Eur J Nutr (2013) 52:869–875
DOI 10.1007/s00394-012-0393-6

ORIGINAL CONTRIBUTION

Blueberry anthocyanins at doses of 0.5 and 1 % lowered plasma cholesterol by increasing fecal excretion of acidic and neutral sterols in hamsters fed a cholesterol-enriched diet

Yintong Liang · Jingnan Chen · Yuanyuan Zuo · Ka Ying Ma · Yue Jiang · Yu Huang · Zhen-Yu Chen

Received: 23 April 2012 / Accepted: 25 May 2012 / Published online: 9 June 2012

Abstract
Purpose The present study investigated the underlying mechanism associated with the hypocholesterolemic activity of blueberry anthocyanins by examining its effect on fecal sterol excretion and gene expression of major receptors, enzymes, and transporters involved in cholesterol metabolism.
Methods Hamsters were divided into three groups and fed a 0.1 % cholesterol diet containing 0 % (CTL), 0.5 % (BL), and 1.0 % (BH) blueberry anthocyanins, respectively, for six weeks. Plasma total cholesterol (TC), triacylglycerols (TAG), and non-high-density lipoproteins cholesterol (non-HDL-C) 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. GC analysis was used to quantify hepatic cholesterol and fecal acidic and neutral sterols.
Results Dietary supplementation of 0.5 and 1.0 % blueberry anthocyanins for 6 weeks decreased plasma TC concentration by 6–12 % in a dose-dependent manner. This was accompanied by increasing the excretion of fecal neutral and acidic sterols by 22–29 % and 41–74 %, respectively. Real-time PCR analyses demonstrated that incorporation of blueberry anthocyanins into diet down-regulated the genes of NPC1L1, ACAT-2, MTP, and ABCG 8. In addition, blueberry anthocyanins were also able to down-regulate the gene expression of hepatic HMG-CoA reductase.
Conclusion The cholesterol-lowering activity of blueberry anthocyanins was most likely mediated by enhancing the excretion of sterols accompanied with down-regulation on gene expression of intestinal NPC1L1, ACAT-2, MTP, and ABCG 8.

Keywords Anthocyanins · Cholesterol · Blueberry · NPC1L1 · Sterol excretion

Y. Liang · Y. Zuo · K. Y. Ma · Z.-Y. Chen (✉)
School of Life Sciences, The Chinese University of Hong Kong, Shatin, NT, Hong Kong, China
e-mail: zhenyuchen@cuhk.edu.hk

J. Chen · Y. Jiang
Department of Biology, Hong Kong Baptist University, Kowloon Tong, Hong Kong, China

Y. Huang
School of Biomedical Sciences, Chinese University of Hong Kong, Hong Kong, China

Introduction

Blueberry is one of the most popular fruits and is also rich in anthocyanins [1]. Consumption of blueberry has been shown to prevent the risk of cardiovascular disease, neurodegenerative diseases, diabetes, and cancer. In obese people, consumption of blueberry improves the metabolic syndrome at dietary achievable doses [2]. Daily consumption of blueberry juice demonstrates an improvement in memory function in older adults [3]. Incorporation of blueberry anthocyanins into diet has been shown to be associated with diet-induced increases in plasma antioxidant status [4]. Overwhelming evidence suggests that blueberry may have beneficial effects against several types of human cancers [5, 6].

Blueberry has also been shown to be capable of reducing plasma cholesterol concentration [7, 8] and improving plasma lipid profile [9]. However, it remains unknown how blueberry anthocyanins exert their cholesterol-lowering activity and more specifically whether they affect gene

expression of cholesterol transporters and enzymes involved in cholesterol absorption and metabolism. These genes mainly include intestinal Niemann-Pick C1 like 1 (NPC1L1), acyl-CoA: cholesterol acyltransferase (ACAT2), microsomal triglyceride protein (MTP) and ATP-binding cassette transporters (ABCG5 and ABCG8) and hepatic 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, low-density lipoprotein (LDL) receptor, cholesterol-7α-hydroxylase (CYP7A1). In brief, NPC1L1 transports the cholesterol from lumen into enterocytes, where cholesterol is converted to cholesteryl ester (CE) by ACAT2. MTP incorporates CE into chylomicrons that are then absorbed via the lymphatic system. ABCG5/8 returns the unabsorbed cholesterol in the enterocytes to the lumen for elimination [10, 11]. HMG-CoA reductase is a regulatory enzyme in cholesterol synthesis pathway, while LDL receptor is responsible for the removal of cholesterol from circulation. CYP7A1 is an enzyme that regulates the conversion of cholesterol to bile acids. In this regard, sterol regulatory element-binding protein 2 (SREBP-2) and liver X receptor-alpha (LXRα) also 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.

