Cholesterol-Lowering Effects of Rice Bran and Rice Bran Oil Fractions in Hypercholesterolemic Hamsters

T. S. KAHLON, R. M. SAUNDERS, R. N. SAYRE, F. I. CHOW, M. M. CHIU, and A. A. BETSCHART

The effects of temperature of extraction and fractionation of rice bran oil were evaluated alone or in combination with defatted rice bran (DFB) to determine the effects on their cholesterol-lowering potential in hypercholesterolemic hamsters. Diets containing full-fat rice bran (FFB), DFB plus rice bran oil (extracted at 4 or 54°C), and 54°C-extracted rice bran oil fractions (gum, wax, and degummed-dewaxed oil) at levels found in FFB were fed to 23-day-old hamsters. All diets contained 10% total dietary fiber, 9% fat, and 3% nitrogen. After 21 days, liver weights and plasma and liver cholesterol and plasma triglycerides were significantly higher in animals fed a diet of 0.3% cholesterol with cellulose (CC) than in animals fed a cholesterol-free cellulose diet. In cholesterol-fed hamsters, significantly lower plasma and liver cholesterol and triglycerides were observed in those fed an FFB diet than in those fed a CC diet. A diet consisting of DFB in combination with degummed-dewaxed rice bran oil resulted in significantly lower liver cholesterol levels than did the CC diet, whereas DFB alone, rice bran oil extracted at either temperature, or DFB plus gum or wax fractions were not significantly different from the respective corn oil controls. Only FFB lowered both plasma and liver cholesterol in hamsters. The data suggest that some of the cholesterol-lowering properties of FFB are present when DFB is recombined with degummed-dewaxed rice bran oil, but some active components appear to be either lost or are deactivated in the fractionation process.

MATERIALS AND METHODS

Male, 23-day-old Syrian golden hamsters (Simonsen Laboratories, Gilroy, CA) were kept individually in wire-bottom cages in a controlled environment (20-22°C, 60% rh, 12-hr light and dark cycles). Animals were assigned by selective randomization to 11 groups of 10 animals each, with test agent diets and water provided ad libitum for the 21-day feeding period. Feed consumption was measured twice a week and animals were weighed once a week. All animal procedures described were approved by the Animal Care and Use Committee of the Western Regional Research Center, USDA, Albany, CA, and conformed to the principles in “Guide for the Care and Use of Laboratory Animals” (Committee on Care and Use of Laboratory Animals 1985).

Stabilized rice bran (Randall et al 1985) obtained from a local milling company was extracted by soaking the rice bran with six changes of hexane at a mean temperature of 4°C for low-temperature extraction and 54°C for high-temperature extraction. The lower temperature was obtained by mixing frozen rice bran with chilled hexane in a 4°C room. The higher temperature resulted from pouring boiling hexane (65°C) over rice bran preheated to the same temperature and allowing the mixture to stand for 10 min (mean temperature 54°C). Hexane was removed from the oil by distillation. A portion of oil extracted at 54°C was heated to 60°C with 1% added water, left overnight at room temperature, and then centrifuged at 20,000 X g for 20 min at 20°C to obtain the gum fraction. Degummed oil was refrigerated (4°C) for four days and centrifuged at 20,000 X g for 20 min at 4°C to obtain the wax fraction. The dry matter contents of the gum and wax obtained were 96.2 and 94.9%, respectively. Defatted brans were desolventized by aerating overnight and heating at 110°C for 20 min. The original moisture content was maintained by adding water. Diet ingredients were analyzed for total dietary fiber (Prosky et al 1988), crude fat (AOAC 1990, method 920.39C), and nitrogen (Kjeldahl procedure). The composition of the full-fat and defatted rice brans is presented in Table 1.

