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Hypocholesterolemic Effect of Capsaicinoids in Rats Fed Diets with or without Cholesterol

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ABSTRACT: The potential mechanism of the hypocholesterolemic effect of capsaicinoids in rats fed with cholesterol-enriched and cholesterol-free diets was determined. Capsaicinoids favorably modified the lipoprotein profile of rats. Capsaicinoids consumption down-regulated the mRNA levels of hepatic 3-hydroxyl-3-methylglutaryl CoA (HMG-CoA) reductase by 0.55-fold and hepatic cholesterol-7α-hydroxylase (CYP7A1) by 0.53-fold in the cholesterol-free diet group ($P < 0.05$) but up-regulated the CYP7A1 level by 1.38-fold in the cholesterol-enriched diet group ($P < 0.05$). It also increased the expression levels of ileal bile acid binding protein and apical sodium-dependent bile acid transporter in the ileum, as well as transient receptor potential vanilloid type-1 in the liver and ileum in the different groups. Capsaicinoids reduced the amount of bile acids in feces by −15.97% and contents of the small intestine by −9.64% in the cholesterol-free diet group ($P < 0.05$) but increased both by 13.06% and 10.20%, respectively, in the cholesterol-enriched diet group. The cholesterol-lowering action of capsaicinoids in the cholesterol-free diet group was attributed to the inhibition of hepatic cholesterol synthesis, whereas that in the cholesterol-enriched diet group was attributed to the stimulation of the conversion of cholesterol to bile acids and the increasing excretions of bile acids in feces.

KEYWORDS: *capsaicinoids, plasma cholesterol, bile acids, cholesterol diets, mechanism of metabolism*

INTRODUCTION

Chili peppers are popularly consumed by many people around the world. Compounds known as capsaicinoids cause the spicy flavor (pungency) of the chili pepper fruit. The primary capsaicinoids in chili peppers is capsaicin, followed by dihydrocapsaicin, nordihydrocapsaicin, homodihydrocapsaicin, and homocapsaicin.[1−3] Capsaicinoids have attracted considerable attention because of their extensive biological and physiological properties. Previous studies suggested that capsaicinoids have potential antioxidant,[4,5] anticancer,[6] pro-energy metabolism and antifat accumulation,[7−11] analgesic,[12] and anti-inflammatory[13] properties.

Cholesterol-lowering through the consumption of capsaicinoids has drawn significant attention. However, the hypocholesterolemic effect of capsaicinoids is still indefinite. Previous studies demonstrated that 5% of red pepper or natural capsaicin from red peppers or synthetic capsaicin equivalent to the amount of capsaicin present in 5% red pepper could significantly reduce the concentration of cholesterol in the plasma and liver of female Wistar rats fed with 1% cholesterol and 0.15% sodium tauroglycocholate.[14] Srinivasan et al.[15] reported that a 0.3 mg % capsaicin supplement has no effect on the cholesterol and phospholipids in the liver and serum of growing female Wister rats fed with a normal diet. Moreover, the administration of 0.015% capsaicin has no effect on the concentration of serum cholesterol but significantly lowers the hepatic cholesterol in rats fed with a cholesterol-free diet. It also has no effect on the concentration of hepatic cholesterol but significantly lowers the serum cholesterol in rats fed with a high cholesterol diet.[16] These discrepancies suggest that capsaicinoids may have different effects on the metabolism of exogenous and endogenous sterol. In addition, the mechanisms underlying the effects of capsaicinoids on cholesterol homeostasis remain unclear. Previous reports indicated that the hypocholesterolemic effect of capsaicinoids is possibly mediated by the inhibition of intestinal cholesterol absorption.[17] Capsaicinoids could also significantly elevate the activity or mRNA level of hepatic cholesterol-7α-hydroxylase (CYP7A1) and stimulate the conversion of cholesterol to bile acids.[18,19]

Although several studies demonstrated the potential effects of capsaicinoids on cholesterol metabolism, their effects on different diets are still unknown. The present study aims to examine the effect of capsaicinoids on the plasma and liver lipids of rats fed with a cholesterol-enriched or cholesterol-free diet. The cholesterol-lowering effects could be achieved by several mechanisms,[20,21] and the relative activity of each mechanism can be assessed by quantifying appropriate cellular biomarkers. To determine the potential mechanism underlying the hypocholesterolemic effect of capsaicinoids, we investigated the gene expression of CYP7A1 and 3-hydroxyl-3-methylglutaryl CoA (HMG-CoA) reductase in the liver, apical sodium-dependent bile acid transporter (ASBT), and ileal bile acid binding protein (IBABP) in the ileum as well as transient receptor potential vanilloid type-1 (TRPV1) in the liver and ileum.