Consumption of blueberry is associated with various health benefits. However, the underlying mechanism related to its hypocholesterolemic activity remains unclear. The present study was, therefore, designed to (1) examine the effect of dietary blueberry anthocyanins on plasma lipids and fecal excretion of neutral and acidic sterols, and (2) investigate the interaction of dietary blueberry anthocyanins with gene expression of hepatic SREBP-2, LXRα, HMG-CoA reductase, LDL receptor, and CYP7A1 as well as intestinal NPC1L1, ACAT2, ABCG5/8, and MTP in hamsters fed a high cholesterol diet.

Materials and methods

Diet

The control diet (CTL) 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; and cholesterol, 1. The two experimental diets were similarly prepared except that 0.5 % (BL) and 1.0 % (BH) blueberry anthocyanins were added. To ensure the even incorporation in diet, blueberry anthocyanins was firstly dissolved into lard at 60 °C and thoroughly mixed. All three powdered diets were mixed with a gelatin solution (20 g/L) in a ratio of 200 g diet per liter. Once the gelatin had set, the diets were cut into pieces of approximately 10 g cubes and stored frozen at −20 °C before feeding the hamsters. All three 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. A 0.1 % cholesterol diet was chosen in the present study as it could induce plasma cholesterol concentration to around 240 mg/dl or 6.2 mM, which is a cut off value for the diagnosis of human hypercholesterolemia.

Hamsters

Male Golden Syrian hamsters ($n = 30$, aged 2.5 months) were divided into three groups ($n = 10$ each) and fed one of the three diets. They were housed in an animal room with two hamsters per cage 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 to food and water. Blood sample was taken from the retro-orbital sinus under light anesthesia using a mixture of ketamine, xylazine, and saline (v/v/v; 4:1:5) 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 whole fecal output for 7 days from each cage was collected at weeks 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.

HPLC analysis of blueberry anthocyanins

Blueberry anthocyanins extract used in the present study was a gift from Tianjin Jianfeng Natural Product Co., Ltd, Tianjin, China. As we previously described [12], the individual anthocyanins were separated and quantified on a C-18 (250 × 4.6 mm, 5 μm) column using a HPLC system with a UV detector at 520 nm. The column temperature and flow rate were set at 30 °C and 0.8 ml/min, respectively. The gradient mobile phase consisted of 0.5 % H_3PO_4 (solvent A) and H_2O: acetonitrile: acetic acid: phosphoric acid (50:48:5:1:0.5, solvent B). The ratio of A to B was programmed 4:1–2:3 in 26 min and then back to 4:1 in 4 min and then was held for another 5 min. The blueberry anthocyanins extract mainly contained cyanidin-3-O-glu (49 % peak areas) and petunidin-3-O-glu (20 %) (Fig. 1).

Analysis of plasma lipids

Plasma total cholesterol (TC) and total triacylglycerols (TAG) were quantified using their respective commercial

