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Hypocholesterolemic effect of physically refined rice bran oil: studies of cholesterol metabolism and early atherosclerosis in hypercholesterolemic hamsters

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Abstract

Physically refined rice bran oil containing 2–4% nontriglyceride components as compared to other vegetable oils appears to be associated with lipid lowering and antiinflammatory properties in several rodent, primate and human models. These experiments were designed to investigate possible mechanisms for the hypocholesterolemic effect of the physically refined rice bran oil and to examine its effect on aortic fatty streak formation. In the first experiment, 30 hamsters were fed, for 8 weeks, chow-based diets plus 0.03% added cholesterol and 5% (wt/wt) coconut, canola, or physically refined rice bran oil (COCO, CANOLA or PRBO animal groups, respectively). Both plasma total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) were significantly reduced in PRBO but not in CANOLA relative to COCO. PRBO also showed a significant 15–17% reduction in cholesterol absorption and significant 30% increase in neutral sterol (NS) excretion with no effect on bile acid (BA) excretion. Both CANOLA and PRBO showed a significant 300–500% increase in intestinal 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and significant (>25%) decrease in hepatic HMG-CoA reductase activities with respect to COCO. In a second experiment, 36 hamsters were fed chow-based diets with 0.05% added cholesterol, 10% coconut oil and 4% additional COCO, CANOLA or PRBO. Relative to COCO and CANOLA, plasma TC and LDL-C were significantly reduced in PRBO. Early atherosclerosis (fatty streak formation) was significantly reduced (48%) only in PRBO, relative to the other two. These results suggest that the lipid lowering found in PRBO is associated with decreased cholesterol absorption, but not hepatic cholesterol synthesis, and that the decrease in fatty streak formation with this oil may be associated with its nontriglyceride components not present in the other two diets.

Keywords: Rice bran oil; Cholesterol; Absorption; HMG-CoA reductase activity; Bile acids; Neutral sterols; Fatty streak area

1. Introduction

Elevations in serum total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) increase the risk of atherosclerosis and coronary heart disease [1]. Numerous studies have demonstrated that oils containing particular saturated fatty acids (SFA) raise serum TC, and in particular, LDL-C [2,3], while those enriched in unsaturated fatty acids [4,5] lower LDL-C when replacing SFA. More recently, there has been a renewed interest in the nontriglyceride components of dietary oils, especially following the discovery of the hypocholesterolemic effect of rice bran [6,7] and rice bran oil [8–14]. Rice bran oil has greater amounts of plant sterols, oryzanol and tocotrienols (all unsaponifiable, nontriglyceride components) (Table 1) than most highly refined vegetable oils [15–18] but also contains more SFA. The hypocholesterolemic effect of rice bran oil can be reproduced by feeding the extracted unsaponifiable lipids alone to animals [10,19]. The significance of this unsaponifiable fraction is supported by studies in nonhuman

Abbreviations: HDL-C, High-density lipoprotein cholesterol; IDL-C, Intermediate-density lipoprotein cholesterol; LDL-C, Low-density lipoprotein cholesterol; VLDL-C, Very low-density lipoprotein cholesterol; LDL-C, TC minus HDL-C; TC, Total cholesterol; TG, Triglyceride; HMG-CoA reductase, 3-hydroxy-3-methylglutaryl coenzyme A reductase; NS, Neutral sterol; BA, Bile acid.

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Table 1
Fatty acid composition and plant sterol composition of the experimental fats

Fatty acid	Canola (%)	Physically refined rice bran oil (%)	Coconut oil (%)
SFA	7.1	19.7	86.5
6:0	n.d.	n.d.	0.6
8:0	n.d.	n.d.	7.5
10:0	n.d.	n.d.	6
12:0	n.d.	n.d.	44.6
14:0	n.d.	0.7	16.8
16:0	4	16.9	8.2
18:0	1.8	1.6	2.8
20:0	0.7	n.d.	n.d.
22:0	0.4	n.d.	n.d.
24:0	0.2	n.d.	n.d.
MUFA	58.9	39.3	5.8
C16:1	0.2	0.2	n.d.
C18:1	56.1	39.1	5.8
PUFA	29.6	35.0	1.8
C18:2	20.3	33.4	1.8
18:3	9.3	1.6	n.d.
Plant sterol content	0.35±0.05	2.58±0.36	Trace

n.d., not detectable; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
Plant sterols: β-sitosterol, campesterol and stigmasterol). Values for fatty acid concentrations are from USDA National Nutrient Database for Standard Reference, Release 16-1. Values for plant sterol concentrations were measured in our laboratory in duplicate according to the procedure in Materials and methods.

