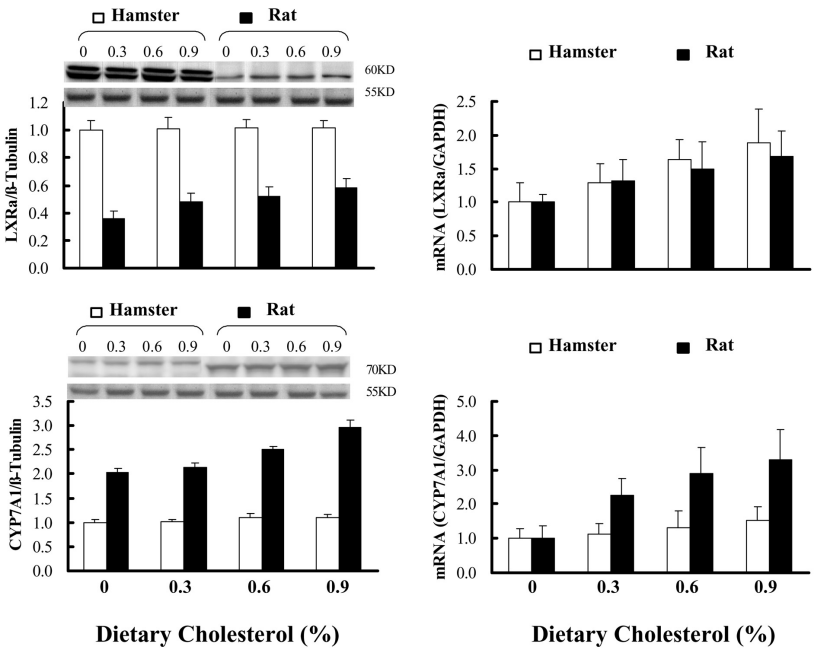

Figure 5. Western blot, real-time PCR and dose-dependent analyses of each target protein with the increasing cholesterol in diet. Rats and hamsters fed the same diets containing 0, 0.3, 0.6, and 0.9% cholesterol, respectively. Data are expressed as means ± SD for ten rats or hamsters. LEFT (Western): Liver X receptor (LXR): p_{trend} for hamsters < 0.05, p_{trend} for rats > 0.05; Cholesterol 7α-hydroxylase (CYP7A1): p_{trend} for hamsters > 0.05, p_{trend} for rats < 0.05. RIGHT (Real Time-PCR): LXR: p_{trend} for hamsters < 0.01, p_{trend} for rats < 0.01; CYP7A1: p_{trend} for hamsters < 0.05, p_{trend} for rats < 0.01. *Means for the same diet groups differ significantly between rats and hamsters at $p < 0.05$.

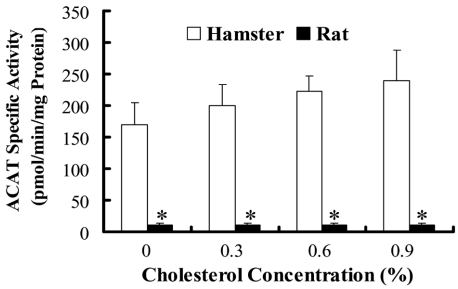

Figure 6. Changes in intestinal ACAT in rats and hamsters fed one of the four diets containing 0, 0.3, 0.6, and 0.9% cholesterol, respectively. p_{trend} for rats < 0.01; p_{trend} for hamsters > 0.05. Data are expressed as means ± SD for 10 rats or hamsters. *Means for the same diet groups differ significantly between rats and hamsters at $p < 0.05$.

The results showed that the intestinal ACAT activity in hamsters was increased in a dose-dependent manner (p for trend < 0.01) whereas that in rats remained unchanged with the increasing dietary cholesterol (Fig. 6).

