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Lower Weight Gain and Hepatic Lipid Content in Hamsters Fed High Fat Diets Supplemented with White Rice Protein, Brown Rice Protein, Soy Protein, and their Hydrolysates

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ABSTRACT: The physiological effects of the hydrolysates of white rice protein (WRP), brown rice protein (BRP), and soy protein (SP) hydrolyzed by the food grade enzyme, alcalase2.4 L, were compared to the original protein source. Male Syrian Golden hamsters were fed high-fat diets containing either 20% casein (control) or 20% extracted proteins or their hydrolysates as the protein source for 3 weeks. The brown rice protein hydrolysate (BRPH) diet group reduced weight gain 76% compared with the control. Animals fed the BRPH supplemented diet also had lower final body weight, liver weight, very low density lipoprotein cholesterol (VLDL-C), and liver cholesterol, and higher fecal fat and bile acid excretion than the control. Expression levels of hepatic genes for lipid oxidation, *PPARα*, *ACOX1*, and *CPT1*, were highest for hamsters fed the BRPH supplemented diet. Expression of *CYP7A1*, the gene regulating bile acid synthesis, was higher in all test groups. Expression of *CYP51*, a gene coding for an enzyme involved in cholesterol synthesis, was highest in the BRPH diet group. The results suggest that BRPH includes unique peptides that reduce weight gain and hepatic cholesterol synthesis.

KEYWORDS: white rice protein, brown rice protein, cholesterol, bile acid, body weight, *CYP7A1*, *CYP51*

INTRODUCTION

Dietary protein not only is the source of essential amino acids required for growth or maintenance but also contains amino acid sequences for peptides that have antimicrobial, blood pressure-lowering (ACE inhibitory), antioxidative, and cholesterol-lowering properties.[1] Small peptides resulting from pancreatic enzyme hydrolysis are absorbed from the small intestine more readily than free amino acids, and some peptide hormones resist digestion and retain their physiological activity.[2] For example, thyrotropin reducing hormone (TRH), a tripeptide, luteinizing-hormone releasing hormone (LHRH), a decapeptide, and insulin, a 51 amino acid peptide, were physiologically active after enteral administration.[3] The hypocholesterolemic properties of peptides are mainly due to their ability to displace cholesterol from bile acid micelles during transport within the intestinal lumen and decrease absorption of cholesterol. This mechanism was supported in reports that rice protein enhanced the fecal excretion of neutral steroids.[4,5] Furthermore, soy protein hydrolysates (SPH) have been shown to up regulate the LDL receptor in hepatocytes *in vitro*.[6,7] All of these studies suggest that multiple mechanisms must be considered to explain the hypocholesterolemic properties of peptides in dietary protein hydrolysates.

Grains are a significant source of dietary protein particularly in developing countries. While wheat is the grain predominately consumed in western countries, rice (*Oryza sativa*) accounts for about 25% of the world cereal grain production and is the staple food in most Asian countries, which like wheat is typically consumed after removing the bran and germ.[8] Whole grain intake has been associated with lowered risk for cardiovascular disease. However, white rice protein (WRP) has been shown to have hypocholesterolemic properties in several studies.[4,5,9,12]

Most reported hypocholesterolemic peptides are prepared by digestion with pepsin or pancreatin.[13,14] Broad specificity proteases such as alcalase have produced bioactive peptides different from those peptides produced by pepsin and pancreatin. Therefore, the aim of the present study is to investigate the effects of both plant intact proteins and their alcalase hydrolysates (WRPH, BRPH, and SPH) on cholesterol and bile acid metabolism in male Syrian Golden hamsters, an animal model widely used to study dietary phytochemicals and lipid metabolism. Syrian Golden hamsters and humans have identical primary bile acids, presence of cholesterol ester transfer protein, similar lipoprotein profiles, and susceptibility to hypercholesterolemia by dietary cholesterol intake.[15−17]

MATERIALS AND METHODS

Protein Hydrolysates Preparation. Soy protein (SP) was purchased from Solae LLC (Supro670, St. Louis, MO) and extracted by Soxhlet apparatus with ethanol to remove isoflavones. WRP and brown rice protein (BRP) were extracted from California medium grain rice by a proprietary process (CRM, Stockton, CA). The protein concentrations of WRP and BRP were 67% and 65%, respectively. Alcalase 2.4 L (Sigma-Aldrich, St. Louis, MO) was used to hydrolyze a 5% suspension of the plant proteins at 50 °C, pH 8.5. The reaction was maintained at a constant pH by addition of 1 M NaOH by an autotitrator (TitraLab TiM840, Radiometer, Lyon, France). The degree of hydrolysis (DH) was determined by the pH stat method.[18] The DH was