MATERIALS AND METHODS

Chemicals and Reagents. Cholesterol was purchased from Dingguo Bis-biotech Co., Ltd. (Beijing, China). Capsaicinoids were purchased from Henan Bis-biotech Co., Ltd. (Henan, China).

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Table 1. Compositions of Experimental Diets (g/kg Diet)

ingredient	cholesterol-free diet	cholesterol diet	ingredient	cholesterol-free diet	cholesterol diet
corn starch	449.5	429.5	mineral mixture[a]	35	35
soy bean oil	100	100	vitamin mixture[b]	10	10
casein	200	200	L-cystine	3	3
sucrose	100	100	choline chloride	2.5	2.5
cellulose	100	100	cholesterol		20

[a]AIN-76 mineral mixture (% mixture). [b]AIN-76 vitamin mixture (% mixture).

Table 2. Primer Sequence and Product Size

	primer sequence		
gene	sense	antisense	product size(bp)
β-actin	ACGGTCAGGTCATCACTATCG	GGCATAGAGGTCTTTACGGATG	155
TRPV1	CAGAGAGCCATCACCATCCTG	AGTTTACCTCGTCCACCCTGAA	148
CYP7A1	GAGGGATTGAAGCACAAGAACC	ATGCCCAGAGAATAGCGAGGT	139
HMG-CoA R	GACCAACCTTCTACCTCAGCAAG	ACAACTCACCAGCCATCACAGT	131
IBABP	CAGACTTCCCCAACTATCACCAG	TCAAGCCACCCTCTTGCTTAC	110
ASBT	GTGACATGGACCTCAGTGTTAGC	GTAGGGGATCACAATCGTTCCT	125

Capsaicin and dihydrocapsaicin were purchased from Sigma-Aldrich (St. Louis, MO). Commercial solid diets were obtained from Chongqing Tengxin Inc. (Chongqing, China). Total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) commercial diagnostic kits were obtained from Beihai Biotechnology (Shanghai, China). Total mRNA extract, reverse transcriptase, and SYBR Premix Ex *Taq*II were from TaKaRa Biotechnology Co., Ltd. (Dalian, China). All other chemicals used were of analytical grade.

High-Performance Liquid Chromatography (HPLC) Analysis of Capsaicinoids. The capsaicinoids were quantified on a Zorbax SB-C18 (4.5 mm ×250 mm, 5 μm) column using an HPLC system with an ultraviolet detector at 280 nm. The column temperature and flow rate were set at 30 °C and 1 mL/min, respectively.

Animals and Diets. Four-week-old Sprague−Dawley rats weighing 180 g ± 10 g were purchased from Chongqing Tengxin Inc. (Chongqing, China). They were individually housed in stainless steel screen-bottomed cages in a room maintained at 23 °C ± 2.0 °C with approximately 50% relative humidity. The room was illuminated from 8:00 a.m. to 20:00 p.m. The rats were acclimated by feeding a commercial solid diet (Chongqing Tengxin Inc., Chongqing, China) for 1 week. After acclimation, 32 rats were randomly divided into four groups of eight (four females and four males per group). All animals received one of the following diets: cholesterol-free diet (C), cholesterol-free diet and gavage with 10 mg/kg/body weight capsaicinoids (CAP), 2% cholesterol-enriched diet (Ch), and cholesterol-enriched diet and gavage with 10 mg/kg/body weight capsaicinoids (Ch+CAP). The rats were given free access to food and water for 28 days. The dietary components are shown in Table 1. All experimental procedures were performed in accordance with the protocols approved by the Institutional Animal Care and Research Advisory Committee.

Sampling and Analytical Procedures. Food intake was recorded daily, whereas body weight was measured every 3 days. Feces were collected on the last 3 days of the experimental period, freeze-dried, weighed, and then milled. On the last day of the experimental period, the rats were starved for 12 h, and their blood was collected from the neck of each rat into a blood collection tube (Vacutainer; Liuyang City Medical Instrument Factory, Hunan, China) containing heparin as an anticoagulant. The plasma was separated by centrifugation at 1,400*g* at 4 °C for 15 min and the serum stored at −80 °C until analysis. The liver was immediately perfused with ice-cold saline (9 g NaCl/L), removed, washed with cold saline, blotted dry on filter paper, weighed, frozen in liquid N_2, and then stored at −80 °C until analysis. After liver collection, the small intestine was removed and weighed, and its contents were collected by flushing with 30 mL of ice-cold saline into a preweighed tube, freeze-dried, and then weighed. Subsequently, the ileum was removed from the small intestine, weighed, frozen in liquid N_2, and stored at −80 °C until further analysis.