and human primates showing that the capacity of rice bran oil to lower serum TC and LDL-C is greater than predicted based on its fatty acid composition [2,12,13,20].

Crude rice bran oil consists of up to 4% unsaponifiables, but refining decreases these amounts depending on the source and the degree of processing. Elimination of the normally used alkali extraction step in the oil refining process results in the production of physically refined rice bran oil (as used in this study) with a higher content of unsaponifiables as compared to alkali-refined rice bran oil [15,16]. Although the plant sterol content, which can account for over half of the unsaponifiables, is not significantly influenced by the processing technique, oryzanol, a family of ferulic acid esters of triterpene alcohols, and plant sterols can be enriched up to 10× more in the physically refined (average 9000 ppm) [21] as compared to the alkali-refined oils [15,16]. Retention of the oryzanol content in the physically refined rice bran oil is important in view of its cholesterol-lowering properties in animals [14,22,23] and humans [14,24–26] and its antioxidant activity [27]. Other unsaponifiables analyzed in physically refined rice bran oil were tocotrienols, analogs of tocopherol (700 ppm), and tocopherol (400 ppm) that were reduced >85% with alkali refinement. Retention of these two components may be beneficial because of the tocotrienol-related inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity [28] and the antioxidant properties of tocopherol [29].

The specific mechanism by which physically refined rice bran oil exerts its effects on plasma TC and LDL-C levels has not been definitively elucidated in humans or experimental animals. The first experiment was designed to investigate the effects of physically refined rice bran oil on dietary cholesterol absorption, HMG-CoA reductase activity (as a proxy for hepatic or intestinal cholesterol synthesis) and fecal neutral sterol (NS) and bile acid (BA) excretions in hypercholesterolemic hamsters previously fed a coconut oil-based diet. The second experiment evaluated whether these effects on plasma lipids and cholesterol metabolism were associated with beneficial changes at the level of the blood vessel wall and the accumulation of Oil-Red-O-positive macrophage-derived foam cells in the aortic arch. Golden Syrian hamsters were used for these experiments since they have shown similar characteristics of lipoprotein and cholesterol metabolism as compared with humans [30]. Portions of this work were previously reported in abstract form [31].

2. Materials and methods

2.1. Experiment 1

2.1.1. Animals

Thirty male 8-week-old F_1B golden hamsters (Biobreeder, Fitchburg, MA) were used in this experiment to assess the impact of coconut oil, canola oil and physically refined rice bran oil on plasma lipid, lipoprotein and cholesterol metabolism. The study was approved by the Institutional Animal Care and Use Committees at University of Massachusetts-Lowell and at the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University. Animals were individually housed in hanging cages in a 12/12-h light/dark cycle with the dark cycle starting at 03:00 h. All diets and water were fed ad libitum throughout the study.

2.1.2. Diets

The basal hypercholesterolemic diet for all animals in all groups contained 0.03% cholesterol and 5% (wt/wt) coconut oil (Pureco 76, Capital City) added to the Purina Rodent Chow (Purina 5001, Purina Mills, St. Louis, MO). A chow-based, rather than a semipurified diet, was used because published data from our laboratory [32] and those from another [33] have demonstrated that the chow-based diet produced a lipoprotein profile (predominately LDL-C) [defined as TC minus high-density lipoprotein cholesterol (HDL-C)] more similar to humans. Subsequently, three experimental diets were used in the study. The experimental diet for the coconut oil group was the same as the basal hypercholesterolemic diet. The experimental diet for the canola oil group (CANOLA) contained 5% canola oil (Select Origins, Southampton, NY) in place of coconut oil, and the physically refined rice bran oil group (PRBO) contained 5% physically refined rice bran oil (TSUNO, Osaka, Japan) in place of coconut oil, with the rest of the components,