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Table 1. Diet Composition (Grams)[a]

	control	WRP	WRPH	BRP	BRPH	SP	SPH
butter	80.0	80.0	80.0	80.0	80.0	80.0	80.0
corn oil	100.0	81.6	81.6	64.8	64.8	90.7	90.7
fish oil	20.0	20.0	20.0	20.0	20.0	20.0	20.0
cholesterol	1.0	1.0	1.0	1.0	1.0	1.0	1.0
cellulose	50.0	41.0	41.0	31.0	31.0	50.0	50.0
casein	200.0						
rice or soy		330	330	353	353	245	245
corn starch	498	406	406	418	418	473	473
DL methionine	3.0	5.9	5.9	5.5	5.5	3.0	3.0
L-proline		10.5	10.5	10.3	10.3	10.4	10.4
L-lysine		5.7	5.7	5.4	5.4		
choline bitartrate	3.0	3.0	3.0	3.0	3.0	3.0	3.0
mineral mix	35.0	35.0	35.0	35.0	35.0	35.0	35.0
vitamin mix	10.0	10.0	10.0	10.0	10.0	10.0	10.0
energy (kcal)	4590	4580	4580	4570	4570	4590	4590

[a] Butter (Odell's); Buffalo corn starch (ConAgra foods food ingredients company); corn oil obtained from local supermarket; fish oil, cholesterol, L-proline (Sigma-Aldrich); casein, choline bitartrate, mineral mix, vitamin mix, cellulose from Dyets, Inc. (Bethlehem, PA); L-lysine from GNC; DL-methionine (ICN).

calculated by the following equation

$$\mathrm{DH} = \frac{h}{h_{\mathrm{tot}}} = \frac{B \times N_{\mathrm{b}}}{\alpha \times h_{\mathrm{tot}} \times M_{\mathrm{p}}} \times 100\% \quad (1)$$

where B is the base consumption in mL, N_{b} is the normality of the base, α is the average degree of dissociation of the α-NH_2 groups, M_{P} is the mass of protein ($N \times 5.95$) in g, h is the hydrolysis equivalents in meqv g^{-1} protein, and h_{tot} is the total number of peptide bonds in the protein substrate (7.72 meqv g^{-1} rice protein).

When the DH value reached 18%, the reaction was terminated by heating to 85 °C for 10 min. The hydrolysates were adjusted to pH 7.0, freeze-dried, and stored in a desiccator. The molecular weight ranges of most bioactive peptides have been reported to be about 800,[13] and corresponds to a DH value of proteins in this study of about 18%.

Animals and Diets. Male Syrian Golden hamsters were purchased from Charles River Laboratories (Wilmington, MA). The animals were housed individually in wire-bottom cages in a room maintained at 20−22 °C, 60% relative humidity, and a 12 h alternating light-dark cycle. Hamsters were acclimatized for 1 week and fed a ground commercial chow (Purina Rodent Laboratory Chow). After 1 week, the hamsters were randomized into 7 groups of 8−10 hamsters. Hamsters were fed high fat and cholesterol diets, containing 20% dietary protein *ad libitum* for 3 weeks (Table 1). Body weights were recorded weekly, and food intake was monitored twice per week. The study was approved by the Animal Care and Use Committee, Western Regional Research Center, USDA, Albany, CA.

Blood, Liver, and Feces. Hamsters were feed deprived for 12 h and anesthetized with isoflurane (Phoenix Pharmaceutical, St. Joseph, MO), and blood was collected via cardiac puncture with syringes rinsed with potassium EDTA solution (15 wt %/v). The blood was transferred to 5 mL polypropylene tubes (containing 5 μL 15% potassium EDTA), gently rocked for a few minutes, and then stored on ice. The blood was then centrifuged at 3500 rpm for 40 min at 4 °C to separate the plasma. Livers were excised, weighed, and separated into two parts. One part was immediately frozen in liquid nitrogen for RT-PCR analysis, and the other part was lyophilized, milled, and stored at −20 °C for lipid extraction. Feces were collected during the last 3 consecutive days of the feeding period and were lyophilized, milled, and stored at −20 °C.

