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Hypocholesterolemic activity of grape seed proanthocyanidin is mediated by enhancement of bile acid excretion and up-regulation of CYP7A1

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Abstract

Interest in grape seed proanthocyanidin (GSP) as a cholesterol-lowering nutraceutical is growing. This study was to investigate the effect of GSP on blood cholesterol level and gene expression of cholesterol-regulating enzymes in Golden Syrian hamsters maintained on a 0.1% cholesterol diet. Results affirmed supplementation of 0.5% or 1.0% GSP could decrease plasma total cholesterol and triacylglycerol level. Western blot and real-time polymerase chain reaction analyses demonstrated GSP did not affect sterol regulatory element binding protein-2 and low-density lipoprotein receptor; however, it increased mRNA 3-hydroxy-3-methylglutaryl coenzyme A reductase. GSP had no effect on the protein mass of liver X receptor alpha (LXRα) but it decreased mRNA LXRα. Most importantly, GSP increased not only the protein level of cholesterol-7α-hydroxylase (CYP7A1) but also mRNA CYP7A1. It was concluded that the hypocholesterolemic activity of GSP was most likely mediated by enhancement of bile acid excretion and up-regulation of CYP7A1.

Keywords: Cholesterol; Grape seed proanthocyanidin; CYP7A1; LDL receptor; HMG-CoA reductase; SREBP-2; Liver X receptor

1. Introduction

Grape seed proanthocyanidins (GSP) refer to a group of procyanidins consisting of a mixture of dimers and oligomers of catechin and epicatechin and their gallic acid esters. GSP has been a subject of the extensive investigations for its various biological activities. It has been reported that GSP is anti-atherosclerosis [1,2], anti-carcinogenesis [3], hypotensive [4] anti-lipogenesis [5], antioxidant [6,7] and antihyperglycemic [8]. GSP has been shown to be hypocholesterolemic in both humans and animals [1,9], although the underlying mechanism remains poorly understood.

Blood total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) correlate directly with risk of heart diseases, whereas high-density lipoprotein cholesterol (HDL-C) correlates inversely with the risk. Maintenance of cholesterol homeostasis is very complex. In this regard, cholesterol metabolism is partially governed at the transcriptional level by sterol regulatory element-binding protein 2 (SREBP-2) and liver X receptor-alpha (LXRα) in a coordinated manner [10]. The former regulates the transcription of 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase (HMG-CoA-R) and low-density lipoprotein receptor (LDL-R), with HMG-CoA-R acting as a rate-limiting enzyme in cholesterol biosynthesis and LDL-R being responsible for the removal of LDL-C from the circulation. In contrast, the latter governs the transcription of a gene encoding cholesterol-7α-hydroxylase (CYP7A1), a rate-limiting enzyme in conversion of cholesterol to bile acids in the liver [10].

Despite extensive research on GSP, little is known of how it interacts with the genes and proteins involved in lipoprotein metabolism in vivo. The present study was therefore undertaken to (i) characterize the interaction of dietary GSP with SREBP-2, LXRα, HMG-CoA-R, LDL-R, and CYP7A1, and (ii) investigate the effect of dietary GSP on fecal excretion of individual neutral and acidic sterols in hamsters.

2. Materials and methods

2.1. Extraction of GSP and analysis

GSP (brand name: Grajfnol) was prepared by Jianfeng Natural Product R&D Co, Ltd (Tianjin, China). According to the Supplier, the grape seeds (variety: Chardonnay) were crushed and ground followed by extraction using 35% ethanol solution. After filtration, the extracts were loaded onto a macroreticular resin column (type:AB-8) and eluted with 30% ethanol solution. The resultant GSP was concentrated and spray-dried.

Individual components in GSP were determined using high-performance liquid chromatography (HPLC). GSP sample was injected into an HPLC column (YMC C_{18}, 5 μm, 250×4.6 mm i.d.) and quantified on a Shimadzu LC-10AT HPLC system equipped with a UV detector at 280 nm. The elution profile was programmed at a flow rate of

