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ARTICLE

Hepatic Gene Expression Related to Lower Plasma Cholesterol in Hamsters Fed High-Fat Diets Supplemented with Blueberry Peels and Peel Extract†

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This study analyzed plasma lipid profiles, genes related to cholesterol and bile acid metabolism, and inflammation in liver as well as adipose tissue from Syrian Golden hamsters fed high-fat diets supplemented with blueberry (BB) pomace byproducts including 8% dried whole blueberry peels (BBPWHL), 2% dried extract of peels (BBPX; 95% ethanol extract), and 6% residue from extracted peel (BBPEXT) compared to a diet containing 5% (w/w) microcrystalline cellulose (control). All BB diets significantly lowered plasma very low density lipoprotein cholesterol and total cholesterol concentrations. Interestingly, BB diets increased fecal lipid excretion. Hepatic *CYP7A1* expression was up-regulated by all BB diets, and the expression of *CYP51* was up-regulated by BBPX and BBPEXT diets, suggesting that both bile acid and cholesterol synthesis were increased. No significant changes in adipocyte gene expression related to inflammatory markers were observed with any BB diet. These data suggest that hepatic modulation of bile acid and cholesterol synthesis primarily contributes to the cholesterol-lowering effect of BB pomace byproducts.

KEYWORDS: Blueberry pomace byproduct; bile acid; cholesterol; CYP7A1; CYP51; inflammatory marker

INTRODUCTION

Chronic inflammation is associated with the onset and development of cardiovascular disease (CVD). As an alternative to pharmaceutical medications, diets containing natural bioactive compounds with anti-inflammatory and antioxidant properties have recently received much attention for the reduction of CVD risk. Blueberries (BB; fruits of various *Vaccinium* species) are particularly rich in antioxidant and anti-inflammatory phytochemicals such as anthocyanins, proanthocyanidins, phenolic acid derivatives, and flavonols (*1*−*4*). Suggested mechanisms of hypocholesterolemic effects of phenolics and flavonoids include inhibition of low-density lipoprotein (LDL) oxidation, platelet aggregation and adhesion, alteration of hepatic cholesterol absorption, triglyceride assembly, and inhibition of triglyceride secretion (*5*−*9*).

Consumption of freeze-dried BB powder improved plasma antioxidant capacity in humans (*10*, *11*). A recent study (*12*) using a pig model reported that LDL oxidation was not related to the hypocholesterolemic effect of freeze-dried BB powder supplementation to a cereal diet. To our knowledge, the mechanism for cholesterol-lowering effect of BB supplementation is not fully defined. It is therefore our objective to understand the possible mechanism(s) underlying the cholesterol-lowering effects of BB by analyzing gene expression associated with lipid metabolism.

BB pomace remaining after juice extraction represents about 20% of the initial BB weight and contains substantial amounts of polyphenolic bioactive compounds such as anthocyanins, procyanidins, and flavonols (*13*). BB peel residues left after juice extraction include a high concentration of anthocyanins and flavonols (especially quercetin), whereas BB pulp has a greater amount of procyanidins (*14*). Procyanidins in pomace are not well absorbed because of their high molecular weights (*15*). Identification and recovery of the effective bioactive polyphenolic compound from this byproduct could benefit producers and processors economically and provide opportunity for the development of food products that promote health.

The Syrian Golden hamster (*Mesocricetus auratus*) has been extensively used as a model to study cholesterol metabolism because of its similarity to humans in terms of lipid profiles and high susceptibility to dietary cholesterol resulting in hypercholesterolemic plasma profiles (*16*−*19*). In the present study, the lipid-lowering effect of whole blueberry peels (BBPWHL) was investigated using male Syrian Golden hamsters. In the interest of "fractionating" the activity, the BBPWHL was extracted with 95% ethanol. Presumably, the alcohol extract (BBPX) contains most of the polyphenols, and the residue from alcohol extraction residue (BBPEXT) contains most of the dietary fiber. The total

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dietary fiber content was controlled across all diets to determine if lipid-lowering activity of blueberry byproducts was due to the polyphenolic content. Plasma lipid profiles and hepatic and adipose tissue gene expression as well as fecal total lipid content were determined to elucidate the potential cholesterol-lowering mechanism of BB pomace byproducts after supplementation to high-fat diets.

MATERIALS AND METHODS

Animals and Diets. Male Golden Syrian hamsters (approximately 80 g, LVG strain, Charles River, Wilmington, MA) were acclimatized and given free access to water and rodent chow for 1 week prior to the initiation of the experimental diets. The study was reviewed and approved by the Animal Care and Use Committee, Western Regional Research Center, USDA, Albany, CA. Hamsters were fed high-fat diets containing either 8% (w/w) blueberry pomace (BBPWHL), 6% blueberry pomace ethanol extract (BBPX), 2% blueberry residue from ethanol extract (BBPEXT), or 5% microcrystalline cellulose (MCC, Dyets Inc., Bethlehem, PA) for 3 weeks with water available ad libitum. MCC, the dietary fiber control diet, is an insoluble fiber that has little effect on sterol metabolism (*20*). The amounts of BBPX and BBPEXT in the diet were equivalent to the amounts BBPWHL before fractionation. Diets consisted of 18% protein, 45% carbohydrate, and 37% fat on a caloric basis supplemented with 0.15% cholesterol (**Table 1**). Total dietary fiber contents were controlled at 5% across all diets; 82.8 g of BBPWHL was needed to provide 5% total dietary fiber content. Body weights were recorded weekly, and food intake was monitored twice a week.