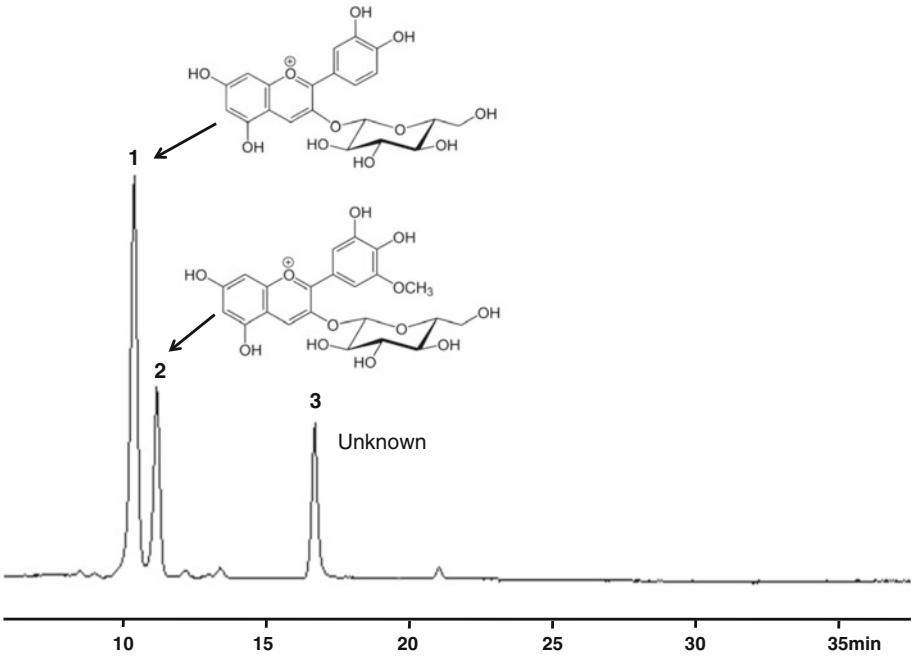

Fig. 1 HPLC chromatogram of blueberry anthocyanins extract. *Peak 1*, cyanidin-3-O-glucose; *Peak 2*, petunidin-3-O-glucose

enzymatic kits from Infinity (Waltham, MA, USA) and Stanbio Laboratories (Boerne, TX, USA), respectively. To quantify plasma high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) were firstly precipitated with phosphotungstic acid and magnesium chloride using a commercial kit (Stanbio Laboratories) as previously described [13]. HDL-C in the supernatant was determined similarly as for TC. Non-HDL-C was calculated by deducing HDL-C from the TC.

Liver cholesterol

Total liver cholesterol was determined as previously described [14, 15]. In brief, the liver lipids were extracted using a solvent mixture of chloroform: methanol with 5α-cholestane being used as an internal standard. The resulting liver lipids were saponified and its cholesterol was converted to its trimethylsilyl-ether (TMS) derivative. The analysis of cholesterol-TMS derivative was performed in a fused silica capillary column (SAC^{TM}-5, 30 m × 0.25 mm, i.d.; Supelco, Inc., Bellefonte, PA, USA) using a Shimadzu GC-14 B Gas–Liquid Chromatograph equipped with a flame ionization detector. Total liver cholesterol was then calculated according to the amount of internal standard 5α-cholestane added.

Fecal total neutral and acidic sterols

Total fecal sterols, a sum of fecal neutral and acidic sterols, were determined as previously described [16]. Fecal sterols are classified into two type's namely neutral and acidic sterols. In brief, 300 mg of fecal samples was weighed with addition of 100 µl of 5α-cholestane solution (5 mg/ml in chloroform) and 1 ml of hyodeoxycholic acid solution (0.6 mg/ml in chloroform) with the former being as an internal standard for the quantification of fecal neutral and the latter for the quantification of acidic sterols, respectively. The sample was saponified in 8 ml of 1 N NaOH in 90 % ethanol at 90 °C for 1 h, and the unsaponified neutral sterols were extracted in 8 ml of cyclohexane. After evaporation, the total fecal neutral sterols were subjected to TMS derivation in 200 µl of TMS reagent at 60 °C for 1 h. The individual neutral sterol-TMS derivatives were separated in a fused silica capillary column (SAC^{TM}-5, 30 m × 0.25 mm, i.d.; Supelco, Inc., Bellefonte, PA, USA) using a Shimadzu GC-14 B Gas–Liquid Chromatograph equipped with a flame ionization detector as previously described. Helium was used as a carrier gas at a flow rate of 1 ml/min. The column temperature was set at 285 °C and held for 30 min. Injector and detector temperatures were set at 300 °C.

Total aqueous phase containing acidic sterols after cyclohexane extraction above were added with 1 ml of 10 N NaOH solution and further saponified at 120 °C for 3 h. After addition of 5 ml of distilled H2O and 3 ml of HCl (25 %), the mixture was extracted with 7 ml of diethyl ether twice. Afterward, 2 ml of methanol, 2 ml of dimethoxypropane, and 40 µl of 37 % HCl solution were added and kept at room temperature overnight. Followed by the solvent evaporation, the individual fecal acidic sterols were subjected to TMS derivation in 300 µl of TMS reagent at

60 °C for 1 h. The individual acidic sterol-TMS derivatives were similarly analyzed in a SAC-5 column. Helium was used as a carrier gas at a flow rate of 1 ml/min. The column temperature was programmed from 230 to 280 °C at a rate of 1 °C/min. Injector and detector temperatures were set at 260 and 300 °C, respectively.