Table 2
Dietary cholesterol and plant sterol content

Groups	Cholesterol[a]		Plant sterol[b]	
	(mg/g diet)	(%)	(mg/g diet)	(%)
COCO	0.51±0.02	0.05	0.61±0.02	0.06
CANOLA	0.48±0.01	0.05	0.80±0.01	0.08
PRBO	0.53±0.04	0.05	1.90±0.02	0.19

Plant sterols in the coconut oil diet contained 18.1% campesterol, 8.7% stigmasterol, 67.5% β-sitosterol and 5.7% unknowns. Plant sterols in the canola oil diet contained 19.9% campesterol, 6.2% stigmasterol, 66% β-sitosterol and 7.9% unknowns. Plant sterols in the rice bran oil diet contained 13.2% campesterol, 8.1% stigmasterol, 43.7% β-sitosterol and 35.0% unknowns.

[a] The Purina Rodent diet naturally contains 0.02% cholesterol. With the 0.03% added cholesterol, the total in the diet becomes 0.05%.

[b] Composition of plant sterols in each diet using procedures in Materials and methods: Plant sterols in the coconut oil diet reflect the plant sterol composition of chow, since coconut oil itself contains no plant sterols.

including 0.03% added cholesterol, identical to the basal hypercholesterolemic diet. Table 1 shows the fatty acid and plant sterol contents of the three oils used in the study. Table 2 shows the content of dietary cholesterol and total plant sterols in the diets as well as the percent composition of the plant sterols within each of these three diets.

2.1.3. Experimental design

Animals were acclimatized for 3 days with regular Purina Rodent Chow and then fed the basal diet for 6 weeks to assure that hamsters were hypercholesterolemic. Baseline values of plasma TC, HDL-C and triglyceride (TG) concentrations were measured at both 4 and 6 weeks during the basal period. The LDL-C in this study, comprising very low-density (VLDL-C), intermediate-density (IDL-C) and low-density (LDL-C) lipoprotein cholesterol, was calculated as the difference between TC and HDL-C. Following the basal period, animals were divided into three experimental groups—COCO, CANOLA and PRBO ($n=10$ for each group)—based on the average LDL-C values of the two measurements; the average LDL-C for each of the three groups was similar. Animals were then fed the three experimental diets for the next 8 weeks and body weight was measured weekly. Measurement of plasma TC, LDL-C, HDL-C and TG was performed at both 4 and 6 weeks of the experimental period. At 6.5 weeks, food intake was measured for three successive days, and a total fecal collection was carried out during the same period. After cholesterol absorption was measured during week 7, animals were euthanized and their livers and small intestines collected for measurement of HMG-CoA reductase activity. Animals were euthanized in the middle of the dark cycle (12/12-h light/dark cycle) when HMG-CoA reductase was at the peak of its diurnal rhythm [34].

2.1.4. Plasma lipid and lipoprotein determination

After an overnight fast, hamsters were tranquilized with 50% CO_2/O_2, and blood samples were drawn from the retroorbital sinus into Eppendorf tubes containing 5 μl of heparin. After centrifugation at 4000 rpm for 8 min, plasma was collected for measurements of TC, TG and LDL-C as previously described [35].

2.1.5. Measurement of cholesterol absorption

The percent absorption of dietary cholesterol was measured by the dual-isotope plasma ratio method described by Zilversmit et al. [36–38], with minor modifications as described in Rong et al. [35]. The percentage cholesterol absorption was calculated based on the plasma isotope ratio of the oral (^{14}C) and intravenous (^{3}H) doses [37].

2.1.6. Food intake measurement and fecal collection

Food samples were weighed and fed to animals for three successive days. Food remaining in the cage the next day was dried to a constant weight as well as a representative aliquot of the food offered. Food consumed was calculated as the difference between offered minus remaining. All fecal materials excreted during the same 3-day period was collected daily and stored at −70°C.