Analysis of Lipoproteins and Triglycerides (TG) in Hamster Plasma. Very low-density lipoprotein cholesterol (VLDL-C), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were separated by size-exclusion chromatography and cholesterol concentration quantitated by postcolumn reaction as previously described.[19] Plasma (15 μL) was injected onto a Superose 6HR HPLC column (Pharmacia LKB Biotechnology, Piscataway, NJ). The lipoproteins were eluted with 0.15 M NaCl and 0.02% (w/v) sodium azide at a flow rate of 0.5 mL/min. Cholesterol in the lipoprotein fractions was reacted with a cholesterol reagent (Roche Diagnostics, Indianapolis, IN) at a flow rate of 0.2 mL/min in a postcolumn reactor, consisting of a knitted mixing coil (1615-50 Bodman, Aston, PA) and a temperature-controlled water jacket (Aura Industrials, Staten, NY). Bovine cholesterol lipoprotein standards (Sigma Aldrich, St. Louis, MO) were used to calibrate the signal on the basis of peak areas. Plasma TG was determined by an enzyme assay kit (Genzyme Diagnostics PEI Inc., PE, Canada).[20]

Hepatic and Fecal Lipids. Freeze-dried and powdered liver and feces were extracted with hexane/isopropanol as described earlier.[21] Approximately 0.5 g was sandwiched between sand layers in an extraction cell and extracted at 60 °C, ∼ 2175 psig with 3:2 (v/v) hexane/isopropanol with the Dionex ASE 200. The lipid extract was evaporated to dryness at 37 °C under nitrogen. The lipid residue was brought to constant weight and weighed to determine the total lipid concentration. The entire sample of residue was dissolved in a 5:2 (v/v) chloroform/methanol. An aliquot (0.55 mL) was transferred to a vial and solubilized in 2.2 mL, 1% Triton X-100 in chloroform. The mixture was mixed well and evaporated to dryness at 37 °C under nitrogen. Approximately 1 mL of deionized water was added to the residue, and the mixture was mixed thoroughly and incubated at 37 °C for 1 h in a water bath. TG concentration of liver and feces was determined with an assay kit, as described above for plasma. Total and free cholesterol concentrations of liver and feces were determined using an enzyme assay kit (Wako Chemicals, Richmond, VA).

Fecal Bile Acid and Nitrogen Content. Total fecal bile acids were determined by an enzymatic method.[22] Briefly, about 50 mg freeze-dried feces was extracted with 1 mL, 50% aqueous tert-butanol for 15 min at 37 °C. At the beginning, in the middle, and at the end of the reaction period, the tubes were vortexed for 10 s. The suspension was then centrifuged at 10 000*g* for 2 min. The supernatants (40 μL) were transferred to 13 × 100 mm glass tubes, and bile acid concentrations were determined using an enzyme recycling rate assay kit (DZ042A-K, Diazyme, San Diego, CA) by measuring the absorbance at 540 nm using a Nanodrop 2000 C spectrophotometer (Thermo Scientific , USA). The nitrogen concentration of freeze-dried feces was determined using a combustion nitrogen analyzer (Elementar Americas Inc., Vario Macro, Mt. Laurel, NJ).

Real-Time PCR. Total RNA from livers was extracted using TRIzol plus RNA purification kit (Invitrogen, Life Technologies, Carlsbad, CA), quantified spectrophotometrically, checked for quality by gel electrophoresis, and used to synthesize cDNA using GeneAmp RNA PCR kit (Applied Biosystems, Foster City, CA) per the manufacturer's protocol. Approximately 1 μL of diluted cDNA (1:10) was used in each real time-PCR using SYBR Green Supermix (Bio-Rad, Hercules, CA) with an Mx3000P instrument (Stratagene, Cedar Creek, TX). The cycle conditions were: 5 min at 95 °C followed by 40 cycles of incubation at 94 °C for 30 s, then 58−62 °C for 1 min, and 72 °C for 30 s. PCR reactions were followed by dissociation curve analysis. The results were analyzed using the software provided with the Mx3000P QPCR system. Differences in mRNA expression were calculated using the ΔΔCT method.[23] The sequences of the primers used for this study were described previously.[24,25]

Statistical Analysis. All data are expressed as means ± SE. Differences among groups were determined by one-way ANOVA

Table 2. Effects of Dietary Protein Supplemented High Fat Diets on Body Weight, Food Intake, Liver, and Adipose Tissue Weight[a]

	control	WRP	WRPH	BRP	BRPH	SP	SPH
body weight (g)	115.1a ± 2.5	115.6a ± 3.8	113.1a ± 3.4	112.8a ± 4.5	101.5b ± 3.8	109.1a ± 3.6	112.8a ± 4.7
body weight gain(g)	15.0a ± 0.9	15.6a ± 1.7	14.2a ± 1.9	13.3a ± 1.8	3.6b ± 2.1	11.0ab ± 2.0	12.5ab ± 1.6
food intake(g/day)	6.6a ± 0.1	7.0a ± 0.2	6.6a ± 0.2	7.0a ± 0.2	6.8a ± 0.1	6.2a ± 0.2	6.4a ± 0.2
liver weight (g)	5.2a ± 0.2	5.0a ± 0.3	4.6a ± 0.2	4.7a ± 0.2	3.7b ± 0.2	4.4ab ± 0.2	4.4ab ± 0.3
RA (g)	0.8a ± 0.05	0.79a ± 0.03	0.74a ± 0.1	0.64a ± 0.09	0.73a ± 0.1	0.67a ± 0.11	0.66a ± 0.07
EA (g)	1.5ab ± 0.1	1.5ab ± 0.09	1.4ab ± 0.16	1.7a ± 0.1	1.3b ± 0.13	1.3ab ± 0.07	1.4ab ± 0.15

