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ORIGINAL CONTRIBUTION

Capsaicinoids lower plasma cholesterol and improve endothelial function in hamsters

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Abstract
Purpose Capsaicinoids are the active compounds in chili pepper. The present study investigated the effect of capsaicinoids on plasma lipids, functionality of aorta including atherosclerotic plaque development, cholesterol absorption biomarker, fecal sterol excretion, and gene expression of major receptors, enzymes, and transporters involved in cholesterol metabolism.
Methods Hamsters were divided into five groups and fed a high-cholesterol diet containing 0 % (CON), 0.010 % (LD), 0.015 % (MD), 0.020 % (HD), and 0.030 % (VD) capsaicinoids, respectively, for 6 weeks. Plasma lipids were measured using the enzymatic kits, and the gene expression of transporters, enzymes, and receptors involved in cholesterol absorption and metabolism was quantified using the quantitative PCR. Endothelial function was assessed by measuring the acetylcholine-induced endothelium-dependent relaxations in aorta.
Results Capsaicinoids reduced plasma total cholesterol, non-high-density lipoprotein cholesterol, and triacylglycerols with high-density lipoprotein cholesterol being unaffected. All four experimental groups had a decrease in the atherosclerotic plaque compared with CON. Dietary capsaicinoids increased the fecal excretion of total acidic sterols possibly mediated by up-regulation of cholesterol 7α-hydroxylase and down-regulation of liver X receptor alpha. Plasma sterol analysis demonstrated that capsaicinoids decreased the ratio of plasma campesterol/cholesterol, suggesting they decreased cholesterol absorption. Capsaicinoids could improve the endothelium-dependent relaxations and reduce the endothelium-dependent contractions by inhibiting the gene expression of COX-2. However, no dose-dependent effect of capsaicinoids on these parameters was seen.
Conclusion Capsaicinoids were beneficial in improving lipoprotein profile and aortic function in hamsters fed a high-cholesterol diet.

Keywords Cholesterol · Capsaicin · Capsaicinoids · Aorta · CYP7A1 · COX-2 · Vascular reactivity

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Introduction

Capsaicinoids refer to a group of pungent compounds found in chili peppers. When they come into contact with mammal tissues, capsaicinoids elicit a sensation of burning. Recently, capsaicinoids have attracted much attention due to their effectiveness in weight management and prevention of the chronic diseases. It has been shown that

capsaicinoids can enhance lipid oxidation and promote weight reduction by increasing adrenergic activity and energy expenditure [1]. Accumulated evidence suggests that capsaicinoids have potential beneficial effect on human cardiovascular system [2, 3]. It has also been reported that capsaicinoids possess the antitumor activity [4]. In addition, capsaicinoids are an antioxidant with an ability to prevent the oxidation of human low-density lipoproteins [5].

Previous reports have indicated that red pepper and capsaicinoids decrease blood cholesterol concentration [6, 7], possibly mediated by inhibition on intestinal cholesterol absorption [8]. The cholesterol absorption is a complex process that requires several transporters and enzymes, namely Niemann-Pick C1 like 1 (NPC1L1), acyl CoA: cholesterol acyltransferase (ACAT2), microsomal triglyceride protein (MTP), and ATP-binding cassette transporters (ABCG5 and ABCG8). NPC1L1 transports cholesterol into enterocytes, where cholesterol is converted to cholesteryl ester (CE) by ACAT2. Subsequently, MTP packs CE into chylomicrons which carry CE into circulation via lymphatic pathway. ABCG5 and ABCG8 return the unabsorbed cholesterol to the lumen for elimination [9, 10]. Cholesterol synthesis occurs mainly in the liver where 3-hydroxy-3-methylglutary-CoA (HMG-CoA) reductase acts as a regulatory enzyme. Blood cholesterol is mainly located in low-density lipoprotein (LDL), and the removal of LDL cholesterol from circulation into the liver is mainly mediated by LDL receptor. Once cholesterol enters the liver, it is metabolized to bile acids by cholesterol-7α-hydroxylase (CYP7A1). Bile acids are then disposed to the lumen via bile fluid. In this regard, sterol regulatory element-binding protein 2 (SREBP-2) and liver X receptor alpha (LXRα) play a key role in cholesterol homeostasis, because the former governs the transcription of LDL receptor and HMG-CoA reductase, while the latter regulates the transcription of CYP7A1. It remains unknown if capsaicinoids affect these transporters, enzymes, proteins, and receptors involved in the cholesterol absorption and homeostasis.

Previous research also suggests that a high prevailing cholesterol concentration is associated with endothelial dysfunction and contributes to the increased risk of coronary heart disease [11]. The correlation of endothelial dysfunction and hypercholesterolemia has been previously demonstrated [12], suggesting that reducing cholesterol concentration could improve the endothelial cell function.

The present study was carried out further to examine the effect of capsaicinoids on plasma and liver lipids, atherosclerotic plaque development, endothelial function, and the gene expression of hepatic SREBP-2, LXRα, HMG-CoA reductase, LDL receptor, and CYP7A1 as well as NPC1L1, ACAT2, ABCG5/8, and MTP in the intestine in hamsters fed a high-cholesterol diet.

