

Rice bran oil and oryzanol reduce plasma lipid and lipoprotein cholesterol concentrations and aortic cholesterol ester accumulation to a greater extent than ferulic acid in hypercholesterolemic hamsters[☆]

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Abstract

Our laboratory has reported that the hypolipidemic effect of rice bran oil (RBO) is not entirely explained by its fatty acid composition. Because RBO has a greater content of the unsaponifiables, which also lower cholesterol compared to most vegetable oils, we wanted to know whether oryzanol or ferulic acid, two major unsaponifiables in RBO, has a greater cholesterol-lowering activity. Forty-eight F₁B Golden Syrian hamsters (*Mesocricetus auratus*) (BioBreeders, Watertown, MA) were group housed (three per cage) in cages with bedding in an air-conditioned facility maintained on a 12-h light/dark cycle. The hamsters were fed a chow-based hypercholesterolemic diet (HCD) containing 10% coconut oil and 0.1% cholesterol for 2 weeks, at which time they were bled after an overnight fast (16 h) and segregated into 4 groups of 12 with similar plasma cholesterol concentrations. Group 1 (control) continued on the HCD, group 2 was fed the HCD containing 10% RBO in place of coconut oil, group 3 was fed the HCD plus 0.5% ferulic acid and group 4 was fed the HCD plus 0.5% oryzanol for an additional 10 weeks. After 10 weeks on the diets, plasma total cholesterol (TC) and non-high-density lipoprotein cholesterol (HDL-C) (very low- and low-density lipoprotein) concentrations were significantly lower in the RBO (−64% and −70%, respectively), the ferulic acid (−22% and −24%, respectively) and the oryzanol (−70% and −77%, respectively) diets compared to control. Plasma TC and non-HDL-C concentrations were also significantly lower in the RBO (−53% and −61%, respectively) and oryzanol (−61% and −70%, respectively) diets compared to the ferulic acid. Compared to control and ferulic acid, plasma HDL-C concentrations were significantly higher in the RBO (10% and 20%, respectively) and oryzanol (13% and 24%, respectively) diets. The ferulic acid diet had significantly lower plasma HDL-C concentrations compared to the control (−9%). The RBO and oryzanol diets were significantly lower for plasma triglyceride concentrations compared to the control (−53% and −65%, respectively) and ferulic acid (−47% and −60%, respectively) diets. Hamsters fed the control and ferulic acid diets had significantly higher plasma vitamin E concentrations compared to the RBO (201% and 161%, respectively) and oryzanol (548% and 462%, respectively) diets; the ferulic acid and oryzanol diets had significantly lower plasma lipid hydroperoxide levels than the control (−57% and −46%, respectively) diet. The oryzanol-fed hamsters excreted significantly more coprostanol and cholesterol in their feces than the ferulic acid (127% and 120%, respectively) diet. The control diet had significantly greater aortic TC and FC accumulation compared to the RBO (115% and 89%, respectively), ferulic acid (48% and 58%, respectively) and the oryzanol (74% and 70%, respectively) diets. However, only the RBO and oryzanol diets had significantly lower aortic cholesterol ester accumulation compared to the control (−73% and −46%, respectively) diet. The present study suggests that at equal dietary levels, oryzanol has a greater effect on lowering plasma non-HDL-C levels and raising plasma HDL-C than ferulic acid, possibly through a greater extent to increase fecal excretion of cholesterol and its metabolites. However, ferulic acid may have a greater antioxidant capacity via its ability to maintain serum vitamin E levels compared to RBO and oryzanol. Thus, both oryzanol and ferulic acid may exert similar antiatherogenic properties, but through different mechanisms.

