Use of rice bran oil in patients with hyperlipidaemia

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ABSTRACT

Background. The quantity and type of dietary fat is known to affect plasma lipid concentration and hence the choice of cooking oil is important to lower the risk of coronary heart disease. Rice bran oil, which was not popular worldwide, is slowly being recognized as a 'healthy' oil in India. We assessed if rice bran oil had hypolipidaemic effects in subjects with elevated lipid levels.

Methods. The study had a cross-over design with subjects (n= 14) randomly assigned to consume either rice bran oil or refined sunflower oil in their homes, for a period of 3 months (period 1). After a washout period of 3 weeks, they were crossed over to the other oil (period 2). The serum lipid values were estimated at the beginning, on day 45 and day 90 of each phase. Additional parameters assessed included anthropometry, dietary and physical activity patterns.

Results. The use of rice bran oil significantly reduced plasma total cholesterol and triglyceride levels compared with sunflower oil. The reduction in plasma LDL-cholesterol with rice bran oil was just short of statistical significance ($p=0.06$). HDL-cholesterol levels were unchanged.

Conclusion. The use of rice bran oil as the main cooking oil significantly reduced serum cholesterol and triglyceride levels. The use of rice bran oil together with dietary and lifestyle modifications may have implications for reducing the risk of cardiovascular disease.

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INTRODUCTION

India is on the threshold of an epidemic of cardiovascular disease, and surveys in urban areas show that coronary risk factors are widespread.[1] Of all the ethnic groups, people of Indian origin have among the highest incidence of cardiovascular and coronary artery disease (CAD),[2,3] often occurring at an early age. Indian immigrants living in various parts of the world have a documented

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increased predisposition to cardiovascular disease as compared to native populations.[4,5] There also appears to be a higher incidence of hospitalization, morbidity and mortality among Indians compared with other ethnic groups.[6] The prevalence of premature CAD in Indians is up to 3 times higher when compared with that among individuals of similar age groups in the western world.[7]

The conventional risk factors for cardiovascular diseases include hypertension, diabetes mellitus, smoking, hyperlipidaemia, low high density lipoprotein (HDL)-cholesterol, tobacco consumption and central obesity, while emerging risk factors include high triglyceride levels, insulin resistance, the metabolic syndrome, elevated homocysteine levels and plasma fibrinogen.[8] The metabolic syndrome is a combination of insulin resistance, dyslipidaemia, hypertension and obesity;[9] and Asian Indians, irrespective of their geographic location, have shown a high prevalence of excess body fat, adverse body fat patterning, hypertriglyceridaemia and insulin resistance.[10]

The risk of CAD can be reduced by lowering serum cholesterol levels[11] and this can be achieved by drug therapy and dietary manipulation. The use of dietary manipulation is less likely to cause undesirable consequences when compared with drug therapy. For instance, the American Heart Association Guidelines for treating hypercholesterolaemia have focused on dietary cholesterol and fat reduction.[12]

The quantity and quality of a dietary fat plays a crucial role in the plasma cholesterol and lipid levels.[13] The traditional cooking oils used in India include groundnut, coconut, sesame, mustard, safflower and sunflower oil, while newer sources include rice bran oil (RBO), palmolein and soya bean oil. RBO obtained during the milling of rice is used widely in Japan, Korea, China, Taiwan and Pakistan as an edible oil. RBO, which can be obtained both by chemical extraction using solvents and by physical refining, has been investigated for its chemical, nutritional and toxicological properties and has been found to be safe for human consumption.[14] The chemical composition of RBO is similar to groundnut oil. Both the oils have about 35% linoleic acid, while RBO has, in addition, about 2.2% of linolenic acid. The special feature of RBO, compared with other vegetable oils, is its high content of the unsaponified fraction which varies from 4.5% when it is obtained by physically refined methods to 3% by chemical refining, as compared with 1% in other edible oils.[15] RBO, with its unsaponifiable matter, which is rich in phytosterols (1.8%), gamma oryzanol (1.6%) and total tocopherol (81.3 mg%), has been shown to have blood cholesterol-lowering abilities compared with other oils, in both animals and humans.[16] Oryzanol is a mixture of ferulic acid esters of triterpene alcohols, while tocopherols and tocotrienols provide oxidative stability to RBO and improve its shelf-life.[17] Oryzanol decreases the absorption of cholesterol and, in hamsters, was shown to inhibit aortic fatty streak formation.[14]