Materials and methods

Diet

Capsaicinoids with a purity of 95 % were obtained from Henan Bis-Biotech Co., Ltd (Henan, China). HPLC analysis found that capsaicinoids consisted of 51.3 % capsaicin, 36.2 % dihydrocapsaicin, and 7.8 % other capsaicinoids. Five diets were prepared by modifying the formulation we previously described [12]. The control diet was prepared by mixing all powdered ingredients (g/kg): cornstarch, 508; casein, 242; lard, 50; sucrose, 119; mineral mix, 40, vitamin mix, 20; DL-methionine, 1; cholesterol, 1. The four experimental diets were similarly prepared except that capsaicinoids were firstly added into lard with their final concentration in diet being 0.010 % (LD), 0.015 % (MD), 0.020 % (HD), and 0.030 % (VD) capsaicinoids, respectively. All five diets contained 0.1 % cholesterol by weight and had 68.2, 26.4, and 5.4 % energy from carbohydrate, protein, and fat, respectively, with no fiber being added.

Hamsters

Male Golden Syrian hamsters ($n = 50$, age 6 months) were divided into five groups ($n = 10$ each) and fed one of the five diets. They were housed in an animal room at 23 °C with 12/12-h light–dark cycles. Fresh diets were given daily, and uneaten food was discarded. Food intake was measured daily, and body weight was recorded twice a week. The hamsters were allowed freely access food and water. Blood sample was taken from the retro-orbital sinus under light ether anesthesia after overnight fasting at week 0, 3, and 6. At the end of week 6, all the hamsters were sacrificed; the liver was removed, washed with saline, weighed, and frozen in liquid nitrogen. All samples were stored at −80 °C freezer prior to cholesterol analysis. The fecal output from each hamster was collected and pooled at week 1 and 6, followed by being freeze-dried, ground, and saved for neutral and acidic sterol analyses. Experiments were approved and conducted in accordance with the guidelines set by the Animal Experimental Ethical Committee, The Chinese University of Hong Kong.

Analysis of plasma lipids

Plasma total cholesterol (TC) and total triacylglycerols (TAG) were quantified using their respective commercial enzymatic kits from Infinity (Waltham, MA, USA) and Stanbio Laboratories (Boerne, TX, USA). To quantify plasma, high-density lipoprotein cholesterol (HDL-C), LDL cholesterol (LDL-C), and very low-density lipoprotein (VLDL) were firstly precipitated with phosphotungstic acid and magnesium chloride using a commercial kit (Stanbio

Laboratories) as described by the Provider [13]. HDL-C in the supernatant was determined similarly as for TC. Non-HDL-C was calculated by deducing HDL-C from the TC.

Measurement of atherosclerotic plaque

Atherosclerotic plaque on endothelial layer was quantified as we previously described [12]. In brief, thoracic aorta was cut opened vertically and then stained with 0.5 g Sudan III in 10 mL ethanol for 3 h. Afterward, the dye was washed with distilled water for 3 times and scanned with a table scanner. The area of atherosclerotic plaque was measured with the aid of computer images analyzing program.

Analysis of plasma lathosterol and campesterol

To investigate the effect of capsaicinoids on cholesterol synthesis and absorption, plasma campesterol/cholesterol and lathosterol/cholesterol were used as biomarkers of cholesterol absorption and synthesis, respectively [14]. In brief, total lipids were extracted using 5 mL of chloroform/methanol (2:1, vol/vol) from serum sample (200 μL). 5α-Cholestane was added as an internal. The solvent was evaporated to dryness under a gentle stream of nitrogen gas. The serum lipids were then mildly saponified using 5 mL of 1 N NaOH in 90 % ethanol at 90 °C for 1 h. The cholesterol, lathosterol, and campesterol were converted into their trimethylsiyl-ether (TMS) derivatives and subjected to the GC analysis.

Analysis of hepatic cholesterol

Total cholesterol was determined as we previously described [15]. Total lipids were extracted using a mixture of chloroform/methanol (2:1, vol/vol) with 5α cholestane being as an internal standard. The lipid extracts were saponified, and the free cholesterol was converted to their TMS-ether derivatives by a commercial TMS reagent. Analysis of the cholesterol TMS-ether derivative was performed in a SAC^{TM}-5 column in a Shimadzu GC-14B GLC equipped with a flame-ionization detector. Hepatic cholesterol was calculated according to the amount of internal standard 5α-cholestane added.

Analysis of fecal neutral and acidic sterols

Both fecal neutral and acidic sterols were determined as previously described [15]. 5α-Cholestane was used as an internal standard for quantification of fecal neutral sterols, while hyodeoxycholic acid was used as an internal standard for quantification of total acidic sterols. Freeze-dried fecal samples were saponified, and the total neutral sterols were extracted into cyclohexane. Afterward, the neutral sterols were converted into their TMS derivatives. Total fecal acidic sterols in the aqueous phase after cyclohexane extraction were also extracted and similarly converted into their TMS derivatives. Individual, neutral, and acidic sterol TMS derivatives were then subjected to the GC analysis.

Real-time PCR analysis of mRNA of hepatic SREBP-2, LDLR, HMG-CoA Reductase, LXRα, CYP7A1, and small intestine NPC1L1, ABCG5, ABCG8, ACAT2, MTP

Each mRNA level was determined as previously described [16]. After extraction and isolation of total using Trizol® Reagent (Invitrogen, Carslbad, CA), total RNA from the liver and intestine was converted to its cDNA using a high capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA). Reverse transcription was carried out in a thermocycler (Gene Amp® PCR system 9700, Applied Biosystems). The resultant cDNA was stored at −20 °C. Real-time PCR analysis was run on a Fast Real-time PCR System 7500 (Applied Biosystems). Primers and TaqMan® probes were used for real-time PCR analysis of liver GAPDH, CYP7A1, HMG-CoA reductase, LDL-R, SREBP-2, and LXRα, while for intestinal NPC1L1, ABCG5, ABCG8, ACAT2, MTP, and cyclophilin, SYBR green was used as a fluorophore. Data were analyzed using the Sequence Detection Software version 1.3.1.21 (Applied Biosystems). Each gene expression was calculated according to the comparative Threshold cycle (C_T) method (Applied Biosystems).