All diets were formulated to contain 10% total dietary fiber, 9% fat, and 3% nitrogen. DFB fractions were incorporated in

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the diets at the same concentration at which they were present in the crude oil in the FFB diet. Corn oil was used as the supplementary source of fat. The composition of the diets is shown in Table II. Two diets were cholesterol-free and contained either 10% cellulose (C) or 50% stabilized, full-fat rice bran (RB). The remaining diets contained 0.3% cholesterol and either 10% cellulose (CC); 10% cellulose plus 9% rice bran oil extracted at 54°C (CCRO54); 10% cellulose plus 9% rice bran oil extracted at 4°C (CCRO4); 50% stabilized, full-fat rice bran (FFB); 41% defatted rice bran, 4°C (DFB4); 42% defatted rice bran, 54°C (DFB54); or 42% defatted rice bran, 54°C, plus either 0.9% rice bran oil gum (DFBG), 0.2% rice bran oil wax (DFBW), or 7.9% degummed-dewaxed rice bran oil (DFBO-WG). All diets were stored at 4°C.

After 21 days, the animals were fasted for 16 hr and anesthetized with CO2 for sample collection. Blood was drawn by cardiac puncture using ethylenediaminetetraacetic acid dipotassium salt (0.3 mg/ml plasma) as the anticoagulant and centrifuged at 1,300 × g for 20 min at 4°C to obtain plasma. Livers were excised, rinsed with water, blotted, weighed, and kept on dry ice. Livers and plasma aliquots were stored at −70°C. Plasma samples were analyzed by enzymatic colorimetric procedures for cholesterol (Sigma diagnostic kit 352, Sigma Chemicals, St. Louis, MO) and triglycerides (Gilford diagnostic kit 232422, Gilford Systems, Oberlin, OH). Plasma values were determined using standard curves obtained by running several concentrations of calibrators corresponding to the respective kits. Aliquots of liver were extracted by the procedure of Folch et al (1957) and analyzed for cholesterol and triglycerides as described previously (Kahlon et al 1990), using Sigma kit 352 for cholesterol and Sigma kit 405 for triglycerides. Liver values were determined from standard curves obtained by running National Bureau of Standards reference materials (cholesterol, SRM 911b; tripalmitin, SRM 1595) through the procedures as described for the samples. All samples were analyzed in triplicate. Data were statistically analyzed using Duncan's new multiple range test (Steel and Torrie 1960). A value of $P < 0.05$ was considered as the criterion of significance.

### RESULTS

Means and standard errors of means of initial weights (59.3 ± 0.3 g), final weights (121.4 ± 0.9 g), weight gains (3.0 ± <0.1 g per day), feed intakes (9.3 ± 0.1 g per day), and feed efficiencies (3.2 ± <0.1, feed/gain) were similar for all treatment groups. In hamsters fed 0.3% cholesterol diets, liver weights were significantly higher than in hamsters fed cholesterol-free diets (Table III). Among cholesterol-fed animals, liver weight per 100 g of fasting body weight was significantly lower with all treatments except DFB4 and DFBW, compared with those fed CC diets. Liver weights for FFB and DFBWO-WG groups also were significantly lower than for DFB4 and DFBW groups.

Plasma cholesterol levels were significantly higher in all cholesterol-fed animals than in those fed cholesterol-free diets (Table III). Animals fed full-fat rice bran with cholesterol (FFB) had significantly lower plasma cholesterol compared with the control (CC) and all other cholesterol-fed groups.

Plasma triglyceride levels were significantly elevated in hamsters fed 0.3% cholesterol diets for 21 days (Table III). Significantly lower plasma triglycerides were observed in groups fed full-fat rice bran with or without cholesterol (FFB and RB), compared with their respective cellulose controls (CC and C). Plasma triglycerides in the FFB group also were significantly lower than in the DFBW, DFBWO-WG, CCRO54, and CCRO4 groups.

Liver cholesterol (total and milligrams per gram) values were significantly elevated in all cholesterol-fed groups compared with cholesterol-free groups (C and RB). In animals fed cholesterol, liver cholesterol values were significantly lower in the FFB or

### TABLE I

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total Dietary Fiber (%)</th>
<th>Nitrogen (%)</th>
<th>Fat (%)</th>
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* Analyzed by the procedure of Kjeldahl procedure.
* Analyzed by ether extraction (AOAC 1990, method 920.39c).