Biochemical Analysis. The concentrations of TC, TG, LDL-C, and HDL-C in the plasma were determined using commercial diagnostic kits (Beihai biotechnology, Shanghai, China). The concentrations of hepatic TG and TC were extracted with chloroform/methanol (2:1, v/v) according to the method of Folch et al.[22] and determined using commercial diagnostic kits. The total lipid content in the liver was determined gravimetrically after extraction. The concentrations of total bile acids in the feces and small intestine contents were determined enzymatically by the 3α-hydroxysteroid dehydrogenase assay method of Sheltaway et al.,[23] using taurocholic acid as the standard.

Histological Analysis. Parts of the small intestine tissues were fixed in Bouin solution and then stored in 70% ethanol until histological analysis. A portion of the stored small intestine was embedded in paraffin and then cut into 5 μm thick semiserial histological sections using a microtome (Microtone Leica EG 1150H, Wetzlar, Germany). We used hematoxylin and eosin to highlight the possible macrovesicular steatosis. Images were captured using a high-resolution digital camera (Nikon H550L, Tokyo, Japan) coupled to an Olympus B×40 microscope.

RNA Extraction and RT-PCR Analysis of Gene Expression. Total RNA was extracted from the frozen liver and ileum according to the method described by Chomczynski et al.[24] RNA was quantified using spectrophotometric analysis at OD260, where purity was assessed by the A260/A280 ratio. RNA integrity was verified by agarose gel electrophoresis using Oligotex-dT30 (Takara Bio, Shiga, Japan). A total of 1 μg of purified mRNA was used for cDNA synthesis using reverse transcriptase (TaKaRa Biotechnology Co., Ltd., Dalian, China) according to the manufacturer's instructions. The mRNA expression for TRPV1, CYP7A1, HMG-CoAR, ASBT, IBABP, and β-actin as a housekeeping gene for normalization was determined by real-time monitoring of PCR using a Light Cycler Instrument (Roche Diagnostics, Mannheim, Germany). cDNA (2 μL) was amplified in a total volume of 20 μL using the SYBR Premix Ex *Taq*II (TaKaRa Biotechnology Co., Ltd., Dalian, China) and specific primers at 0.4 μM each. The reaction mixture was incubated for initial denaturation at 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s and 58 °C for 20 s. The sequences of the gene-specific primers (Sangon Biological Engineering, Shanghai, China) used in the study are listed in Table 2. Relative gene expression was calculated using the crossing point of each target gene; the β-actin gene was used as the reference.

Statistical Analysis. The results were expressed as the means ± standard error. Variance analysis was established by one-way ANOVA. Significant differences between groups were calculated using Duncan's multiple range tests. Significance was considered at the $P \leq 0.05$ level.

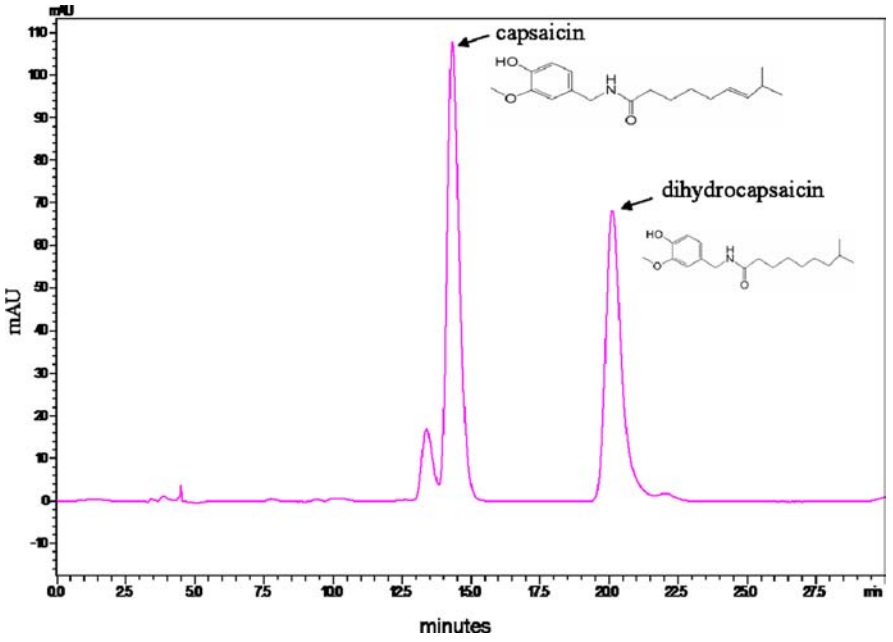

Figure 1. HPCL chromatograms of capsaicinoids and structures of capsaicin and dihydrocapsaicin.