Blueberry Samples. Frozen rabbiteye blueberries (*Vaccinium ashei* var. Tifblue) were obtained from the U.S. Highbush Blueberry Council frozen blueberry bank, from the 2004 harvest. After 24 h of thawing, the berries were placed in a 42 gal bladder press. The bladder was expanded to 3 bar (43.5 psi) of pressure and maintained for 15 min. The semidry peels were then reconfigured in the bladder press and brought to 3 bar of pressure for 10 min. The mostly dry peels were transferred to a food grade plastic liner and tied. The plastic liner was then placed in a freezer unit overnight after enclosure in a second food grade plastic liner. The double-lined bag of peels was placed in a foam cooler and shipped to the USDA, ARS, NPURU, in Oxford, MS. The frozen peels were lyophilized. Three kilograms of the lyophilized peels (BBPWHL) was extracted with 95% ethanol (300 g of peels per 1.2 L of ethanol, three extractions) in a Waring heavy-duty blender (Eberbach Corp., Ann Arbor, MI). The combined extracts were filtered under vacuum using a Buchner funnel lined with filter paper. The extract was concentrated in a rotary evaporator and finally dried in a lyophilizer (BBPX) to yield 27.7%. The extracted peels (BBPEXT) were dried in a vacuum desiccator at room temperature. BBPWHL, BBPX, and BBPEXT byproducts were supplemented into high-fat diets for the animal study. The freeze-dried pomace (BBPWHL) was analyzed for protein by combustion nitrogen analysis (vario Macro, Elementar, Hanau, Germany), fat by solvent extraction (accelerated solvent extraction; Dionex, Sunnyvale, CA), total dietary fiber by TDF-10 kit (Sigma-Aldrich, St. Louis, MO), ash (muffle furnace 600 °C, overnight, AACC method 08-01.01), and carbohydrate by difference. Macronutrient and total dietary fiber content analysis shows that BBPWHL contains 3.82 ± 0.12% fat, 5.57% protein, 0.58% ash, 67.4 ± 0.57% total dietary fiber, and 22% carbohydrate (by difference) on a dry weight basis.

Plasma and Tissue Collection. Hamsters were fasted for 12 h and anesthetized with isoflurane (Phoenix Pharmaceutical, St. Joseph, MO). Blood was collected by cardiac puncture with syringes previously rinsed with potassium EDTA solution (15% w/v), and plasma was separated after centrifugation at 1500 rpm for 30 min at 4 °C. Livers and adipose tissues were collected, weighed, and immediately frozen in liquid nitrogen for analysis.

Plasma Lipoprotein Analysis. Plasma lipoprotein cholesterol concentrations were determined by size exclusion chromatography as previously described (*21*). Briefly, an Agilent 1100 chromatograph was employed with a postcolumn derivatization reactor, consisting of a mixing coil (1615-50 Bodman, Aston, PA) in a temperature-controlled water jacket (Aura Industrials, Staten, NY). A Hewlett-Packard (Agilent, Palo Alto, CA) HPLC pump 79851-A was used to deliver cholesterol reagent (Roche Diagnostics, Indianapolis, IN) at a flow rate of 0.2 mL/min. Bovine cholesterol lipoprotein standards (Sigma Aldrich, St. Louis, MO) were used to calibrate the signal on the basis of peak areas. Fifteen microliters of plasma was injected via an Agilent 1100 autosampler onto a Superose 6HR HPLC column (Pharmacia LKB Biotechnology, Piscataway, NJ). The lipoproteins were eluted with a pH 7.0 buffer solution containing 0.15 M NaCl and 0.02% sodium azide at a flow rate of 0.5 mL/min. Plasma triglyceride level was determined by a kit (Genzyme Diagnostics PEI Inc., PE, Canada) using an enzyme assay.

Table 1. Diet Composition (Grams)

diet ingredient	control	BBPWHL	BBPX	BBPEXT
butter	80	80	80	80
corn oil	100	97.2	100	100
fish	20	20	20	20
cholesterol	1.5	1.5	1.5	1.5
casein	222.2	214.9	214.9	214.9
MCC[a]	52.6	0	52.6	0
BB	0	82.8[b]	22.9[c]	59.9[d]
corn starch	553.3	531.6	531.6	546.6
DL-methionine	3	3	3	3
choline bitartrate	3	3	3	3
mineral mix	35	35	35	35
vitamin mix (w/o vitamin E)	10	10	10	10

[a] MCC (microcrystalline cellulose, control): nonviscous water-insoluble fiber. [b] Whole blueberry peels. [c] Blueberry peel ethanol extracts. [d] Extracted peels (i.e., residue from ethanol extraction).

Hepatic Lipid Content Analysis. Hepatic total and free cholesterol were determined by enzyme assays using kits (Wako Chemicals, Richmond, VA). Hepatic triglycerides were determined using a kit (Genzyme Diagnostics PEI Inc.).