### TABLE II

<table>
<thead>
<tr>
<th>Diet</th>
<th>Abbreviation</th>
<th>Cellulose</th>
<th>Rice Bran</th>
<th>Defatted Rice Bran</th>
<th>Corn Oil</th>
<th>Rice Bran Oil</th>
<th>Rice Bran Oil Gum</th>
<th>Rice Bran Oil Wax</th>
<th>Degummed-Dewaxed Rice Bran Oil</th>
<th>Casein</th>
<th>Corn Starch</th>
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<td>20.0</td>
<td>55.7</td>
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</tbody>
</table>

* All diets contained 3.5% mineral mix (American Institute of Nutrition [AIN] 1980), 1.0% vitamin mix (AIN 1980), 0.3% methionine, and 0.2% choline bitartrate.
* Diets were equal in total dietary fiber (10%), fat (9%), and nitrogen (3%).
* Defatted rice bran and rice bran oil were obtained by hexane extraction of full-fat rice bran at 4 or 54°C. Fatty acid composition of 4, 54°C, and defummed-dewaxed rice bran oil was similar with mean values: saturates, 18.1% (palmitic, 15.9%; stearic, 1.6%; arachidic, 0.6%); monounsaturates, 40.9% (oleic, 40.1%; vaccenic, 0.8%); and polyunsaturates, 40.3% (linoleic, 36.8%; linolenic, 1.5%).
* Rice bran oil extracted at 54°C contained 9.48% gum, 2.46% wax, and 88.07% degummed-dewaxed oil.
DFB-WG groups than in the CC control and all other cholesterol-fed groups. Significantly lower liver triglyceride values (total and milligrams per gram) were observed in the FFB, DFBG, and DFBW groups, compared with the CC group.

**DISCUSSION**

The significant elevation in liver weights in hamsters fed the CC diet compared with those fed the D diet is in agreement with our previous report (Kahlon et al 1991). Similar observations of increased liver weight with cholesterol feeding have been made in rats (Ayano et al 1980, Seetharamaiah and Chandrasekhar 1988). The increase in liver weight is apparently due to fatty infiltration (Beynen et al 1986, Chanutin and Ludewig 1933). Significantly lower liver weights in hamsters fed FFB, DFB-WG, or CCRO54 compared with liver weights in hamsters in the CC group suggest that FFB and some of its fractions may reduce liver lipid accumulation with hypercholesterolemic diets.

Significant plasma cholesterol reductions by stabilized FFB in cholesterol-fed hamsters is consistent with our previous reports (Kahlon et al 1990, 1991). Significantly lower plasma cholesterol values for the FFB group compared with any of the DFB and/or RBO fraction treatments suggest that intact RBO has hypocholesterolemic properties and also support the previous observation (Kahlon et al 1991) that the maximum cholesterol-lowering potential of rice bran is in the intact, stabilized FFB.

FFB also has been reported to lower serum cholesterol in cholesterol-fed chicks (Newman et al 1990). However, plasma cholesterol levels were not reduced by 50% rice bran diets in cholesterol-fed monkeys (Malinow et al 1976) nor in swine fed a 40% FFB diet (Mixon et al 1990). In human studies, Hesteg et al (1990) observed serum cholesterol reductions with stabilized rice bran in mildly hypercholesterolemic subjects, and Suzuki (1982) reported that supplementation of a hypercholesterolemic diet with unpolished rice resulted in lower serum cholesterol elevations. However, the consumption of 30 g of rice bran per day (Ranhotra et al 1989) or 60 g of rice bran per day (Kestin et al 1990) did not significantly lower blood total cholesterol levels in mildly hypercholesterolemic men.

