ORIGINAL ARTICLE

Effect of low calorie diet with rice bran oil on cardiovascular risk factors in hyperlipidemic patients

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Background: Cardiovascular disease remains the leading cause of death and disability in industrialized and developing countries. The aim of this research was to determine the effect of rice bran oil, with a low-calorie diet, on lipid profiles, in hyperlipidemic patients. **Materials and Methods:** This study was a parallel groups' randomized clinical trial with a pre- and post-test design. Fifty hyperlipidemic patients of both sexes and age range of 25 – 65 years had participated. The patients received a low-calorie diet based on 1400 kcal energy, 17% protein, 26% fat, and 57% carbohydrate per day for four weeks. The treatment group received a low-calorie diet including rice bran oil (30 g / day). Blood samples were obtained after an overnight (12 hours) fasting period before the study and on the last day of the intervention period. Anthropometric indices and levels of serum triacylglycerol, total cholesterol, low-density lipoprotein, and high-density lipoprotein were measured before and after the intervention. **Results:** In both groups, weight, body mass index, waist, and hip circumferences were significantly reduced ($P < 0.05$) after four weeks. In comparison with the control group, the results of treatment with rice bran oil, with a low-calorie diet, showed that at the end of the fourth week, total cholesterol, low-density lipoprotein, and atherogenic ratio of total cholesterol / high-density lipoprotein were significantly decreased ($P < 0.05$). **Conclusions:** The results confirm that rice bran oil, when consumed as part of a healthy diet, is effective in improving risk factors for cardiovascular disease.

Key words: Cardiovascular disease, diet, low density lipoprotein, rice bran oil, total cholesterol

INTRODUCTION

Cardiovascular diseases (CVD) have emerged as a major health burden worldwide in recent times. Observational studies on distinct populations support the existence of a linear relationship between plasma lipid levels and cardiovascular disease-induced death rate. This is found to be true, even if the elevated plasma cholesterol is at the borderline level.[1] The composition of the diet plays an important role in the management of lipid and lipoprotein concentrations in the blood.[2] Rice bran oil (RBO) is now emerging as an everyday oil.[3] Rice bran is a by-product of the rice milling industry, separated from the white portion of the rice in the polishing process. Human consumption has been traditionally limited because of the bran's instability and the rapid onset of rancidity produced by a high lipase activity in the bran causing deterioration of the lipids.[4] However, improved oil extraction methods have reduced this problem.[5] Also, high levels of unsaponifiable material are found in RBO.[6] This is composed of plant sterols, triterpene alcohols, ferulic acid esters (γoryzanol), and vitamin E isomers (tocopherols and tocotrienols). In addition, RBO contains up to 20% saturated fatty acids (SFA) and equal amounts of mono unsaturated fatty acids (MUFA) and poly unsaturated fatty acids (PUFA).[3] RBO has a mild flavor and high smoking point, making it suitable for use in many cooking methods.[7] Consumption of a diet with RBO induces a decrease in the plasma level of total cholesterol (TC) and low-density lipoprotein (LDL) in healthy subjects,[8-10] in hyperlipidemic patients,[11,12] in nephrotic syndrome children,[13] and also in hamsters,[14] but there has been no significant effect on plasma triacylglycerol (TAG).[9] However, a three-month diet supplemented with RBO in hyperlipidemic patients showed a significant reduction in plasma TAG, but high-density lipoprotein (HDL) and LDL levels were unchanged.[15] Moreover, in a recent study[16] on mildly hypercholesterolemic individuals, a dietary intervention of RBO spread over twelve weeks was reported to have had no significant change on TAG and HDL, although Lichtenstein *et al*.[17] observed that the dietary supplement of RBO in middle-aged and elderly subjects for 32 days

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resulted in a significant reduction in HDL. In our previous studies, a low-calorie diet with soy protein in hyperlipidemic type 2 diabetic patients[18] and in hyperlipidemic patients,[19] also dietary intervention with canola oil,[20] as a part of healthy diet, improved the lipid profile. The effect of RBO, with a low-calorie diet, on blood lipids in hyperlipidemic patients has not been studied thoroughly. Hence, the objective of this study was to investigate the effect of RBO, with low-calorie diet, on blood lipids, TAG, TC, LDL, and HDL in hyperlipidemic patients.

