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Hypolipidemic Effect of Oils with Balanced Amounts of Fatty Acids Obtained by Blending and Interesterification of Coconut Oil with Rice Bran Oil or Sesame Oil

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Blended oils comprising coconut oil (CNO) and rice bran oil (RBO) or sesame oil (SESO) with saturated fatty acid/monounsaturated fatty acid/polyunsaturated fatty acid at a ratio of 1:1:1 and polyunsaturated/saturated ratio of 0.8–1 enriched with nutraceuticals were prepared. Blended oils (B) were subjected to interesterification reaction using sn-1,3 specific Lipase from *Rhizomucor miehei*. Fatty acid composition and nutraceutical contents of the blended oil were not affected by interesterification reaction. Male Wistar rats were fed with AIN-76 diet containing 10% fat from CNO, RBO, SESO, CNO+RBO blend (B), CNO+SESO(B), CNO+RBO interesterified (I), or CNO+SESO(I) for 60 days. Serum total cholesterol (TC), low-density lipoprotein cholesterol, and triacylglycerols (TAGs) were reduced by 23.8, 32.4, and 13.9%, respectively, in rats fed CNO+RBO(B) and by 20.5, 34.1, and 12.9%, respectively, in rats fed CNO+SESO(B) compared to rats given CNO. Rats fed interesterified oils showed a decrease in serum TC, low-density lipoprotein cholesterol (LDL-C), and TAGs in CNO+RBO(I) by 35, 49.1, and 23.2 and by 33.3, 47, and 19.8% in CNO+SESO(I), respectively, compared to rats given CNO. Compared to rats fed CNO+RBO blended oils, rats on CNO+RBO interesterified oil showed a further decrease of 14.6, 24.7, and 10% in TC, LDL-C, and TAG. Rats fed CNO+SESO interesterified oils showed a decrease in serum TC, LDL-C, and TAG by 16.2, 19.6, and 7.8%, respectively, compared to rats given blended oils of CNO+SESO (B). Liver lipid analysis also showed significant change in the TC and TAG concentration in rats fed blended and interesterified oils of CNO+RBO and CNO+SESO compared to the rats given CNO. The present study suggests that feeding fats containing blended oils with balanced fatty acids lowers serum and liver lipids. Interesterified oils prepared using Lipase have a further lowering effect on serum and liver lipids even though the fatty acid composition of blended and interesterified oils remained same. These studies indicated that the atherogenic potentials of a saturated fatty acid containing CNO can be significantly decreased by blending with an oil rich in unsaturated lipids in appropriate amounts and interesterification of blended oil.

KEYWORDS: Coconut oil; rice bran oil; sesame oil; blended oils; interesterified oils; serum lipids; liver lipids

INTRODUCTION

Cardiovascular disease (CVD) is on the rise in Asian countries like India (*1–3*) , which may account for at least one-third of all the deaths by the year 2015 (*4, 5*) and replace infectious diseases as the major causes of death in India. Risk factors identified for CVD include high concentrations of lipids, cholesterol, and low-density lipoprotein (LDL) cholesterol, lower concentrations of high-density lipoprotein (HDL) cholesterol, hypertriglyceridemia, and elevated concentrations of lipoprotein (a), apolipoprotein (apo) B, and total homocysteine in the plasma (*1*). Studies have shown that diet (*6*) and, in particular, dietary lipids have important roles in controlling the concentrations of these risk factors (*7, 8*). The quality of a dietary fat is dependent on the type of fatty acid chain length, unsaturation, geometry (*9, 10*), and position on the glycerol backbone. The minor components present in oils may also provide beneficial effects to reduce risk factors for CVD. It is well-known that saturated fat in the diet increases cholesterol concentration (*9, 11–13*) by suppressing the LDL-receptor activity and thus decreasing the clearance of LDL from circulation (*14*). Even though polyunsaturated fatty acids (PUFAs) in dietary lipids are essential in helping to decrease the serum cholesterol concentration, their consumption in excessive amounts results in exerting oxidative stress (*15*) if not properly balanced with antioxidants.