2.1.7. Measurement of cholesterol and plant sterol content in diets

Food samples were lyophilized to dryness and ground to a fine powder before extraction. Food samples (100 mg) in triplicate were extracted according to Jones et al. [39]. To each extraction tube, 5α-cholestane (0.5 mg) was added as a recovery standard.

2.1.8. Gas chromatographic analysis of cholesterol and plant sterols content in food samples

Neutral sterol and plant sterol standards, including 5α-cholestane, coprostanol and coprostanone, were purchased from Steraloids (Wilton, NH); cholesterol, campesterol, stigmasterol and β-sitosterol were from Sigma (St. Louis, MO). The NS extracts of the food samples were dissolved in 0.5 ml of hexane and then quantified by gas chromatography (Autosystem, Perkin-Elmer, Norwalk, CT) using a capillary column (RTX7-225, 30 m, 0.25 mm ID, 0.25-μm film thickness, Restek, Bellefonte, PA), accomplished with the aid of Turbochrom software (Turbochrom 3.3, Perkin-Elmer). Samples were analyzed under the following conditions: 1.0 μl of NS sample was injected into the column at 220°C oven temperature. After remaining at this temperature for 1 min, the oven temperature was increased to 238°C (20°C/min), held for 1.0 min and further increased to 248°C (0.7°C/min). The injector and detector were set at 250°C and 252°C, respectively. The carrier gas (helium) flow was 20 psi with the inlet splitter set at 4.12:1.

2.1.9. Measurement of fecal NS and BA content

Fecal NS and BA were extracted and isolated according to Ausman et al. [40] using 200 mg samples (in duplicate) and with 5α-cholestane (0.5 μg) used as recovery standard

for the NS. A recovery factor (71.34% for BA) was calculated based on the recovery of lithocholic acid from four fecal samples (in duplicate) with and without added lithocholic acid before extraction.

2.1.10. Gas chromatographic analysis of fecal NS

The fecal NS extractants were dissolved in 1.0 ml of hexane and then separated and quantified by gas–liquid chromatography (Autosystem) under conditions identical to those of the food cholesterol and plant sterol analyses. All fecal NS data were corrected for recovery of the internal standard for each sample. The mean recovery of the 5-α cholestane from fecal material was 87.8±14.7% (mean±S.D.).

2.1.11. Gas chromatographic analysis of fecal BA

Bile acid standards, including lithocholic acid, deoxycholic acid, ursodeoxycholic acid and cholic acid were from Sigma; chenodeoxycholic acid was from Calbiochem (La Jolla, CA). Fecal BA were quantified as described by Setchell et al. [41] with modifications as described by Jones et al. [39].

2.1.12. Preparation of hepatic and intestinal microsomes

Hepatic microsomes were prepared according to Harwood et al. [42] with minor modifications described by Rong et al. [35]. Intestinal microsomes for total HMG-CoA reductase activity were prepared from intestinal mucosal scrapings based on the method described by Harwood et al. [43] with minor modification described by Rong et al. [35].

2.1.13. Measurement of hepatic and intestinal HMG-CoA reductase activity

The activity of reductase (HMG-CoA reductase) (EC 1.1.1.34) was measured as described by Harwood et al. [43] with modifications detailed by Rong et al. [35]. HMG-CoA reductase activity is expressed as picomoles of mevalonate formed per minute per milligram microsomal protein.

2.2. Experiment 2

2.2.1. Animals

Thirty-six 10-week-old male F_1B golden hamsters (Biobreeder) were used in this experiment to assess the effect of coconut, canola and physically refined rice bran oil-containing diets on early atherosclerosis. Other details were the same as for experiment 1.

2.2.2. Diet

Animals were fed for 10 weeks a chow-based diet, for reasons given in the first experiment, composed of a base containing 0.05% cholesterol and 10% (wt/wt) coconut oil to which was added either 4% more coconut oil, canola oil or physically refined RBO. Again, these diet groups will be termed COCO, CANOLA and PRBO.

2.2.3. Plasma lipid and lipoprotein measurement

At 4, 8 and 10 weeks of diet, blood samples were withdrawn and plasma was prepared and analyzed for TC, HDL-C, LDL-C and TG as described in experiment 1.