Table 3. Effects of Capsaicinoids on Serum Lipids in Rats[a]

	C	CAP	Ch	Ch + CAP
TC (mmol/L)	2.12 ± 0.05	1.89 ± 0.07	4.09 ± 0.23[b]	2.64 ± 0.11[c]
TG (mmol/L)	0.44 ± 0.05	0.33 ± 0.02[b]	0.74 ± 0.06[b]	0.59 ± 0.02
HDL-C (mmol/L)	0.86 ± 0.02	1.03 ± 0.01[b]	1.00 ± 0.02[b]	1.05 ± 0.02
non-HDL-C (mmol/L)	1.26 ± 0.05	0.86 ± 0.06[b]	3.09 ± 0.22[b]	1.60 ± 0.10[c]
AI	1.48 ± 0.09	0.84 ± 0.05[b]	3.11 ± 0.23[b]	1.52 ± 0.09[c]
AAI	0.41 ± 0.01	0.55 ± 0.02[b]	0.25 ± 0.01[b]	0.40 ± 0.01[c]

[a]Rats were fed different diets for 28 days. Results are expressed as the means ± SE, $n = 8$. AI: atherogenic index was calculated as {(TC) − (HDL-C)}/(HDL-C). AAI: antiarterial hardness indices were calculated as (HDL-C)/TC. [b]The value was significantly different from that of the C group at $P < 0.05$. [c]The value was significantly different from that of the Ch group at $P < 0.05$.

Table 4. Effects of the Capsaicinoids on Liver Lipids in Rats[a]

	C	CAP	Ch	Ch + CAP
liver weight (g)	4.25 ± 0.40	4.40 ± 0.24	6.33 ± 0.32[b]	5.51 ± 0.43[c]
TC (μmol/g liver)	10.13 ± 0.58	9.63 ± 0.28	17.43 ± 0.95[b]	16.79 ± 0.94
TC (μmol/liver)	41.45 ± 2.95	42.79 ± 2.84	110.45 ± 11.77[b]	91.05 ± 5.75
TG (μmol/g liver)	20.16 ± 0.72	19.47 ± 0.54	27.46 ± 1.29[b]	28.43 ± 1.30
TG (μmol/liver)	86.77 ± 11.54	86.95 ± 6.51	176.66 ± 8.80[b]	159.77 ± 19.33
lipid concentration(mg/g)	70.78 ± 5.71	50.63 ± 4.02[b]	179.29 ± 8.92[b]	137.86 ± 4.27[c]
total lipids (g/liver)	0.29 ± 0.04	0.23 ± 0.03	1.17 ± 0.09[b]	0.77 ± 0.08[c]

[a]Rats were fed different diets for 28 days. Results are expressed as the means ± SE, $n = 8$. [b]The value was significantly different from that of the C group at $P < 0.05$. [c]The value was significantly different from that of the Ch group at $P < 0.05$.

All statistical analyses were conducted using the 12.0 SPSS software for Windows (SPSS, Chicago, IL, USA).

RESULTS

Quantitative Analysis. The standard curves for capsaicin and dihydrocapsaicin are $y = 0.0001x + 1.4125$ ($R^2 = 0.9991$) and $y = 0.0002x + 1.5312$ ($R^2 = 0.9990$), respectively. The chromatogram of the capsaicinoids is shown in Figure 1. The contents of capsaicin and dihydrocapsaicin were 355.42 and 592.74 g/kg, respectively.

Serum Lipid Profile. Compared with the C group, the plasma TC, TG, and non-HDL-C concentrations in the CAP group decreased by 10.85% ($P = 0.198$), 25% ($P = 0.065$), and 31.75% ($P = 0.026$), respectively, whereas the HDL-C concentration increased by 19.77% ($P < 0.01$; Table 3). High cholesterol feeding for 4 weeks dramatically increased the plasma TC, and this increase was observed mainly in the non-HDL-C associated fraction. The increase in non-HDL-C was nearly 2.5-fold ($P < 0.01$). Dietary capsaicinoids countered the extent of hypercholesterolemia. The plasma TC and non-HDL-C in the Ch + CAP group were lowered significantly by 35.45% ($P < 0.01$) and 48.22% ($P < 0.01$), respectively, compared with those in the Ch group. The cholesterol-enriched diet also dramatically increased the TG and HDL-C concentrations ($P < 0.01$). However, these concentrations were lowered by the

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capsaicinoid supplements (0.74 ± 0.06 vs 0.59 ± 0.02, $P = 0.02$; 1.00 ± 0.02 vs 1.05 ± 0.02, $P = 0.081$).