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In India, dietary habits, especially the fat consumption, vary according to geographical region and availability of fat. Each oil used in our cooking is unique in its fatty acid composition and also in its minor components. Coconut oil (CNO) is the predominant dietary fat in Kerala and in coastal regions of Karnataka in south India. CNO is rich in saturated fatty acids (SFAs). Mustard oil is used in the northern part of the country whereas sunflower oil, ground nut oil, and palm oil are used in most parts of the country. Sesame oil (SESO) is used in southern India. The newer source of edible oil, like rice bran oil (RBO), is currently in use in southern India. These oils contain more unsaturated fatty acids.

To get optimal benefits from oils, the Indian Council of Medical Research recommended that the SFA, monounsaturated fatty acid (MUFA), and PUFA in the oils be consumed in equal proportions and that the fat in such a diet may provide 20–25% of the energy (*16*, *17*). These guidelines are in tune with the ones promoted by the American Heart Association (*18*, *19*). However no single oil obtained from natural sources can provide the fatty acids in the recommended proportions. Therefore, blending of saturated fats with unsaturated oils becomes an alternative approach to give an oil with balanced fatty acids.

Blended oils represent a physical mixture of two or more oils. Blending of oils has been used as an approach to enhance the oxidative and thermal stability of oils (*20*). The genetic modification of plants to alter the fatty acid composition of plant lipids has been attempted in the case of high oleic sunflower oil and canola to improve the thermal stability and nutritional value of oils. But such approaches require sustained efforts by the growers (*21*–*23*). Even though one can balance the fatty acid composition to the desired proportions by blending suitable oils, it may not always result in oils with the desired physicochemical (*24*) or nutritional properties (*25*) because the physical characteristics of individual oils will be retained in the blended oil. This may be overcome by rearranging the fatty acids in the triacylglycerol molecules mediated by the enzymatic or chemical interesterification process. Lipase catalyzed interesterification is specific and has become a useful tool for the production of designer lipids to enhance the oxidative stability (*26*), to improve the physicochemical properties (*27*, *28*) such as the melting profile of butter, table spread, and margarine, and to incorporate long chain fatty acids such as docosahexaenoic acids and eicosapentaenoic acid for synthesizing specialty fats (*29*). Rearrangement of fatty acid on the glycerol moiety catalyzed by lipases may change the physical (*25*) and nutritional properties of the oil (*30*). The new TAG molecules formed in the interesterified oil may be absorbed differently than the original TAG from the native oil or in the blended oil (*31*).

The minor components present in the oils are shown to have independent health benefits. These minor constituents are unique to each oil. For example, tocopherol (vit-E), present in almost all edible oils containing unsaturated fatty acids, is a potent antioxidant. It inhibits LDL oxidation in smokers (*32*), reduces atherogenic effects of saturated fats (*33*), and prevents ischemic heart disease (*34*). Tocotrienol, which is present mainly in palm oil and RBO, reduces cholesterol oxidation (*35*) and has been shown to have antiatherogenic effects (*36*, *37*). γ-Oryzanol, ester of ferulic acid, is uniquely present in RBO and has hypocholesterolemic activity (*38*) by suppressing the HMG Co-A reductase. Sesamin, the lignan molecule in SESO, is a potent and specific inhibitor of Δ-5 desaturase (*39*) and has hypocholesterolemic properties (*40*). It is a precursor of enterolactone (*41*), which reduces the risk of acute coronary events (*42*). Sesamolin, a metabolic product of sesamin, inhibits lipid peroxidation (*43*). Thus, the reports in the literature suggest that both saponifiable and unsaponifiable fractions of oils can exhibit beneficial effects.

The aim of the present study is to examine the effect of blended oils with balanced amounts of fatty acids, along with minor components, on serum and tissue lipids. Further, the effect of interesterification on the blended oil in modulating serum and tissue lipids was also examined.