[a] Data presented as means ± SE. Different letters indicate significant difference at $P < 0.05$. RA = retroperitoneal adipose; EA = epididymal adipose; WRP = white rice protein; WRPH = white rice protein hydrolysates; BRP = brown rice protein; BRPH = brown rice protein hydrolysates; SP = soy protein; SPH = soy protein hydrolysates.

analysis of variance using the Minitab 15 statistical program (Minitab INC., State College, PA). The significance was defined at the 95% confidence level. The correlations were determined by the SPSS16.0 statistical program (IBM INC., New York).

RESULTS

Body and Organ Weights. There were no significant differences in final body weight and weight gain between the treatment and the control after 3 weeks feeding except for 12% lower final body weight and 76% lower weight gain in the BRPH diet group (Table 2). There were no differences in food intake, but the lower weight gain of the BRPH diet translated into lower food efficiency, 0.02 g wt gain/g food compared with 0.05 g wt gain/g food for the control. Compared with casein, all the plant dietary proteins reduced the liver weights of the hamsters, but only hamsters fed BRPH supplemented diet had significant lower liver weight (29%). There were no differences of epididymal or retroperitoneal adipose weights among all groups.

Plasma and Liver Lipids. Total plasma cholesterol was lowered about 8% by the diets of BRPH and SP, and 19% by the diet of SPH compared with the control (Table 2). Total plasma cholesterol concentration of the BRPH diet group was lower than the BRP diet group ($P < 0.05$). Plasma and hepatic total cholesterol, VLDL-C, and LDL-C concentrations in WRP, BRP, and SP diet groups tended to be higher than their hydrolysates. The WRPH, BRPH, SP, and SPH diets reduced VLDL-C concentration (23−63%) compared with the control. Hepatic total lipid concentration tended to be lower in hamsters fed the plant dietary proteins supplemented diets, but only the WRPH and SPH diet groups had lower hepatic total lipid concentration ($P < 0.05$). Except for the WRP supplemented diet, all other diets reduced hepatic total and esterified cholesterol concentrations (Table 3). Hepatic total and esterified cholesterol concentrations of hydrolysates diet groups tended to be lower than intact proteins diet groups. Hepatic TG concentration was higher in BRPH and SPH diet groups.

Fecal Lipids and Nitrogen. Total fecal excretion was significantly higher (74%) in BRPH diet group. Total fecal lipid excretion was 93%, 94%, 280%, and 64% higher in the WRPH, BRP, BRPH, and SPH diet groups, respectively, compared with the control. Fecal nitrogen excretion also increased (about 41−163%) in all other diets compared with the control. Fecal nitrogen excretion of the BRPH diet group was also 49% higher than the BRP diet group. Fecal free and total cholesterol concentrations of the WRPH and BRPH diets were significantly higher than the control. Fecal TG concentration was significantly higher in the BRPH diet group compared with the control. All plant protein diets increased fecal bile acid excretion (26−75%) compared with the control (Table 4).

Hepatic Gene Expression. Expression of hepatic genes related to fatty acid metabolism was determined by real-time PCR (Figure 1). Real-time PCR was limited to comparison of the diets of hydrolysates and BRP to the control due to resource constraints. Expression levels of genes involved in fatty acid oxidation including the nuclear transcription factor, peroxisome proliferator activated receptor α (*PPARα*), peroxisomal acyl-coenzyme A oxidase 1 (*ACOX1*), and the mitochondrial carnitine palmitoyltransferase 1 (*CPT1*) were about 1.5−2 fold higher relative to the control. The expression level of stearoyl CoA desaturase-1 (*SCD1*), a gene involved in fatty acid synthesis, was down-regulated in all treatment diets with the BRPH supplemented diet causing the lowest expression relative to the control. However, the mRNA level of fatty acid synthase (*FAS*) was higher in WRPH, BRP, BRPH, and SPH diet groups compared with the control and the mRNA level of sterol receptor element binding protein-1c (*SREBP-1C*) was higher in WRPH, BRP, and SPH diet groups relative to the control.