The variation tendency of the atherogenic index (AI) and antiarterial hardness index (AAI) was in accordance with the variation of non-HDL-C. Compared with the C group, AI decreased by 43.24% ($P = 0.001$), whereas AAI increased by 34.14% ($P < 0.01$) in the CAP group. Compared with the Ch group, AI decreased by 51.13% ($P < 0.01$), whereas AAI increased by 60% ($P < 0.01$) in the Ch + CAP group.

Liver Lipid Profile. The cholesterol-enriched diet dramatically increased the liver weight, TC, and TG. However, capsaicinoid consumption had no effect on either the cholesterol-free or cholesterol-enriched diet groups.

The cholesterol-enriched diet promoted the accumulation of lipids in the liver, whereas the dietary capsaicinoids treatment countered this tendency ($P < 0.01$; Table 4). Compared with the C group, the lipid concentration in the liver was lower by 28.47% ($P = 0.020$) in the CAP group. Compared with the Ch group, the lipid concentration and total lipids was lower by 23.11% and 34.19% in the Ch + CAP group, respectively ($P < 0.01$).

Hepatic and Ileal Gene Expression. To understand the mechanism of the hypocholesterolemic action of capsaicinoids, the expressions of hepatic and ileal genes involved in cholesterol homeostasis in the body were measured by real-time polymerase chain reaction (Figures 2 and 3). Compared with the C group, dietary capsaicinoids up-regulated the hepatic and ileal mRNA expressions of TRPV1 by 2.02-fold and 1.58-fold, respectively, in the CAP group ($P < 0.01$). Compared with the C group, the mRNA expressions of hepatic HMG-CoA R and CYP7A1 were significantly down-regulated by 0.55-fold and 0.53-fold ($P < 0.01$), respectively, whereas the mRNA expression of IBABP was significantly up-regulated by 1.67-fold ($P < 0.01$) in the CAP group.

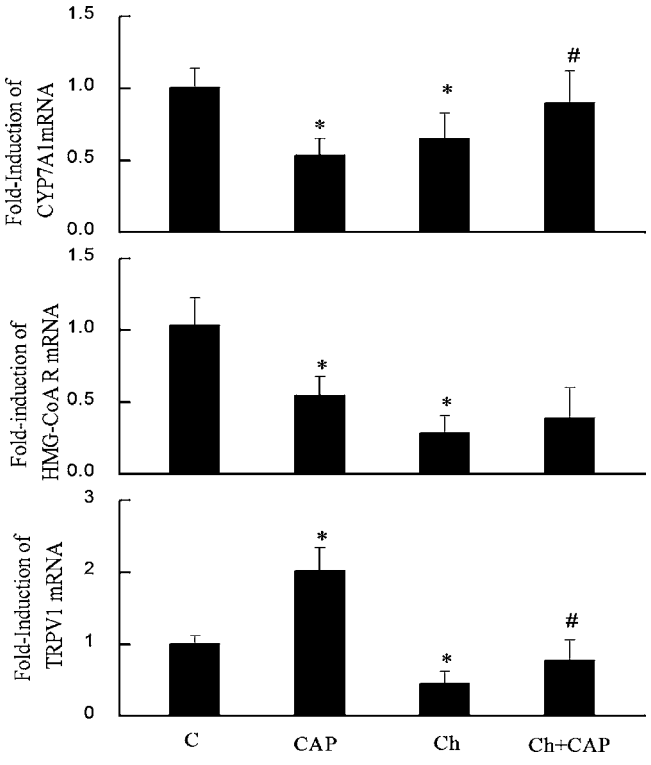

Figure 2. Effect of dietary capsaicinoids on mRNA levels of hepatic CYP7A1, HMG-CoA reductase, and TRPV1 in rats. Rats were fed different diets for 28 d, and results are expressed as the means ± SE, $n = 8$. C, control group fed the cholesterol-free diet; CAP, capsaicinoids group fed the cholesterol-free diet with capsaicinoids (10 mg/kg/body weight); Ch, high cholesterol group fed the cholesterol diet (2%); Ch + CAP, high cholesterol with capsaicinoids group fed the cholesterol diet with capsaicinoids (10 mg/kg/body weight). *, the value was significantly different from that of the C group at $P < 0.05$; #, the value was significantly different from that of the Ch group at $P < 0.05$.