MATERIALS AND METHODS

Participants

This study was a parallel groups' randomized clinical trial with a pre- and post-test design. The study was conducted on 50 hyperlipidemic patients who were healthy in other respects and were recruited from the Nutrition and Diet Therapy Clinic, in Qazvin, Iran, in 2011. The inclusion criteria required the age to be 25 – 65 years. The subjects had to hsave at least one of the blood lipid indices, including TC ³ 200, HDL £ 40, LDL ³ 130, and TAG ³ 150 mg / dl. Exclusion criteria included pregnancy and lactation, smoking, steroid therapy, taking lipid-lowering drugs, hormone and a personal history of nephropathy, cardiovascular disease, diabetes, and other chronic disease.

Protocol

Fifty subjects (42 females and eight males) participated in this study. For sample size determination we consider total cholesterol (TC) as the target parameter with a mean of ≈ 225 mg / dl and standard deviation ≈ 30 mg / dl, according to our previous study.[19] For α=0.0, β=0.2, d = 25 mg / dl (relative error = 11%), we got a sample size for each group, n = 23. We made 25 blocks with two subjects in each. The subjects in each block were similar (match). By two color cards, the subjects in each block were randomly assigned to the case and control groups. All subjects received a low-calorie diet, 1400 Kcal energy per day for four weeks, including 26% fat, 17% proteins, and 57% carbohydrates, from a registered dietitian. The treatment group received the low-calorie diet including (30 g / day) RBO. Rice bran oil was prepared by the Arian Top Noosh Company, Tehran, Iran, for rice bran oil (King rice bran oil, Bangkok, Thailand) dedication. The rice bran oil used had a national quality control certificate number 6650. The low-calorie diet with rice bran oil was well-tolerated and accepted in all cases. Oral and written instructions for recording foods were given to all subjects by the clinical nutritionist. All patients gave informed consent for their participation in the study after reading the protocol of this experiment and receiving information about rice bran oil consumption. Consent was obtained from each patient. They could quit the study freely. All participants provided informed written consent. The protocol was approved by the Research Council and Ethical Committee of the Qazvin University of Medical Sciences. This clinical trail is registered in ClinicalTrail.gov by number, NCD: Seven-day food records and the main daily nutrient intake record, completed during the last week of the study by subjects. All these records were reviewed by the clinical nutritionist for checking the diet compliance. Subjects´ compliance was assessed by analyzing seven days' diet records. It was analyzed by using a computerized nutrient database (Dorosty Food Processor-DFP, version 2003, Shahid Beheshti University, Tehran, Iran), which was mainly based on the national nutrient composition data. Blood samples were obtained after an overnight (12 hours) fasting period before the study and on the last day of the intervention period.

Measurements

Anthropometric indices and levels of serum TAG, TC, LDL, and HDL were measured before and after the intervention. Serum TC and TAG concentration were measured by commercially available enzymatic reagents (Pars Azmoon, Tehran, Iran) adopted to the Selectra autoanalyzer (Vital Scientific, Spankeren, Netherlands). HDL-cholesterol was measured after precipitation of the apolipoprotein B containing lipoproteins with phosphotungistic acids.[21] Inter- and intra-assay coefficients of variation were both less than 5% for all these measurements. Body weight was measured when minimally clothed, without shoes, with digital scales. Height was measured in a standing position, with the shoulders in a normal state, without shoes, using a tape meter. Waist circumference was measured to the nearest 0.1 cm at the narrowest level over light clothing, using an unstretched tape measure.[22] During the study, the participants were asked not to change their habitual physical activity levels.

Statistical analysis

Results are presented as mean ± standard deviation. Data were analyzed with the SPSS package Version 13 (SPSS Inc., Chicago. IL). The paired t-test was applied to determine the difference between the before and after measurements and two independent sample t- tests were used for analysis of differences between the groups. The Kolmogorov Smirnov test for checking the normality of lipid profiles was used. P-Value < 0.05 was accepted as statistically significant for all tests.