Keywords: Rice bran oil; Oryzanol; Ferulic acid; Plasma cholesterol; Aortic cholesterol

1. Introduction

There have been numerous studies in humans and animals that have demonstrated that oils containing saturated fatty acids (SATS) raise serum total cholesterol (TC) and,

in particular, low-density lipoprotein cholesterol (LDL-C) levels [1–3], whereas those enriched in unsaturated fatty acids [1–9] lower LDL-C when replacing saturated fat. In general, the predictive equations of Keys et al. [2] and Hegsted et al. [3] demonstrated that the fatty acid components and cholesterol in the diet are the primary determinants of diet-induced hypo- or hypercholesterolemia. However, a review of several studies also indicated a hypocholesterolemic effect of some unsaponifiables, in particular, plant sterol components [10]. Several investigators reported that not only can plant sterols significantly lower LDL-C levels even at relatively low intakes [11–16], but also some plant sterols are more active than others [14–16]. In addition, cholesterol-lowering effects of other unsaponifiables such as tocotrienols [17–21], analogs of tocopherol and oryzanol, a ferulate ester of phytosterols and triterpene alcohols, also have been reported [22–26].

Particularly germane to the study reported in this communication is the finding that crude rice bran oil (CRBO) contains an unusually high content of unsaponifiables (up to 4.4%) [27], which is several-fold greater than most other vegetable oils. The unsaponifiables of CRBO are composed of plant sterols (43%), 4-methyl sterols (10%), triterpene alcohols (29%) and less polar components such as squalene or tocotrienols (19%) [28]. In addition, rice bran oil (RBO) contains up to 20% SATS and approximately equal amounts of polyunsaturated (40%) and monounsaturated fatty acids (40%) [29], a fatty acid profile quite different from other often-utilized hypocholesterolemic vegetable oils. In recently reported studies, the hypocholesterolemic action of RBO has been attributed to its yet poorly characterized unsaponifiables [30,31]. For example, studies in rats [32,33] have shown that the unsaponifiables of RBO lowered serum TC and LDL-C and raised high-density lipoprotein cholesterol (HDL-C), and that these alterations in lipoprotein cholesterol were associated with increased fecal excretion of neutral sterols and total bile acids. Our first study in monkeys confirmed the LDL-C and apo B-lowering properties of RBO and suggested that the fatty acid composition of RBO did not entirely explain its cholesterol-lowering properties [34]. One study in humans fed 35–40 g of either RBO or some combination of coconut oil, palm oil or ground nut oil showed 25–30% reductions in serum TC levels in the RBO group [35]. However, this study needs to be qualified because of the highly SATS nature of the oils used and the limited information on ground nut oil. A report of a human trial of RBO [36] compared to other vegetable oils also demonstrated significant serum LDL-C-lowering properties of RBO, which was not entirely explained by the predictive equations of either Keys et al. [2] or Hegsted et al. [3] based on the fatty acid composition of RBO. Thus, the reports in the literature suggest a significant contribution of the unsaponifiable fraction of RBO to its cholesterol-lowering properties. The aim of the current study is to know whether oryzanol or ferulic acid, two major unsaponifiable components of RBO, have a greater cholesterol-lowering

activity and possibly different mechanism(s) of action with regard to their potential antiatherosclerotic property.