In studies involving rats, RBO produced hypolipidaemia.[18] The hypocholesterolaemic effect of RBO in humans was first reported from Japan[19] and later in a cross-sectional, short term study (30 days) from India,[13] of 21 subjects.

We aimed to assess the hypolipidaemic effect of RBO and whether it persisted for a longer time period (3 months) when the oil was introduced into a household.

METHODS

Subjects

Fourteen subjects with hyperlipidaemia participated in the study. The sample size was calculated based on data from the study by Raghuram *et al.*[13] to detect a minimum reduction of at least 20% in the mean serum cholesterol level (247.3 mg/dl), with a standard deviation of 10 mg/dl. A beta value of 0.2 and and α level of 0.05 was used to calculate the required sample size of 14 subjects, with 7 in each group. The subjects were recruited from the staff of St John's Medical College and Hospital. Normal, healthy subjects (9 men and 5 women) who did not have any organ dysfunction, were not on any medication, and satisfied the following criteria were included in the study: age 40–60 years, serum cholesterol levels >200 mg/dl, serum triglycerides >150 mg/dl, predominantly ate home food, and were willing to participate in the study. The exclusion criteria were intake of any form of lipid-lowering medication and proven cardiovascular disease. The subjects were not on any diuretics or beta-blockers that could elevate the lipid levels. All subjects were told about the alternative use of lipid-lowering drugs at the time of recruitment. Hence, the patients participating in the study made the informed choice of trying out a lifestyle change-based method of lowering their lipid levels. The exact protocol of the study was explained to the subjects and written consent was obtained from each of them. The study was approved by the Institutional Ethical Review Board of St John's Medical College.

Anthropometric and skinfold measurements

Anthropometric and skinfold measurements were done at the beginning, every month and at the end of each phase of the study. Subjects were weighed in minimal clothing using a digital scale, (Afcoset, India), which had a precision of 0.01 kg. All weights were measured twice and the mean expressed to the nearest 0.01 kg. The height of the subjects was recorded without footwear, using a vertically mobile scale (Holtain, Crymych, UK) to the nearest 0.1 cm. Body mass index (BMI) was calculated as follows; BMI=weight (kg)/height^2 (m^2). Waist circumference was measured at the mid-point between the lower border of the rib cage and iliac crest, while the hip circumference was measured at the maximum extension of the buttocks.

Skinfold measurement was done in triplicate in the standing position. The measurements were made to the nearest 0.2 mm using skinfold calipers (Holtain, Crymych, UK). The mean of biceps, triceps, subscapular and suprailiac skinfold measurements were taken for further calculations. All measurements were standardized and carried out according to the *Anthropometric standardization reference manual*.[20] The logarithm of the sum of the 4 skinfolds was used in age- and gender-specific equations to obtain the body density, from which estimates of the percentage of body fat were calculated.[21]

Experimental protocol

A cross-over study design was used with the 14 subjects being randomized into 2 groups (either RBO or refined sunflower oil first). The subjects received the first oil for 3 months (period 1), and then, after a washout period of 3 weeks, were crossed over to the other oil group for another 3 months (period 2). The subjects who received RBO in period 1 and refined sunflower oil in period 2 constituted group 1, while those who received refined sunflower oil in period 1 and RBO in period 2 constituted group 2.