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

Each mRNA level was determined as previously described [14]. Total RNA from liver or intestine was extracted and isolated 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).

Statistical analysis

Data were expressed as mean and standard deviation. One-way analysis of variance (ANOVA) followed by using post hoc LSD method will be used statistically to evaluate differences among the three groups. A simple linear regression analysis was carried out in SPSS to assess the dose-dependent effect of blueberry anthocyanins on plasma TC, fecal sterol expression, and mRNA of intestinal NPC1L1, ACAT2, MTP, and ABCG 8 and hepatic HMG-CoA reductase. The difference was considered significant when P was less than 0.05.

Results

Food intake, body, and organ weights

The three groups of hamsters had an average food intake of 9.9 g daily and had a body weight gain of 11 g during the whole experimental period. However, no differences in food intake, body weight, and relative weights of heart, kidney, testes, and perirenal and epididymal fat pad were seen among the three groups (data not shown).

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

Three groups of hamsters had similar concentrations of plasma TC, HDL-C, non-HDL-C, and TAG at week 0 (Table 1). When the experiment reached the end of week 3, BH group started to show a decrease in plasma TC and non-HDL-C, and the ratio of non-HDL-C to HDL-C compared with the CTL group. At the end of week 6, plasma TC, non-HDL-C, and the ratio of non-HDL-C to HDL-C concentrations in BH group were similarly decreased compared with those in the CTL group (Table 1). The linear regression analysis clearly demonstrated the effect of blueberry anthocyanins on plasma TC and non-HDL-C concentrations was dose-dependent (Table 1). However, no significant difference in plasma HDL-C and TAG was seen among the three hamsters across the entire study period (Table 1).

Liver cholesterol concentration

In contrast to the control, the two experimental groups had hepatic cholesterol concentration decreased by 22–24 % (Table 1).

Fecal total sterols and apparent sterol retention

Cholesterol in colon is subjected to the microbial bioconversion, producing series of cholesterol microbial metabolites with coprostanol and dihydrocholesterol being as the major products, while the cholesterol in liver is converted to bile acids and eliminated mainly as cholic and chenodeoxycholic acids via bile duck. Like cholesterol, these two primary bile acids were subjected to microbial bioconversion, producing secondary bile acids lithocholic and deoxycholic acids in large colon. At week 1, the two experimental groups showed a similar excretion of neutral and acidic sterols (data not shown). When the experiment reached the end of week 6, the two experimental groups demonstrated a dose-dependent increase in excretion of neutral ($P < 0.005$) and acidic sterols ($P < 0.001$) (Table 2). The sterol balance analysis clearly demonstrated that the blueberry anthocyanins significantly decreased the apparent sterol retention in a dose-dependent manner (Table 2).

Real-time PCR mRNA

RT-PCR analysis demonstrated that dietary blueberry anthocyanins produced a dose-dependent decrease in

Table 1 Changes in serum total cholesterol (TC), triacylglycerols (TAG), HDL-cholesterol (HDL-C), non-HDL-cholesterol (non-HDL-C), and liver cholesterol concentration in hamsters fed the control (CTL) and the two experimental diets containing 0.5 % blueberry (BL) and 1 % blueberry (BH) for 0, 3, 6 weeks