2. Materials and methods

2.1. Animals and experimental design

Forty-eight F₁B Golden Syrian hamsters (*Mesocricetus auratus*) (BioBreeders, Watertown, MA) were used. They were group housed (three per cage) in hanging cages with bedding in a temperature-controlled room (25°C) maintained on a 12-h light/dark cycle. Hamsters were given food and water ad libitum. Hamsters were fed Purina Rodent Chow (Ralston Purina, St. Louis, MO) for 1 week in order to acclimate them to the facility. The hamsters were then fed a nonpurified hypercholesterolemic diet (HCD) containing 10% coconut oil and 0.1% cholesterol for 2 weeks, at which time they were bled after an overnight fast (16 h) and segregated into 4 groups of 12 with similar plasma cholesterol concentrations. A nonpurified diet, rather than a semipurified diet, was used because published data from our laboratory [37] and those from another [38] indicated that hamsters on the nonpurified diet are more responsive to various cholesterol-lowering interventions and a resultant lipoprotein profile (non-HDL-C > HDL-C), which is similar to that of humans. Group 1 was continued on the HCD, group 2 was fed the HCD containing 10% crude RBO (Riceland Foods, Stuttgart, AR) in place of the coconut oil, group 3 was fed the HCD containing 0.5% ferulic acid (>99% pure by HPLC from Sigma-Aldrich, St. Louis, MO) and group 4 was fed the HCD containing 0.5% oryzanol (TSUNO, Osaka, Japan). The RBO contained 15.8 mg of oryzanol per gram of RBO and 500 µg of tocopherols per gram of RBO. The major tocopherols were α-tocopherol and α-tocotrienol. The amount of oryzanol used in the current study is similar to the amount used in a previous study from our laboratory [39]. The oryzanol used in the current study contained approximately 16% campesterol and 7% β-sitosterol, 30% cycloartenol, 23% 24-methylene-cycloartenol and 22% cyclobranol esters with ferulic acid. Treatment diets were fed for 10 weeks. Food disappearance and body weights were monitored on a weekly basis. The animals were maintained in accordance with the guidelines of the Committee on Animal Care of the University of Massachusetts Lowell Research Foundation and the guidelines prepared by the Committee on Care in Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (DHEW publication no. 85-23, revised 1985).

2.2. Plasma lipid determinations

Blood samples were taken at 8 and 10 weeks from food-deprived hamsters (12 h) and collected via the retroorbital sinus into heparinized capillary tubes under ultrapure CO₂/O₂ (50:50) gas (Northeast Airgas, Salem, NH) anesthesia. Plasma was harvested after centrifugation at 1500×g at room temperature for 20 min, and plasma TC [40] and

Table 1

Initial and final body weights (grams) and food consumption (grams per day) of hamsters after 10 weeks of dietary treatment

Diet	Initial body weight	Final body weight	Food consumption
Coconut oil	93.5±8.69	125.5±23.9	14.7±2.20
RBO	91.5±11.7	130.2±24.8	14.9±1.41
Ferulic acid	94.7±11.2	129.4±24.5	14.7±1.28
Oryzanol	95.2±8.20	128.0±27.3	14.9±2.13

Values are expressed as mean±S.D., $n=12$.

ANOVA with Student–Newman–Keuls was used.

triglycerides (TGs) [41] concentrations were measured enzymatically. Plasma very low-density lipoprotein cholesterol (VLDL-C) and LDL-C, which we combined and termed *non-HDL-C*, were precipitated with phosphotungstate reagent [42], and HDL-C was measured in the supernatant. The concentration of non-HDL-C was calculated as the difference between plasma TC and HDL-C.

2.3. Plasma vitamin E analyses

Plasma vitamin E concentrations were determined by adding 200 μ l of plasma with 10 μ l of retinyl acetate (internal standard, 10 μ g/ml) with 200 μ l of ethanol containing butylated hydroxytoluene (BHT) (10 mg/L) and 1.0 ml of hexane followed by vortex mixing. The samples were centrifuged at 500 \times g for 5 min, and the organic layer was transferred to a 7.0-ml brown borosilicate screw top vial. The sample residues were reextracted with 1.0 ml of hexane, and the organic layers were combined. The organic layer was evaporated under N_2 and reconstituted with 200 μ l of ethanol containing BHT (10 mg/dl) and injected into an HPLC. The HPLC system is a Model 5600 CoulArray eight-channel system with two Model 580 pumps, a high-pressure gradient mixer, a PEEK pulse damper, a Model 540 autoinjector, a CoulArray Thermostatic Chamber and a serial array of eight coulometric electrodes (ESA Laboratories, Chelmsford, MA). The column is a 3.0 \times 150-mm, 3- μ M, Supelcosil LC-18 (Supelco, Bellefonte, PA). The mobile phase consisted of methanol/1 M propanol/1 M ammonium acetate (78:20:2 vol/vol/vol) at a flow rate of 0.8 ml/min. The concentration of vitamin E was determined by external standardization using purified solutions (Sigma, St. Louis, MO).