2.2.4. Fixation of the aorta and aortic morphometric analysis

The procedures used were the same as described in Rong et al. [35]. An image analysis system (Image Technology, Cresskill, NJ), which was attached to a light microscope, was used to measure the total Oil-Red-O-stained macrophage-derived foam cells that constituted the fatty streak in the aortic arch [44–46]. Units of measurement of fatty streak area are $\mu m^2/mm^2 \times 10^3$ of aortic tissue.

2.2.5. Statistical analysis

Statistical Analysis System (SAS-PC) [47] was used for all statistical analyses for both experiments 1 and 2. Descriptive statistics and graphs were used to summarize the overall effects of the diets and distributions of the outcome measures. When violations of the basic testing assumptions were noted, transformations of the data were used. When these were not successful, a signed rank test with Bonferroni's correction for multiple comparisons was utilized. Where possible, analysis of variance followed by Tukey's t test for multiple comparisons ($P<.05$) was used to determine the statistical significance of mean values among three groups for all variables.

3. Results

3.1. Experiment 1

3.1.1. Effects of physically refined rice bran oil on plasma lipids, lipoprotein cholesterol and body weight

Table 3 lists the detailed lipid profile at week 6 for hamsters on the three experimental diets. At week 6, plasma TC levels in PRBO were significantly lower (21%) than in COCO. This difference in plasma TC was attributed almost solely to the LDL-C in PRBO, which was significantly decreased (29%) from COCO. The plasma TC and LDL-C

Table 3
Plasma lipids, lipoprotein cholesterol and body weight at week 6

	Groups		
	COCO	CANOLA	PRBO
TC (mmol/L)	4.14±0.26[a]	3.59±0.18[ab]	3.26±0.21[b]
LDL-C (mmol/L)	2.84±0.26[a]	2.28±0.18[ab]	2.02±0.18[b]
HDL-C (mmol/L)	1.29±0.05	1.32±0.03	1.24±0.05
TG (mmol/L)	3.43±0.27	3.82±0.28	3.96±0.27
Body weight (g)	149±4	153±3	145±3

Data are presented as means±S.E.M. with $n=10$ for each group except for COCO ($n=9$). Means sharing the same superscript are not significantly different at $P<.05$. LDL-C, TC minus HDL-C. Lipid values (mean±S.E.M. for 70 hamsters) for hamsters on a "chow"-based diet are the following: TC, 3.36±0.21; LDL-C, 1.50±0.16; HDL-C, 1.89±0.21; TG, 1.81±0.23.

Table 4
HMG-CoA reductase activities and cholesterol absorption

	Groups		
	COCO	CANOLA	PRBO
Liver HMG-CoA reductase (pmol/min per mg protein)			
Expressed	5.8±0.5[a]	3.6±0.4[b]	3.6±0.2[b]
Total	13.9±0.8[a]	10.6±0.6[b]	10.4±0.8[b]
Small intestine total*			
HMG-CoA reductase (pmol/min per mg)	4.5±1.1[a]	17.3±2.5[b]	28.8±5.2[b]
Cholesterol absorption (%)	65.5±2.6[a]	63.9±2.5[a]	54.6±2.3[b]

Data are expressed as mean±S.E.M., with $n=10$ for each group except for COCO ($n=9$). Means sharing the same superscript are not significantly different at $P<.05$.

* Data log transformed prior to statistical analysis.

levels in CANOLA were intermediate and not significantly different from either COCO or PRBO. There were no significant differences in plasma HDL-C and TG and body weight among the three groups during the course of the experiment.

3.1.2. Cholesterol absorption

Following 7 weeks of dietary treatment, cholesterol absorption in PRBO was significantly decreased (~17%) as compared to both COCO and CANOLA (Table 4).

3.1.3. Hepatic and intestinal HMG-CoA reductase activities

Both the expressed (nonphosphorylated) and total (phosphorylated) hepatic HMG-CoA reductase activities were significantly decreased (~38% and 24%, respectively) in both PRBO and CANOLA as compared to COCO (Table 4). The mean intestinal HMG-CoA reductase activities for CANOLA and PRBO were significantly greater (2.8 and 5.4 times), respectively, than for COCO (Table 4). The ANOVA and Tukey's HSD test carried out on the log 10 transformed data indicated that the activities in CANOLA and PRBO were not quite statistically significant ($P<.058$).