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1 ml/min, while the gradient mobile phase composed of solvent A (2% acetic acid) and solvent B (acetonitrile:water:acetic acid, 80:19.6:0.4; v/v/v). The ratio of A to B was programmed: 0–3 min, 100:0; 3–6 min, changed to 96:4; 6–15 min, changed to 90:10; 15–30 min, changed to 85:15; 30–50 min, changed to 77–23; 50–60 min, changed to 75:25; 60–66 min, changed to 70:30; 66–80 min, changed to 50:50; 80–83 min, changed to 20:80; 83–85 min, changed to 100:0 and then was held for additional 5 min (Fig. 1). On the basis of peak areas, GSP contained 6.04% dimmer procyanidins (Peaks 1 and 2), 26.50% catechin (Peak 3), 10.53% procyanidin B2 (Peak 4), 31.01% epicatechin (Peak 5), 10.23% dimmers esterified gallic acid (Peaks 6 and 9), 4.10% trimers esterfied with gallic acid (Peaks 7 and 8), and 3.63% trimers esterified with gallic acid (Peak 10).

2.2. Diets

Three diets were prepared as previously described [11]. The control diet contained the following ingredients in proportion (g/kg diet): cornstarch, 508; casein, 242; lard, 50; sucrose, 119; mineral mix AIN-76, 40; vitamin mix AIN-76A, 20; DL-methionine, 1; cholesterol, 1 (Table 1). The two experimental diets were prepared by adding 0.5% and 1.0% GSP (w/w) into the control diet, respectively. The powered diets were mixed with a gelatin solution (20g/L) in a ratio of 200g diet per liter of solution (Table 1). Once the gelatin has set, the diets were cut into pieces of approximately 10-g cubes and stored frozen at −20°C. AIN-76 diet provide all nutrients to meet the nutrition requirements as AIN-93 diet because the feeding study lasted only 8 weeks and no kidney calcification had been reported to occur in hamsters [12].

2.3. Animals

Thirty-three (113±6 g) male adult Golden Syrian hamsters (*Mesocricetus auratus*) were randomly divided into three groups (n=11) and housed individually in wire-bottomed cages at 23°C in a 12-hour light-dark cycle animal room. Before the experiment, all the hamsters were acclimated on a 0.1% cholesterol diet (control diet) for 2 weeks. During the following 6 weeks, one group were continued to be fed the control diet and the other two groups were fed one of the two experimental diets containing 0.5%GSP or 1.0%GSP. During the study, food was given daily, any uneaten food was discarded, the amount of food consumed was measured each day and feces were collected. The hamsters had free access to food and distilled water, and their body weights were recorded weekly. Blood (0.5 ml) was collected from the retro-orbital sinus into a heparinized capillary tube under light anesthetization, using a mixture of ketamine, xylazine and saline (v/v/v, 4:1:5), at the end of Weeks 0, 3 and 6 after food deprivation for 14 h. The blood was centrifuged at 1000×g for 10 min and the plasma was collected and stored at −20°C until analysis. After a 3-day recovery, all hamsters were sacrificed by carbon dioxide. Blood was collected from abdominal artery into a vacuum heparinized tube and centrifuged at 1000×g for 10 min, the plasma was collected and stored at −20°C until analysis. The liver and heart were removed, washed in saline, weighed, flash frozen in liquid nitrogen and stored at −80°C until analysis. Experiments were conducted following the approval and guidelines set by the Animal Experimental Ethical Committee, The Chinese University of Hong Kong.

2.4. Determination of serum lipoproteins

Plasma TC and total triacylglycerols (TG) were quantified using commercial enzymatic kits from Thermo (Waltham, MA, USA) and Stanbio Laboratories (Boerne, TX, USA), respectively. For measurement of plasma HDL-C, LDL-C and very LDL-C were first precipitated with phosphotungstic acid and magnesium chloride in a commercial kit (Stanbio Laboratories, Boerne, TX, USA). Non-HDL-C was calculated by deducting HDL-C from TC.

Table 1
Composition (%) of the control and two experimental diets containing 0.5% grape seed proanthocyanidin (0.5% GSP) and 1.0% grape seed proanthocyanidin (1.0% GSP)

Component	Control	0.5% GSP	1.0% GSP
Cornstarch	50.8	50.8	50.8
Casein	24.2	24.2	24.2
Sucrose	11.9	11.9	11.9
Lard	5	5	5
Mineral mix	4	4	4
Vitamin mix	2	2	2
Gelatin	2	2	2
DL-Methionine	0.1	0.1	0.1
Cholesterol	0.1	0.1	0.1
GSP	–	0.5	1.0

2.5. Measurement of organ cholesterol

Cholesterol in organs was determined using a method as previously described [11]. In brief, total lipids were extracted from 300 mg of organ sample using chloroform-methanol (2:1, v/v). After saponification, cholesterol in the non-saponified substances was converted to their trimethylsilyl (TMS)-ether derivatives by a commercial TMS reagent (Sigma-Sil-A, Sigma, St. Louis, MO, USA). The analysis of cholesterol TMS-ether derivative was performed in a fused silica capillary column (SAC-5, 30 m ×0.25 mm, i.d.; Supelco, Bellefonte, PA, USA) using a Shimadzu GC-14 B gas liquid chromatograph (GLC) equipped with a flame ionization detector as previously described [11].