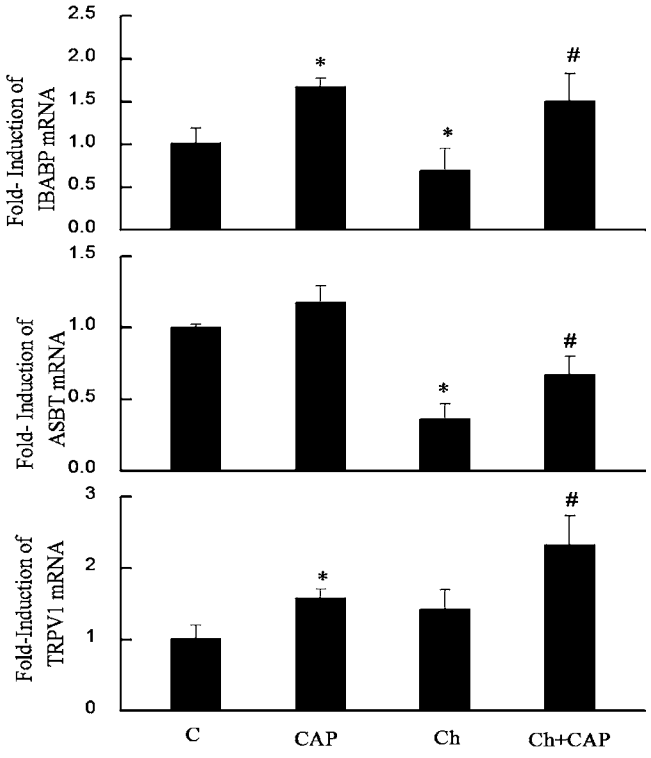

Figure 3. Effect of dietary capsaicinoids on mRNA levels of ileal IBABP, ASBT, and TRPV1 in rats. Rats were fed different diets for 28 d. Results are expressed as the means ± SE, $n = 8$. C, control group fed the cholesterol-free diet; CAP, capsaicinoids group fed the cholesterol-free diet with capsaicinoids (10 mg/kg/body weight); Ch, high cholesterol group fed the cholesterol diet (2%); Ch + CAP, high cholesterol with capsaicinoids group fed the cholesterol diet with capsaicinoids (10 mg/kg/body weight). *, the value was significantly different from that of the C group at $P < 0.05$; #, the value was significantly different from that of the Ch group at $P < 0.05$.

Effect of Capsaicinoids on the Histology of the Small Intestine. To confirm the histologic changes in the small intestine after the intake of capsaicinoids, hematoxylin and eosin staining was performed. The small intestines of rats in the C and Ch groups showed no significant histologic differences. However, dietary capsaicinoids significantly increased the length and perimeter of microvilli in rats fed with a cholesterol-free or cholesterol-enriched diet (Figure 4).

Total Bile Acid Levels in the Small Intestinal Contents and Feces. The dry weights of the small intestinal contents and excreted feces were not affected by the diets (Table 5). The cholesterol-enriched diet promoted the secretion of bile acids

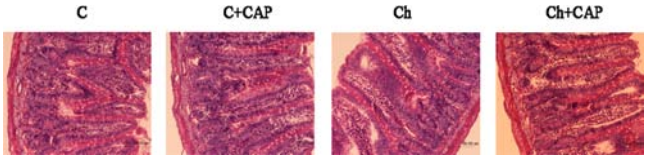

Figure 4. Effect of dietary capsaicinoids on the histology of small intestines from rats. Hematoxylin- and eosin-stained small intestines sections. C, control group fed the cholesterol-free diet; CAP, capsaicinoids group fed the cholesterol-free diet with capsaicinoids (10 mg/kg/body weight); Ch, high cholesterol group fed the cholesterol diet (2%); Ch + CAP, high cholesterol with capsaicinoids group fed the cholesterol diet with capsaicinoids (10 mg/kg/body weight).

by 26.33% ($P < 0.01$) and 36.82% ($P < 0.01$) in the small intestinal contents and excreted feces, respectively, in the Ch group. The amounts of bile acids in the small intestinal contents (from 34.14 ± 0.79 to 30.85 ± 0.54, $P = 0.01$) and excreted feces (from 11.08 ± 0.41 to 9.31 ± 0.28) were remarkably decreased ($P < 0.01$) by capsaicinoids in the cholesterol-free diet. By contrast, they were remarkably increased ($P < 0.01$) in the cholesterol-enriched diet (from 43.13 ± 0.61 to 47.53 ± 0.61, and from 15.16 ± 0.38 to 17.14 ± 0.37, respectively).