Vascular reactivity

Aortic functionality was assessed according to the method previously described [17]. In brief, the thoracic aortas were dissected out and placed in Krebs–Henseleit solution (KHS) containing (mmol/L): NaCl (119), $NaHCO_3$ (25), $MgCl_2$ (1), KCl (4.7), KH_2PO_4 (1.2), $CaCl_2$ (2.5), and D-glucose (11.1). After the surrounding fatty tissues were removed, each aorta was cut into several ring segments (∼1.0 mm long each) followed by mounting each between two tungsten wires in chambers of a Multi Myograph System for the continual recording of changes in isometric force. Each chamber was filled with 5 mL-KHS gassed by 95 % O_2 and 5 % CO_2 and maintained at 37 °C. The rings were stretched to a previously determined optimal resting tension of 10 mN. Thirty minutes after setting up the preparation, the rings were contracted in 60 mmol/L KCl to test their contractility, then washed in normal KHS, and finally allowed to equilibrate for 30 min. The first set of experiments examined the change in endothelium-dependent relaxations in hamster aortas from three treatment

Table 1 Change in food consumption, body weight, and relative organ weights in hamsters fed the control (CON) and the four experimental diets containing 0.010 % (LD), 0.015 % (MD), 0.020 % (HD), and 0.030 % capsaicinoids (VD) by weight

	CON	LD	MD	HD	VD
Food intake	9.0 ± 0.5	8.5 ± 0.8	9.0 ± 0.7	9.0 ± 0.7	9.0 ± 0.6
Body weight (g)					
Initial	120.5 ± 7.1	120.5 ± 13.0	115 ± 12.2	118.0 ± 10.9	117.5 ± 12.5
Final	112.5 ± 20.0	112.0 ± 8.7	110.0 ± 12.8	114.5 ± 16.7	111.0 ± 14.7
Relative organ weight (% body weight)					
Liver	6.0 ± 0.6[a]	5.4 ± 0.8[b]	5.8 ± 0.6[b]	5.8 ± 0.6[b]	5.9 ± 0.7[b]
Kidney	1.2 ± 0.5	1.0 ± 0.1	1.1 ± 0.2	1.1 ± 0.2	1.1 ± 0.1
Heart	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
Testis	2.3 ± 0.6	2.4 ± 0.7	2.4 ± 0.8	2.5 ± 0.4	2.3 ± 0.9
Epididymal fat pad	1.5 ± 0.4	1.3 ± 0.4	1.3 ± 0.2	1.4 ± 0.3	1.3 ± 0.4
Perirenal fat pad	1.0 ± 0.3[a]	0.7 ± 0.2[b]	0.7 ± 0.2[b]	0.7 ± 0.4[b]	0.8 ± 0.3[b]

Data were expressed as mean ± SD; $n = 10$ each group. Means at the same row with different superscript letters differ significantly at $P < 0.05$

Table 2 Changes in plasma total cholesterol (TC), triacylglycerols (TG), HDL-cholesterol (HDL-C), non-HDL-cholesterol (non-HDL-C), liver total cholesterol, aortic cholesterol, atherosclerotic plaque, plasma lathosterol/cholesterol, and campesterol/cholesterol in hamsters fed the control (CON) and the experimental diets containing 0.010 % (LD), 0.015 % (MD), 0.020 % (HD), and 0.030 % (VD) capsaicinoids