2.4. Plasma lipid hydroperoxide analyses

Cayman Chemical Company's Lipid Hydroperoxide Assay Kit was used to measure plasma lipid hydroperoxides

Table 2

Plasma lipids and lipoprotein cholesterol concentrations (millimoles per liter) in hamsters fed treatment diets for 10 weeks (average of weeks 8 and 10 bleeds)

Diet	TC	non-HDL-C	HDL-C	TC/HDL-C	TG
Coconut oil	16.32±0.49 ^a	14.97±0.49 ^a	1.35±0.04 ^a	12.41±0.47 ^a	7.40±0.44 ^a
RBO	5.95±0.53 ^b	4.47±0.54 ^b	1.48±0.02 ^b	4.05±0.39 ^b	3.45±0.44 ^b
Ferulic acid	12.67±0.35 ^c	11.44±0.38 ^c	1.23±0.05 ^c	10.88±0.77 ^c	6.55±0.61 ^a
Oryzanol	4.93±0.21 ^b	3.40±0.21 ^b	1.53±0.04 ^b	3.33±0.21 ^b	2.61±0.15 ^b

Values are means±S.E.M., $n=12$.

Values in a column not sharing a superscript are significantly different at $P<.05$.

ANOVA with Student–Newman–Keuls was used.

(LPHs) by ELISA [43]. This assay measures the hydroperoxides directly utilizing the redox reactions with ferrous ion. The resulting ferric ions were detected using thiocyanate ion as the chromogen.

2.5. Aortic tissue collection, aortic cholesterol extractions and measurements

At the time of sacrifice, week 10, hamsters were anesthetized with an intraperitoneal injection of sodium pentobarbital (62.5 mg/ml at a dosage of 0.2–0.25 ml/200 g body weight) (Henry Schein, Port Washington, NY), and aortic tissue was obtained for aortic cholesterol analysis as described [44]. A pilot study was conducted to evaluate the extent to which this procedure removed tissue cholesterol. Aortic cholesterol concentrations were determined after tissue was placed in solvent (4 ml of methanol and 8 ml of chloroform) overnight with frequent vigorous mixing and compared with the concentrations obtained following tissue minced or homogenization as reported [45]. No significant differences in aortic cholesterol content were observed between the different cholesterol extraction procedures.

2.6. Fecal neutral sterol measurements

Fecal samples were collected over the final 3 days of the exposure period, freeze dried (lyophilized) and ground prior to analysis as described [44]. Extraction efficiency for neutral sterols following this protocol was approximately 94%.

2.7. Statistical analysis

SigmaStat software was used for all statistical evaluations (Jandel Scientific, San Rafael, CA) [46]. A repeated measures two-way analysis of variance (ANOVA) was used to analyze plasma lipid and lipoprotein cholesterol data between treatment groups and time of measurements. A one-way ANOVA was performed between treatment groups for all other data. When statistical significance was found by ANOVA, the Student–Newman–Keuls separation of means was used to determine group differences. All values were expressed as mean±S.E.M., and statistical significance was set at the minimum $P<.05$.

3. Results

All hamsters in each group survived for the entire length of the study. No significant differences were observed

Table 3

Plasma γ - and α -tocopherol (millimoles per liter) and LHP (micromoles per liter) concentrations in hamsters fed the treatment diets for 10 weeks

Diet	γ -Tocopherol	α -Tocopherol	LHP
Coconut oil	4.36±0.52 ^a	8.54±0.51 ^a	6.15±2.68 ^a
RBO	1.06±0.29 ^b	3.23±0.24 ^b	5.69±3.01 ^{ab}
Ferulic acid	3.47±0.31 ^a	7.71±0.45 ^a	2.65±0.89 ^b
Oryzanol	0.44±0.27 ^b	1.55±0.15 ^b	3.29±1.59 ^b

Values are mean±S.E.M., $n=12$.

Values in a column not sharing a superscript are significantly different at $P<.05$.