The initial blood biochemistry investigations included lipid profile and haemoglobin. About 2 ml of whole blood was collected in an EDTA vacutainer by trained personnel under aseptic conditions. Haemoglobin was measured using Sysmax 1800, US. The blood was then centrifuged in a refrigerated centrifuge at 4 °C to obtain the plasma, which was analysed for lipid levels. The total cholesterol, triglyceride and HDL-cholesterol in plasma were estimated by colorimetric assays performed on the Dade Dimension RxL. Cholesterol was measured by the oxidation of cholesterol (liberated by cholesterol esterase), by cholesterol oxidase to 7-hydroxycholesterol. Triglyceride was measured by pre-incubating the sample with a lipase enzyme reagent which converts triglycerides into free glycerol and fatty acids.[22] All these assays were calibrated by use of the Dade Dimension human calibrator. The analytical CV% for total cholesterol, triglycerides and HDL-cholesterol were 4.1%, 4.7% and 4.1%, respectively. LDL-cholesterol was calculated from primary measurements using the empirical formula of Friedewald equation.[23] Dietary assessment was carried out using a food frequency questionnaire with 129 food items; this has been developed for and applied specifically among the urban population.[24] A validated physical activity questionnaire[25] was used to assess the physical activity of the patient. The subjects were asked to continue with their routine eating and physical activity pattern. The subjects were provided oil for the entire family for a period of 1 month at a time. The subjects reported weekly to the Nutrition Service and their body weight and a 24-hour dietary recall of the food intake was recorded. Blood tests were done initially at baseline (day 0), and on days 45 and 90 of each period. The food frequency and physical activity questionnaires were administered at baseline and day 90 of each phase.

Statistical analysis

The data are presented as mean (SD). A paired *t* test was used to look for significant differences in the physical characteristics, physical activity levels and food intake of the subjects. The physical characteristics of the day 0 value in period 1 were compared with the day 0 value in period 2 (post-washout value) to assess whether the values were similar at entry level in both the oil groups.

Paired *t* test analysis was used to assess whether the lipid parameters on day 0 in the two periods (within a group) were different from each other. Independent sample *t* test was done to ascertain that the day 0 values between the two groups in both time periods were not significantly different. The Wilcoxon rank sum test was used to assess the change in parameters across period 1, period 2 and total change in both oil groups, and the median and range were used as summary measures. All these analyses were carried out using SPSS version 13 (SPSS Inc, USA). Values were considered significant if $p<0.05$.

RESULTS

The 14 subjects were between 40 and 60 years of age. Thirteen of the subjects (93%) were non-vegetarians and 1 was a vegetarian. Only 1 of the subjects was a smoker (about 2 cigarettes per day). Six of the subjects (43%) consumed about 90 ml of alcohol on a weekly basis. Eight of the subjects (57%) used sunflower as the main cooking oil prior to the study, 2 (14%) used groundnut oil and the remaining 4 (29%) used a combination of oils.

The physical characteristics of the subjects were similar throughout the study as well as before each period of the study (Table I). There were no significant differences in lipid levels between the baseline values for each period in either of the randomization groups (paired *t* test) and also between the randomization groups (independent *t* test) at entry levels of each period (Table II).

The Wilcoxon rank sum test showed that the reduction in total cholesterol and serum triglycerides was significantly higher for the RBO group compared with the refined sunflower oil group, while the reduction in LDL-cholesterol in the RBO group was short of significance (p=0.08). There was no change in the HDL-cholesterol values (Table III).

There were no significant changes in the intake of nutrients or of physical activity of the subjects from baseline to day 90 during each of the interventions (Table IV).