	CON	LD	MD	HD	VD
Week 0					
TC (mmol/L)	5.10 ± 0.70	4.95 ± 0.57	5.02 ± 0.44	5.06 ± 0.85	5.02 ± 0.89
TAG (mmol/L)	2.97 ± 0.66	3.04 ± 0.69	3.16 ± 0.85	3.00 ± 0.70	3.13 ± 0.89
HDL-C (mmol/L)	1.93 ± 0.20	1.82 ± 0.19	1.85 ± 0.19	1.96 ± 0.18	1.98 ± 0.19
Non-HDL-C (mmol/L)	3.17 ± 0.56	3.13 ± 0.50	3.17 ± 0.45	3.11 ± 0.76	3.15 ± 0.71
Non-HDL-C/HDL-C	1.64 ± 0.26	1.67 ± 0.25	1.69 ± 0.29	1.68 ± 0.26	1.62 ± 0.25
Week 3					
TC (mmol/L)	5.51 ± 0.53[a]	4.90 ± 0.46[b]	4.87 ± 0.56[b]	4.88 ± 0.66[b]	4.77 ± 0.30[b]
TAG (mmol/L)	4.10 ± 0.73[a]	3.25 ± 0.62[b]	2.99 ± 0.70[b]	3.24 ± 0.95[b]	3.18 ± 0.58[b]
HDL-C (mmol/L)	2.31 ± 0.24	2.31 ± 0.33	2.27 ± 0.30	2.28 ± 0.17	2.26 ± 0.19
Non-HDL-C (mmol/L)	3.20 ± 0.48[a]	2.59 ± 0.50[b]	2.61 ± 0.52[b]	2.61 ± 0.52[b]	2.50 ± 0.18[b]
Non-HDL-C/HDL-C	1.31 ± 0.12[a]	1.15 ± 0.31[ab]	1.17 ± 0.30[ab]	1.14 ± 0.17[ab]	1.11 ± 0.10[b]
Week 6					
TC (mmol/L)	6.54 ± 0.95[a]	5.56 ± 0.55[b]	5.57 ± 0.93[b]	5.59 ± 0.85[b]	5.51 ± 0.62[b]
TAG (mmol/L)	4.39 ± 0.89[a]	3.41 ± 1.04[b]	3.28 ± 0.54[b]	3.32 ± 0.97[b]	3.27 ± 0.58[b]
HDL-C (mmol/L)	2.23 ± 0.43	2.37 ± 0.38	2.38 ± 0.32	2.35 ± 0.25	2.40 ± 0.40
Non-HDL-C (mmol/L)	4.32 ± 0.67[a]	3.19 ± 0.67[b]	3.19 ± 1.00[b]	3.24 ± 1.00[b]	3.11 ± 0.73[b]
Non-HDL-C/HDL-C	2.00 ± 0.48[a]	1.39 ± 0.43[ab]	1.38 ± 0.53[b]	1.42 ± 0.55[ab]	1.34 ± 0.44[b]
Liver cholesterol (μmol/g)	86.89 ± 10.34	96.20 ± 31.03	99.30 ± 20.17	94.65 ± 24.57	84.30 ± 17.84
Aortic cholesterol (μmol/g)	3.10 ± 0.78[a]	2.59 ± 0.52[b]	1.81 ± 0.26[b]	2.33 ± 0.78[b]	2.07 ± 0.52[b]
Aortic atherosclerotic plaque (% area)	16.56 ± 3.80[a]	10.32 ± 3.30[b]	9.35 ± 2.08[b]	9.75 ± 2.24[b]	10.87 ± 2.81[b]
Serum lathosterol/cholesterol ($\times 10^{-3}$)	1.01 ± 0.33	0.93 ± 0.32	0.90 ± 0.52	0.97 ± 0.55	0.87 ± 0.33
Plasma campesterol/cholesterol ($\times 10^{-3}$)	3.05 ± 0.90[a]	1.38 ± 0.38[b]	1.67 ± 0.64[b]	1.70 ± 0.23[b]	1.52 ± 0.15[b]

Data were expressed as mean ± SD; $n = 10$ each group. Means at the same row with different superscript letters differ significantly at $P < 0.05$

groups. The second series of experiments tested endothelium-dependent contractions in hamster aortas with endothelium. To visualize endothelium-dependent contractions, aortic rings needed to be exposed for 30 min to 100 μmol/L L-NAME aiming to eliminate the relaxant effect of endothelium-derived nitric oxide (NO) before application of

Table 3 Cholesterol intake and changes in fecal sterol profile in hamsters fed the control (CON) and the experimental diets containing 0.010 % (LD), 0.015 % (MD), 0.020 % (HD), and 0.030 % (VD) at week 6

	CON	LD	MD	HD	VD
Cholesterol intake (mg/day)	10.35 ± 0.7	9.17 ± 0.74	9.32 ± 0.93	10.29 ± 0.12	9.48 ± 1.40
Fecal output (g/day)	0.27 ± 0.05	0.34 ± 0.09	0.36 ± 0.10	0.30 ± 0.09	0.33 ± 0.07
Fecal neutral sterols (mg/day)					
Coprostanol	0.66 ± 0.20	0.69 ± 0.09	0.75 ± 0.37	0.87 ± 0.62	0.97 ± 0.23
Coprostanone	0.02 ± 0.00	0.03 ± 0.03	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.02
Cholesterol	0.37 ± 0.16	0.56 ± 0.23	0.90 ± 0.40	0.72 ± 0.46	0.70 ± 0.50
Dihydrocholesterol	0.27 ± 0.05^{b}	0.30 ± 0.09^{ab}	0.46 ± 0.14^{a}	0.36 ± 0.17^{ab}	0.37 ± 0.15^{ab}
Campesterol	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
Stigmasterol	<0.01	<0.01	<0.01	<0.01	<0.01
Sitosterol	<0.01	<0.01	<0.01	<0.01	<0.01
Total sterol	1.35 ± 0.39	1.60 ± 0.37	2.14 ± 0.76	2.00 ± 1.22	2.09 ± 0.88
Fecal acidic sterols (mg/day)					
Lithocholic acid	1.21 ± 0.40^{b}	2.22 ± 0.49^{a}	2.50 ± 0.92^{a}	2.84 ± 0.05^{a}	1.99 ± 0.48^{ab}
Deoxycholic acid	0.40 ± 0.15	0.46 ± 0.11	0.50 ± 0.17	0.43 ± 0.02	0.56 ± 0.26
Chenodeoxycholic acid + cholic acid	0.45 ± 0.20	0.39 ± 0.14	0.49 ± 0.14	0.28 ± 0.11	0.26 ± 0.07
Ursocholic acid	0.02 ± 0.01	0.05 ± 0.04	0.05 ± 0.03	0.01 ± 0.01	0.02 ± 0.01
Total acid sterol	2.08 ± 0.44^{b}	3.12 ± 0.59^{ab}	3.41 ± 1.0^{a}	3.29 ± 0.54^{a}	2.82 ± 0.59^{ab}

Mean values within a row unlike superscript letters are significantly different ($P < 0.05$). The total fecal output from 7 days was pooled, and the data were expressed as an average per day

acetylcholine (0.1–10 μmol/L). This contraction was absent in rings without endothelium as we previously described [18]. The effects of each of the following inhibitors were examined including SC560 (COX-1 inhibitor) and NS398 (a COX-2-specific inhibitor). Acetylcholine-induced endothelium-dependent contractions were obtained following a period of 30-min incubation with each inhibitor. Specificity of these inhibitors and antagonists at concentration used for each inhibitor was tested before, and they did not affect contractions induced by 60 mmol/L KCl [18]. Endothelium-dependent contractions were expressed as active tension [force recorded/(2 × ring's length)].