	CTL	BL	BH	Regression R	*P* value
Week 0					
TC (mmol/l)	2.87 ± 0.44	2.92 ± 0.41	2.90 ± 0.23	0.031	0.883
TAG (mmol/l)	2.74 ± 0.52	2.59 ± 0.39	2.72 ± 0.41	−0.020	0.916
HDL-C (mmol/l)	1.47 ± 0.18	1.50 ± 0.13	1.50 ± 0.10	0.016	0.939
Non-HDL-C (mmol/l)	1.40 ± 0.36	1.45 ± 0.34	1.42 ± 0.28	0.028	0.894
Non-HDL-C:HDL-C	0.94 ± 0.23	0.96 ± 0.21	0.96 ± 0.22	−0.035	0.868
Week 3					
TC (mmol/l)	6.65 ± 0.49[a]	6.39 ± 0.59[ab]	5.97 ± 0.62[b]	−0.448	0.028
TAG (mmol/l)	4.24 ± 0.98	3.88 ± 0.91	3.57 ± 0.78	−0.268	0.206
HDL-C (mmol/l)	2.56 ± 0.23	2.56 ± 0.36	2.61 ± 0.08	0.058	0.789
Non-HDL-C (mmol/l)	4.06 ± 0.44[a]	3.83 ± 0.36[ab]	3.36 ± 0.59[b]	−0.539	0.007
Non-HDL-C:HDL-C	1.59 ± 0.22[a]	1.53 ± 0.23[a]	1.28 ± 0.21[b]	−0.500	0.013
Week 6					
TC (mmol/l)	6.62 ± 0.39[a]	6.26 ± 0.80[ab]	5.84 ± 0.41[b]	−0.524	0.012
TAG (mmol/l)	5.12 ± 1.32	5.20 ± 1.40	4.91 ± 0.91	−0.092	0.698
HDL-C (mmol/l)	2.69 ± 0.43	2.66 ± 0.38	2.59 ± 0.32	−0.084	0.709
Non-HDL-C (mmol/l)	3.96 ± 0.59[a]	3.57 ± 0.67[ab]	3.26 ± 0.39[b]	−0.483	0.023
Non-HDL-C:HDL-C	1.57 ± 0.47[a]	1.34 ± 0.23[ab]	1.27 ± 0.16[b]	−0.478	0.033
Liver cholesterol (μmol/g)	129. ± 16[a]	98 ± 28[b]	101 ± 28[a]	−0.438	0.022

[ab] Mean values within a row unlike superscript letters are significantly different ($P < 0.05$). A simple linear regression analysis was conducted to assess the dose-dependent activity of blueberry anthocyanins; *R* correlation coefficient

mRNA levels of intestinal NPC1L1, ACAT2, MTP, and ABCG 8 with ABCG5 being unaffected (Fig. 2). In the liver, blueberry anthocyanins only down-regulated dose dependently mRNA HMG-CoA reductase (Fig. 2) and had no effect on mRNA levels of SREBP-2, LXRα, CYP7A1, and LDL receptor (Data not shown).

Discussion

The present study clearly demonstrated supplementation of blueberry anthocyanins in diet could modulate favorably plasma lipids by reducing plasma TC and non-HDL-C with HDL-C and TAG being unaffected. Results of the present study were in agreement with those reported in hamsters [6], pigs [8], mice [17], and rats [18]. The present study was taken further to examine the underlying mechanisms by which dietary blueberry anthocyanins reduced plasma TC concentration. It was found that dietary blueberry anthocyanins reduced plasma TC possibly mediated by increasing fecal excretion of both acidic and neutral sterols.

The present study found that the hypocholesterolemic activity of blueberry anthocyanins was associated with at least two mechanisms. First, supplementation of 0.5 and 1.0 % blueberry anthocyanins into diet significantly increased the fecal excretion of bile acids by 37 and 66 %, respectively, in a dose-dependent manner. In view that excess cholesterol in mammals is usually disposed via biliary excretion or by conversion to bile acids, the results suggest that based on the significant increase in bile acid excretion, this may be a primary mechanism. Despite the evidence above, we did not observed any effect of dietary blueberry anthocyanins on gene expression of hepatic CYP7A1 and LXRα, suggesting the effect on bile acid did not occur at gene level rather than at the physical interaction of blueberry anthocyanins with bile acids in the intestine. Second, addition of blueberry anthocyanins into diet also increased neutral sterol excretion by 24–30 %, suggesting that enhancement of neutral sterol excretion would be an additional mechanism underlying the cholesterol-lowering activity of blueberry anthocyanins. The present study was the first time to study the effect of blueberry anthocyanins on gene expression of intestinal NPC1L1, ACAT2, MTP, and ABCG 5/8. Consistent with greater excretion of fecal total neutral sterols, dietary blueberry anthocyanins could decrease the mRNA levels of intestinal NPC1L1, ACAT2, MTP, and ABCG 8, indirectly suggesting that blueberry anthocyanins could affect the cholesterol absorption, possibly mediated by its interaction with gene expression of these transporters and enzymes