3.1.4. Food, cholesterol and plant sterol intake and fecal excretion

On average, PRBO consumed a slightly greater amount of food and thus cholesterol per day than CANOLA, with results from COCO intermediate and not significantly different than either group (Table 5). All groups consumed significantly different amounts of plant sterols. Finally, the fecal weight in PRBO was significantly greater than for either COCO or CANOLA.

Table 5
Food, cholesterol and plant sterol intake and fecal weight

Components	Groups		
	COCO	CANOLA	PRBO
Food intake (g/kg·day)	34.3±1.0[ab]	32.4±1.3[a]	36.8±0.1[b]
Cholesterol intake (mg/kg·day)	17.4±0.5[ab]	15.8±0.6[a]	18.4±0.2[b]
Plant sterol intake (mg/kg·day)	20.8±0.6[a]	26.0±1.0[b]	69.9±0.9[c]
Fecal weight (g/kg·day)	5.2±0.2[a]	5.0±0.2[a]	5.7±0.1[b]

Both food intake and fecal weight are expressed on a dry weight basis. Data are expressed as mean±S.E.M., with $n=10$ for each group except for COCO ($n=9$). Means sharing the same superscript are not significantly different at $P<.05$.

Table 6
Fecal NS and BA excretion

Components	Group		
	COCO	CANOLA	PRBO
	(mg/kg body weight per day)		
Fecal NS	7.5±0.3[a]	7.5±0.4[a]	9.8±0.7[b]
Cholesterol	2.5±0.2[a]	2.4±0.2[a]	4.0±0.6[b]
Coprostanol	4.8±0.2[a]	4.9±0.2[ab]	5.5±0.3[b]
Coprostanone	0.2±0.02[a]	0.3±0.03[a]	0.4±0.02[b]
Fecal BA	15.5±1.0	16.1±1.6	16.9±0.8
Lithocholic acid	3.8±0.4	4.0±0.4	4.1±0.3
Deoxycholic acid	4.4±0.5	4.5±0.5	5.2±0.4
Chenodeoxycholic acid	0.2±0.04	0.3±0.04	0.3±0.04
Cholic acid	0.2±0.02	0.2±0.01	0.2±0.02
Ursodeoxycholic acid	0.4±0.06	0.4±0.07	0.6±0.04
Unknowns	6.4±0.3	6.8±0.6	6.5±0.2
Fecal NS+BA	23.0±1.1[a]	23.7±1.4[ab]	26.7±1.2[b]
Cholesterol intake	17.4±0.5[ab]	15.8±0.6[a]	18.4±0.2[b]
Sterol balance	5.6±1.1	7.9±1.2	8.4±1.2
TC absorbed	11.3±0.4	10.1±0.4	10.3±0.5
Unabsorbed exogenous cholesterol	6.0±0.6[a]	5.7±0.5[a]	8.1±0.5[b]
Fecal sterols — Unabs. Chol.	16.9±1.3	17.9±1.2	18.7±1.3
Unabsorbed endogenous cholesterol	1.4±0.4	1.8±0.6	1.7±1.0

Data are expressed as mean±S.E.M., with $n=10$ for each group except for COCO ($n=9$). Means sharing the same superscript are not significantly different at $P<.05$.
Unabs. Chol.=unabsorbed dietary cholesterol.