MATERIALS AND METHODS

Materials. CNO and SESO were purchased from a local market. RBO was provided by Foods, Fats & fertilizers Ltd. (A.P., India). Lipozyme IM-60 was a gift from NOVO Nordisk Bioindustrial Inc. (Danbury, CT, U.S.A.). Palmitic acid, BF_3 in methanol, dipalmitoyl phosphatidylcholine, and cholesterol were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Choline chloride, DL-methionine, magnesium oxide, manganese carbonate, ferric citrate, calcium phosphate, potassium citrate monohydrate, and vitamins were purchased from Himedia Laboratories (Mumbai, India). Heparin, manganese chloride, and sodium metaperiodate were obtained from Sisco Research Laboratory (Mumbai, India). Ferric chloride, ammonium thiocyanate, bipyridyl pyridine, and pyrrogallol were purchased from Qualigens (Mumbai, India). Sodium selinite, manganese carbonate, and potassium sulfate were from Loba chemie (Mumbai, India). Casein was purchased from Nimesh Corporation (Mumbai, India). All solvents used were of analytical grade and distilled before use.

Preparation of Blended Oils. Blended oils were prepared by mixing approximately 20% CNO with 80% RBO or 22% CNO with 78% SESO to get SFA/MUFA/PUFA in near equal proportions (1:1:1). The blended oils were stirred at 40 °C for 1 h using a magnetic stirrer after flushing nitrogen. The mixing efficiency was monitored by periodically withdrawing the blended oil and estimating the fatty acid composition by gas chromatography (GC). The theoretical and experimental values were compared to determine the mixing efficiency.

Preparation of Interesterified Oils. Interesterified oils were prepared from blended oils using lipozyme IM RM *Rhizomucor miehei* at 1% (w/w, specific activity of lipase 6.2 $\pm$ 0.2 μmol/mg protein). Incubations were carried out in a shaking water bath (BS-31) at a speed of 160 rpm for 72 h at 37 °C. After the interesterification reaction, the oil sample was decanted and the enzyme was washed with hexane and dried for reuse. The qualities of oils were checked after interesterification. There was no significant change in the peroxide value of the interesterified oils compared to those of the blended oils. Free fatty acid concentration was increased marginally in interesterified oils. There was no significant change in the nutraceutical contents and fatty acid composition of the oils.

Fatty Acid Composition of Oils. Fatty acid composition of blended and interesterified oils was analyzed by GC (Fisons GC fitted with a flame ionization detector). (*44*) The oils were saponified with 0.5 M KOH and methylated with 40% BF_3 in methanol. The fatty acid methyl esters were separated using a fused silica capillary column 25 m $\times$ 0.25 mm (Parma bond FFAP-DF-0.25, Machery-Negal GmbH co., Duren, Germany). The operating conditions were as follows: initial column temperature 120 °C, raised by 15 °C/min to 220 °C, injection temperature 230 °C, and detector temperature 240 °C. Nitrogen was used as the carrier gas. Individual fatty acid was identified by comparing with the retention times of standards (Nuchek Prep, Elysin, MN, U.S.A.) and was quantified by an online Chromatopac CR-6A integrator.

Tocopherol, Oryzanol, and Lignan Contents in the Oil. Tocopherol contents in the oils were analyzed by HPLC using a Phenomenex C_{18} column of 250 $\times$ 4.60 mm, 5 micron particle size (*45*). Calibration curves were generated using pure standards of the α-, β-, γ-, and δ-tocopherols and tocotrienols. Oryzanol content in RBO and its blends and interesterified samples was estimated spectrophotometrically at 340 nm in a UV–visible spectrophotometer (Shimadzu UV-1601) and quantitated using the molar extinction coefficient as described earlier (*46*). Sesame lignans (sesamin, sesamolin, and sesamol) in SESO and its blends and interesterified samples were separated by HPLC using a mobile phase of 70% methanol and analyzed at 295 nm (*47*).