Table 2 Daily fecal excretion of neutral and acidic sterols as well as sterol balance in hamsters fed the control (CTL) and the experimental diets containing 0·5 % blueberry (BL) and 1 % blueberry (BH) at week 6

	CTL	BL	BH	Regression *R*	*P* value
Neutral sterols (μmol/day)					
Coprostanol	0.87 ± 0.13[b]	1.00 ± 0.31[ab]	1.23 ± 0.28[a]	0.528	0.011
Coprostanone	0.10 ± 0.05	0.10 ± 0.03	0.08 ± 0.05	−0.255	0.252
Cholesterol	1.16 ± 0.34	1.55 ± 0.57	1.50 ± 0.18	0.340	0.122
Dihydrocholesterol	0.39 ± 0.08	0.44 ± 0.08	0.46 ± 0.08	0.327	0.138
Campesterol	0.02 ± 0.00	0.02 ± 0.02	0.02 ± 0.00	0.185	0.409
Stigmasterol	0.10 ± 0.02[b]	0.12 ± 0.02[ab]	0.12 ± 0.02a	0.641	0.001
Sitosterol	0.07 ± 0.00[b]	0.07 ± 0.02[ab]	0.10 ± 0.02[a]	0.474	0.026
Total	2.72 ± 0.42[b]	3.31 ± 0.44[b]	3.52 ± 0.44[a]	0.625	0.002
Acidic sterols (μmol/day)					
Lithocholic acid	3.03 ± 0.61[b]	4.70 ± 1.67[a]	6.13 ± 0.88[a]	0.752	<0.001
Deoxycholic acid	1.12 ± 0.18	1.32 ± 0.33	1.38 ± 0.41	0.351	0.109
Chenodeoxycholic acid + cholic acid	0.19 ± 0.08	0.18 ± 0.09	0.19 ± 0.03	−0.031	0.893
Ursocholic acid	0.24 ± 0.07	0.27 ± 0.10	0.24 ± 0.02	0.176	0.434
Total	4.58 ± 0.93[b]	6.48 ± 1.66[a]	7.95 ± 1.00[a]	0.726	<0.001
Calculated cholesterol intake (μmol/day)	26.10 ± 1.53	26.12 ± 1.11	26.74 ± 1.68	0.178	0.427
Net sterol retention (μmol/day)	18.79 ± 2.44[a]	16.33 ± 1.20[b]	15.28 ± 2.03[b]	−0.625	0.002
Apparent sterol retention (%)	72.02 ± 9.36[a]	62.52 ± 4.60[b]	57.12 ± 7.60[b]	−0.783	<0.001

[ab] Mean values within a row unlike superscript letters are significantly different ($P < 0.05$). Sterol Retention = [calculated cholesterol intake]—[Total neutral sterols + Total acidic sterols]. Apparent sterol retention = [Sterol retention ÷ Cholesterol intake] × 100. A simple linear regression analysis was conducted to assess the dose-dependent activity of blueberry anthocyanins; *R* correlation coefficient