RBO (extracted at 4 or 54°C) combined with cellulose resulted in similar plasma cholesterol levels compared with corn oil with cellulose, in agreement with our previous observation (Kahlon et al 1991), as well as that reported by Edwards and Radcliffe (1991). RBO has been reported to significantly lower cholesterol in rats (Sharma and Rukmini 1986, 1987), monkeys (Nicolosi et al 1991), and humans (Suzuki and Oshima 1970a, 1970b; Raghuram et al 1989). The lack of effect of extracted rice bran oil in our studies in hamsters may be attributable to the fact that we are comparing rice bran oil to corn oil, which may also have hypocholesterolemic properties. Nicolosi et al (1990) found that total cholesterol reductions were similar with RBO and corn oil in monkeys. With corn oil, both low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol were significantly reduced, whereas rice bran oil significantly lowered LDL cholesterol without significant reductions in HDL cholesterol. In the present study, HDL cholesterol data have not been included because of variable discrepancies in results obtained in three pilot studies with dextran sulfate or phosphotungstic acid-MgCl₂ precipitation methods compared to gradient density ultracentrifugal separation of HDL cholesterol.

Non-significant plasma cholesterol reductions with defatted rice bran diets (DFB4 or DFB54) in this study are in agreement with our previous reports (Kahlon et al 1990, 1991). Similar observations with DFB have been made in chicks (Newman et al 1990) and in swine (Mixon et al 1990). However, studies in rats have shown hypocholesterolemic effects with hemicellulose (Aoe et al 1989) and neutral detergent fiber from DFB (Ayano et al 1980).

Adding the gum or wax from RBO or degummed-dewaxed rice bran oil to DFB resulted in no additional plasma cholesterol-lowering effects. These results differ from other reports that showed serum cholesterol reductions with isolated fractions of RBO, such as wax fed to rats (Ishibashi and Yamamoto 1980), unsaponifiables (especially oryzanol) in rats (Sharma and Rukmini 1987; Seetharamaiah and Chandrasekhar 1988, 1989) or humans (Yoshino et al 1989), and B-sitosterol in humans (Best et al 1954).

The variability in response to rice bran and its fractions may be attributable to differences in the extraction and refining techniques employed, the dietary concentrations of the components tested, casein versus plant protein, and in the species used in the studies, as well as initial cholesterol status.

Plasma triglycerides were significantly elevated with cholesterol feeding, in agreement with our previous results (Kahlon et al 1991). Elevations in plasma triglycerides with cholesterol feeding may be due to inhibition of hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase (Singhal et al 1983). Hypertriglyceridemia in hamsters with HMG-CoA reductase inhibition has been reported (Anin et al 1988) where values increased from 318 to 2,752 mg/dl. FFB with or without cholesterol resulted in significantly lower plasma triglycerides compared to cellulose with or without cholesterol, respectively (FFB versus CC, RB versus C). This differs from our previous study (Kahlon et al 1991), as well as other reports (Malinow et al 1976, Hesteg et al 1990, Kestin et al 1990), where FFB had no significant plasma triglyceride-lowering effect. In the present study, plasma triglyceride levels were not influenced by rice bran oil with cellulose, which could be attributable to the fact that we are comparing rice bran oil to corn oil, which may also have hypocholesterolemic properties.

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**TABLE III**

<table>
<thead>
<tr>
<th>Diet</th>
<th>Abbreviation</th>
<th>Liver Weight (g)</th>
<th>Liver Fasting Body Weight (g)</th>
<th>Liver (mg/liver)</th>
<th>Liver (mg/g)</th>
<th>Cholesterol (mg/dl)</th>
<th>Plasma (mg/dl)</th>
<th>Cholesterol Triglycerides (mg/g)</th>
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<td>Cholesterol-free diets</td>
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<tr>
<td>Cellulose</td>
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<td>4.5±0.1 d</td>
<td>162±6.3 e</td>
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<td>RB</td>
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<td>2.2±0.1 d</td>
<td>278±26 f</td>
<td>43±3.9 b</td>
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<td>6.7±0.2 a</td>
<td>5.8±0.1 a</td>
<td>324±19.2 a-c</td>
<td>242±6.9 a-b</td>
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<td>71±12.0 a-b</td>
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<td>Rice bran</td>
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<td>282±11.5 c</td>
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<td>6.3±0.2 a-b</td>
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<td>298±13.8 bc</td>
<td>232±0.9 a-b</td>
<td>35.7±1.2 ab</td>
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<td>1,309±217 a</td>
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* Values are means±SEM; n = 10. Means with different letters within a column differ significantly (P<0.05).
is consistent with our previous results (Kahlon et al 1991). However, other investigators have reported significant serum triglyceride reductions with rice bran oil or oryzanol in humans (Raghuram et al 1989, Yoshino et al 1989) or with oryzanol in rats (Seetharamaiah and Chandrasekhar 1988).