DISCUSSION

The present study clearly demonstrated that capsaicinoids supplements could favorably modulate serum lipids by reducing plasma TC and non-HDL-C in rats fed with a cholesterol-free or cholesterol-enriched diet. Considering that the diet of the C and CAP groups did not contain cholesterol, the level of cholesterol in the serum must be attributed to the changes in endogenous sterol metabolism. However, considering that the diet of the Ch and Ch + CAP groups contained 2% cholesterol, their hypocholesterolemic effect must be influenced by endogenous and exogenous cholesterol metabolism.

Plasma cholesterol levels are tightly controlled by the regulation of endogenous cholesterol synthesis, absorption of exogenous cholesterol, and induction of cellular LDL uptake by LDL receptors.[25,26] The liver is the main organ for endogenous cholesterol synthesis, in which HMG-CoA reductase is the rate-limiting enzyme.[27] The present data indicated that the mRNA level of hepatic HMG-CoA reductase was significantly down-regulated in the CAP group compared with that in the C group (Figure 2). Acetyl-CoA reductase is the precursor of cholesterol synthesis in the liver and also the startup material of the tricarboxylic acid cycle.[28] It is the catabolic product of sugar, protein, and fat. We speculated that capsaicinoids might increase thermogenesis in the cholesterol-free diet group by promoting the tricarboxylic acid cycle. As a result, the conversion and utilization of acetyl-CoA are accelerated, thereby reducing the amount of cholesterol synthesis precursors. Therefore, capsaicinoids could inhibit cholesterol biosynthesis in the liver. Moreover, blood cholesterol is mainly located in LDL, and the removal of LDL-C from the circulation into the liver is mainly mediated by LDL receptors.[19] Unfortunately, we did not measure the mRNA levels of LDL receptors in the present study. However, Choi et al.[29] suggested that the inhibition of HMG-CoA reductase could deplete the intracellular pool, leading to the up-regulation of cell surface LDL receptors. Hence, we conjectured that capsaicinoids could induce the expression of LDL receptors and enhance the cellular uptake of LDL-C from the circulation. In addition, the diet of the C and CAP groups did not contain cholesterol; thus, the circulation did not have supplements of exogenous cholesterol. These data suggested that capsaicinoids reduced plasma cholesterol content by decreasing hepatic endogenous cholesterol synthesis through the depression of HMG-CoA reductase in rats fed with the cholesterol-free diet. The cholesterol-enriched diet inhibited cholesterol synthesis in the liver (Figure 2). This result is in agreement with the finding of Jones[28] and Yang,[30] who found that HMG-CoA reductase activities in the cholesterol-enriched diet group are lower than those in the cholesterol-free diet group. In the present study, capsaicinoids showed no effect on the mRNA expression of hepatic HMG-CoA reductase in the Ch + CAP group (Figure 2). Therefore, the hypocholesterolemic effect of capsaicinoids in rats fed with the cholesterol-enriched diet may not be explained by the suppression of cholesterol synthesis. This result supports the findings of Liang et al.[19] that capsaicinoids have no significant effect on HMG-CoA reductase in rats fed a 1% cholesterol diet.

Excessive cholesterols are eliminated via the following two mechanisms. First, cholesterols can incorporate into bile fluid and be eliminated as fecal neutral sterols. Second, cholesterols are converted to bile acids and eliminated as fecal bile acids.[31] CYP7A1 is the first rate-limiting enzyme responsible for the conversion of cholesterol to bile acids in the liver.[32] In the present study, capsaicinoids down-regulated the mRNA level of CYP7A1 in the cholesterol-free diet group but up-regulated that in the cholesterol-enriched diet group (Figure 2). This discrepancy may be explained by different cholesterol intakes. No supernumerary cholesterol was found for the synthesis of bile acids in the liver when exogenous cholesterol deficiency and synthesis of endogenous cholesterol was suppressed in the CAP group. Thus, the mRNA level of CYP7A1 and the bile acid concentration in the small intestinal contents and excreted feces decreased. Conversely, although cholesterol synthesis was inhibited, the cholesterol-enriched diet significantly increased the accumulation of free cholesterol in the liver. The

Table 5. Effect of the Capsaicinoids on Total Bile Acids Level of Feces and Small Intestine Contents in Rats[a]