Relative expression of lanosterol 14 α-demethylase (*CYP51*), coding for the first committed step of cholesterol synthesis, was 3-fold higher in the BRPH supplemented diet group than the control. Expression of cholesterol 7 α-hydroxylase (*CYP7A1*), a gene encoding the rate-limiting enzyme in the classical pathway of bile acid synthesis, was up-regulated by 9-, 8-, 6-, and 6-fold by WRP, BRP, BRPH, and SPH diets, respectively, compared with the control. In addition, BRPH and SPH supplemented diets also induced the expression of the LDL receptor (*LDLR*), hepatocyte nuclear factor 4α (*HNF4α*), a modulator of bile acid synthesis, and the ATP-binding cassette, subfamily B member 11 (*ABCB11*). The relative mRNA levels of liver X receptor (*LXRα*) were not different from the control.

DISCUSSION

The purpose of this study was to investigate the effects of alkaline extracted brown and white rice proteins and their alcalase hydrolysates on lipid metabolism in a hypercholesterolemic animal model. Peptides may decrease cholesterol absorption in the intestinal lumen or modulate the expression of genes related to cholesterol metabolism in hepatocytes.[6,7] In the intestinal lumen of rats, hydrophobic peptides from milk and soy have been shown to displace cholesterol from bile acid and phosphatidylcholine micelles which potentially reduces cholesterol absorption.[14,26] In this study, we found that BRPH, but not BRP, WRP, or WRPH, supplemented high fat diet had an antiobesity

Table 3. Effects of Dietary Protein Supplemented High Fat Diets on Plasma and Hepatic Lipid, Cholesterol, and TG Concentrations in Hamsters[a]

	control	WRP	WRPH	BRP	BRPH	SP	SPH
			Plasma Lipids				
VLDL-C (mmol/L)	13.2ab ± 2.6	10.2ab ± 1.2	7.9b ± 0.7	16.6a ± 3.3	4.8b ± 1.1	7.6b ± 0.6	6.4b ± 1.1
LDL-C (mmol/L)	59.2b ± 4.2	70.5a ± 5.6	51.7b ± 5.2	80.9a ± 6.8	68.5ab ± 8.4	50.7b ± 3.6	42.6b ± 5.4
HDL-C (mmol/L)	103.6a ± 4.2	106.2a ± 6.0	115.8a ± 5.2	95.8a ± 4.6	88.8a ± 6.1	102.7a ± 3.5	93.2a ± 3.3
TC (mmol/L)	176.0ab ± 4.2	186.9ab ± 7.9	175.5ab ± 5.9	193.3a ± 5.7	162.1b ± 14.1	161.0b ± 3.1	142.2b ± 6.9
TG (mmol/L)	69.3b ± 11.7	65.7b ± 4.6	70.4b ± 6.1	104.8a ± 7.8	66.5b ± 6.6	61.2b ± 4.3	72.1b ± 7.4
			Hepatic Lipids				
total lipid (%)	20.3a ± 1.2	17.7ab ± 0.3	16.9b ± 0.5	18.5ab ± 0.9	17.4ab ± 0.8	18.7ab ± 1.4	16.8b ± 0.6
TC (mg/g liver)	30.4a ± 2.5	32.0a ± 2.2	23.5b ± 2.1	23.5b ± 2.3	14.9b ± 2.8	21.2b ± 3.5	14.4b ± 2.2
FC (mg/g liver)	8.7a ± 0.7	8.4a ± 0.3	8.1a ± 0.4	8.0a ± 0.5	7.7a ± 0.6	8.0a ± 0.6	7.0a ± 0.5
TG (mg/g liver)	131.4b ± 12.9	130b ± 15.8	129.5b ± 11.9	137.4b ± 14	180.1a ± 15.8	114.6b ± 17.2	171.8a ± 16.9

[a] Data presented as means ± SE. Different letters indicate significant difference at $P < 0.05$. TC = total cholesterol; TG = triglyceride; FC = free cholesterol; WRP = white rice protein; WRPH = white rice protein hydrolysates; BRP = brown rice protein; BRPH = brown rice protein hydrolysates; SP = soy protein; SPH = soy protein hydrolysates.