Table 1. Fatty Acid Composition (wt %) of the Dietary Fats[a]

dietary fat	CNO	RBO	CNO+RBO(B)	CNO+RBO(I)	SESO	CNO+SESO(B)	CNO+SESO(I)
8:0	2.5 ± 0.6	nd	nd	nd	nd	nd	nd
10:0	4.7 ± 0.4	nd	nd	nd	nd	nd	nd
12:0	50.6 ± 0.6	nd	8.4 ± 0.1	8.8 ± 0.3	nd	14 ± 0.1	13.6 ± 0.9
14:0	21.6 ± 0.4	1.4 ± 0.3	5.1 ± 0.1	4.7 ± 0.7	nd	5.7 ± 0.2	5.6 ± 0.2
16:0	9.1 ± 0.5	20.9 ± 2.7	19.0 ± 0.1	18.2 ± 1.4	9.6 ± 0.6	10 ± 0.4	9.6 ± 0.1
18:0	2.7 ± 0.3	2.5 ± 0.5	2.3 ± 0.1	2.4 ± 0.3	5.7 ± 0.6	5.4 ± 0.5	5.1 ± 1.5
18:1	7.0 ± 0.5	41.0 ± 3.5	35.6 ± 0.1	36.0 ± 0.1	41.0 ± 1.2	31.0 ± 0.5	31.3 ± 0.8
18:2	1.8 ± 0.1	34.2 ± 1.0	29.0 ± 0.7	28.9 ± 0.6	44.0 ± 2.8	34.0 ± 1.0	34.3 ± 0.3
SFA	91.5	24.8	34.8	34.7	15.3	35.1	34.3
MUFA	7.0	41.0	350.6	36.0	41.0	30.9	31.4
PUFA	1.8	34.2	29.0	28.9	43.6	34.0	34.3
P/S ratio	0.02	1.5	0.82	0.83	2.8	0.96	1.0
S:M:P	1:0.07:0.02	1:1.83:1.4	1:1.01:0.83	1:1.03:0.83	1:2.67:2.8	1:0.9:0.98	1:0.9:1

[a] Values are the mean of the triplicate samples; nd, not detected (limit of detection; 0.3%); B, blended oils; I, interesterified oils.

Experimental Animals. Male Wistar rats [OUBT-Wistar, IND-cft (2c)] (*Rattus norvegicus*) weighing 45 ± 2 g were grouped (four rats in each group) by random distribution and housed in individual cages, under a 12 h light/dark cycle, and in an approved animal house facility at the Central Food Research Institute in Mysore, India. Animals were given a fresh diet daily, and leftover food was weighed and discarded. The gain in body weight of animals was monitored at regular intervals. The animals had free access to food and water throughout the study. Each group of rats was fed for a total of 60 days, AIN-76 diet containing 10% fat from CNO, RBO, SESO, CNO+RBO(B), CNO+SESO (B), CNO+RBO(I), or CNO+SESO(I), 60% sucrose, 20% casein, 5% cellulose, 3.5% mineral mix, 1.0% vitamin mix, 0.2% choline chloride, and 0.3% methionine (*48*). After 60 days of feeding, rats were fasted overnight and sacrificed under diethyl ether anesthesia. Blood was drawn by cardiac puncture, and serum was separated by centrifuging at 700*g* for 20 min at 4 °C. The liver was removed, rinsed with ice-cold saline, blotted, weighed, and stored at −20 °C until analyzed. The experimental protocol was approved by the institutional animal ethics committee.

Analysis of Lipid Parameters. *Serum Lipid Extraction.* Serum lipids were extracted according to Bligh and Dyer (*49*). Methanol and chloroform were added to the serum separately in proportions of 2:2:0.8 (v/v) and mixed well, and the extract was filtered using Whatman no. 1 filter paper. The filtrate was allowed to settle, and the lower chloroform layer was separated and used for further analysis.

Liver Lipid Extraction. Liver lipid was extracted by the method of Folch et al. (*50*). One gram of liver was homogenized with 1.0 mL of 0.74% potassium chloride, and 20 mL of chloroform and methanol (2:1 v/v) was added and homogenized for 1 min. The mixture was left overnight and filtered through a Whatman no. 1 filter paper. Three milliliters of 0.74% potassium chloride was added and mixed well. The solution was allowed to stand at room temperature. The upper aqueous layer was removed carefully, and then the lower phase was washed with 3 mL of chloroform/methanol/water (3:48:47 v/v) mixture. The chloroform layer was used for lipids analysis.