2.6. Determination of fecal neutral and acidic sterols

Individual fecal neutral and acidic sterols were quantified as previously described [11,13]. Total fecal sample from each hamster was freeze-dried, ground and well mixed. In brief, stigmasterol was added in the sample as an internal standard for quantification of neutral sterols, whereas hyodeoxycholic acid was added as an internal standard for quantification of acidic sterols. The total neutral sterols were extracted using cyclohexane and were then converted to their corresponding TMS-ether derivatives for GLC analysis. The acidic sterols in the remaining aqueous layer were saponified and extracted into diethyl ether. The acidic sterols were similarly converted to their TMS-ether derivatives for GLC analysis.

2.7. Western blotting analyses

Total liver proteins were extracted according to the method described previously by Vaziri and Liang with some modification [14]. The total protein was centrifuged and the pellet (both membrane and nuclear proteins) was obtained. Membrane and

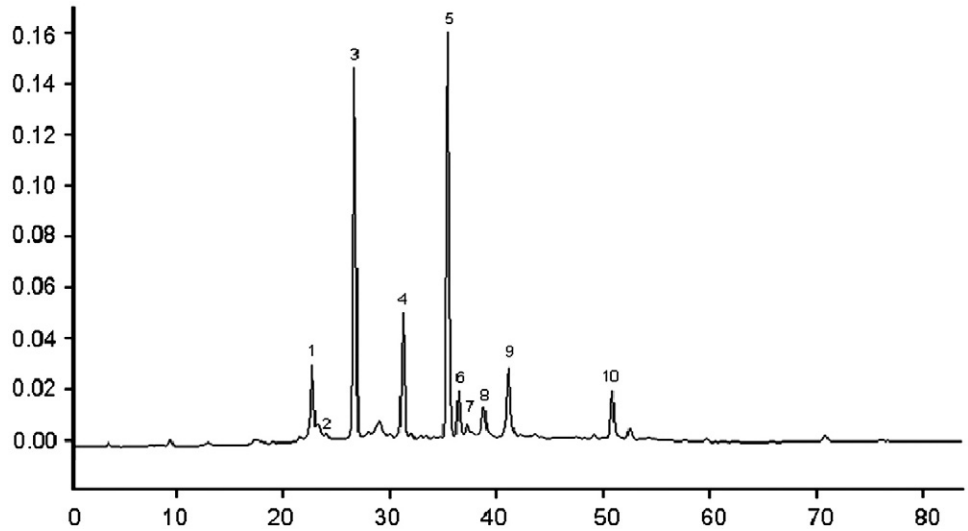

Fig. 1. HPLC chromatogram of GSP. Identification of peaks: Peaks 1 and 2, dimmer procyanidins; Peak 3, catechin; Peak 4, procyanidin B2; Peak 5, epicatechin; Peaks 6 and 9; dimmers esterified gallic acid; Peaks 7 and 8, trimers esterfied with gallic acid; and Peak 10, trimers esterified with gallic acid.

Table 2
List of oligonucleotide primers and probes used to amplify mRNA by real-time PCR

Gene	Accession number	Forward primer 5′ → 3′	Reverse primer 5′ →3′	TaqMan probe 5′
GAPDH	DQ403055	GAACATCATCCCTGCATCCA	CCAGTG AGCTTCCCGTTCA	CTTGCCCACAGCCTTGGCAGC
CYP7A1	L04690	GGTAGTGTGCTGTTGTATATGGGTTA	ACAGCCCAGGTATGG AATCAAC	CACCTGCTTTCCTTCTCC
HMG-CoA-R	X00494	CGAAGGGTTTGCAGTGATAAAGGA	GCCATAGTCACATGAAGCTTCTGT A	ACGTGCGAATCTGCT
LDL-R	M94387	GCCGGGACTGGTCAG ATG	ACAGCCACCATTGTTGTCCA	GCACTCATTGGTCCT GCAGTCCTT
SREBP-2	U12330	GGACTTGGTCATGGGAACAGATG	TGTAATCAATGGCCTTCCTCAGAAC	CCAAGATGCACAA ATC