Table 4. Effects of Different Dietary Protein Supplemented High Fat Diets on Fecal Lipid, Bile Acid, and Nitrogen Contents in Hamsters[a]

	control	WRP	WRPH	BRP	BRPH	SP	SPH
dry feces weight (g/d)	0.69b ± 0.1	0.71b ± 0.03	0.87ab ± 0.1	0.7b ± 0.1	1.2a ± 0.1	0.8ab ± 0.1	1.1a ± 0.09
total lipid(mg/d)	15.3c ± 1.9	22.2bc ± 1.5	36.5b ± 4.8	41.3b ± 3.2	61.2a ± 6.1	25.9b ± 4.8	31b ± 5.2
TC (mg/d)	1.6b ± 0.15	1.8b ± 0.22	4.9a ± 0.62	2.8ab ± 0.15	4.5a ± 0.06	1.9b ± 0.17	2.0b ± 0.18
FC (mg/d)	1.4b ± 0.11	1.5b ± 0.18	4.2a ± 0.59	2.3b ± 0.09	3.6a ± 0.02	1.4b ± 0.11	1.5b ± 0.18
TG (mg/d)	0.55c ± 0.06	0.71bc ± 0.05	0.86bc ± 0.1	1.0b ± 0.07	1.8a ± 0.04	0.58bc ± 0.08	0.69bc ± 0.05
TBA (mmol/d)	1.2b ± 0.17	2.0a ± 0.17	2.3a ± 0.22	1.5b ± 0.19	2.1a ± 0.25	2.1a ± 0.25	1.7ab ± 0.22
N content (mg/d)	33.6c ± 3.4	56.2b ± 2.5	53.2b ± 7.4	59.3b ± 4.4	88.4a ± 9.9	47.3bc ± 5.0	58.7b ± 5.0

[a] Data presented as means ± SE. Different letters indicate significant difference at $P < 0.05$. TC = total cholesterol; FC = free cholesterol; TG = triglyceride; TBA = total bile acid; WRP = white rice protein; WRPH = white rice protein hydrolysates; BRP = brown rice protein; BRPH = brown rice protein hydrolysates; SP = soy protein; SPH = soy protein hydrolysates.

and hypocholesterolemic effect on hamsters. Hamsters fed the BRPH supplemented diet had lower weight gain (−76%) and final body weight (−12%) compared with the control which was not due to differences in food intake. White rice is high in glutelin (70−85%) while the bran present only in brown rice is high in albumin (37%) and globulin (36%).[27] Lower body weight gain of the BRPH diet group might be due to the differences in protein fractions as well as alcalase hydrolysates. Yang et al.[5] reported that rice proteins caused lower weight gain and food intake in young but not mature rats and two varieties of rice that differed in glutelin and prolamin contents did not affect the plasma total cholesterol content in rats. Lower weight gain in the BRPH diet group could also be attributed to higher total daily fecal excretion, fecal lipid, and nitrogen content (Table 4). Fecal dry weight, lipid, and nitrogen content were negatively correlated, $r = -0.785$, $r = -0.871$, and $r = -0.795$, respectively, with final body weight ($P < 0.05$). Indigestible, hydrophobic peptides have been reported to disrupt micellar lipid transport and possibly digestion and absorption.[28] We observed not only higher fecal lipid excretion from WRPH, BRP, and BRPH diet groups but also higher fecal TG excretion compared with the control. A negative correlation of fecal TG concentration and final body weight was observed ($r = -0.815$, $P < 0.05$). Increased fecal TG level may indicate lipid malabsorption due to induced inefficiency of fat digestion since the mammalian pancreatic lipase−colipase system also requires bile acid as a cofactor. Fecal fat excretion may also be affected by dietary fat composition since saturated fats that were high in our study are less easily digested. A rice protein supplemented high fat and cholesterol diet was reported to reduce plasma cholesterol and weight gain in growing rats.[5] Feeding dietary obese rats a diet containing 20% alacalase hydrolyzed corn gluten, a byproduct of corn processing that has none of the functional or allergenic properties of wheat gluten, for one month caused a weight loss.[29] The corn gluten hydrolysate contained 70% free amino acids, in particular free leucine, and the authors attributed some of the effects to negative nitrogen balance and possible catabolism of lean body mass.[29] Another study found increasing dietary leucine intake reduced weight gain in mice on high fat but not chow diets due to increasing energy expenditure, not decreases in food intake.[30] In this study, all cereal based diets were made equivalent by supplementation with sufficient amino acids (Table 1) to meet AIN93 M levels.[31] Compared with most other proteins, SP is reported to have the highest levels of leucine, but in our study WRP (8.56%) and BRP (8.38%) tended to have higher leucine content than SP (8.13%).