Total Cholesterol Extraction. The TC in the serum and liver was quantified by the method of Searcy and Bergquist (*51*). An aliquot from the chloroform extract was dried under a stream of nitrogen followed by the addition of 1.5 mL of ferric chloride–acetic acid reagent (504 mg/L). After mixing thoroughly, it was left at room temperature for 15 min. Concentrated sulfuric acid (1 mL) was added, mixed, and left at room temperature in the dark for 45 min. The color intensity was measured in the spectrophotometer (Shimadzu 1601 model) at 540 nm. The cholesterol concentration in the sample was quantitated from a standard curve generated with AnalaR cholesterol (30–150 μg).

HDL Cholesterol Estimation. HDL cholesterol was estimated after precipitating LDL-C with a heparin (5000 units/mL)–manganese chloride reagent (2 M) (*52*). The solution was mixed well and kept at 4 °C overnight. This was then centrifuged at 3500*g* for 20 min. HDL cholesterol was measured as described earlier after extracting the supernatant with acetone/alcohol (1:1 v/v).

LDL+VLDL Cholesterol Estimation. The precipitate obtained from serum after adding heparin and manganese chloride contained LDL+VLDL. The precipitate was dissolved in saline, and cholesterol was extracted with acetone alcohol (1:1 v/v) and estimated as described earlier.

TAG Estimation. TAGs were estimated by the method of Fletcher, (*53*) using tripalmitin as the reference standard (30–300 μg). An aliquot from chloroform extract was evaporated and redissolved in isopropanol. TAG purifier was added, mixed, and centrifuged, and the supernatant was saponified with 0.6 mL of 5% potassium hydroxide in isopropanol/water (2:3 v/v) at 65 °C for 15 min. A 1 mL amount of sodium metaperiodate (for working solution, 12 mL of 0.025 M stock solution of sodium metaperiodate in 1 N acetic acid and 20 mL of isopropanol and brought up to 100 mL with 1 N acetic acid) was added and mixed, and 0.5 mL of acetyl acetone was added, mixed, and incubated at 50 °C for 30 min. The color intensity was measured at 405 nm in a Shimadzu 1601 spectrophotometer.

Phospholipid Estimation. Phospholipids were estimated by a ferrous ammonium thiocyanate method (*54*) using dipalmitoylphosphatidyl choline (15–100 μg) as the reference standard. The lipid extract in chloroform was evaporated and redissolved in 2 mL of chloroform. Ferrous ammonium thiocyanate (2 mL) was added and vortexed for 1 min. Following the phase separation, absorbance of the chloroform phase was measured at 488 nm in a Shimadzu 1601 spectrophotometer.

Fatty acid composition of dietary lipids, serum, and liver were analyzed as methyl esters by GC (*44*) as described earlier.

Statistical Analysis. The results were analyzed statistically using ANOVA (*55*). A P value of <0.01 was not considered to be significantly different.

RESULTS

Fatty Acid Composition of Dietary Lipids. Analysis of the lipids in the diets showed that CNO-containing diets had 92% SFAs; RBO and SESO diets contain 75% and 85% unsaturated fatty acids, respectively (**Table 1**). The SFA/MUFA/PUFA ratio of CNO is 1:0.07:0.02, that of RBO is 1:1.8:1.4, and that of SESO is 1:2.7:2.8 showing that none of the oils used in the study contain fatty acids in the proportions desired by nutritionists. When CNO was blended with RBO or SESO in selected amounts, the SFA/MUFA/PUFA ratios of the resulting blends were in the proportion of 1:1:0.8 and 1:0.9:0.98 for CNO+RBO and CNO+SESO, respectively. The fatty acid composition of interesterified oils was not different from their respective blends.