TABLE I. Physical characteristics of the subjects based on the order of oil intervention

Type of oil	Period 1 (initial phase of randomization)		Period 2 (post-washout phase)	
	Day 0	Day 90	Day 0	Day 90
	Rice bran oil (n=7)		*Refined sunflower oil (n=7)*	
Weight (kg)	65.6 (5.6)	65.4 (5.1)	65.3 (5.2)	65.4 (5.3)
Height (cm)	161.3 (5.4)	161.3 (5.4)	161.3 (5.4)	161.4 (5.6)
Body mass index (kg/m²)	25.2 (2.1)	25.2 (2.3)	25.1 (2.2)	25.2 (2.2)
Mid-arm circumference (cm)	28.1 (2.3)	28.4 (2.6)	28.2 (2.2)	28.2 (2.0)
% Fat	28.9 (4.2)	28.8 (4.8)	28.8 (4.6)	28.9 (4.6)
Waist circumference (cm)	87.3 (2.8)	87.4 (3.1)	87.0 (3.0)	87.1 (3.0)
Hip circumference (cm)	92.8 (2.7)	92.6 (2.9)	92.8 (2.8)	92.8 (2.7)
Waist–hip ratio	0.94 (0.04)	0.94 (0.06)	0.94 (0.06)	0.95 (0.04)
Systolic blood pressure (mmHg)	130.6 (8.5)	130.5 (8.9)	130.4 (8.7)	130.3 (9.2)
Diastolic blood pressure (mmHg)	81.7 (10.3)	81.9 (10.3)	81.8 (10.5)	82.0 (10.4)
	Refined sunflower oil (n=7)		*Rice bran oil (n=7)*	
Weight (kg)	62.0 (8.8)	62.1 (8.1)	62.3 (8.2)	62.0 (8.3)
Height (cm)	161.0 (7.6)	161.0 (7.8)	161.0 (7.8)	161.0 (7.6)
Body mass index (kg/m²)	24.1 (4.0)	24.3 (4.0)	24.2 (3.9)	24.1 (3.9)
Mid-arm circumference (cm)	26.9 (1.2)	27.0 (1.3)	27.0 (1.3)	27.1 (1.2)
% Fat	33.5 (3.5)	33.3 (3.7)	33.4 (3.7)	33.4 (3.7)
Waist circumference (cm)	86.0 (7.8)	86.1 (7.5)	86.1 (7.5)	86.1 (7.5)
Hip circumference (cm)	95.8 (5.0)	96.3 (2.6)	96.6 (2.8)	96.8 (5.1)
Waist–hip ratio	0.90 (0.08)	0.89 (0.08)	0.89 (0.08)	0.89 (0.09)
Systolic blood pressure (mmHg)	113.3 (9.3)	113.7 (9.8)	113.9 (9.7)	114.6 (9.5)
Diastolic blood pressure (mmHg)	74.9 (8.5)	75.3 (8.6)	75.6 (8.6)	76.1 (8.1)

Values are mean (SD). No significant difference was observed within each period (day 0 *v.* day 90) and also between the baseline and post-washout (day 0 of period 1 *v.* day 0 of period 2) using paired *t* test analysis.

TABLE II. Mean serum lipid values of the subjects at day 0, day 45 and day 90 of the two groups based on order of oil intervention

Type of oil	Period 1 (initial phase of randomization)			Period 2 (post-washout phase)		
	Day 0	Day 45	Day 90	Day 0	Day 45	Day 90
	Rice bran oil (n=7)			*Refined sunflower oil (n=7)*		
Total cholesterol (mg/dl)	239.9 (17.9)	223.7 (22.1)	227.7 (17.3)	236.4 (29.4)	236.0 (35.5)	232.0 (24.5)
LDL-cholesterol (mg/dl)	156.0 (30.1)	139.0 (21.5)	143.6 (30.0)	156.7 (24.9)	164.1 (34.2)	145.9 (31.2)
HDL-cholesterol (mg/dl)	48.0 (10.3)	47.0 (11.5)	45.4 (10.9)	43.0 (12.0)	42.9 (10.0)	50.0 (16.4)
Serum triglycerides (mg/dl)	244.7 (54.0)	126.1 (30.7)	144.3 (58.4)	216.7 (93.7)	181.0 (77.1)	180.1 (85.6)
	Refined sunflower oil (n=7)			*Rice bran oil (n=7)*		
Total cholesterol (mg/dl)	242.9 (20.5)	264.6 (45.0)	248.0 (37.4)	256.7 (33.5)	236.7 (42.0)	225.4 (39.3)
LDL-cholesterol (mg/dl)	154.3 (16.0)	175.3 (37.6)	162.6 (24.8)	164.3 (23.2)	149.0 (38.8)	140.9 (30.8)
HDL-cholesterol (mg/dl)	50.6 (8.5)	47.6 (13.3)	43.4 (12.0)	44.3 (5.3)	43.7 (9.9)	44.1 (8.1)
Serum triglycerides (mg/dl)	214.9 (46.3)	207.9 (50.4)	209.9 (65.6)	212.9 (37.9)	167.3 (32.4)	174.3 (29.7)