nuclear proteins were separated on a 7% sodium dodecyl sulfate polyacrylamide gel and transferred to polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA) using a semi-dry transfer system. Membranes were then blocked in 5% nonfat milk Tris-buffered saline with Tween-20 for 1 hour and overnight at 4°C in the same solution containing anti-LDL-R antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-HMG-CoA-R (Upstate USA, Lake Placid, NY, USA), anti-CYP7A1 (Santa Cruz Biotechnology), anti-LXRα , or anti-SREBP-2 antibody (Santa Cruz Biotechnology) [13]. The membrane was then incubated for one hour at 4°C in diluted horseradish peroxidase-linked Goat anti-rabbit IgG, donkey anti-rabbit IgG or goat anti-mouse IgG. The membranes were developed with ECL enhanced chemiluminescence agent and subjected to autoradiography on SuperRX medical X-ray film. Densitometry was quantified using the BioRad Quantity one software. Data on abundance of SREBP-2, LDL-R, HMG-CoA-R, LXRα and CYP7A1 were normalized with β-tubulin [13].

2.8. Real time PCR analyses

Total mRNA levels for liver SREBP-2, LDL-R, HMG-CoA-R, LXRα and CYP7A1 were quantified as previously described [15]. Briefly, total liver mRNA was extracted and isolated using Tizol Reagent (Invitrogen, Carslbad, CA, USA) according to the manufacturer's instruction. Total RNA was converted to complementary DNA (cDNA) using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA, USA). Real-time PCR analysis was carried out on a Fast Real-time PCR System 7500 (Applied Biosystems). All primers and TaqMan probes used for real-time PCR for these genes were listed in Table 2 (11) except for those of LXRα, which was purchased from Applied Biosystems). Real-time PCR was performed using a TaqMan Fast Universal PCR Master Mix (Applied Biosystems). The expressions of target genes were normalized with that of GAPDH.

2.9. Statistics

Data were expressed as Mean±Standard Deviation (SD). The group means were statistically analyzed using one-way analysis of variance (ANOVA) and post hoc LSD test on SigmaStat Advisory Statistical Software (SigmaStat version 14.0, SPSS Inc., Chicago, IL, USA). Significance was defined as *P*-value less than .05.

Table 3
Changes in serum TC, TG, HDL-C, non-HDL-C and organ cholesterol in hamsters fed the control and the two experimental diets containing 0.5% grape seed proanthocyanidin (0.5%GSP), 1.0% grape seed proanthocyanidin (1.0%GSP) for the 6 weeks

	Control	0.5%GSP	1.0%GSP
Week 0			
TC (mmol/L)	6.46±0.82	6.41±0.59	6.43±0.65
HDL-C (mmol/L)	2.74±0.19	2.81±0.27	2.69±0.20
Non-HDL-C (mmol/L)	3.72±0.83	3.60±0.49	3.74±0.58
Non-HDL-C/HDL-C	1.37±0.36	1.29±0.18	1.40±0.23
TG (mmol/L)	3.28±1.54	2.44±0.57	3.41±1.81
Week 3			
TC (mmol/L)	6.36±0.80	6.14±0.49	5.62±0.66
HDL-C (mmol/L)	2.57±0.35	2.71±0.30	2.46±0.28
Non-HDL-C (mmol/L)	3.79±0.85	3.40±0.45	3.13±0.70
Non-HDL-C/HDL-C	1.51±0.40	1.26±0.22	1.28±0.34
TG (mmol/L)	4.01±1.59^{a}	2.87±0.85^{ab}	2.36±0.98^{b}
Week 6			
TC (mmol/L)	6.30±0.63^{a}	6.05±0.85^{ab}	5.39±0.63^{b}
HDL-C (mmol/L)	2.66±0.15	2.63±0.40	2.55±0.18
Non-HDL-C (mmol/L)	3.64±0.59^{a}	3.45±0.72^{ab}	2.85±0.57^{b}
Non-HDL-C/HDL-C	1.37±0.23	1.32±0.23	1.12±0.22
TG (mmol/L)	4.62±1.36^{a}	4.08±0.98^{ab}	3.23±1.10^{b}
Organ cholesterol			
Liver (mmol/kg)	212.49±19.12^{a}	207.59±19.16^{a}	151.31±18.31^{b}
Heart (mmol/kg)	4.64±0.78	4.45±0.24	4.63±0.56
Adipose (mmol/kg)	2.89±0.29	2.82±0.29	2.88±0.52

Data are mean±S.D.
^{a,b}Means in a row with different letters differ significantly (P<.05).