Proteins or peptides may modulate the activities of hepatic enzymes related to lipid metabolism.[4,30] In this study epididymal and retroperitoneal adipose weights were similar for all treatments. However, hepatic expression levels of genes related to fat oxidation, *ACOX1* and *CPT1*, were higher in all the hydrolysates

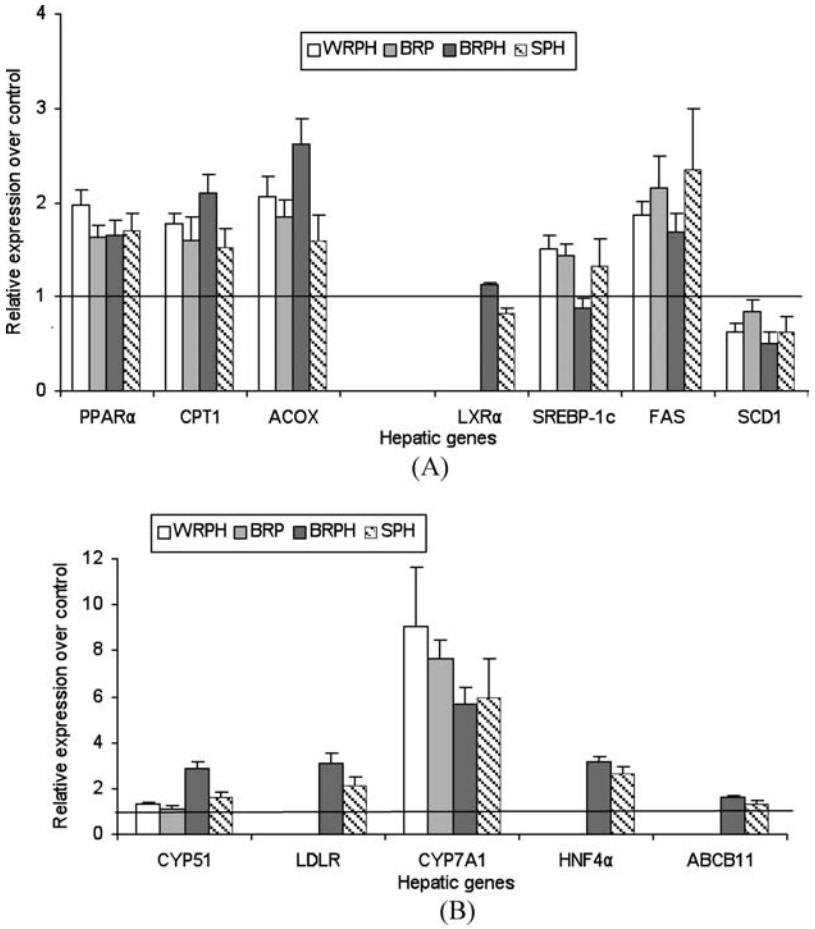

Figure 1. (A). Hepatic mRNA expression of lipid metabolism-related genes including peroxisome proliferator activated receptor α (*PPARα*), peroxisomal acyl-coenzyme A oxidase 1 (*ACOX1*), the mitochondrial carnitine palmitoyltransferase 1 (*CPT1*), fatty acid synthase (*FAS*) stearoyl CoA desaturase-1 (*SCD1*), sterol receptor element binding protein-1c (*SREBP-1C*), and liver X receptor (*LXRα*) in male Golden Syrian hamsters fed high-fat diets containing white rice protein hydrolysates (WRPH), brown rice protein (BRP), brown rice protein hydrolysates (BRPH), and soy protein hydrolysates (SPH) for 3 weeks. Each mRNA was normalized to endogen actin and is expressed relative to the control level. Data are expressed as mean ± SE.(B). Hepatic mRNA expression of cholesterol and bile acid metabolism-related genes including lanosterol 14 α-demethylase (*CYP51*), cholesterol 7 α-hydroxylase (*CYP7A1*), the LDL receptor (*LDLR*) and hepatocyte nuclear factor 4α (*HNF4α*), and ATP-binding cassette, subfamily B member 11 (*ABCB11*) in male Golden Syrian hamsters fed high-fat diets containing white rice protein hydrolysates (WRPH), brown rice protein (BRP), brown rice protein hydrolysates (BRPH), and soy protein hydrolysates (SPH) for 3 weeks. Each mRNA was normalized to endogen actin and is expressed relative to the control level. Data are expressed as mean ± SE.

diet groups but highest in the BRPH diet group, which suggested higher energy expenditure. There were negative correlations between the final body weight and expression levels of genes for *CPT1* ($r = -0.883$, $P = 0.1$) and *ACOX1* ($r = -0.869$, $P = 0.1$). Correlations between fecal total lipid concentration and mRNA levels of *CPT1* ($r = 0.946$, $P < 0.05$) and *ACOX1* ($r = -0.883$, $P < 0.05$) were observed. Although no significant change in the expression level of *LXR α* occurred, its target gene *SCD1*, the enzyme that catalyzes the rate-limiting step in the biosynthesis and storage of monounsaturated fatty acid from saturated fatty acids,[32] was significantly down-regulated by the BRPH diet group. In this study, *SREBP-1C* and *LXR α* mRNA levels did not correlate with *SCD1* expression indicating regulation may occur post-transcriptionally possibly through protein processing.[33] *SCD1* deficient animals have been shown to increase energy expenditure and reduce body adiposity, hepatic TG, cholesterol esters, and phospholipids content.[32−35] The significant up-regulation of genes for hepatic lipolytic *CPT1* and *ACOX1* and significant down-regulation of the gene for hepatic lipogenic *SCD1* as well as the fecal lipid excretion could contribute to the decreased body of hamsters fed the BRPH diet. Additionally, because *FAS* and *SCD1* appeared to be regulated differently, a common mechanism for both does not appear to be at work in this situation.