The significant liver cholesterol reductions with full-fat rice bran in this study are consistent with our previous reports (Kahlon et al 1990, 1991) where full-fat rice bran resulted in significant liver cholesterol reductions in hamsters fed 0.5 or 0.3% cholesterol. No significant effect of DFB on liver cholesterol is consistent with a previous study (Kahlon et al 1991) but not with earlier results (Kahlon et al 1990), where defatted stabilized rice bran resulted in significant liver cholesterol reductions in 0.5% cholesterol-fed hamsters. The difference in effect appears to be attributable to the higher cholesterol content of the diets in the earlier study.

Adding degummed-dewaxed rice bran oil to defatted rice bran (DFBO-WG) resulted in a significant reduction in liver cholesterol compared to all cholesterol diets except FFB, whereas defatted rice bran fed separately, or in combination with rice oil gum or wax, resulted in no significant liver cholesterol reductions compared with the CC control. The data suggest that the cholesterol-lowering activity of rice bran oil is present in the degummed-dewaxed oil and that rice oil gum and wax have no cholesterol-lowering effects.

Non-significant liver cholesterol reductions with rice bran oil in combination with cellulose is consistent with our previous report (Kahlon et al 1991), although Seetharamaiah and Chandrasekhar (1989) and Sharma and Rukmini (1987) have observed significantly lower liver cholesterol in rats fed 10% RBO compared to peanut oil. Our consistent findings of no significant cholesterol reductions with crude RBO may be attributable to its comparison with corn oil or to its gum and wax content. The lack of effect of the wax fraction of RBO in our study is in disagreement with Ishibashi and Yamamoto (1980), who found that a 10% rice wax diet significantly lowered liver cholesterol in rats. The much lower level (0.2% of diet) of wax used in our study, which reflects the level found in the FFB diet, compared with a 50-fold higher wax content in the former study, may be partly responsible for this discrepancy.

Total liver triglycerides were significantly higher in the CC diet than in the C diet, in contrast to our previous study (Kahlon et al 1991) in which cholesterol feeding did not result in significant liver triglyceride elevations. Liver triglyceride concentrations were significantly lower with FFB or defatted rice bran combined with gum or wax from rice bran oil (DFBG or DFBW). In addition to the FFB, DFBG, and DFBW groups, total liver triglycerides also were significantly lower in the DFBO-WG group. The lack of effect by the DFBO-WG diet on liver triglyceride concentrations (milligrams per gram) may in part be due to significantly lower liver weight in this group. Seetharamaiah and Chandrasekhar (1988, 1989) reported reductions in liver triglyceride levels in rats with 0.5% oryzanol or 10% RBO; Sharma and Rukmini (1987) also observed liver triglyceride reductions with 10% RBO or with 0.4% unsaponifiables from RBO, and insignificant reductions with 10% RBO in an earlier study (Sharma and Rukmini 1986).

In conclusion, under the conditions of this study, only FFB significantly lowered both plasma and liver cholesterol in hypercholesterolemic hamsters. Degummed-dewaxed rice bran oil when recombined with defatted rice bran at levels found in FFB lowered liver cholesterol. Defatted rice bran, RBO extracted at 4 or 54°C, and wax or gum fractions of RBO had no significant influence on cholesterol status compared with the respective corn oil controls. The data suggest that some of the cholesterol-lowering properties of FFB are also present when degummed-dewaxed rice bran oil is recombined with defatted rice bran, but some active components appear to be either lost or are deactivated in the fractionation process.

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