3. Results

3.1. Body weight and food intake

The body weight gain was similar among the three groups (Data not shown). GSP did not affect the weight gain compared with the control hamsters. Food intake ranged 11.9-12.0 g/day/hamster among the three groups. No significant difference in food intake was seen between the control and the two experiment diet groups.

3.2. Serum TC, TG and HDL-C

No differences in TC, HDL-C, non-HDL-C, ratio of non-HDL-C/HDL-C and TG were seen at the beginning of the experiment (Table 3). At the end of week 3, there was a decreasing trend in TC, non-HDL-C and TG in response to the increasing amount of GSP in diet. At the end of week 6, plasma TC in the two experimental groups decreased significantly compared with that in the control. A significant decreasing trend in plasma non-HDL-C and TG in response to the increasing amount of GSP was observed. To be specific, plasma TC in 0.5%GSP and 1.0%GSP groups was reduced by 4% and 16%, while non-HDL-C was reduced by 5% and 22%, respectively. Similarly, plasma TG in 0.5 and 1.0 GSP was reduced by 12% and 30%, respectively, compared with that in the control at the end of Week 6.

3.3. Organ cholesterol

Total cholesterol in the liver decreased significantly in the 1.0%GSP group compared with the control group (Table 3). No difference in hepatic cholesterol level was seen between the control and 0.5%GSP group. There were no significant differences in the cholesterol level of heart and adipose among the three groups.

Table 4
Fecal excretion of neutral and acidic sterols (mg/hamster/day) in hamsters fed the control and the two experimental diets containing 0.5% grape seed proanthocyanidin (0.5% GSP), and 1.0% grape seed proanthocyandin (1.0% GSP) for the 6 weeks

	Control	0.5%GSP	1.0%GSP
Coprostanol	1.13±0.75	0.64±0.22	0.46±0.31
Coprostanone	0.05±0.02	0.05±0.02	0.08±0.01
Cholesterol	0.37±0.12	0.36±0.15	1.63±1.25
Dihydrocholesterol	0.27±0.07	0.26±0.07	0.22±0.04
Campersterol	0.07±0.03	0.07±0.01	0.07±0.02
Total neutral sterols	2.08±0.95	1.39±0.33	2.25±1.55
Lithocholic acid	0.46±0.11^{b}	2.23±1.58^{ab}	2.57±1.21^{a}
Deoxycholic acid	0.15±0.08^{b}	0.66±0.41^{a}	0.94±0.53^{a}
Chenodeoxycholic acid+cholic acid	0.26±0.13^{b}	1.11±0.64^{ab}	1.62±0.80^{a}
Ursodeoxycholic acid	0.14±0.04	0.38±0.25	0.54±0.35
Total acidic sterols	1.00±0.32^{b}	4.38±2.86^{ab}	5.67±1.98^{a}

Data are mean±S.D.
^{a,b}Means in a row with different letters differ significantly (P<.05).

Table 5
Cholesterol balance in hamsters fed the control diet, and two experimental diets supplemented with containing 0.5% grape seed proanthocyanidin (0.5% GSP) and 1.0% grape seed proanthocyandin (1.0% GSP) for the 6 weeks

	Control	0.5GSP	1.0GSP
Cholesterol intake (mg/day)	11.21±0.69	12.35±0.54	12.79±0.49
Fecal total neutral sterol output (mg/day)	2.08±0.95	1.39±0.33	2.25±1.55
Fecal total acidic sterol output (mg/day)	1.00±0.32	4.38±2.86	5.67±1.98
Net cholesterol equivalent retained (mg/day)	8.13±0.73	6.59±2.52	4.87±2.86
Apparent cholesterol absorption (% intake)	72.80±8.67[a]	53.68±20.62[ab]	38.22±22.14[b]

Data are mean±S.D.
[a,b]Means in a row with different letters differ significantly ($P<.05$).