RESULTS

The mean age of subjects was 41.86 ± 9.65 years and the average of weight, waist, and hip were 83.87 ± 14.15 kg, 107.16 ± 13.18 cm, and 112.28 ± 16.90 cm, respectively. The mean body mass index (BMI) and waist-to-hip ratio (WHR) were 33.06 ± 4.44 kg / m^2 and 0.45 ± 0.06, respectively [Table 1]. There were no significant differences in age, weight, height, waist, hip, BMI, WHR, TC, TAG, LDL, HDL, LDL / HDL or atherogenic ratio of TC / HDL between the two groups (treatment and

control) at the baseline [Table 2]. The Kolmogorov Smirnov test was used for checking the normality of Cholesterol, TAG, LDL, and HDL in two groups (treatment and control). All P-values were between 0.160 and 0.854, which indicated that the normality assumption was accepted. Both groups lost weight significantly ($P < 0.05$), and BMI and waist and hip circumferences were significantly reduced ($P < 0.05$) after four weeks of treatment [Table 3]. There were no significant differences at the beginning of dietary intervention (baseline) between the plasma lipid concentrations. A low calorie-diet including RBO intervention resulted in significantly lower levels of TC, LDL, and ratio of TC / HDL than in the baseline (231.92 ± 25.3 mg / dl versus 206.92 ± 25.50 mg / dl, 132.41 ± 14.73 mg / dl versus 122.34 ± 19.78 mg / dl, and 5.30 ± 1.21 versus 4.78 ± 1.04, respectively), ($P < 0.05$). No significant change occurred in HDL or LDL / HDL. Lipid profiles before and after intervention, in both groups, are summarized in Table 3. Total cholesterol was significantly decreased only in the treatment group, the differences (before minus after) between the two groups were significant [$P = 0.030$, Table 3].

DISCUSSION

Contrary to traditional notions of cardiovascular disease (CVD) as a 'western' disease of 'affluence', more than three-quarters of global CVD mortality now occurs in the middle- and lower-income nations.[23] Elevations in serum TC and LDL increase the risk of atherosclerosis and coronary heart disease.[14] Dietary modification, for example, the dietary approach to stop hypertension (DASH)-diet is rich in fruits, vegetables, and whole grains and low in saturated fat, total fat, and cholesterol,[24] and is the preferable form of treatment for most types of hyperlipidemia. The present study shows that treatment with a diet containing RBO, for four weeks, results in a significant decrease in the levels of TC, LDL, and atherogenic ratio of TC / HDL by 10.3, 8.6, and 7.1%, respectively. These findings are in agreement with a recent report, which shows that consumption of RBO spread for four weeks significantly reduces blood TC and LDL, in mildly hypercholesterolemic individuals. [16] In several studies, similar to the result of this study, diet

Table 1: Characteristics of all patients

Characteristics	Minimum	Maximum	(Mean ± SD)
Age (years)	26.0	63.0	41.86 ± 9.65
Weight (kg)	59.30	114.0	83.87 ± 14.15
Height (cm)	143.5	178.0	159.22 ± 7.87
Waist (cm)	85.0	144.0	107.16 ± 13.18
Hip (cm)	47.0	143	112.28 ± 16.90
BMI (Kg / m^2)	26.31	47.45	33.06 ± 4.44
WHR (Ratio)	0.78	1.05	0.45 ± 0.06
TC (mg/dl)	162.0	296.0	224.62 ± 29.23
TAG (mg/dl)	75.0	690.0	206.30 ± 111.90
LDL (mg/dl)	80.0	175.0	127.02 ± 22.42
HDL (mg/dl)	24.0	70.0	44.36 ± 10.86
LDL/HDL	1.31	5.38	3.03 ± 0.92
TC / HDL	2.43	8.17	5.32 ± 1.31

Data represent mean ± standard deviation, TAG = Triacylglycerol, TC = Total cholesterol, LDL = Low-density lipoprotein, HDL = High-density lipoprotein, WHR = Waist-to-hip ratio, BMI = Body mass index

Table 2: Baseline characteristics of patients

Characteristics	Treatment	Control	*P* value
Age (years)	44.56 ± 8.65	39.16 ± 10.0	0.05
Weight (kg)	80.41 ± 13.74	86.83 ± 14.19	0.14
Height (cm)	157.90 ± 7.34	160.54 ± 8.25	0.23
Waist (cm)	106.48 ± 11.30	107.84 ± 15.04	0.71
Hip (cm)	111.32 ± 10.45	113.24 ± 11.56	0.53
BMI (Kg/m^2)	32.34 ± 4.21	33.77 ± 5.66	0.31
WHR (Ratio)	0.96 ± 0.05	0.95 ± 0.07	0.73
TC (mg/dl)	231.92 ± 25.30	217.32 ± 31.52	0.08
TAG (mg/dl)	205.08 ± 134.81	207.52 ± 86.21	0.94
LDL (mg/dl)	132.41 ± 14.73	122.06 ± 23.46	0.11
HDL (mg/dl)	45.66 ± 10.30	43.16 ± 11.44	0.43
LDL/HDL	3.04 ± 0.83	3.03 ± 1.00	0.97
TC/HDL	5.30 ± 1.21	5.33 ± 1.42	0.95