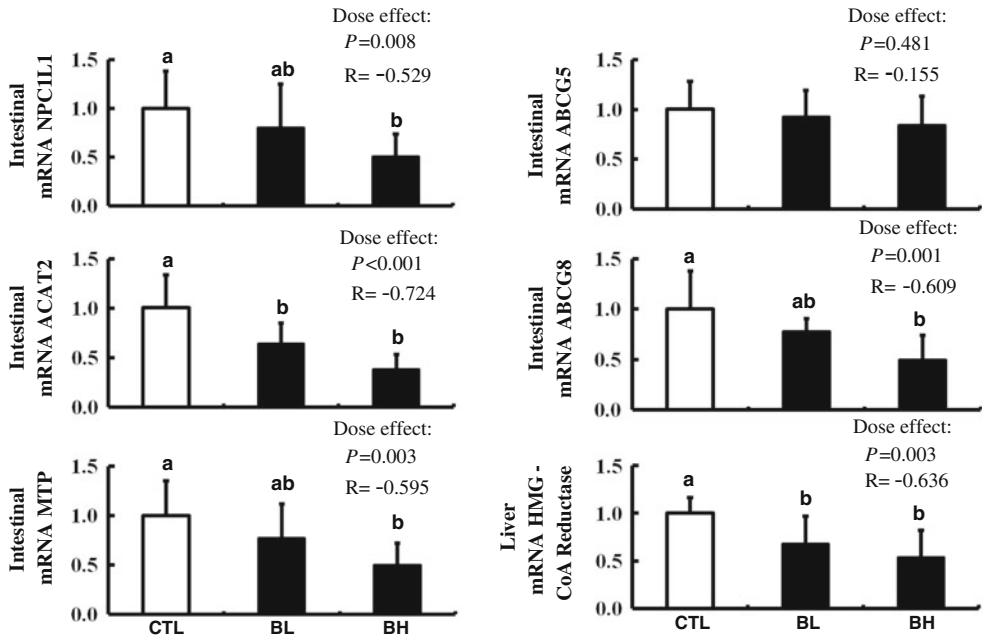

Fig. 2 Effect of blueberry anthocyanins on mRNA of intestinal Niemann-Pick C1 like 1 (NPC1L1), acyl-CoA: cholesterol acyltransferase 2 (ACAT2), microsomal triacylglycerol transport protein (MTP), ATP-binding cassette transporters sub-family G member 5 and 8 (ABCG5/8) and hepatic 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase in hamsters fed diets supplemented with 0 % (CTL), 0.5 % (BL) and 1.0 % (BH) blueberry anthocyanins. [a,b]Mean values with unlike *superscript letters* are significantly different between two groups ($P < 0.05$). A simple linear regression analysis was conducted to assess the dose-dependent activity of blueberry anthocyanins; *R* correlation coefficient

involved in intestinal cholesterol absorption. The apparent sterol retention rate could be calculated by using the following equation: Apparent sterol retention = [Calculated cholesterol intake -Fecal total sterols]/Calculated cholesterol intake. At week 6, incorporation of blueberry anthocyanins into diet produced a dose-dependent reduction in sterol retention rate. These data also indirectly suggested that blueberry anthocyanins increased the excretion of fecal bile acids or decreased the sterol absorption.

Anthocyanin intake in humans presents great variations. It is estimated that humans consume about 160–900 mg total flavonoids with 5–25 mg being anthocyanins [19]. Qin et al. [9] studied the effect of berry anthocyanins on serum lipid profile and found that dyslipidemic subjects given 160 mg anthocyanins twice daily (equivalent to 320 mg/2,000 kcal) had LDL cholesterol decreased by 13.6 % and HDL-cholesterol increased by 13.7 % with serum TC being unchanged. Regarding the commercially available anthocyanin supplement, each tablet or capsule in market contains 300–600 mg anthocyanin extract. In general, supplement manufacturers recommend 3 tablets or capsules daily, which is equivalent to 900–1,800 mg/2,000 kcal. In the present study, 0.5 % anthocyanins in diet was equivalent to 2,500 mg/2,000 kcal, which was higher than the amount recommended by such supplement manufacturers. In this regard, the concentration of blueberry anthocyanins used in the present study could achieve its cholesterol-lowering activity under the normal physiological conditions in humans if the data could be extrapolated to humans. In addition, such high doses used in the present study had an advantage because it could maximize the biological activity of blueberry anthocyanins so that the underlying mechanisms could be thoroughly investigated.

In summary, we systematically studied the effect of dietary blueberry anthocyanins on plasma TC and intestinal NPC1L1, ACAT-2, MTP, and ABCG 5/8 as well as hepatic SREBP-2, LDL receptor, HMG-CoA reductase, LXRα, and CYP7A1 in hamsters fed a high cholesterol diet. It was found that blueberry anthocyanins dose dependently decreased plasma TC concentration. This was accompanied by marked increase in bile acid excretion and decrease in sterol retention with down-regulation of intestinal NPC1L1, ACAT, MTP, and ABCG 8.