Statistical analysis

One-way analysis of variance followed by using Fisher's LSD method will be used statistically to evaluate differences in plasma lipids, aortic functions, atherosclerotic plaque, cholesterol absorption biomarker, and gene expression of cholesterol transporters and enzymes involved in the cholesterol metabolism among the five groups.

Results

Food intake, body, and organ weights

No differences in food intake, body weight, and relative weights of heart, kidney, testes, and epididymal fat pad were seen among the five groups (Table 1). The relative liver and perirenal fat pat were smaller in the four experimental groups compared with the control hamsters. However, no significant difference was seen among the four experimental groups.

Plasma TC, HDL-C, non-HDL-C, and TAG

Five groups of hamsters had similar concentrations of plasma TC, HDL-C, non-HDL-C, and TAG at week 0 (Table 2). When the experiment reached the end of week 3 and 6, the four experimental groups had plasma TC, non-HDL-C, and TAG concentrations significantly lower than the control hamsters. No effect on HDL-C was seen between the control and the four experimental groups. No significant differences in plasma TC, HDL-C, and TAG were seen among the four experimental groups (LD, MD, HD, and VD groups) (Table 2).

Aortic cholesterol, plasma sterols, and atherosclerotic plaque

Four experimental diets decreased aortic cholesterol and atherosclerotic plaque compared with the control diet; however, no significant difference was seen among the four experimental groups (Table 2). Results showed that capsaicinoids decreased plasma campsterol/cholesterol ratio, indicating they decreased the cholesterol absorption. Dietary capsaicinoids had no effect on liver cholesterol

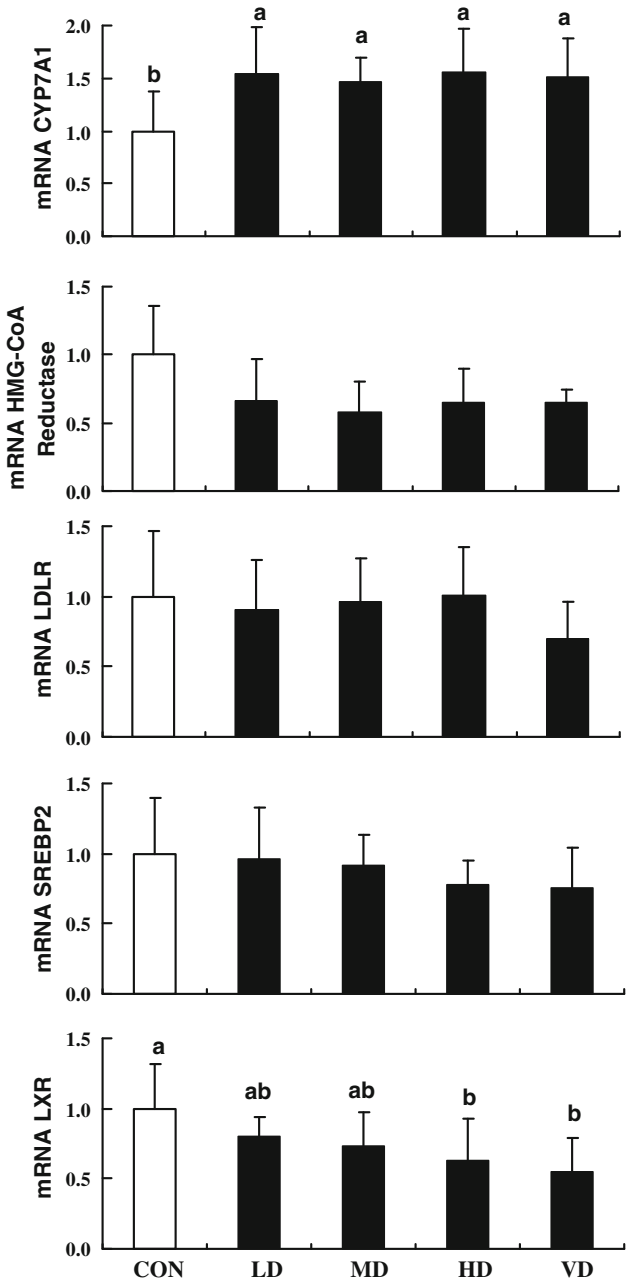

Fig. 1 Effect of dietary capsaicinoids on mRNA levels of hepatic sterol regulatory element-binding protein-2 (SREBP-2), liver X receptor alpha (LXR), 3-hydroxy-3-methylglutary-CoA (HMG-CoA) reductase, LDL receptor (LDLR), and cholesterol-7α-hydroxylase (CYP7A1) in hamsters fed the control (CON), and four experimental diets containing 0.010 (LD), 0.015 % (MD), 0.020 % (HD), and 0.030 % (VD) capsaicinoids. Means with different *superscript letters* differ significantly, $P < 0.05$

concentration and plasma ratio of lathosterol/cholesterol, indicating they had no effect on de novo cholesterol synthesis (Table 2).