3.1.5. Fecal NS and BA excretion

Coprostanol, the major bacterial metabolite of colonic cholesterol, accounted for about 60% (range, 56–65%) of the total daily NS excreted in all three groups (Table 6) with only a small amount of coprostanone; the remainder was chiefly preformed cholesterol. Total NS, cholesterol and coprostanone were significantly increased in PRBO as

Table 7
Plasma lipids, lipoprotein cholesterol and fatty streak area in F_1B hamsters

	Groups		
	COCO	CANOLA	PRBO
TC (mmol/L)	10.29±1.06[a]	7.81±0.23[a]	5.92±0.21[b]
LDL-C (mmol/L)	8.64±1.06[a]	6.21±0.23[a]	4.42±0.18[b]
HDL-C (mmol/L)	1.68±0.05[a]	1.63±0.03[ab]	1.50±0.05[b]
TG (mmol/L)	11.66±1.94[a]	5.80±0.32[b]	5.83±0.35[b]
Fatty streak area ($\mu m^2/mm^2\times10^3$)	15±2[a]	13±2[a]	8±1[b]

Values represent means±S.E.M. ($n=12$ for each group). Statistics were carried out using a signed rank test with Bonferroni's correction for multiple comparisons. Means sharing the same superscript are not significantly different at $P<.05$.

compared to both COCO and CANOLA. Daily fecal excretion of coprostanol was significantly greater in PRBO than in COCO but not as compared to CANOLA.

Daily fecal BA excretion, which was identified as lithocholic acid, deoxycholic acid, chenodeoxycholic acid, cholic acid, ursodeoxycholic acid and unknowns (approximately 40% of the total BA), showed no group differences for either the pattern or the amount of the individual components or for the sum of total daily BA excretions (Table 6). Total daily sterol excretion, which includes total daily NS and BA, showed a trend of PRBO> CANOLA>COCO, in which PRBO was significantly greater (16%) than COCO with no significant difference between the intermediate CANOLA and the other two groups.

3.2. Experiment 2

3.2.1. Plasma lipids, body weight and fatty streak area

Plasma lipids (TC, LDL-C and TG) in each of the three groups were normally distributed. Nevertheless, the large variability of the COCO group made it impossible to carry out statistics based on a normal distribution, even with a transformation. Therefore, comparisons with these three variables between COCO and the other two groups were done with a signed rank *t* test with Bonferroni's correction for multiple comparisons. Plasma TC and LDL-C were significantly reduced (42% and 48%, respectively) for PRBO as compared to COCO and CANOLA with no difference between the latter two groups (Table 7). High-density lipoprotein cholesterol in PRBO was significantly reduced from COCO with CANOLA intermediate. Plasma TG were significantly reduced (~50%) in both PRBO and CANOLA relative to COCO. Aortic fatty streak area was significantly reduced (>40%) in PRBO relative to COCO and CANOLA with no significant difference between the latter two groups. Finally, there were no significant diet treatment effects on body weight (data not shown).

4. Discussion

In experiment 1, rice bran oil-containing diets had a hypocholesterolemic effect on plasma TC and LDL-C somewhat greater than that of the less SFA-containing canola oil diet when both were compared to the coconut oil diet. In experiment 2, with a greater cholesterol and fat load, the circulating lipids amongst COCO, CANOLA and PRBO were all significantly different. Thus, this is suggestive that a dose response effect may be operative.

The mechanisms for the cholesterol lowering in both groups are not necessarily similar. The decreased SFA content of the canola oil with respect to the coconut oil probably accounted for the decreased circulating TC and LDL-C concentrations. However, the lowest circulating lipids were observed in both experiments by animals fed physically refined rice bran oil, which has a greater amount of SFA than canola, suggesting that something other than SFA was playing a role. In this case, the decrease in cholesterol absorption (55% vs. 64%) is one likely explanation. It has been established in both humans and experimental animals that feeding dietary plant sterols in pharmacologic amounts can decrease serum cholesterol levels [48–52] under conditions in which the dietary fatty acid pattern is kept constant. It has also been demonstrated that both plant sterols and triterpene alcohols can inhibit cholesterol absorption [53–57]. When plant sterols were mixed with radiolabeled cholesterol, the uptake of this cholesterol from intestine was inhibited, and the degree of inhibition increased as the ratio of dietary plant sterol to cholesterol increased. Rong et al. [35] have also shown that oryzanol in the test dose, as well as in all diets fed, has been associated with even greater reductions of cholesterol absorption than observed in the current experiment.