ANOVA with Student–Newman–Keuls was used.

between dietary treatments for initial or final body weights. All groups did gain significant amounts of body weight during the 10-week study ($P<.05$) (average weight gain of 65% for all treatments) (Table 1). At the same time, no significant difference for food consumption between the treatment diets was observed (Table 1).

Plasma lipid and lipoprotein cholesterol concentrations between weeks 8 and 10 were not significantly different within dietary treatments, and therefore, the values were averaged (Table 2). Plasma TC and non-HDL-C (very low- and low-density lipoprotein) concentrations were significantly lower ($P<.05$) in the hamsters fed the RBO (−64% and −70%, respectively), the ferulic acid (−22% and −24%, respectively) and the oryzanol (−70% and −77%, respectively) diets compared to the coconut oil-fed hamsters (Table 2). Plasma TC and non-HDL-C concentrations were significantly lower ($P<.05$) in the hamsters fed the RBO (−53% and −61%, respectively) and the oryzanol (−61% and −70%, respectively) diets compared to the ferulic acid-fed hamsters (Table 2). Compared to the coconut oil- and ferulic acid-fed hamsters, plasma HDL-C concentrations were significantly higher ($P<.05$) in hamsters fed the RBO (10% and 20%, respectively) and oryzanol (13% and 24%, respectively) diets (Table 2). Also, the hamsters fed the ferulic acid diet had significantly lower ($P<.05$) plasma HDL-C concentrations compared to the coconut oil-fed hamsters (−9%) (Table 2). The plasma TC/HDL-C ratio followed the same pattern as the plasma TC. Plasma TC/HDL-C ratio was significantly lower ($P<.05$) in the hamsters fed the RBO, ferulic acid and the oryzanol diets compared to the coconut oil-fed hamsters (Table 2). The plasma TC/HDL-C ratio was significantly lower ($P<.05$) in the hamsters fed the RBO and

the oryzanol diets compared to the ferulic acid-fed hamsters (Table 2). The hamsters fed the RBO and oryzanol diets had significantly lower ($P<.05$) plasma TG concentrations compared to the hamsters fed the coconut oil (−53% and −65%, respectively) and ferulic acid (−47% and −60%, respectively) diets (Table 2). The hamsters fed the RBO and oryzanol diets were not significantly different from each other, nor were the hamsters fed the coconut oil and ferulic acid diets significantly different from each other with regard to plasma TG concentrations (Table 2).

The plasma γ -tocopherol concentrations were significantly higher ($P<.05$) in the coconut oil- and ferulic acid-fed hamsters compared to the hamsters fed the RBO (311% and 227%, respectively) and oryzanol (891% and 689%, respectively) (Table 3). The hamsters fed the RBO and oryzanol diets were not significantly different from each other, nor were the hamsters fed the coconut oil and ferulic acid diets significantly different from each other for plasma γ -tocopherol concentrations (Table 3). The same pattern was observed for plasma α -tocopherol concentrations. Plasma α -tocopherol concentrations were significantly higher ($P<.05$) in the coconut oil- and ferulic acid-fed hamsters compared to the hamsters fed the RBO (164% and 139%, respectively) and oryzanol (451% and 397%, respectively) diets (Table 3). The hamsters fed the RBO and oryzanol diets again were not significantly different from each other, nor were the hamsters fed the coconut oil and ferulic acid diets significantly different from each other for plasma α -tocopherol concentrations (Table 3). Even though the coconut oil-fed hamsters had significantly higher plasma tocopherol concentrations, they had significantly higher ($P<.05$) plasma LPH concentrations compared to the hamsters fed the ferulic acid (132%) and oryzanol (87%) diets (Table 3). Although not significant, the hamsters fed the RBO had higher plasma LPH concentrations compared to the hamsters fed the ferulic acid (115%) and oryzanol (73%) diets (Table 3). The hamsters fed the ferulic acid and oryzanol diets were not significantly different from each other, nor were the hamsters fed the coconut oil and RBO diets for plasma LPH concentrations (Table 3).