Real-Time RT-PCR. Total RNA from livers and adipose tissues was extracted using TRIzol plus RNA purification kit (Invitrogen, Life Technologies, Carlsbad, CA), and cDNA was synthesized using a GeneAmpRNA PCR kit (Applied Biosystems, Foster City, CA) in accordance with the manufacturer's protocol. One microliter of diluted cDNA (1:10) was used in each 25 μL of real-time RT-PCR reaction using SYBR Green Supermix (Bio-Rad, Hercules, CA) with an Mx3000P instrument (Stratagene, Cedar Creek, TX). The cycle conditions were as follows: 5 min at 95 °C followed by 40 cycles of incubation at 94 °C for 15 s, then 55−60 °C for 1 min, and 72 °C for 30 s. **Table 2** provides the sequences of the primers used for this study. The primers were validated by size and sequencing of PCR products. No accumulation of nonspecific products and primer−dimers was observed in a gel electrophoresis test of the PCR reaction products. Results were analyzed using the software provided with the Stratagene Mx3000P QPCR system. Differences in mRNA expression were calculated after normalization to 18S and β-actin expression for liver and adipose tissue, respectively.

Fecal Bile Acids and Sterol Analysis. Feces were collected for three consecutive days immediately prior to sacrifice and lyophilized, milled, and stored at −20 °C. Fecal total lipid contents were determined by a gravity method as described previously (*22*).

Statistical Analysis. All data are expressed as mean ± SE. Differences among groups were determined by one-way analysis of variance using the JMP7 statistical program (SAS Institute, Cary, NC). The significance was defined at the 95% confidence level.

RESULTS

Metabolic Effect of BB Byproduct Supplementation on Hamsters. Hamsters fed the high-fat diets supplemented with BB pomace byproducts (BBPWHL, BBPX, and BBPEXT) significantly lowered plasma concentrations of very low density lipoprotein cholesterol (VLDL-C) (~ 44%) and total cholesterol (22−29%) compared to control animals fed high-fat diets with 5% MCC (**Figure 1**). Compared with control, the plasma concentration of LDL-C was lowered by BB pomace byproduct diets, but a statistically significant reduction (34%) was observed only with BBPX diet (**Figure 1**).

Table 2. Sequences of PCR Primers

gene[a]	product size (bp)	primer pair	primer sequence (5′−3′)
β-ACTIN	96	forward	ACGTCGACATCCGCAAAGACCTC
		reverse	TGATCTCCTTCTGCATCCGGTCA
18S	86	forward	GGTCATAAGCTTGCGTTGAT
		reverse	GAGGGCCTCACTAAACCATC
ACOX	134	forward	TTACATGCCTTTGTTGTCCCTATC
		reverse	CGGTAATTGTCCATCTTCAGGTA
ABCB11	136	forward	AACAACGCATTGCTATTGCTC
		reverse	GTCCGACCCTCTCTGGCTTT
ABCG5	131	forward	CCCCTCACTTAATTGGAGAAT
		reverse	GTTTCTGATAAATCCAGATCCAA
CD68	219	forward	CAAGCATAGTTCTTTCTCCAG
		reverse	GCTGGTAGGTTGATTGTCGTCT
CRP	136	forward	CGTGTTGTCATTATGTAGGTCTTA
		reverse	GTAGCTTTATTGACTCATGGACC
CYP7A1	154	forward	ACTGCTAAGGAGGATTTCACTCT
		reverse	CTCATCCAGGTATCGATCATATT
CYP51	195	forward	GAGAGAAGTTTGCCTATGTGCC
		reverse	TGTAACGGATTACTGGGTTTTCT
GRP78	183	forward	CAACTGGTGAAGAGGATACATCA
		reverse	CCACTTGGGCTATAGCATTTTC
PPARα	133	forward	CTCCACCTGCAGAGCAACCA
		reverse	CGTCAGACTCGGTCTTCTTGAT
SCD1	127	forward	GCCACCTGGCTGGTGAACAGTG
		reverse	GGTGGTAGTTGTGGAAGCCCTCG

[a] ACOX, acyl-CoA oxidase; ABCB11, bile salt export pump, BSEP; ABCG5, ATP binding cassette (*ABC*) half-transporter; CD68, cluster of differentiation 68; CRP, c-reactive protein; CYP7A1, cholesterol 7α-hydroxylase; CYP51, lanosterol 14α-demethylase; GRP78, glucose-regulated protein 78; PPARα, peroxisome proliferator-activated receptor α; SCD1, stearoyl-coenzyme A desaturase.

Fecal total lipid content increased (33%) in all hamsters fed BB supplemented diets, although there were no significant effects (**Figure 2**).

Supplementation of the diet with BB pomace byproducts did not affect body weight, liver weight, or abdominal retroperitoneal adipose tissue weight (**Table 3**). Food intake of the BBPX diet group was significantly increased by 20% compared with the control diet group (**Table 3**).

Addition of BB pomace byproducts to high-fat diet did not affect hepatic total lipid, free cholesterol, and triglyceride contents as compared with control (**Figure 3**). Hepatic cholesterol contents in BBPWHL and BBPX diet groups were significantly lowered by 40% compared with control diet group (**Figure 3B**). The BBPEXT diet, although not statistically significant, also lowered hepatic cholesterol content (by 18%, **Figure 3B**).