Both PRBO and CANOLA demonstrated approximately 38% and 24% reductions in the expressed and total hepatic HMG-CoA reductase activities, respectively. This suggests that the reduced plasma TC and LDL-C in PRBO and CANOLA compared to COCO could partially be explained by the suppressed hepatic cholesterol synthesis in these two groups. The replacement of SFA in COCO with unsaturated fatty acids in CANOLA and PRBO might be expected to decrease HMG-CoA reductase activity since greater hepatic HMG-CoA reductase activity associated with saturated fat feeding has been previously reported [58]. The ability of rice bran oil to decrease the hepatic HMG-CoA reductase activity may also be associated with increased levels of tocotrienols. These are analogs of tocopherol previously shown to decrease HMG-CoA reductase activities in vitro [28] and found in abundance in rice bran oil [50]. However, since synthetic activities in both CANOLA and PRBO were similar, this mechanism does not appear to have been in effect during this experiment.

It is noteworthy that the decreased circulating TC and LDL-C concentrations and the decreased hepatic cholesterol synthesis in both CANOLA and PRBO are counteracted, although apparently only partially, by increased intestinal cholesterol synthesis. Nguyen et al. [58] have recently shown that sitosterol feeding (2% of diet) up-regulates jejunal HMG-CoA reductase activity and this was associated with decreased cholesterol absorption and decreased cholesterol flux through the enterocyte. The decreased cholesterol absorption in these experiments is consistent with this interpretation although we were not able to demonstrate the decreased cholesterol flux in these hamsters.

Our study demonstrated that in addition to its effect on cholesterol absorption and hepatic and intestinal synthesis, physically refined rice bran oil significantly increased fecal sterol excretion, in particular, the excretion of fecal NS with respect to the effect of coconut oil with canola oil intermediate. There were no significant diet effects on fecal BA excretion. Our observation of increased NS excretion is consistent with that reported by others [8,59], where rice

bran oil feeding was associated with a greater reduction in serum TC and LDL-C, and an increase in fecal NS and BA excretion compared to a control diet with a similar fatty acid composition. Trautwein et al. [60] have shown in hamsters that sterols and stanols increase fecal cholesterol excretion but have no effect on fecal BA excretion. It is also consistent with the data of others in which dietary cholesterol caused a sixfold increase in NS output whereas BA output generally remained constant [61,62]. Studies in guinea pigs [63] have demonstrated that interventions such as dietary fiber that inhibit cholesterol absorption and increase fecal cholesterol excretion reduce hepatic stores of cholesterol and increase LDL-receptor activity.

The simplest explanation for the decrease in macrophage-derived foam cells that constitute the aortic fatty streak area for PRBO is the reduction in the LDL-C fraction. However, this does not explain the similar aortic fatty streaks of COCO and CANOLA despite widely differing TC and LDL-C levels. Other components in physically refined rice bran oil may be contributing to its antiatherogenic properties. For example, the ferulic acid component in the oryzanol fraction of physically refined rice bran oil has been shown to have antioxidant activity [27]. Similarly, hamsters fed diet containing purified oryzanol showed decreased fatty streaks as compared to controls [35]. Thus, it is possible that the prevention of fatty streak formation in PRBO hamsters could be associated with the inhibition of LDL oxidation by oryzanol, and in particular, the ferulic acid component. The sensitivity of the hamster to the antioxidant activity of tocopherol and probucol as it relates to LDL oxidation and early atherosclerosis has recently been demonstrated [64]. Similarly, tocotrienols from rice bran oil have been shown to reduce the degree of atherosclerotic lesion in apoE-deficient mice [65].

In summary, our studies demonstrate that relative to coconut oil, feeding physically refined RBO to hamsters can cause a reduction in plasma TC and LDL-C comparable or greater to that of canola oil-containing diets. This reduction in plasma cholesterol level is associated with a decrease in percent cholesterol absorption, a decreased hepatic HMG-CoA reductase activity, an increase in intestinal HMG-CoA reductase activity and an increase in fecal NS excretion in comparison to coconut oil fed animals. These metabolic changes in PRBO were also associated with a reduction in early atherosclerosis. The relatively greater unsaponifiable content present in the physically refined rice bran oil is believed to be a dominant factor responsible for the hypocholesterolemic and antiatherogenic properties, although the mechanisms involved have not been definitively shown.

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