Nutraceutical Contents. Unique minor components were present in each oil. Blending of two oils resulted in complementing minor components which were lacking in individual oils. Total tocopherol content of CNO was only 3.1 mg/100 g oil (**Table 2**). However, the tocopherol content in RBO was 100.1 mg/100 g oil and in SESO it was 70.5 mg/100 g oil. When CNO was blended with RBO to balance the fatty acid composition, the resulting blended oil contained 79.9 mg of tocopherols/

Table 2. Minor Constituents of Oils[a]

(a) Oryzanol and Sesame Lignans

	oryzanol (%)	sesame lignans (mg/100 g oil)		
	contents	sesamin	sesamolin	sesamol
CNO	nd	nd	nd	nd
RBO	1.25 ± 0.08	nd	nd	nd
CNO+RBO(B)	1.02 ± 0.07	nd	nd	nd
CNO+RBO(I)	1.02 ± 0.08	nd	nd	nd
SESO	nd	98.2 ± 2.7	72.3 ± 1.3	72.3 ± 1.7
CNO+SSESO(B)	nd	166.4 ± 2.3	122.9 ± 2.1	123.0 ± 1.2
CNO+SSESO(I)	nd	0.75 ± 0.02	0.49 ± 0.04	0.48 ± 0.02

(b) Tocopherols and Tocotrienols

	tocopherols (T, mg/100 g oil)			tocotrienols (T_3, mg/100 g oil)			
	α	β and γ	δ	α	β and γ	δ	total (T+T_3, mg/100 g oil)
CNO	nd	nd	nd	2.3 ± 0.3	0.5 ± 0.07	0.3 ± 0.03	3.1
RBO	12 ± 0.6	20.2 ± 1.2	1.8 ± 0.07	14 ± 0.8	51.3 ± 2.4	0.86 ± 0.2	100.1
CNO+RBO(B)	9.6 ± 0.3	16.1 ± 1.1	1.4 ± 0.04	11.1 ± 0.6	41.1 ± 2.3	0.6 ± 0.02	79.9
CNO+RBO(I)	9.1 ± 0.4	15.9 ± 1.1	1.4 ± 0.08	10.6 ± 0.7	41.5 ± 2.1	0.6 ± 0.03	79.1
SESO	nd	67.2 ± 2.3	3.3 ± 0.4	nd	nd	nd	70.5
CNO+SSESO(B)	nd	51.4 ± 1.6	2.1 ± 0.2	0.33 ± 0.2	0.11 ± 0.04	0.06 ± 0.01	54.0
CNO+SSESO(I)	nd	52.2 ± 1.8	2.5 ± 0.1	0.5 ± 0.01	0.1 ± 0.05	0.06 ± 0.01	55.36

[a] nd, not detected; values are the mean ± SD of the triplicate samples; B, blended oils; I, interesterified oils.

Table 3. Growth and Organ Weights of Rats Fed Native, Blend, and Interesterified Oils[a]

	food intake (g/rat) daily	total gain in body wt (g)	FER	liver wt (g/100 g body wt)	heart wt (g/100 g body wt)	brain wt (g/100 g body wt)
CNO	12.5 ± 0.95	235 ± 20	0.27 ± 0.008	3.3 ± 0.28	0.29 ± 0.02	0.56 ± 0.06
RBO	11.7 ± 1.3	216 ± 49	0.27 ± 0.03	3.4 ± 0.09	0.28 ± 0.05	0.63 ± 0.06
CNO+RBO(B)	12.1 ± 0.69	222 ± 24	0.27 ± 0.01	3.7 ± 0.4	0.28 ± 0.02	0.57 ± 0.06
CNO+RBO(I)	11.9 ± 0.99	219 ± 35	0.27 ± 0.02	3.5 ± 0.2	0.27 ± 0.04	0.61 ± 0.07
SESO	12.2 ± 0.94	219 ± 24	0.27 ± 0.04	3.5 ± 0.02	0.28 ± 0.03	0.56 ± 0.06
CNO+SESO(B)	12.2 ± 1.3	238 ± 27	0.28 ± 0.01	3.5 ± 0.2	0.28 ± 0.01	0.56 ± 0.07
CNO+SESO(I)	12.1 ± 1.0	260 ± 18	0.27 ± 0.02	3.6 ± 0.4	0.27 ± 0.01	0.57 ± 0.06

[a] Values are mean ± SD ($n = 4$ rats); FER (food efficiency ratio) calculated by dividing total gain in body weight to food intake.