	C	CAP	Ch	Ch + CAP
		Small Intestine Contents		
dry weight (g)	0.20 ± 0.02	0.28 ± 0.05	0.23 ± 0.02	0.29 ± 0.01
total bile acids (μmol/contents)	34.14 ± 0.79	30.85 ± 0.54[b]	43.13 ± 0.61[b]	47.53 ± 0.61[c]
		Fecal Excretion		
dry weight (g/d)	0.96 ± 0.13	0.90 ± 0.07	1.33 ± 0.15	1.23 ± 0.10
total bile acids (μmol/d fecal)	11.08 ± 0.41	9.31 ± 0.28[b]	15.16 ± 0.38[b]	17.14 ± 0.37[c]

[a]Rats were fed different diets for 28 days. Results are expressed as the means ± SE, $n = 8$. [b]The value was significantly different from that of the C group at $P < 0.05$. [c]The value was significantly different from that of the Ch group at $P < 0.05$.

administration of capsaicinoids stimulated the conversion of cholesterol to bile acids and increased the concentration of bile acids in the small intestinal contents and excreted feces (Table 5).

Bile acids are the end products of cholesterol metabolism. Plasma cholesterols can be lowered by disrupting bile acid reabsorption. They are synthesized in the liver and secreted in the small intestine. Approximately 95% of bile acids delivered to the duodenum are reabsorbed efficiently through the small intestine (ileum) and returned via a portal vein to the liver then excreted again into the bile (enterohepatic circulation).[33] The intestinal absorption of bile salts is significantly mediated by the ASBT and IBABP in the ileum. However, the present study showed that capsaicinoids up-regulated the mRNA levels of IBABP and ASBT in both cholesterol-free and cholesterol-enriched diet groups (Figure 3). These data suggested that capsaicinoids did not disrupt the enterohepatic circulation but accelerated the reabsorption of bile acids. Previous studies showed that dietary red peppers induce the alterations in the fluidity and passive permeability properties of brush-border membranes, which are associated with the induction of increased microvilli length and perimeter, thereby increasing the absorptive surface of the small intestine.[34] In the present study, the histologic data confirmed that dietary capsaicinoids can significantly increase the length and perimeter of microvilli in rats fed with cholesterol-free diet or cholesterol diet (Figure 4). Therefore, the promotion of bile acid reabsorption in enterohepatic circulation might be attributed to the increased surface of the small intestine.

The liver plays a major role in cholesterol metabolism, including cholesterol synthesis, bile salt homeostasis, plasma protein synthesis, hormone production, and detoxification. Several studies suggested that capsaicinoids are readily absorbed from the gastrointestinal tract and metabolized in the liver by P450 through in vitro incubation or administration in rats.[35,36] The capsaicin receptor TRPV1 was implicated in obesity, diabetes, metabolic syndromes, and cardiovascular diseases.[37,38] The proteins of TRPV1 were also reportedly expressed in hepatocytes and intestinal cells.[39−41] In the present study, the mRNA level of TRPV1 in the liver and ileum was not affected by the addition of cholesterol. However, after orally administering capsaicinoids, the mRNA level of TRPV1 in the liver and ileum in rats fed with a cholesterol-free or cholesterol-enriched diet was significantly up-regulated (Figures 2 and 3). Capsaicin regulates the expressions of PPARγ, SREBP-1, FAS, ABCA1, and LRP1, prevents obesity, and affords protective effects on vascular endothelial cells.[42,43] Blockage of TRPV1 using a TRPV1 RNAi antagonist, such as capsazepin, can be a novel therapeutic tool to attenuate atherosclerosis and obesity.[42,43] In the current study, dietary capsaicinoids significantly up-regulated the mRNA expression of TRPV1 in rats fed with a cholesterol-free or cholesterol-enriched diet. Therefore, we hypothesized that the metabolites of capsaicinoids in the liver and ileum provoke TRPV1 and affect the mRNA expressions of HMG-CoA reductase, CYP7A1, IBABP, and ASBT. However, further studies need to be conducted to prove this hypothesis.

In conclusion, capsaicinoids significantly decreased the plasma cholesterol of rats fed with a cholesterol-free or cholesterol-enriched diet. The cholesterol-lowering action of capsaicinoids in rats fed with a cholesterol-free diet is attributed to the inhibition of the synthesis of hepatic cholesterol, whereas that in rats fed with a cholesterol-enriched diet is attributed to the stimulation of the conversion of cholesterol to bile acids and increasing excretions of bile acids in feces.

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Notes

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ABBREVIATIONS USED

CYP7A1, cholesterol-7α-hydroxylase; TRPV1, transient receptor potential vanilloid type-1; HMG-CoA, hepatic 3-hydroxyl-3-methylglutaryl CoA reductase; IBABP, ileum bile acids binding protein; ASBT, ileal apical sodium-dependent bile acids transporter

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