The results also show that WRPH, BRPH, and SPH supplemented diets reduce plasma cholesterol (VLDL-C and total cholesterol) concentrations. An earlier study reported similarly that plant proteins, particularly SP, lowered levels of atherogenic lipoproteins and sometimes increased antiatherogenic HDL-C as well.[34] Pullen et al.[35] reported that hepatic secretion of TG as lipoprotein was decreased as hepatic TG concentration increased. In this study, hepatic TG concentration had significant ($P < 0.01$) negative correlations with VLDL concentration ($r = -0.73$) and hepatic total cholesterol ($r = -0.947$). This could be related to the higher hepatic TG concentration of hamsters fed WRPH, BRPH, and SPH supplemented diets. Interestingly, there was a positive correlation between hepatic and plasma total cholesterol concentrations ($r = 0.806$, $P < 0.05$). Although plasma cholesterol levels were not affected by most diet

treatments, hepatic total and esterified cholesterol concentrations were lower in all diet treatments except the WRP supplemented diet. Lower hepatic total cholesterol may be related to higher fecal total cholesterol and bile acid concentrations. Sugano et al.[13] reported that rats fed a diet including 20% high molecular weight fractions of SPH had reduced plasma and liver cholesterol levels compared with the unhydrolyzed SP and the low molecular weight fractions. One recent investigation has also shown that the hypocholesterolemic response induced by rice proteins contributed to an increase of fecal excretion of total steroids.[5]

Our hepatic gene expression data indicate that some treatment diet groups change the expression of genes related to cholesterol and bile acid metabolism. In the BRPH diet group, hepatic and fecal total cholesterol concentration were negatively ($r = -0.969, P < 0.05$) and positively ($r = 0.758$, $P = 0.1$) correlated, respectively, with the expression of *CYP 51*. Higher expression of *LDLR*, which carries esterified cholesterol into the cell, was correlated with lower hepatic esterified cholesterol concentration in the BRPH diet group ($r = -0.558$, $P = 0.16$). An amount of bile acid equivalent to that lost in the feces is synthesized from cholesterol, which is accomplished by a system of feedback controls. Bile acid feedback regulates *CYP7A1* transcription through *HNF-4*.[36] In this study, significant fecal bile acid excretion up-regulated the mRNA levels of *CYP7A1* and *HNF-4*. Mice overexpressing *CYP7A1* were protected against high fat diet-induced hypercholesterolemia, obesity, and insulin resistance.[37] The *ABCB11* gene produces a protein found in the liver, the bile salt export pump (BSEP), and mutations in the *ABCB11* gene have been shown to prevent the BSEP protein from effectively transporting bile salts out of the liver. The expression of *ABCB11* was up-regulated (1.6- fold) by the BRPH diet. The mRNA level of *ABCB11* was positively correlated with *CYP7A1* mRNA level ($r = 0.781, P < 0.05$).

In summary, we have demonstrated that supplementation of a high-fat diet with different rice proteins and their hydrolysates results in a reduction of plasma cholesterol (VLDL-C and total cholesterol) and hepatic total cholesterol concentration through an increase of fecal lipid, total cholesterol, and bile acid excretion. The expression levels of *CYP51* and *LDLR*, genes related to decreases in hepatic cholesterol level, were higher in BRPH and SPH diet groups. Up-regulation of *CYP7A1* was observed in WRPH, BRP, BRPH, and SPH diet groups supporting the mechanism of depletion of hepatic bile acids due to increased fecal excretion. The lower body weight gain of the BRPH diet group was correlated with the modification of expression of genes related to fatty acid synthesis and oxidation. In conclusion, hydrolysates of rice proteins by a food grade industrial protease were shown to modulate hepatic lipid metabolism and in some cases resulted in reduced hepatic and plasma cholesterol. BRPH reducing body weight gain might be useful in combination with foods that are high in cholesterol and fat such as eggs, cheese, pizza, hamburger, sandwiches, and other fast foods to mitigate components that increase weight or undesirable plasma lipids.

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