Effects of BB Byproduct Supplementation on Levels of Hepatic Genes Related to Bile Acid and Cholesterol Metabolism. The relative expression of hepatic genes related to cholesterol, bile acid, and fatty acid metabolism was determined. Compared with control diet, the BBPWHL diet decreased the hepatic mRNA level of the *CYP51* gene (0.6-fold), which is involved in the first step of cholesterol synthesis, whereas BBPX and BBPEXT diets induced 2.3- and 2-fold increases of *CYP51* gene expression, respectively, relative to control diets (**Figure 4**). In all BB pomace byproduct supplemented diets, the mRNA levels of *ABCG5*, which encodes biliary cholesterol transporters, were decreased by 0.1−0.4-fold relative to the control diet (**Figure 4**). The mRNA levels of the *CYP7A1* gene encoding the enzyme for the initial rate-limiting step of bile acid synthesis were significantly increased by ∼2-fold in all BB pomace byproduct supplemented diets (**Figure 4**). The mRNA level of peroxisome proliferator-activated receptor (*PPAR*) α, a transcription factor regulating fatty acid β-oxidation, was up-regulated by ∼1.8-fold with the BBPX diet, whereas it was down-regulated by 0.4-fold with the BBPWHL and BBPEXT diets, as compared with control diet (**Figure 4**). The mRNA levels of acyl-CoA oxidase (*ACOX*), a target gene of *PPARα* and encoding rate-limiting enzyme for peroxisomal β-oxidation, were reduced (0.4 ± 0.1- and 0.6 ± 0.1-fold changes by BBPWHL and BBPEXT diets, respectively) as compared with the control diet (**Figure 4**). The relative gene expression of *ACOX* in the BBPX diet was 0.7 ± 0.2 compared with control diet (**Figure 4**). The relative expression level of stearoyl-coenzyme A desaturase (*SCD-1*) mRNA, the gene encoding the enzyme converting saturated long-chain fatty acids to monounsaturated long-chain fatty acids, was not significantly altered among BB byproduct supplemented diets (0.6 ± 0.2-, 0.8 ± 0.5-, and 1.0 ± 0.3-fold changes in BBPWHL, BBPX, and BBPEXT diets, respectively) (**Figure 4**). Hamsters fed the BBPWHL diet had reduced hepatic expression of *CRP* (c-reactive protein) gene by ∼0.3-fold compared with control (**Figure 4**).

The relative gene expression for inflammatory markers *CRP* and *CD68* and early endoplasmic reticulum (ER)-stress responsive genes such as *GRP78* was determined in adipose tissue (**Figure 5**). No BB pomace byproduct diets significantly altered the relative levels of *CRP*, *CD68*, and *GRP78* mRNA compared to the control (**Figure 5**).

DISCUSSION

Dietary BB pomace, the byproduct from juice processing, significantly reduced plasma concentration of VLDL-C (by 44% of control) and total cholesterol (by 22−29% of control) in Golden Syrian hamsters. Plasma concentration of LDL-C was further significantly lowered with 2% BBPX diet. These data demonstrated that natural BB pomace byproducts can protect against hypercholesterolemia induced by a diet similar in 37% calorie fat content to the American diet and containing 0.15% cholesterol and 2% fish oil in an animal model used to study dietary components that affect cholesterol metabolism.

The cholesterol-lowering effects of BB pomace byproducts appear to involve modulation of genes that regulate hepatic bile acid and cholesterol synthesis. The levels of hepatic expression of the *CYP7A1* gene encoding the rate-limiting enzyme for bile acid synthesis were up-regulated in all groups fed BB pomace byproduct diets, indicating a significant increase in bile acid synthesis (**Figure 6**). This is consistent with previous studies reporting that rats fed barley or psyllium husk, two sources of soluble dietary fiber (SDF), for 2 or 3 weeks also had increased *CYP7A1* mRNA levels and decreased plasma total cholesterol levels (*23*,*24*). Hepatic cholesterol synthesis is down-regulated as reflected by reduction of hepatic expression of the *CYP51* gene in the BBPWHL diet. This was unexpected because increased *CYP7A1* expression suggests a need for more substrate, cholesterol, for bile acid synthesis. Alternatively, hepatic expression of *CYP51* was increased in BBPX and BBPEXT diets, indicating an increase in cholesterol synthesis. Cholesterol from de novo synthesis could be, then, redirected to bile acid synthesis, resulting in a reduction of the net hepatic cholesterol level in BBPX and BBPEXT diets. Indeed, levels of hepatic cholesterol content were significantly decreased (by 40%) after BBWHL and BBPX supplementation. BBPEXT diet also showed the trend toward reduced (by 18%)