Data represent mean ± standard deviation, TAG = Triacylglycerol, TC = Total cholesterol, LDL = Low-density lipoprotein, HDL = High-density lipoprotein, WHR = Waist-to-hip ratio, BMI = Body mass index

Table 3: Characteristics of patients before and after intervention

Characteristics	Treatment groups		Control groups		*P* value*
	Before	After	Before	After	
Weight (Kg)	80.41 ± 13.74[a]	78.24 ± 13.27[a]	86.83 ± 14.19[b]	82.86 ± 13.64[b]	0.031
Waist (Cm)	106.48 ± 11.30[a]	101.96 ± 10.35[a]	107.84 ± 15.04[b]	105.96 ± 16.11[b]	0.009
Hip (Cm)	111.32 ± 10.45[a]	108.48 ± 10.24[a]	113.24 ± 11.56[b]	110.28 ± 11.26[b]	0.799
BMI (Kg/m^2)	32.34 ± 4.21[a]	31.28 ± 4.08[a]	33.77 ± 5.66[b]	32.24 ± 5.56[b]	0.029
WHR	0.96 ± 0.05[a]	0.94 ± 0.05[a]	0.95 ± 0.07	0.96 ± 0.86	0.013
TC (mg/dl)	231.92 ± 25.30[a]	206.92 ± 25.50[a]	217.32 ± 31.52	213.28 ± 39.67	0.030
TAG (mg/dl)	205.08 ± 134.81[a]	153.84 ± 85.19[a]	207.52 ± 86.21[b]	136.46 ± 38.28[b]	0.349
LDL (mg/dl)	132.41 ± 14.73[a]	122.34 ± 19.78[a]	122.06 ± 23.46	106.66 ± 43.74	0.756
HDL (mg/dl)	45.66 ± 10.30	45.10 ± 10.57	43.16 ± 11.44	43.74 ± 13.25	0.789
LDL / HDL	3.04 ± 0.83	2.84 ± 0.74	3.03 ± 1.00	3.63 ± 0.88	0.236
TC / HDL	5.30 ± 1.21[a]	4.78 ± 1.04[a]	5.33 ± 1.42	4.88 ± 1.15	0.932

Data represent mean ± standard deviation, TAG = Triacylglycerol, TC = Total cholesterol, LDL = Low-density lipoprotein, HDL = High-density lipoprotein, WHR = Waist-to-hip ratio, BMI = Body mass index, *For comparing the differences (before minus after) in two groups, [a]Significant difference for the characteristics in the treatment group ($P < 0.05$), [b]Significant difference for the characteristics in the control group ($P < 0.05$)

treatment with RBO has resulted significantly in the reduction of TC and LDL.[11,12,25,26] LDL cholesterol has been the focus of much research, as it is conclusively linked to atherosclerosis, coronary heart disease (CHD) development, and acute clinical events, including myocardial infarction (MI) and stroke. Consequently LDL is the primary target for intervention efforts. A decrease of 1 mg / dl in LDL results in about a 1 to 2% decrease in the relative risk for CVD[27] and a 10% reduction in TC would decrease CHD incidence by about 30%.[28] High levels of unsaponifiable material are found in RBO.[6] This is composed of phytosterols (γ-oryzanol), triterpene alcohols, and vitamin E isomers (tocopherols and tocotrienols). In addition, RBO contains up to 20% SFA and equal amounts of MUFA and PUFA.[3]