Fecal neutral and acidic sterols

Cholesterol in colon underwent the microbial bioconversion, producing series of cholesterol microbial metabolites with coprostanol and dihydrocholesterol being as the major products. To simplify the presentation, only data on fecal samples collected from week 6 were reported. As shown in Table 3, the effect of capsaicinoids on the excretion of fecal dihydrocholesterol in MD group was significant compared with the control group. No statistical difference in total neutral sterol excretion was warranted compared with the control (Table 3). Concerning fecal acidic sterols, hamsters from LD, MD, and HD groups had greater excretion of lithocholic acid compared with the control (Table 3). Regarding the total acidic sterols, MD and HD groups had greater excretion than the control hamsters.

Real-time PCR mRNA

RT-PCR analysis demonstrated that dietary capsaicinoids had no effect on mRNA levels of NPC1L1, ACAT2, MTP, and ABCG 5/8 (data not shown). In contrast, capsaicinoids up-regulated mRNA level of CYP7A1 accompanied by down-regulation LXRα but they had no significant effect on that of SREBP-2, HMG-CoA reductase, and LDL receptor (Fig. 1). Results showed that the effect of dietary capsaicinoids on gene expression of CYP7A1 and LXRα was not dose-dependent.

Vascular reactivity

The function of aortas from the control, LD, and HD group was assessed. It was found that aortas from both LD and HD relaxed better than those from the control. Acetylcholine at concentration higher than 1 μmol/L turned relaxation into contraction (Fig. 2). On the other hand, endothelium-dependent contractions were significantly greater in response to acetylcholine in the control than that in both LD and HD hamster (Fig. 2).

Role of COX in endothelium-dependent contractions

To simplify the presentation, only data on endothelium-dependent contractions were shown (Fig. 3). Endothelium-dependent contraction in the control group was eliminated by S18886, a selective thromboxane receptor (TP) receptor antagonist, and largely inhibited by selective COX-2 inhibitor indomethacin, but it was unaffected by COX-1 inhibitor SC560 (Fig. 3). The COX-2 but not COX-1 expression was lower in the aorta of HD hamsters as compared with those from control group (Fig. 3).

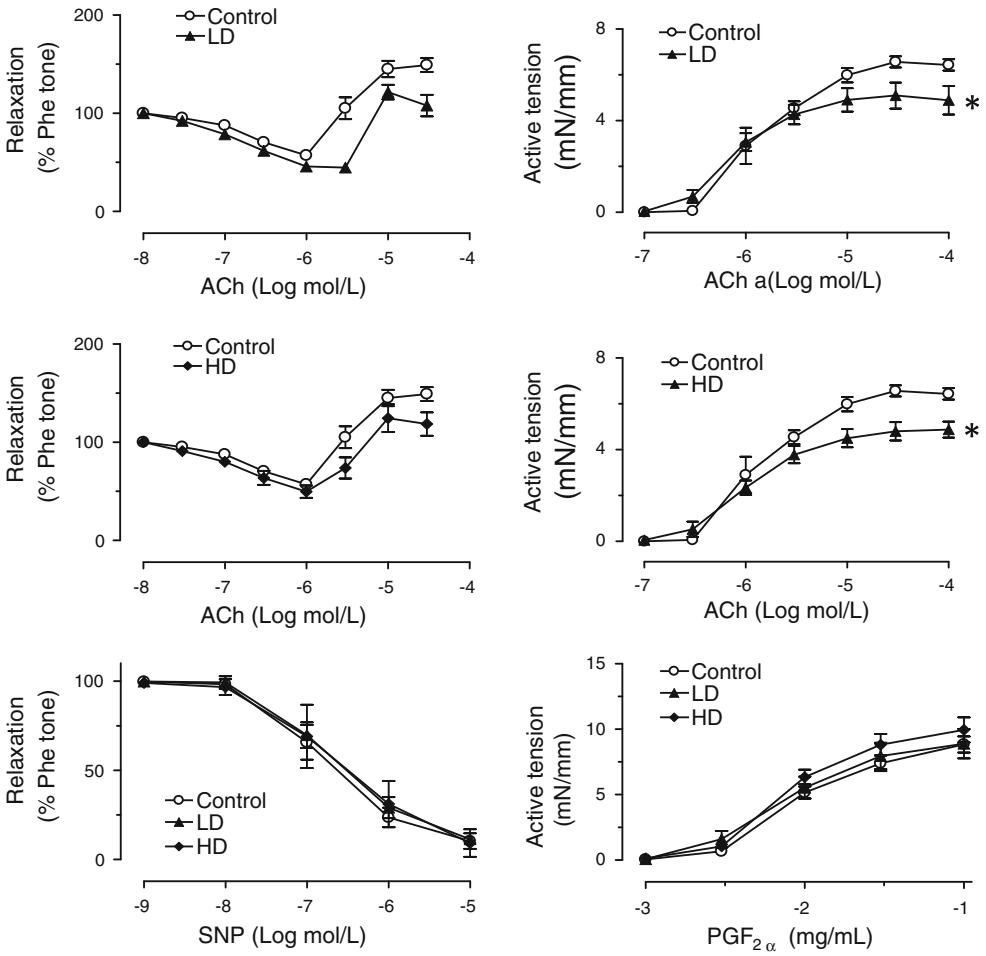

Fig. 2 Effect of dietary capsaicinoids on the acetylcholine (ACh)-induced endothelium-dependent relaxations in aortas with endothelium of hamsters fed the control and the experimental diets containing 0.010 % (LD) and 0.020 % (HD) capsaicin. The ACh-induced endothelium-dependent contractions were observed in rings with endothelium treated with L-NAME (100 μmol/L, 30-min incubation). Endothelium-independent relaxations in response to sodium nitroprusside (SNP) and PGF2α-induced contractile responses were measured in aortic rings without endothelium. Data are means ± SD of 7–8 experiments. *$P < 0.05$, compared with the control between two *curves*