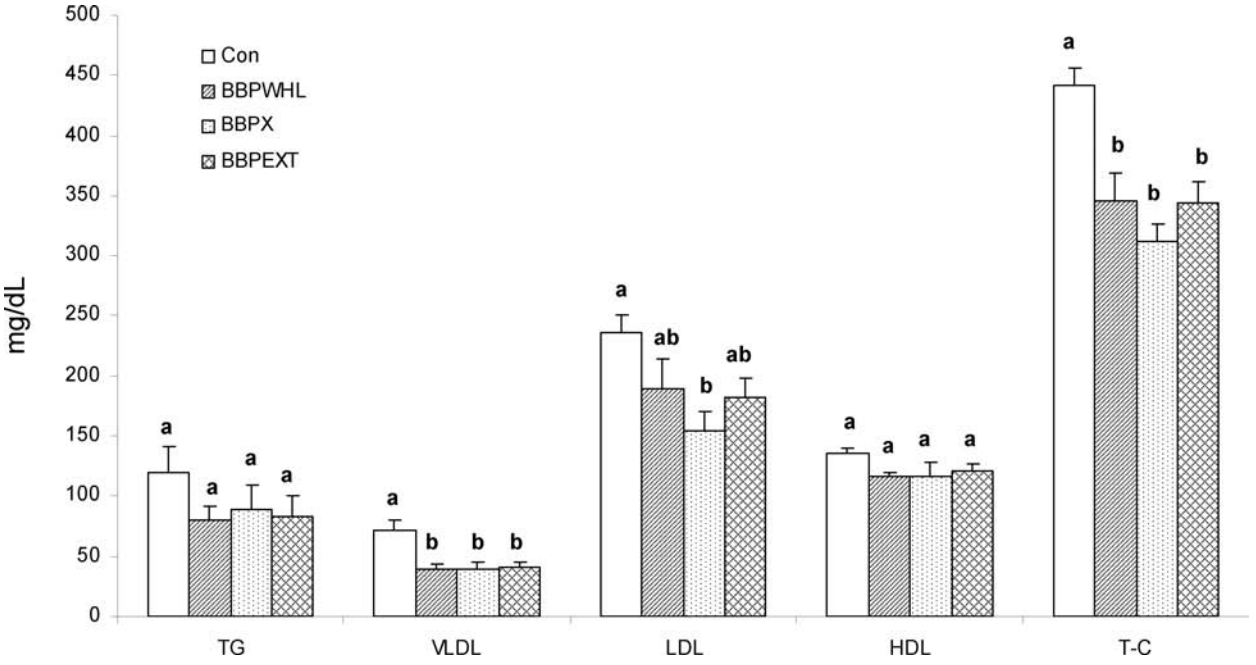

Figure 1. Effect of blueberry pomace byproducts on concentration of plasma lipids. Male Golden Syrian hamsters were fed high-fat diet containing 8% (w/w) whole blueberry peels (BBPWHL), 6% residue from blueberry peel extraction (BBPEXT), 2% blueberry peel ethanol extract (BBPX), or 5% microcrystalline cellulose for 3 weeks, and blood was collected in fasting state. Data are expressed as mean ± SE; $n = 8-10$/group. Different letters indicate significant difference at $P < 0.05$.

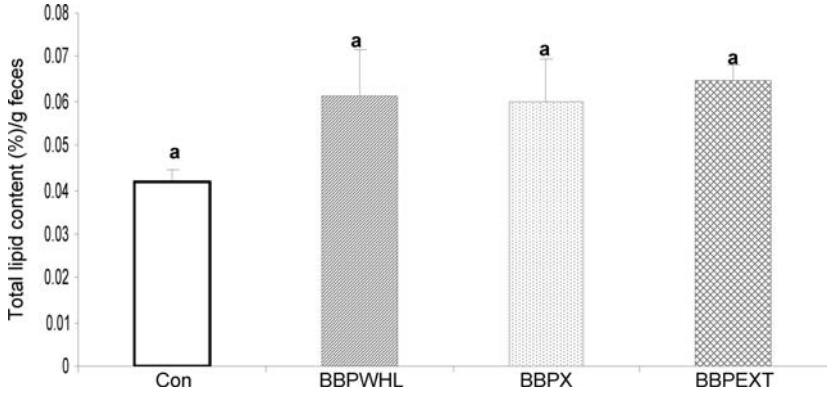

Figure 2. Effect of blueberry pomace byproducts on fecal total lipid content. Male Golden Syrian hamsters were fed high-fat diets containing 8% (w/w) whole blueberry peels (BBPWHL), 6% residue from blueberry peel extraction (BBPEXT), 2% blueberry peel ethanol extract (BBPX), or 5% microcrystalline cellulose for 3 weeks, and feces were collected for three consecutive days prior to sacrifice. Data are expressed as mean ± SE; $n = 8-10$/group. Different letters indicate significant difference at $P < 0.05$.

Table 3. Effects of Blueberry Peel Diets on Body Weight, Food Intake, and Weight of Liver, Intestine, and Adipose Tissue

	control	BBPWHL	BBPX	BBPEXT
body weight (g)	105 ± 3.30	108 ± 3.12	113.4 ± 2.25	110.8 ± 4.17
body weight gain (g)	25.5 ± 1.72	33.6 ± 3.50	26.3 ± 4.58	34.1 ± 3.97
food intake (g/day)	7.4 ± 0.3a	8.1 ± 0.6ab	9.3 ± 2.2b	7.7 ± 1.1ab
liver weight (g)	6.83 ± 0.33	6.21 ± 0.31	5.76 ± 0.25	5.98 ± 0.38
abdominal adipose tissue (g)	3.17 ± 0.32	4.11 ± 0.31	3.45 ± 0.30	3.37 ± 0.35

hepatic cholesterol content compared with control diets. These differences in *CYP51* expression suggest that more than one bioactive component of BB pomace affects sterol metabolism. We recognize the limitation of relying on mRNA expression levels as a measure of pathway flux; however, previous studies have shown a linear relationship between enzyme activity and gene expression for these enzymes (*23*−*25*). It is worth mentioning that the livers of the BB-fed animals appeared dark and smooth compared to the pale and mottled livers of the control animals (data not shown).