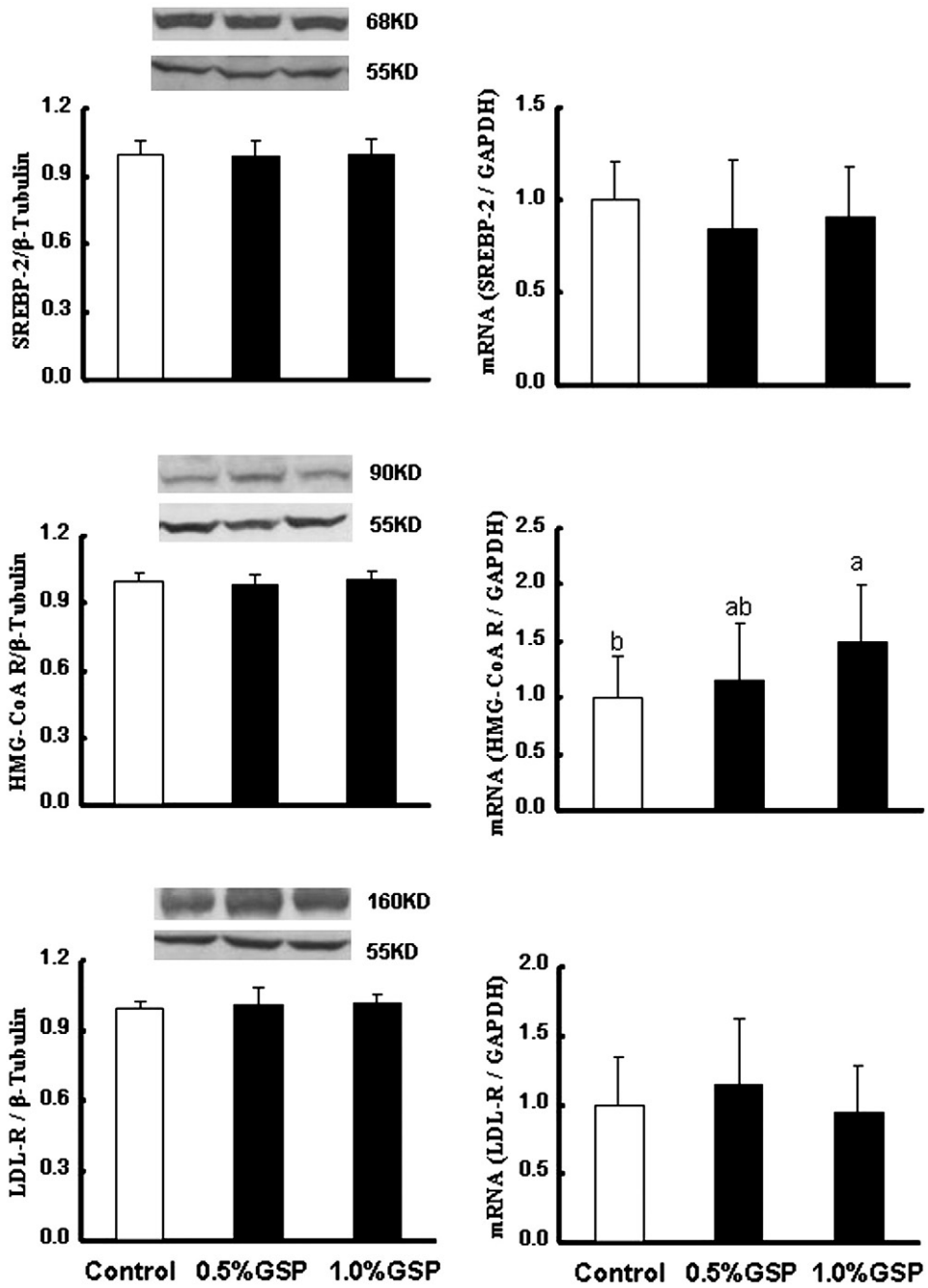

Fig. 2. The relative immunoreactive mass and mRNA of hepatic SREBP-2, HMG-CoA-R, LDL-R in hamsters fed the control diet, and two experimental diets supplemented with containing 0.5% grape seed proanthocyanidin (0.5% GSP) and 1.0% grape seed proanthocyandin (1.0% GSP) for the 6 weeks.

3.4. Fecal neutral and acidic sterols

Supplementation of GSP into diets caused greater fecal excretion of acidic sterols compared with the control diet group (Table 4). The major acidic sterols affected included lithocholic acid, chenodeoxycholic acid, cholic acid and deoxycholic acid. However, no significant difference in fecal excretion of neutral sterols was seen among the control and the two GSP groups.