Discussion

The present study clearly demonstrated supplementation of capsaicinoids in diet could modulate favorably serum lipids by reducing plasma TC and non-HDL-C with HDL-C being unaffected. Result was in agreement with that of Manjunatha and Srinivasan [6], who found that incorporation of 0.015 % capsaicinoids into diet could reduce serum TC, non-HDL-C, and TAG by 23, 44, and 14 %, respectively, in SD rats fed a 30 % fat diet. In our hamster model fed a diet containing 5 % lard and 0.1 % cholesterol, we found reduction in serum TC, non-HDL-C, and TAG could reach 16, 25, and 26 %, respectively. The present study was taken further to examine the effect of dietary capsaicinoids at doses ranging from 0.010 to 0.30 % on plasma lipids, finding that plasma lipids-lowering activity was not dose-dependent. We speculated that capsaicinoids were very effective in plasma lipid reduction and 0.010 % capsaicinoids were probably a saturated dose above which no additional effect on TC and TAG could be seen.

Excess cholesterol in mammals is usually disposed via bile fluid duct or by conversion to bile acids. In this regard, the increase in bile acid excretion is likely the primary mechanism underlying plasma TC-reducing activity of capsaicinoids. First, dietary capsaicinoids were associated with 36–64 % greater excretion of bile acids. Second, dietary capsaicinoids could up-regulate the gene expression of CYP7A1, which is a regulatory enzyme in bile acid synthesis pathway. Result was in agreement with the report of Srinivasan and Sambaiah [19], who found that the activity of hepatic CYP7A1 was significantly elevated in rats given curcumin, capsaicin, ginger, and mustard. LXRα can up- or down-regulate a number of genes including CYP7A1 and ABCG 5/8 dependent on mammal species [20, 21]. The present results demonstrated that dietary capsaicinoids down-regulated the gene expression of liver LXRα, although the effect was not dose-dependent.

The present study was the first time to study the effect of capsaicinoids on fecal neutral sterol profile and gene expression of intestinal NPC1L1, ACAT2, MTP, and ABCG 5/8. The second mechanism underlying plasma TC-reducing activity of capsaicinoids was probably associated with greater fecal neutral sterol excretion. We found that feeding dietary capsaicinoids significantly decreased plasma ratio of campesterol/cholesterol [14], suggesting that capsaicinoids affected the cholesterol absorption.