Values are mean (SD). No significant difference was observed between day 0 of period 1 and day 0 of period 2 (post-washout period) (paired *t* test). No significant differences were observed in the day 0 values between the two groups in both time periods (independent *t* test).

TABLE III. Collated data of subjects on rice bran oil and refined sunflower oil intake

Parameter	Day 90 – Day 0 value		p value
	Rice bran oil Median (range)	Refined sunflower oil Median (range)	
Total cholesterol (mg/dl)			
Group 1	–10.0 (–33 to 4)	–2.0 (–57 to 27)	0.39
Group 2	–35.0 (–44 to –18)	5.0 (–23 to 34)	0.03*
Total	–20.0 (–44 to 4)	1.5 (–57 to 34)	0.02*
LDL-cholesterol (mg/dl)			
Group 1	–10.0 (–56 to 36)	–4.0 (–75 to 12)	1.0
Group 2	–34.0 (–53 to 59)	0 (–14 to 57)	0.03*
Total	–31.5 (–56 to 59)	–0.5 (–75 to 57)	0.08
HDL-cholesterol (mg/dl)			
Group 1	–4.0 (–14 to 9)	7.0 (–3 to 15)	0.09
Group 2	–2.0 (–7 to 11)	–9.0 (–16 to 7)	0.11
Total	–3.0 (–14 to 11)	–1.0 (–16 to 15)	0.8
Serum triglyceride (mg/dl)			
Group 1	–94.0 (–182 to –6)	–40.0 (–269 to 105)	0.15
Group 2	–45.0 (–95 to 38)	–9.0 (–71 to 68)	0.12
Total	–64.5 (–182 to 38)	–17.0 (–269 to 105)	0.05†

*p<0.05, Wilcoxon rank sum test †p=0.05, Wilcoxon rank sum test

DISCUSSION

We found that the use of RBO as the main cooking oil for a period of 3 months significantly reduced the plasma cholesterol and triglyceride levels. The plasma triglyceride values were reduced by 30% on day 90 compared with the baseline value. This is significant, since increased plasma triglyceride level is an important risk factor for atherosclerosis of the coronary arteries and the metabolic syndrome among South Asians. Triglycerides bring about changes in LDL particle size, density, distribution and composition producing smaller, denser and more atherogenic particles.[26] Indians worldwide have been shown to have a triad of high triglycerides with low HDL levels and high LDL levels (dyslipidaemia).[27] The changes in lipid levels were apparent by day 45 and persisted till the end of the 90-day intervention period.

The hypocholesterolaemic activity of RBO is thought to be due to its unsaponification factor.[17] RBO also contains phytosterols which act at the intestinal level by interfering with the absorption of cholesterol from the gut.[14] Oryzanol may inhibit the absorption of cholesterol and also increase the faecal excretion of bile acids. Although oryzanols are present in small amounts in other oils such as soya bean, palm and sesame, RBO is their richest source. Cycloartenol, a triterpene alcohol, has a structure that is similar to cholesterol and it may compete with the binding sites of cholesterol, sequestering cholesterol from the system.[13] Tocotrienols are thought to inhibit the HMG CoA reductase activity in the biosynthetic pathway of cholesterol, and also possess antioxidant, antithrombotic and anticarcinogenic properties.[13]