3.5. Cholesterol balance

Data on the apparent cholesterol absorption was calculated as previously described [11]. Total intake of cholesterol was compared with its excretion of total neutral and acidic sterols (Table 5). Net cholesterol equivalent retained was calculated by difference between intake and excretion of both neutral and acidic sterols. It was found that net cholesterol retention was the most in the control followed by 0.5%GSP and 1.0%GSP in a decreasing order. The apparent cholesterol absorption was calculated using an equation [(cholesterol intake - excretion of neutral and acidic sterols)/cholesterol intake], proving that GSP decreased the apparent cholesterol absorption.

3.6. Immunoblot and mRNA analyses of SREBP-2, LDL-R, HMG-CoA-R, CYP7A1 and LXRα

The western blot and RT-PCR analyses demonstrated that supplementation of GSP into diets had no significant effect on hepatic SREBP-2, LDL-R in hamsters (Fig. 2). Although the immunoblot analysis showed that GSP had no effect on the protein mass of HMG-CoA-R, the RT-PCR analysis demonstrated that GSP could up-regulate the production of mRNA HMG-CoA-R. Results demonstrated that GSP had no effect on the protein level of LXR-α. However, mRNA LXR-α was down-regulated in hamsters fed the 1.0% GSP (Fig. 3). In contrast, GSP up-regulated not only the protein level but also the mRNA level of CYP7A1.

4. Discussion

The present study was to investigate the hypocholesterolemic activity of GSP using hamsters as an animal model. In this regard, supplementation of GSP into diet reduced not only plasma TC and non-HDL-C levels but also plasma TG (Table 3). It was found that supplementation of 1.0 % GSP for six weeks could decrease plasma TC by 15% and TG by 30%. Results are in agreement with those reported in rabbits [1], hamsters [16,17] and rats [18]. To investigate the underlying mechanism, the present study was focused on characterization of the interaction of dietary GSP with SREBP-2, HMG-CoA-R, LDL-R, LXRα and CYP7A1.

Cholesterol is mainly eliminated from the body via conversion of cholesterol to bile acids. The present study clearly demonstrated that dietary GSP was able to increase the excretion of bile acids by three- to fourfold (Table 4). This was partially mediated by up-regulation of CYP7A1 in both transcriptional and translational levels (Fig. 3). We further demonstrated that GSP had no effect on the protein level of LXRα but it decreased its mRNA level. The observation is in agreement with that of del Bas et al. [18], who studied the effect of GSP on the mRNA levels of these proteins and enzymes involved in cholesterol metabolism in rats, finding that GSP was able to up-regulate CYP7A1 with a slight down-regulation of LXRα. Regulation of gene expression of CYP7A1 by LXRα is complex and varies with species. It has been shown that LXRα up-regulates CYP7A1 transcription in rats but not in hamsters [19]. To explain why expression of LXRα was down-