4 Discussion

The present study demonstrated clearly that hamsters and rats had different response to dietary cholesterol. Firstly, like humans, hamsters were hyper-responsive whereas rats were relatively hypo-responsive to the increasing cholesterol (12). Secondly, effect of dietary cholesterol on plasma lipoproteins in hamsters is similar to that in humans. Lin and Connor (13) had demonstrated that in both normocholesterolemic and hepercholesterolemia humans, dietary cholesterol challenging elevated not only plasma TC and LDL-C but also HDL-C. Like humans, hamsters had serum LDL-C and HDL-C increased proportionally, whereas rats had only LDL-C raised with HDL-C being decreased in response to the increasing cholesterol in diet. Thirdly, serum HDL-C in hamsters accounted for 40% serum TC (13), which is closer to that in humans, whereas in rats, it only accounted for 10% TC when dietary cholesterol was increased from 0 to 0.9% in diets. Lastly, serum TG level was raised in a dose-dependent manner in hamsters, whereas it was decreased in rats when cholesterol in diet was increased from 0 to 0.9%. In review of these differences, it is clear that hamsters would be a better model than rats to study the cholesterol-lowering activity of nutraceuticals and functional foods.

The molecular mechanisms responsible for differences in sensitivity to dietary cholesterol were firstly investigated by comparing LDL-R in hamsters with that in rats. Results showed that LDL-R in rats was significantly up-regulated while in hamsters it remained unchanged in response to the increasing dietary cholesterol (Fig. 4). The present result was in agreement with that of Horton *et al*. (14), who found that rats had greater LDL-R activity than hamsters maintained on the same cholesterol diet. The difference in LDL-R between rats and hamsters could be explained by their cholesterol levels in the liver. The higher the hepatic cholesterol is, the lower the LDL-R is. Rats had lesser accumulation of cholesterol in the liver compared with hamsters fed the same amount of dietary cholesterol (Fig. 2) and therefore greater LDL-R was expected. Greater LDL-R level in rats would lead to efficient uptake of LDL-C by the liver and hence serum cholesterol did not rise sharply in response to the increasing cholesterol in diet. In this regard, Yokode

et al. [15] have shown that greater LDL-R level is one of the resistant factors to the dietary cholesterol-induced hypercholesterolemia in mice fed a high cholesterol diet. Interesting, change in protein levels of LDL-R in rats was opposing to that in the LDL-R mRNA, whereas in hamsters protein level of LDL-R did not change but LDL-R mRNA decreased in response to the increasing dietary cholesterol (Fig. 4). In fact, this phenomenon had been previously documented by Horton *et al*. (14), who found that rats had increased LDL-R activity but decreased LDL-R mRNA in response to the increasing cholesterol in diet. Although the underlying mechanism for this phenomenon remains unclear, the present study clearly demonstrated that different response to dietary cholesterol was due, in large part, to difference in the amount of LDL-R between hamsters and rats.

The change in HMG-CoA-R in response to dietary cholesterol was also investigated in both rats and hamsters fed the same cholesterol diets. HMG-CoA-R is regulated mainly by cellular cholesterol level. In response to a high cholesterol level, the expression level of HMG-CoA-R would decrease in order to maintain a constant level of cholesterol [16]. The present study found both hepatic HMG-CoA-R and its mRNA in hamsters and rats decreased in a dose-dependent manner in response to the increasing cholesterol in diets. However, suppression on hepatic HMG-CoA-R in rats by dietary cholesterol was much stronger than that in hamsters (Fig. 3). The present result was consistent with that of Horton *et al* (14), who observed that rats had several folds higher in basal HMG-CoA-R activity than hamsters, and the former had a sharp decrease whereas the latter had no or little change in HMG-CoA-R activity in response to the increase in the dietary cholesterol. It had been suggested that the basal level expression of HMG-CoA-R could be an indicator of hypo-responsiveness to dietary cholesterol [17]. The higher the basal expression of hepatic HMG-CoA-R is, the greater the "cholesterol buffering capacity" and the greater the resistance to dietary cholesterol could be. In fact, the rats have a higher level of hepatic cholesterol biosynthesis and are known to be more resistant to dietary cholesterol than hamsters [18, 19].

The expression of LDL-R and HMG-CoA-R is partially controlled by SREBP-2. The present study was the first report to compare the expression of SREBP-2 in rats and hamsters fed the diets with the increasing cholesterol intake. In rats, hepatic SREBP-2 remained unchanged but its mRNA was suppressed by the increasing cholesterol in diets. In contrast, hepatic SREBP-2 and its mRNA were decreased in a dose-dependent manner in response to the increasing dietary cholesterol in hamsters. The present results indicated clearly that expression of SREBP-2 in rats was not sensitive to dietary cholesterol. However, LDL-R was up-regulated in order to meet the need in the efficient removal of LDL-cholesterol from the blood to the liver. The reverse was obviously seen for hamsters.