A 10-week feeding study was performed in 14 volunteers, who consumed a diet with RBO, substituted for oil that had a fatty acid composition similar to that of RBO.[25] Their results confirmed the previous findings of the total and LDL-cholesterol–lowering effects of RBO in humans. When matching the fatty acids of the RBO with control oil, they showed that the effect of RBO on serum cholesterol concentrations was due to the unsaponifiables present in it and not due to its fatty acid profile.[25] However, Utarwuthipong *et al*. reported that consumption of the rice bran oil / palm oil (3 : 1) mixture of oils rich in oleic acid and equivalent in amounts of linoleic acid and palmitic acid could reduce levels of total cholesterol and LDL-cholesterol,[12] as in our previous intervention study with olive oil (known for its high levels of MUFA), where LDL was significantly decreased.[29] Also, in an intervention study with almonds (rich in MUFA) significant serum LDL and TC were seen.[30]

Consumption of RBO, which is rich in oleic acid has been shown to increase hepatic LDL and CYP7AL (cholesterol 7 a-hydroxylase) expression to a great level. [31] Chen *et al*. speculated that the high MUFA content and unsaponifiable components in RBO may have a synergistic hypercholesterolemic effect. Of the RBO components, phytosterol including gamma oryzanol are thought to be responsible for changes in the blood cholesterol concentration.[9] The increased fecal neutral sterols and bile acids due to RBO-diet consumption can be explained in part by the reduction in cholesterol reabsorption in the intestines. [31] The mechanism of inhibiting cholesterol absorption by phytosterol, whose chemical structure is very similar to that of cholesterol, interferes with the cholesterol movement into micelles and reduces cholesterol absorption in the intestines. In addition, phytosterols are able to increase the excretion of bile acids, which results in the lowering of plasma and liver cholesterol levels.[32]

In the present study, the RBO used contained 2400 ppm oryzanol. Chen and Cheng[31] reported that oryzanol affected biliary secretion and fecal excretion of cholesterol and bile acids. It significantly increased the fecal excretion of bile acids and neutral sterols. They speculated that the observed hypolipidemic effect of the RBO diet may have been due to increased hepatic LDL-receptor expression, which facilitated the lowering of LDL and increased CYP7A1 expression, which facilitated cholesterol catabolism, and then the upregulation of HMG-CoA reductase expression, to synthesize cholesterol for cholesterol homeostasis *in vivo*.[31] Oxidative damage is a major contributor to the development of cardiovascular pathologies, such as, atherosclerosis. [33] The current recommendations for the prevention of atherosclerosis aim at improving the quality of the diet, by increasing dietary antioxidants, such as vitamin E.[34] The vitamin E isomers (tocopherol and tocotrienols) that are also present in RBO, may confer additional health benefits, particularly antioxidant activity, which has been credited to this compound.[35,36] Tocotrienols have been suggested to lower TC concentrations in the blood, possibly because of the inhibition of the activity of 3-hydroxy-3 methyl-glutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme, in endogenous cholesterol synthesis. [37] Arora *et al*. reported that after consumption of RBO for three months in hyperlipidemic subjects, a lower incidence of elevated TAG was seen.[11] In another study, 75 ml of RBO, thrice daily as the cooking medium with breakfast, lunch, and dinner, for a period of 50 days was used and significant reduction in TAG was seen.[10] Our results were not consistent with the observations of Arora *et al*. and Rajnarayana *et al*., which at a higher amount of RBO and longer study period resulted in the reduction of TAG.[10,11] In the present study, no significant change occurred in HDL. This finding is in agreement with the earlier reports. [9,11,15,16,25] Furthermore, in our study that was similar to the result of a recent study,[16] the ability of RBO to reduce the atherogenic ratio of TC / HDL concentration to a great level (7.3%), may be indicative of further health benefits that can be attributed to this product. In some intervention studies, a three-day food record was used,[16,38] to reach a more accurate estimation of energy and macronutrient intake. The patients of this study were asked to record their food intake for one week. Our study had some limitations, such as, the small number of participants and hyperlipidemic patients, who did not receive lipid-lowering drugs. The strengths of the study were that it reproduced real-life conditions with home prepared foods for diet, and use of supplemental foods that were commonly available and consumed by the public. RBO, which contained these compounds, could become an important functional food, with cardiovascular health benefits. The use of RBO, together with dietary modification, may have implications for reducing the risk of cardiovascular disease.

In conclusion, the result of the present study showed that short-term consumption of RBO with a low-calorie diet, as a part of healthy diet, improved the atherogenic lipid profiles and cardiovascular risk factors in hyperlipidemic patients.

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