Several studies have suggested that bile acid pool depletion could trigger alteration of hepatic expression of the *CYP7A1* gene as a compensatory mechanism because levels of hepatic *CYP7A1* mRNA were increased when animals were fed 7.5% dietary psyllium, a fiber that increases bile acid excretion, or 1−1.5% cholestyramine, a bile acid sequestrant (*20*, *26*−*31*). Bile acids can act as signaling molecules for nuclear receptors that regulate bile acids and cholesterol metabolism, such as farnesoid X receptor (FXR) α (*32*). Thus, it is possible that alteration of the enterohepatic pool size of bile acids by BB pomace byproducts affects hepatic *CYP7A1* expression via regulation of nuclear receptors including FXRα. All BB pomace byproduct diets increased total lipid content per gram of feces, about 34%, although it was not significant. There is a significant negative correlation ($P < 0.05$) between total plasma cholesterol concentration and fecal lipid

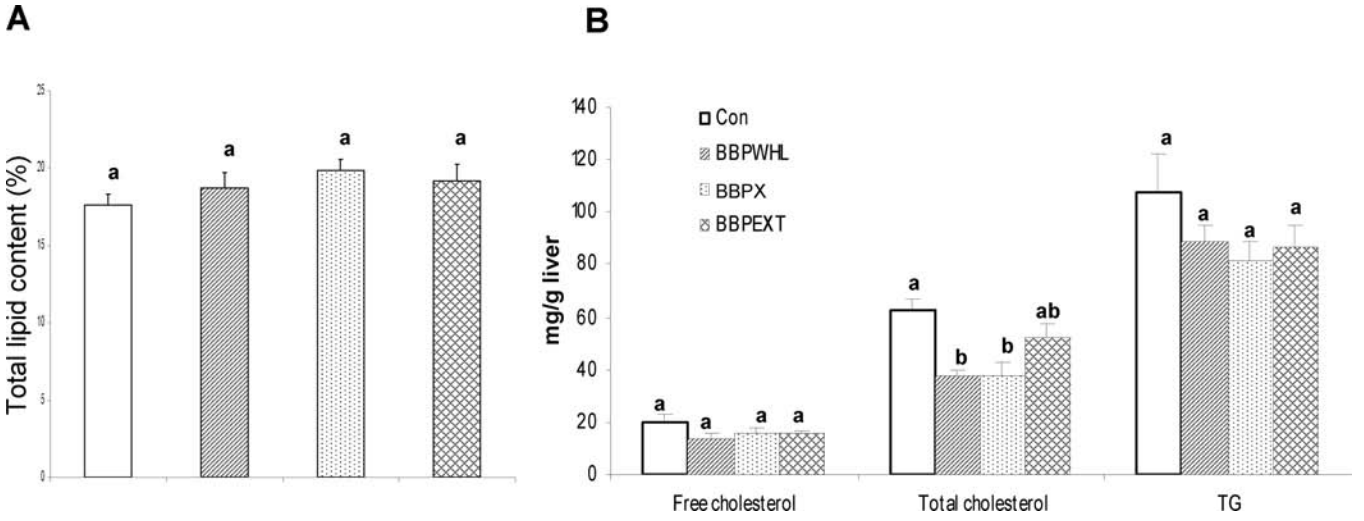

Figure 3. Effect of blueberry pomace byproducts on hepatic total lipid content (**A**) and hepatic triglyceride and cholesterol contents (**B**). High-fat diets containing 8% (w/w) whole blueberry peels (BBPWHL), 6% residue from blueberry peel extraction (BBPEXT), 2% blueberry peel ethanol extract (BBPX), or 5% microcrystalline cellulose were fed to male Golden Syrian hamsters for 3 weeks, and livers were collected in fasting state. Data are expressed as mean ± SE; $n = 8-10$/group. Different letters indicate significant difference at $P < 0.05$.