Our study has confirmed the results of a previous study,[13] which demonstrated that RBO reduces the serum cholesterol and triglyceride levels when used as the main cooking oil. However, the decline in serum cholesterol and triglyceride levels observed in the previous study after a period of 30 days was 25% and 35%, respectively, as compared to 8% and 30% in our study. There are many reasons to explain this difference in the magnitude of the results. The population and the oil that was habitually used prior to the study were different. In the previous study, the habitual oil

TABLE IV. Food intake and physical activity levels of the subjects at the beginning and end of each phase as measured by food frequency and physical activity questionnaires

Parameters	Rice bran oil		Refined sunflower oil	
	Day 0	Day 90	Day 0	Day 90
Energy (kcal/day)	2227.5 (661.2)	1986.5 (593.2)	2131.0 (642.9)	2061.7 (481.6)
Protein (g/day)	62.7 (20.1)	56.9 (17.1)	60.9 (20.7)	60.6 (17.5)
Fat (g/day)	57.9 (23.6)	53.2 (24.4)	57.1 (23.6)	51.9 (17.1)
Carbohydrate (g/day)	363.5 (100.8)	320.1 (82.4)	341.7 (89.7)	336.8 (77.8)
Saturated fat (g/day)	22.3 (9.7)	20.6 (9.1)	20.5 (8.0)	18.5 (6.9)
Cholesterol (mg/day)	146.6 (83.3)	123.4 (63.3)	117.1 (77.6)	104.6 (76.7)
Fibre (g/day)	10.1 (4.4)	8.8 (4.4)	9.8 (3.8)	9.8 (3.2)
Physical activity level	1.6 (0.1)	1.6 (0.1)	1.7 (0.1)	1.6 (0.2)

Values are in mean (SD). No significant changes were observed in any of the parameters.

of most of the subjects was palm oil or groundnut oil, while in our study most of the subjects (57%) consumed sunflower oil. The difference in the fatty acid composition of the habitually used oil may be one of the reasons for the difference in results. In addition, although the amount of edible oil used by the subjects in the previous study was mentioned as 35–39 g/day, it is not clear what percentage of this constituted the daily energy intake. In our study, fat contributed about 24% of the total energy, while the saturated fat intake was about 9% of the total diet. We also observed that there were no significant changes in the lipid levels with the use of sunflower oil. This could be because 57% of the subjects were consuming sunflower oil before the study.

Our study shows that RBO when was used as the main cooking oil has lipid-lowering effects that last for a period of 3 months. The fatty acid composition of RBO along with the minor components of the unsaponifiable fraction may be acting synergistically to produce the hypocholesterolaemic action, indicating that the hypocholesterolaemic activity of a dietary fat depends not only on the fatty acid content of the oil, but also on the minor components of the unsaponification fraction.

We did only one baseline estimation of lipids. We realize that to arrive at a mean value, sequential measurement of lipids is ideal. The shortcoming of a single measurement is that there could be biological variations which could account for intra-individual variations over repeated measurements. However, it was not feasible for us to do more than one baseline measurement of lipids.

In conclusion, since RBO has several advantages and health benefits, it can be used routinely as a cooking oil and has the advantage that it can be consumed by the entire family as against medication which can be used only by the individual. Thus, it could also help in preventing other family members from developing cardiovascular disease and play a wider preventive role.[14] It also has a high heat tolerance and is ideal for frying and stir-frying as it does not burn or smoke. In addition, organoleptic evaluation studies have indicated that RBO products have higher acceptability and keeping qualities than groundnut and palm oil products.[14] In our study, none of the subjects reported any adverse sensory effects, and the oil was well accepted with good compliance. RBO is also cheaper than other oils available in the market. Appropriate measures need to be taken to enhance the production as well as utilization of this healthy oil in India.

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