Hamsters fed the ferulic acid diet excreted significantly less ($P<.05$) total fecal neutral sterols compared to the hamsters fed the coconut oil (−41%), RBO (48%) or

Table 4

Fecal neutral sterols concentrations (milligrams per gram of dry feces) of hamsters fed the treatment diets for 10 weeks

Neutral sterol	Coconut oil	RBO	Ferulic acid	Oryzanol
Coprostanol	1.25±0.14 ^{ab}	1.22±0.36 ^{ab}	0.84±0.14 ^a	1.91±0.55 ^b
Coprostanone	0.10±0.02	0.15±0.04	0.05±0.02	0.19±0.09
Cholesterol	0.64±0.04 ^a	0.60±0.11 ^a	0.35±0.06 ^b	0.77±0.25 ^a
Campesterol	1.79±0.17 ^{ab}	2.27±0.46 ^a	1.03±0.24 ^b	1.72±0.45 ^{ab}
Stigmasterol	0.23±0.02 ^{ab}	0.31±0.05 ^a	0.14±0.02 ^b	0.30±0.15 ^{ab}
Sitosterol	0.74±0.06 ^{ab}	0.97±0.25 ^a	0.41±0.09 ^b	0.86±0.19 ^{ab}
Sitostanol	0.52±0.04	0.48±0.09	0.31±0.08	0.49±0.09
Total	5.28±0.46 ^a	6.00±1.05 ^a	3.13±0.63 ^b	6.24±1.48 ^a

Values are means±S.E.M., $n=4$ pools of 3 animals each.

ANOVA with Student–Newman–Keuls was used.

Table 5

Total cholesterol, FC and CE concentration (micrograms per milligram of tissue) in ascending aortas of hamsters fed the treatment diets for 10 weeks

Diet	TC	FC	CE	FC/CE
Coconut oil	4.65±0.42 ^a	3.48±0.45 ^a	1.17±0.29 ^a	2.71±0.11 ^a
RBO	2.16±0.31 ^b	1.84±0.33 ^b	0.32±0.09 ^b	5.69±1.98 ^b
Ferulic acid	3.14±0.21 ^b	2.20±0.28 ^b	0.94±0.28 ^{ab}	2.31±0.16 ^a
Oryzanol	2.68±0.33 ^b	2.05±0.37 ^b	0.63±0.18 ^b	3.28±0.64 ^{ab}

Values are mean±S.E.M., *n* = 12.

Values in a column not sharing a superscript are significantly different at *P* < .05.

ANOVA with Student–Newman–Keuls was used.

oryzanol (−50%) diets (Table 4). No other dietary treatments were significantly different from each other for total fecal sterol excretion (Table 4). Hamsters fed the oryzanol diet excreted significantly more (*P* < .05) fecal coprostanol and cholesterol, the two major components of cholesterol excretion in feces, compared to the hamsters fed the ferulic acid (127% and 120%, respectively) diet (Table 4). Hamsters fed the coconut oil and RBO diets excreted significantly more (*P* < .05) fecal cholesterol only compared to hamsters fed the ferulic acid (83% and 71%, respectively) (Table 4). The hamsters fed the RBO diet excreted significantly more (*P* < .05) fecal campesterol, stigmaterol and β-sitosterol compared to the hamsters fed the ferulic acid (120%, 121% and 137%, respectively) diet (Table 4).

Despite having higher plasma vitamin E concentrations and excreting high levels of fecal cholesterol, the coconut oil-fed hamsters had significantly higher (*P* < .05) levels of aortic TC and free cholesterol (FC) compared to the hamsters fed the RBO (115% and 89%, respectively), the ferulic acid (48% and 58%, respectively) and the oryzanol (74% and 70%, respectively) diets (Table 5). Also, the hamsters fed the RBO and oryzanol diets had significantly lower (*P* < .05) aortic cholesterol ester compared to the hamsters fed the coconut oil (−73% and −46%, respectively) diet (Table 5). The hamsters fed the RBO, ferulic acid and oryzanol diets were not significantly different from each other for aortic TC, FC or esterified cholesterol concentrations (Table 5). Also, aortic FC/ester cholesterol (EC) ratio in the aortas of hamsters fed RBO was significantly higher than in those fed the coconut oil and ferulic acid diets (Table 5). No other dietary treatments were significantly different from each other for aortic free to ester ratio (Table 5).