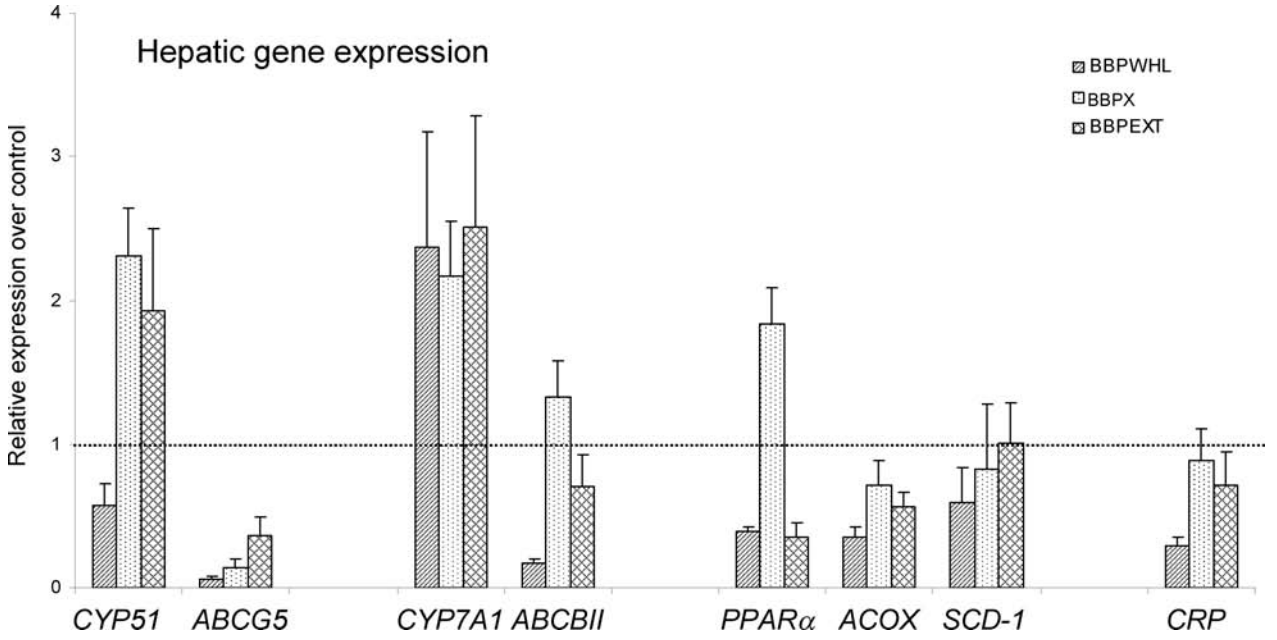

Figure 4. Hepatic mRNA expression of lipid metabolism-related genes including lanosterol 14α-demethylase (*CYP51*), ATP binding cassette (*ABC*) half-transporter (*ABCG5*), cholesterol 7α-hydroxylase (*CYP7A1*), bile salt export pump BSEP (*ABCB11*), peroxisome proliferator-activated receptor (*PPAR*) α, acyl-CoA oxidase (*ACOX*), stearoyl-coenzyme A desaturase (*SCD*)-1, and c-reactive protein (CRP) in male Golden Syrian hamsters fed high-fat diets containing 8% (w/w) whole blueberry peels (BBPWHL), 6% residue from blueberry peel extraction (BBPEXT), 2% blueberry peel ethanol extract (BBPX), or 5% microcrystalline cellulose for 3 weeks. Each mRNA was normalized to 18S and is expressed relative to the control level. Data are expressed as mean ± SE; $n = 8-9$/group.

excretion, suggesting fecal lipid excretion is associated with cholesterol-lowering effect of BB pomace byproducts. Further study is required to determine daily total fecal bile acid excretion following BB pomace byproduct intake.

Recent studies have implied that insulin regulates *CYP7A1* expression. Hepatic *CYP7A1* expression was down-regulated in animal models that were under a hepatic insulin resistant state such as the liver-specific insulin receptor knockout (LIRKO) mice, the high-fat-fed obese mouse, and the leptin-deficient *ob*/*ob* mouse (*33*, *34*). A previous study in our laboratory has shown that insulin resistance in high-fat-fed hamsters was improved with soluble dietary fiber feeding as reflected by a doubling of the glucose infusion rate during the hyperinsulinemic−euglycemic clamp analysis (data not shown). This was paralleled by a reduction of hepatic lipid content. However, in the current study hepatic total lipid and triglyceride contents were not significantly changed by any BB pomace byproduct. In addition, supplementation of BB pomace byproducts into high-fat diets did not affect fasting plasma glucose and insulin concentration. Therefore, it is less likely that there is a link between up-regulation of *CYP7A1* expression and hepatic insulin resistance following BB pomace byproduct intake.

Whole BB supplementation of high-fat diets fed to C57BL/6J mice was reported to improve insulin sensitivity via the

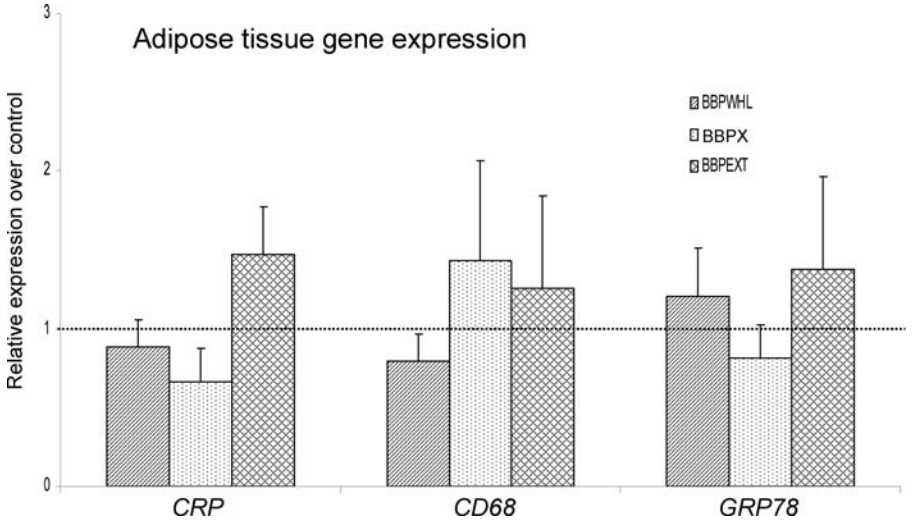

Figure 5. Gene expression in white adipose tissue for gene markers of inflammation (CRP and CD68) and early endoplasmic reticulum (ER) stress (GRP78) in male Golden Syrian hamsters fed high-fat diets containing 8% (w/w) whole blueberry peels (BBPWHL), 6% residue from blueberry peel extraction (BBPEXT), 2% blueberry peel ethanol extract (BBPX), or 5% microcrystalline cellulose for 3 weeks. Each mRNA was normalized to β-actin and is expressed relative to the control level. Data are expressed as mean ± SE; $n = 4-5$/group.