The present results showed that rats eliminated cholesterol and bile acids more efficiently than hamsters. Firstly, rats had greater excretion of fecal total neutral sterols and bile acids than hamsters in response to the increasing dietary cholesterol (Fig. 3). Secondly, rats had greater CYP7A1 and its mRNA than hamsters (Fig. 5). In general, species which are hypo-responsive to dietary cholesterol are able to excrete greater amount of bile acids than those of the hyper-responsive [20]. When dietary cholesterol was increased, bile acid biosynthesis was increased in rats but not in hamsters. The present results supported the view that the increased bile acids biosynthesis accounted for the increased fecal bile acids because CYP7A1, the rate-determining enzyme which controls the conversion of hepatic cholesterol to bile acids, was up-regulated in response to the increasing dietary cholesterol in rats but not in hamsters. Subsequently, bile acids were not efficiently reabsorbed from the intestine *via* the enterohepatic circulation in rats, leading a greater excretion of bile acids. The present result was consistent with that of Horton *et al* [14], who found that rats were much more efficient than hamsters to eliminate the bile acids by up-regulation and activation of cholesterol 7α-hydroxylase. In fact, efficient removal of bile acids in feces further promoted the up-regulation of CYP7A1 because bile acids inhibited the expression of CYP7A1 [21]. In general, rats and mice showed a marked up-regulation whereas humans, hamsters, monkeys, guinea pigs, and rabbits did not respond or even repress CYP7A1 expression with the increasing dietary cholesterol in diets [14, 22, 23]. In this regard, hamsters serve as a better model for studying cholesterol catabolism than rats because the former had bile acid synthesis pattern similar to that in humans.

CYP7A1 are partially regulated at the transcriptional level by LXR-α [24, 25]. The present study examined the effect of dietary cholesterol on LXR-α expression in both rats and hamsters fed the same high cholesterol diets. With the increasing dietary cholesterol, LXR-α was increased in rats but it was not seen in hamsters, although the former had the basal LXR-α level much lower than that in the latter. In addition, up-regulation of LXR-α was well correlated with mRNA/protein expression of CYP7A1 in both rats and hamsters. The regulatory role of LXR-α in regulation of CYP7A1 was demonstrated in the LXR-α knock-out mice (LXR-$\alpha^{-/-}$), which could not up-regulate CYP7A1 in response to the cholesterol feeding [26]. In this regard, LXR-α was not up-regulated in hamsters by the increasing dietary cholesterol and neither was CYP7A1, resulting in less efficient excretion of cholesterol and bile acids compared with that in rats. Unlike LXR-α, farnesoid X receptor (FXR), a bile acid receptor, down-regulates CYP7A1. Bile acids, which return to the liver *via* the enterohepatic circulation, activate FXR followed by down-regulation of CYP7A1 (27). Unfortunately, the present study did not measure the gene expression of FXR. It is therefore impos-

sible to explain the correlation between higher expression of LXR-α and lower expression of CYP7A1 in hamsters without taking FXR into consideration.

Esterification of cholesterol catalyzed by intestinal ACAT is an essential rate-limiting step in intestinal cholesterol absorption [28]. Characterization of the intestinal ACAT was different between hamsters and rats. Firstly, the basal intestinal ACAT activity in hamsters was greater than that in rats (Fig. 6). Secondly, the intestinal ACAT in hamsters but not in rats was dose-dependently increased with the increase in cholesterol in diet. Greater ACAT activity in hamsters might lead to greater cholesterol absorption and lower fecal neutral sterol excretion (Fig. 3).

In summary, the present study emphasized that hamsters and rats metabolized cholesterol differently in many ways. Although the present experiment does not allow us compare the genomic and proteomic profile between hamsters and humans, we have provided here a putative mechanism by which rats are resistant, while hamsters are prone, to hypercholesterolemia induced by excessive dietary cholesterol intake. Unlike rats in which HDL-C accounts very little for serum TC, the lipoprotein profile in hamsters was similar or closer to that in humans. In addition, hamsters handle bile sterol secretion, and synthesize hepatic cholesterol and bile acids in the manner similar to as humans. Together with a fact that rats have dietary cholesterol at level ten times as that for hamsters would develop the same degree of hypercholesterolemia, it is concluded that hamsters but not rats shall be chosen as a model to study the efficacy of cholesterol-lowering nutraceuticals and functional foods.

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5 References

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