4. Discussion

The current study was designed to examine (a) the cholesterol-lowering and antiatherosclerotic activity and possible mechanism(s) of RBO, (b) the cholesterol-lowering and antiatherosclerotic activity and possible mechanism(s) of oryzanol and ferulic acid and (c) which component of RBO unsaponifiables is more efficacious, oryzanol or ferulic acid at equal dietary levels. There are abundant data available from studies with human subjects [1–7], nonhuman primates [47,48] and appropriate rodent species [49,50], which demonstrate the primary effects of SATS and dietary cholesterol upon the circulating levels of lipoproteins.

Despite RBO containing higher amounts of saturated fat and lesser amounts of mono- and polyunsaturated fats than other often-utilized hypocholesterolemic vegetable oils, the reductions in plasma non-HDL-C concentrations observed in the current study were comparable to those of other studies when RBO replaces other oils [35,36,51]. Previous studies indicated that the unsaponifiable component of RBO, that is, the plant sterols and oryzanol, are major cholesterol-lowering factors in RBO [22–26]. In addition, RBO also contains tocotrienols, which were reported to inhibit cholesterol synthesis [17–21]. Thus, it is impossible to state with any certainty which unsaponifiable component or a combination of all three is the contributing component to the hypocholesterolemic response of RBO. In a human study by Lichtenstein et al. [36], in which RBO was compared to other vegetable oils as part of the AHA Step II diet, they also showed the cholesterol-lowering property of RBO. However, in that study [36], plasma HDL-C was decreased with the consumption of RBO, thereby not improving the TC/HDL-C ratio, which was not the case in the current study.

In the current study, we observed that both 0.5% oryzanol and 0.5% ferulic acid lowered plasma TC and non-HDL-C compared to the control hamsters; however, the hamsters fed the oryzanol diet exhibited significantly lowered plasma TC and non-HDL-C than did ferulic acid. Also, the amount of lowering by oryzanol was similar to RBO in the current study. The cholesterol-lowering activity of oryzanol has been demonstrated by other investigators [24–26,52–55]. Shinomiya et al. [26] showed that when 0.5% oryzanol was fed to rats on a high cholesterol diet, plasma TC was significantly decreased after 8 weeks on the dietary treatment; however, plasma LDL-C and VLDL-C were not. In a study reported by Seetharamaiah and Chandrasekhara [54], plasma non-HDL-C was significantly reduced in rats fed 0.2% or more oryzanol after 7 weeks of dietary treatment. They also showed that when oryzanol was added to a cholesterol-free diet, no differences in plasma lipids were observed, suggesting that oryzanol may affect cholesterol metabolism by altering dietary cholesterol absorption.

In the current study, we observed that hamsters fed 0.5% oryzanol increased their fecal excretion of coprostanol and cholesterol, thus, TC excretion, compared to the hamsters fed the ferulic acid diet but not to the control. The hamsters fed the RBO diet excreted more fecal cholesterol than the hamsters fed the ferulic acid diet. Thus, it appears that a major mechanism by which oryzanol and possibly RBO