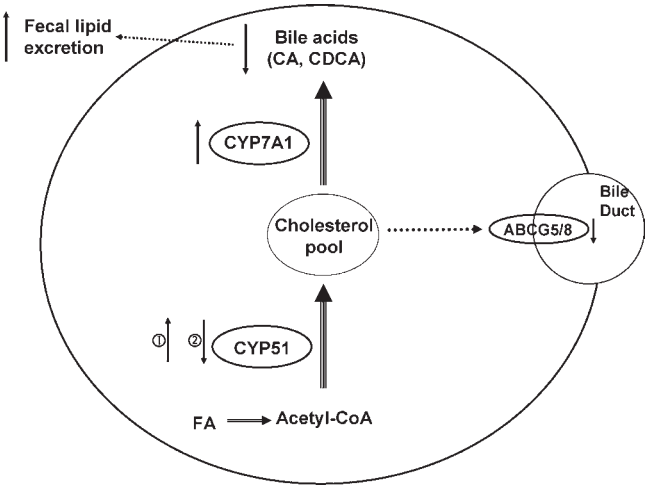

Figure 6. Summary of the proposed mechanism responsible for lipid-lowering effects in hamsters fed BB pomace byproduct diets on hepatic bile acid and cholesterol synthesis. Impediment to absorption or enterohepatic recirculating bile acids, via blueberry pomace byproduct treatment, increases *CYP7A1* mRNA, resulting in increased bile acid synthesis. The mRNA level of *CYP51* is up-regulated, reflecting increased cholesterol synthesis in BBPX and BBPEXT diets indicated by ①. The expression of *CYP 51* gene is down-regulated in BBPWHL diet, indicating decreased cholesterol synthesis shown by ②. The expression of *ABCG5* decreased to meet the demand of cholesterol for bile acid synthesis.

modulation of inflammatory markers in adipose tissue (*35*). In the present study, BB byproduct supplementation did not change expression of *CRP* and *CD68* (anti-inflammatory marker genes) and *GRP78* (early ER-stress responsive gene) genes relative to the control group in adipose tissue. In contrast, hepatic expression of the *CRP* gene was down-regulated with BBPWHL supplementation. Beneficial effects of BB on oxidative and endoplasmic reticulum stress could be less functional in the Golden Syrian hamster, although a good model to study cholesterol metabolism.

ABCG5 (a cholesterol transporter) in the liver mediates cholesterol secretion into the bile, and its up-regulation is considered to increase biliary cholesterol excretion, resulting in lowered plasma cholesterol levels (*36*). In the present study, the expression level of *ABCG5* was significantly lowered in hamsters fed all BB pomace byproducts compared with those fed the control diet, indicating a reduction of biliary cholesterol excretion to direct cholesterol synthesis toward bile acid production. These data suggest adaptive changes in cholesterol metabolism are involved in hamsters fed BB byproducts to meet cholesterol demand for hepatic bile acid synthesis.

Pterostilbene and flavonoids, presumably enriched in the BBPX, have been demonstrated to induce expression of the *PPAR*α gene (*37*−*39*) encoding the transcription factor regulating genes related to fatty acid oxidation. In the current study, the BBPX diet increased hepatic *PPAR*α expression by 1.8-fold over the control diet, whereas expression of the *ACOX* gene, a *PPAR*α-dependent and peroxisomal β-oxidation gene, was not up-regulated. This result suggests *PPAR*α expression is up-regulated with BBPX diets but is not activated. However, further analysis will be needed to dissect the specific bioactive components in the different fractions of BB pomace.

In summary, supplementation of a high-fat diet with BB pomace byproducts resulted in a reduction of plasma cholesterol (VLDL-C and total cholesterol) concentrations. Hepatic gene expression profile showed an up-regulation of *CYP7A1* expression, suggesting an increase in bile acid synthesis (**Figure 6**). Hepatic cholesterol synthesis was also assumed to increase because there was an up-regulation of *CYP51* expression in the BBPX and BBPEXT diets indicated as ① in **Figure 6**. The BBPWHL diet decreased hepatic cholesterol synthesis, shown as ② in **Figure 6**. Fecal lipid excretion correlated with plasma total cholesterol concentration. Anti-inflammatory action of adipose tissue was not observed in hamsters after BB pomace byproduct intake as indicated by unaltered expression of *CRP*, *CD68*, and *GRP78* genes.

In conclusion, BB pomace byproducts modulate hepatic cholesterol and bile acid synthesis and play an important role in their cholesterol-lowering effect. Our data suggest that dietary applications of natural BB pomace byproducts can provide cardiopreventive benefits.

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