References

1. Prior RL, Cao G, Martin A, Sofic E, McEwen J, O'Brien C, Lischner N, Ehlenfeldt M, Kalt W, Krewer G, Mainland M (1998) Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity and variety of Vaccinium species. J Agric Food Chem 46:2586–2593
2. Basu A, Du M, Leyva MJ, Sanchez K, Betts NM, Wu M, Aston CE, Lyons TJ (2010) Blueberries decrease cardiovascular risk factors in obese men and women with metabolic syndrome. J Nutr 140(9):1582–1587
3. Krikorian R, Shidler MD, Nash TA, Kalt W, Vinqvist-Tymchuk MR, Shukitt-Hale B, Joseph JA (2010) Blueberry supplementation improves memory in older adults. J Agric Food Chem 58:3996–4000
4. Kay CD, Holub BJ (2002) The effect of wild blueberry (*Vaccinium angustifolium*) consumption on postprandial serum antioxidant status in human subjects. Br J Nutr 88(4):389–397
5. Seeram NP (2008) Berry fruits for cancer prevention: current status and future prospects. J Agric Food Chem 56:630–635
6. Adams LS, Phung S, Yee N, Seeram NP, Li L, Chen S (2010) Blueberry phytochemicals inhibit growth and metastatic potential of MDA-MB-231 breast cancer cells through modulation of the phosphatidylinositol 3-kinase pathway. Cancer Res 70(9):3594–3605
7. Kim H, Bartley GE, Rimando AM, Yokoyama W (2010) Hepatic gene expression related to lower plasma cholesterol in hamsters fed high-fat diets supplemented with blueberry peels and peel extract. J Agric Food Chem 58:3984–3991
8. Kalt W, Foote K, Fillmore SA, Lyon M, van Lunen TA, McRae KB (2008) Effect of blueberry feeding on plasma lipids in pigs. Br J Nutr 100(1):70–78
9. Qin Y, Xia M, Ma J, Hao Y, Liu J, Mou H, Cao L, Ling W (2009) Anthocyanin supplementation improves serum LDL- and HDL-cholesterol concentrations associated with the inhibition of cholesteryl ester transfer protein in dyslipidemic subjects. Am J Clin Nutr 90:485–492
10. Chen ZY, Jiao R, Ma KY (2008) Cholesterol-lowering nutraceuticals and functional foods. J Agric Food Chem 56:8761–8773
11. Ostlund RE Jr, Lin X (2006) Regulation of cholesterol absorption by phytosterols. Curr Atheroscler Rep 8:487–491
12. Peng C, Zuo Y, Kwan KM, Liang Y, Ma KY, Chan HYE, Huang Y, Yu H, Chen ZY (2012) Blueberry extracts prolongs lifespan of *Drosophila melanogaster*. Exp Gerontol 47:170–178
13. Warnick GR, Benderson J, Albers N (1982) Dextran sulfate-Mg^{2+}precipitation procedure for quantitation of high-density lipoprotein cholesterol. Clin Chem 28:1379–1388
14. Ma KY, Yang N, Jiao R, Peng C, Guan L, Huang Y, Chen ZY (2011) Dietary calcium decreases plasma cholesterol by down-regulation of intestinal NPC1L1 and MTP and up-regulation of CYP7A1 and ABCG 5/8 in hamsters. Mol Nutr Food Res 55:247–258
15. Liang YT, Tian XY, Chen JN, Peng C, Ma KY, Zuo Y, Jiao R, Lu Y, Huang Y, Chen ZY (2012) Capsaicinoids lower plasma cholesterol and improve endothelial function in hamsters. Eur J Nutr. doi:10.1007/s00394-012-0344-2
16. Liang YT, Wong WT, Guan L, Tian XY, Ma KY, Huang Y, Chen ZY (2011) Effect of phytosterols and their oxidation products on lipoprotein profiles and vascular function in hamster fed a high cholesterol diet. Atherosclerosis 219:124–133
17. Prior RL, Wu X, Gu L, Hager T, Hager A, Wilkes S, Howard L (2009) Purified berry anthocyanins but not whole berries normalize lipid parameters in mice fed an obesogenic high fat diet. Mol Nutr Food Res 53(11):1406–1418
18. Li YC, Li BX, Geng LJ (2011) Hypolipidemic and antioxidant effects of total flavonoids from blueberry leaves. Eur Food Res Technol 233(6):897–903
19. Cassidy A, O'Reilly EJ, Kay C, Sampson L, Franz M, Forman JP, Curham G, Rimm EB (2011) Habitual intake of flavonoid subclassess and incident hypertension. Am J Clin Nutr 93:338–347