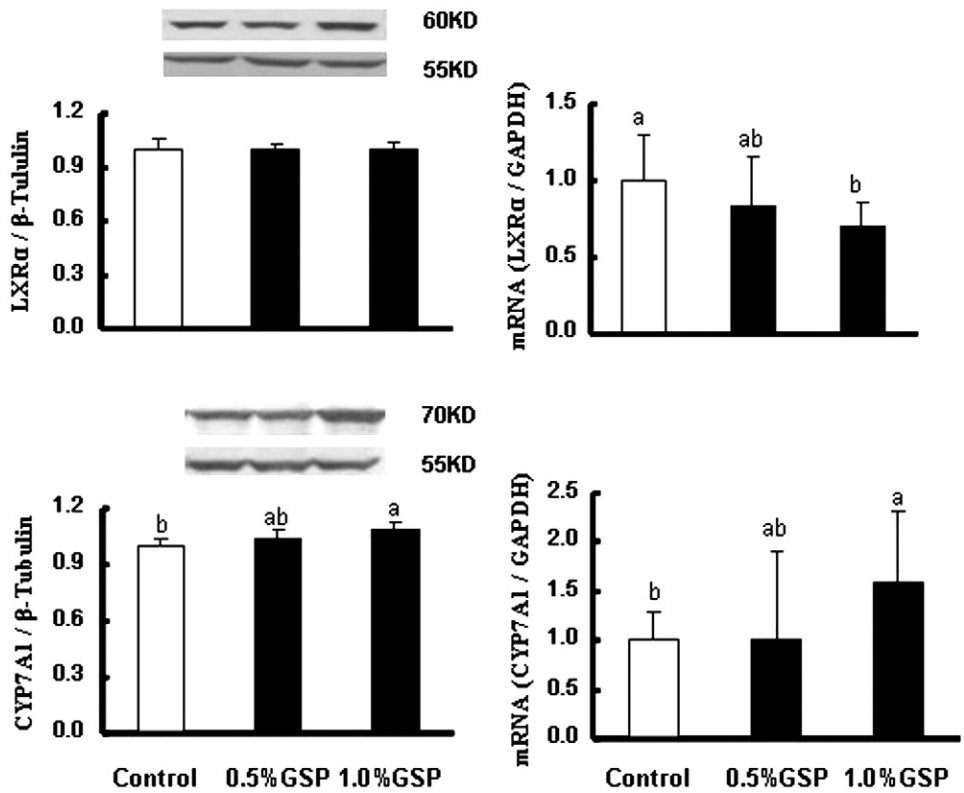

Fig. 3. The relative immunoreactive mass and mRNA of hepatic liver X receptor (LXR) and cholesterol- 7α-hydroxylase (CYP7A1) in hamsters fed the control diet, and two experimental diets supplemented with containing 0.5% grape seed proanthocyanidin (0.5% GSP) and 1.0% grape seed proanthocyandin (1.0% GSP) for the six weeks.

regulated while that of CYP7A1 was up-regulated in response to dietary GSP, we had quantified the cholesterol concentrations in various organs, finding that supplementation of GSP significantly decreased the liver cholesterol concentration (Table 3). Thus, down-regulation on expression of LXRα by GSP is well explained because LXRα is a cholesterol sensor in the liver [20]. GSP decreased the hepatic cholesterol concentration, which, in turn, down-regulated the expression of LXRα. It should be pointed out that the CYP7A1 transcription is regulated by not only LXRα but also other factors including farnesoid X receptor, a repressor of CYP7A1 gene expression [18]. Therefore, dietary GSP could up-regulate the expression of CYP7A1 without necessarily up-regulating LXRα.

We investigated the effect of dietary GSP on SREBP-2, LDL-R and HMG-CoA-R, finding that GSP had no effect on their protein and mRNA levels except it increased only the mRNA HMG-CoA-R. This is in agreement with the results of del Bas et al. [18], who demonstrated that GSP could up-regulate the mRNA HMG-CoA-R by >40%. The observed increase in the mRNA HMG-CoA-R could be explained by the decrease in hepatic cholesterol concentration in 1.0GSP group. The HMG-CoA-R is sensitive to the cholesterol concentration in the liver and become up-regulated if the liver cholesterol is reduced. In addition, hamsters have a diurnal pattern in expression of SREBP-2, LDL-R and HMG-CoA-R with their activity in midnight being several times higher than that in midday [21]. Perhaps, the abundance in HMG-CoA-R, SREBP-2 and LDL-R was already very low because hamsters in the present study were sacrificed between 9–11 a.m., so that cholesterol catabolism rate was nil and no effect of dietary GSP in these proteins could be seen in hamsters after the overnight fasting. In this regard, we are currently investigating the possible effect of dietary GSP on these proteins in hamsters with a full stomach.

The present study is the first of its kind to study the effect of GSP on cholesterol balance. It is known that excessive cholesterol is mainly eliminated by mammals in forms of fecal bile neutral and bile acids. This will allow us to obtain information on the quantity of cholesterol intake, cholesterol elimination (sum of both fecal neutral and acidic sterols), and the net body accumulation of cholesterol relative to dietary cholesterol [11]. The present study found that the net cholesterol retention or the apparent cholesterol absorption was the highest in the control followed by 0.5 GSP, and 1.0 GSP groups in a decreasing order, suggesting that GSP shift the cholesterol balance in a way towards greater cholesterol elimination (Table 5).

In summary, we systematically studied the effect of dietary GSP on SREBP-2, LDL-R, HMG-CoA-R, LXRα and CYP7A1 at both transcriptional and translational levels in hamsters. We found that GSP at 1.0% level markedly increased the fecal excretion of bile acids by two- to threefolds mediated by up-regulation of CYP7A1. In addition, dietary 1.0% GSP reduced significantly the apparent cholesterol absorption from 72.80–38.22%. The data suggest that enhancement of bile acid excretion is the major mechanism by which GSP decreases plasma cholesterol concentration.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jnutbio.2009.10.007.

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