lower blood cholesterol concentrations is via increased fecal excretion of cholesterol and its metabolic products. Previous studies have also shown that oryzanol and RBO increase fecal excretion of cholesterol. In one study [55], when oryzanol was injected (intraperitoneally) to mice, it caused an increased excretion of fecal sterols-¹⁴C and total bile acids-¹⁴C derived from cholesterol-¹⁴C injection. A previous study by our laboratory [39] demonstrated that oryzanol feeding in hamsters caused a decreased dietary cholesterol absorption, and that this suppressed cholesterol absorption was at least partially responsible for the lower plasma TC and non-HDL-C concentrations that were observed. Another study by our laboratory [56] demonstrated that RBO feeding prevented hypercholesterolemia induced by a high-cholesterol diet in hamsters by suppressing cholesterol absorption and enhancing fecal sterol excretion. It has also been reported that feeding the unsaponifiable lipids extracted from RBO to rats caused an increase in fecal sterol excretion [57]. Although it appears that the blood cholesterol-lowering activity of oryzanol is predominantly through prevention of cholesterol absorption and increased fecal excretion, the blood cholesterol-lowering mechanism of ferulic acid remains unknown at this time.

The current study showed that feeding 0.5% ferulic acid and oryzanol reduced plasma LPH formation compared to the control diet, whereas RBO feeding did not. Previous work showed that ferulate [58] and oryzanol [59] possess antioxidant activity. Andreasen et al. [60] demonstrated a significant antioxidant activity of ferulate from rye in inhibiting low-density lipoprotein oxidation. Oryzanol has been shown to inhibit linoleic acid oxidation [61,62] and cholesterol oxidation [63] to a greater extent than the vitamin E components in other studies. Furthermore, oryzanol components, namely, cycloartenyl ferulate and 24-methyl-encycloartenyl ferulate, were shown to act as antioxidants in methyl linoleate bulk and multiphase lipid systems and as radical scavengers [64]. Also in the current study, at this dietary level, ferulic acid feeding prevented a significant reduction in plasma vitamin E concentrations compared to RBO and oryzanol feeding. It is unknown at this time why RBO and oryzanol-feeding would result in decreases in plasma vitamin E concentrations, and why this decrease in plasma vitamin E concentrations in the oryzanol-fed hamsters still produced a significant decrease in plasma LPH concentrations. One possible reason for the reduction in plasma vitamin E concentrations in hamsters fed the RBO diet is that the crude RBO we used may contain ingredients that inhibit vitamin E absorption, along with cholesterol. Dietary ingredients that inhibit cholesterol absorption, possibly through disruption of the formation of micelles [65,66], may also inhibit the absorption of other lipid soluble products, including vitamin E. The same may be true for the hamsters fed the oryzanol diet. Because ferulic acid feeding did not change cholesterol excretion, it probably did not disrupt micelle formation and, thus, did not inhibit vitamin E absorption. Another possible explanation of why

the oryzanol produced significantly lower plasma LPHs while reducing plasma vitamin E levels simultaneously is the possibility that oryzanol is metabolized by digestive enzymes in the GI tract of hamsters into free ferulate and free sterols [67], of which the free ferulate is then absorbed and acts as an antioxidant within the plasma, and the free sterol inhibits the cholesterol absorption within the GI tract, thereby lowering blood cholesterol levels [68,69].

The current study also showed that the feeding of RBO, oryzanol and ferulic acid decreased the amount of cholesterol accumulation in the aortic arch of hamsters compared to control. Although all three treatments reduced TC and FC accumulation, only the RBO and oryzanol treatments significantly reduced cholesterol ester accumulation compared to control. A recent study published by our laboratory in hamsters demonstrated that the reductions in plasma non-HDL-C concentrations with oryzanol feeding were associated with similar decreases in aortic fatty streak formation [39].

In conclusion, the current study suggests that at equal dietary levels, oryzanol has a greater effect on lowering plasma non-HDL-C levels and raising plasma HDL-C compared to ferulic acid, possibly through a greater extent to increase fecal excretion of cholesterol and its metabolites. However, ferulic acid may have a greater antioxidant capacity by maintaining plasma vitamin E levels, whereas oryzanol and RBO reduce it. Thus, although both oryzanol and ferulic acid may have similar antiatherogenic properties, as shown by reductions in aortic cholesterol accumulation, it appears that their antiatherosclerotic potentials are through several, yet some different, mechanisms of action. It is also noteworthy that the greatest effect on aortic cholesterol accumulation was due to RBO.

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