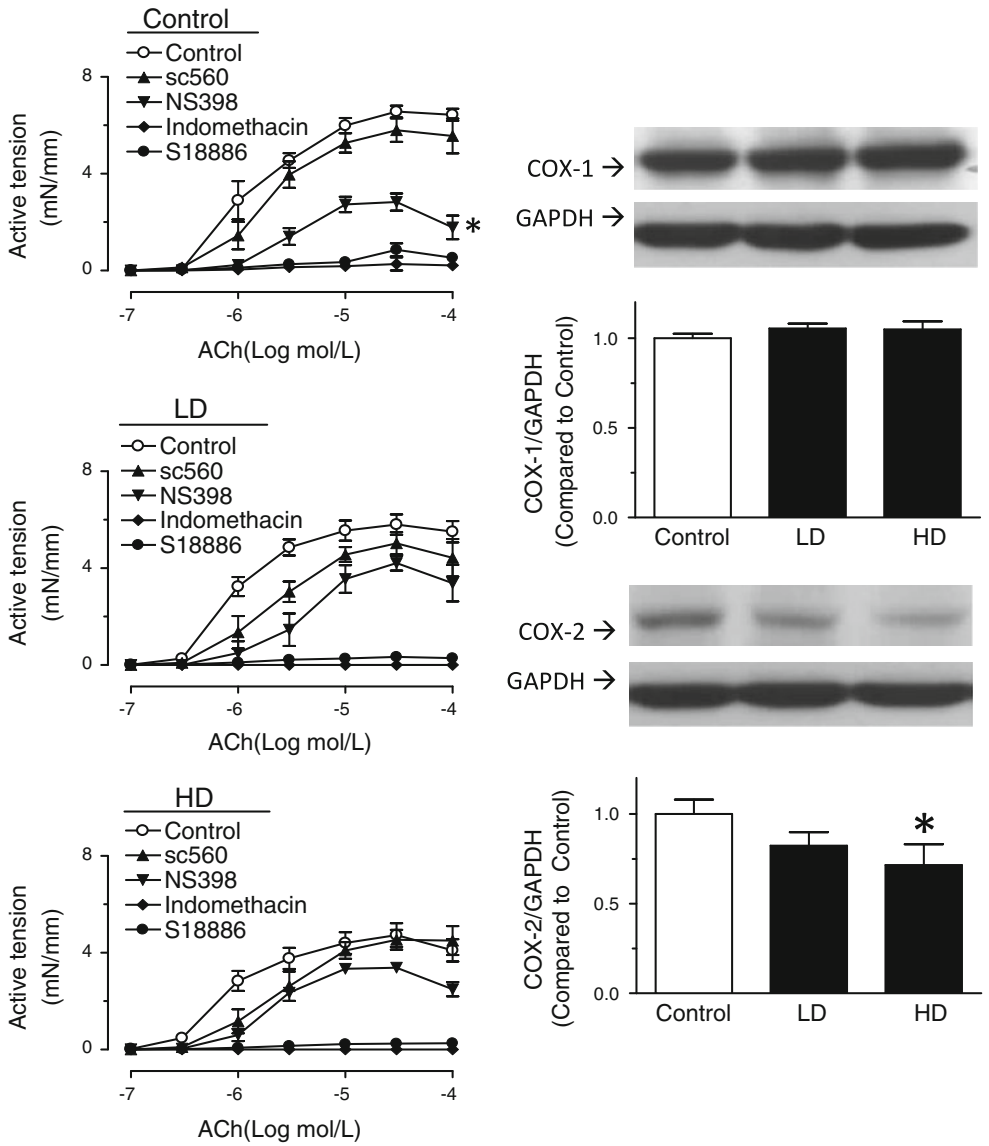

Fig. 3 Effects of TP receptor antagonist S18886 (100 nmol/L), COX-1 inhibitor SC560 (300 nmol/L), COX-2 inhibitor NS398 (10 μmol/L), and non-selective COX inhibitor indomethacin (1 μmol/L) on endothelium-dependent contractions to acetylcholine (Ach) in L-NAME-treated aortas and protein expressions of COX-1 and COX-2 in hamsters fed the control (CON) or the experimental diets containing 0.010 % (LD) and 0.020 % (HD) capsaicinoids. Data are mean ± SEM of 7–8 experiments. *$P < 0.05$, compared with the control between two *curves*

A detail analysis on fecal individual sterols demonstrated a trend that addition of capsaicinoids into diet increased the fecal cholesterol and its microbial metabolite dihydrocholesterol. However, a significant difference was not seen. This discrepancy on the data between the biomarker and fecal neutral sterol excretion was probably due to a large variation in the analysis of fecal neutral sterols among the individual hamsters. Nevertheless, the claim that capsaicinoids decreased cholesterol absorption was supported by the report of Seo et al. [8], who investigated the effect of intraduodenally infused capsaicinoids on the lymphatic absorption of [^{14}C]-cholesterol in SD rats, finding that capsaicinoids in lumen could inhibit the intestinal absorption of cholesterol. Despite of the evidence above, we did not observe any effect of dietary capsaicinoids on gene expression of intestinal NPC1L1, ACAT2, MTP, and ABCG 5/8, suggesting the effect on sterol absorption did not occur at gene level rather than at the physical interaction of capsaicinoids with cholesterol in the intestine. Because of their non-polar nature, it is speculated that capsaicinoids may have a hydrophobic interaction with cholesterol in the intestine, hindering the sterol absorption.

The present study had also examined the effect of dietary capsaicinoids on functionality of artery in vivo. It was found that capsaicinoids could improve the endothelium-dependent relaxations and reduce the endothelium-dependent

contractions. It was likely that the beneficial effect of capsaicinoids on aorta was correlated with their inhibition on the formation of atherosclerotic plaque because capsaicinoids have been shown to possess some antioxidant activity and inhibit the LDL oxidation [5]. Up-regulation of COX-2 was associated with endothelial dysfunction, atherosclerotic plaque, and inflammation in hypertension and diabetes [22]. The previous study has shown that the increased COX-2 expression and activity contributes to the impaired endothelium-dependent relaxation and the enhanced endothelium-dependent contraction in hypertension [23]. COX-2 and TP may also have a proatherogenic role [24]. In the present study, enhanced endothelium-dependent contraction in aorta was markedly attenuated by COX-2 inhibitor NS398, abolished by TP receptor antagonist s18886, while only slightly reduced by COX-1 inhibitor sc560, indicating that COX-2 mainly contributes to the production of endothelium-derived contracting factors (EDCFs) in aorta from the hypercholesterolemic hamster; however, the involvement of COX-1, albeit to a lesser degree in triggering endothelium-dependent contractions, cannot be excluded. Of note, non-selective COX inhibitor indomethacin eliminated endothelium-dependent contractions, suggesting that the production of EDCFs may be an additive or synergistic action of both COX-2 and COX-1. Acetylcholine-induced less endothelium-dependent contractions in aorta from capsaicinoids-treated HD group as compared with non-treated HD group. In addition, the expression of COX-2 was down-regulated by capsaicinoids with COX-1 expression being unaltered in the HD group, suggesting that the favorable vascular effect of capsaicinoids was at least in part mediated through their inhibition on either activity or expression of COX-2.

No data in humans are available to show that capsaicinoids are hypocholesterolemic. The dose used in the present study was slightly higher than that of actual human consumption. It has been estimated that the consumption of chili pepper in Indian, Thailand, and Mexico could reach 2.5, 5, and 20 g/person/day, respectively [25]. Such consumption of chili pepper is equivalent to 0.5–4 mg capsaicinoids/kg body weight/day provided that capsaicinoids in chili pepper are 1 % by weight. In the present study, LD group was given a diet containing 0.010 % capsaicinoids, which was equivalent to 7 mg/kg body weight/day [25]. In this regard, the concentration of capsaicinoids used in the present study could achieve its cholesterol-lowering and vascular activity under the normal physiological conditions if the data in hamsters could be extrapolated to those in humans.

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