Table 4. Serum Lipid Profile (mg/dL) of Rats Fed Native, Blend, and Interesterified Oils[a]

	CNO	RBO	CNO+RBO(B)	CNO+RBO(I)	SESO	CNO+SESO(B)	CNO+SESO(I)
total cholesterol	66.2 ± 3.6 a	42.7 ± 1.8 b	50.4 ± 3.0 c	43.0 ± 2 b	50.7 ± 2.1 c	52.6 ± 3.4 c	44.1 ± 2.8 b
HDL-cholesterol	17.1 ± 2.1 a	18.0 ± 1.4 a	17.2 ± 1 a	18.1 ± 1 a	20.5 ± 1.0 b	20.3 ± 1.0 b	18.7 ± 1.0 a
LDL+VLDL	49.1 ± 2.1 a	24.8 ± 1.8 b	33.2 ± 2.3 c	25.0 ± 1 b	30.2 ± 1.6 c	32.4 ± 2.4 c	26.0 ± 2.0 b
triacyglycerols	147 ± 5.9 a	110 ± 2.5 b	127 ± 2.6 c	114 ± 1.6 b	128 ± 2.9 c	130 ± 3.7 c	118 ± 1.5 b
phospholipids	103 ± 8.0 a	104 ± 5.9 a	102 ± 8.0 a	93 ± 2.4 a	101 ± 7.8 a	90 ± 5.8 a	94 ± 5.9 a

[a] Values show the mean ± SD ($n = 4$ rats); values in the same row with common letters are not significantly different at $P < 0.01$; B, blended oils; I, interesterified oils.

100 g oil. When CNO was blended with SESO, the tocopherol concentration was found to be 54 mg/100 g oil. The oryzanol content in RBO was 1.25% (**Table 2**). Oryzanol was not found in any other oil. When CNO was blended with RBO, the blended oil contained 1.02% oryzanol. The major lignans present in SESO are sesamin, sesamolin, and sesamol at 98.2, 166.4, and 0.75 mg/100 g oil, respectively (**Table 2**). These lignans were not found in other oils. Blending CNO with SESO resulted in enriching the blended oil with sesamin, sesamolin, and sesamol content at 72.3, 122.9, and 0.49 mg/100 g oil, respectively, which was not present in CNO. The content of these minor constituents in interesterified oils was similar to that found in blended oils.

Effect of Dietary Lipids on Growth Parameters. The amount of food consumed by all rats in each group was similar. The average food intake was 12.1 ± 0.23 g/rat daily (combined mean ± SD of all groups, **Table 3**). There was no significant change in the food efficiency ratio measured by the gain in body weight to the amount of food consumed. The weights of the liver and heart were similar in rats given different dietary fats. Though marginally higher weight for the brain was observed in rats given RBO, it was not statistically significant. Histological examination of organs showed no abnormalities. This study indicated that feeding blended and interesterified oils had no adverse effect on growth and general health of animals.

Effect of Pure Oils on Lipid Parameters in Serum. The type of fat consumed altered the cholesterol concentration in serum. Rats fed CNO had serum TC concentration of 66.2 mg/dL (**Table 4**) while those fed RBO had serum cholesterol concentration of 42.7 mg/dL. Thus, a 35.5% decrease in serum cholesterol concentration was observed in rats given RBO compared to those fed CNO. Rats fed SESO had a serum cholesterol concentration of 50.7 mg/dL, which was 23.4% lower than that observed in rats fed CNO. The LDL-C and TAG were decreased by 49.6 and 25.4%, respectively, in rats fed RBO, and these lipids in rats fed SESO were reduced by 38.6 and 22.6%, respectively, as compared to the rats given CNO.

Table 5. Liver Lipid Profiles (mg/g Tissue) of Rats Fed Native, Blend, and Interesterified Oils[a]

	CNO	RBO	CNO+RBO(B)	CNO+RBO(I)	SESO	CNO+SESO(B)	CNO+SESO(I)
total cholesterol	6.2 ± 0.2 a	4.6 ± 0.4 b	5.2 ± 0.16 c	4.5 ± 0.1 b	5.5 ± 0.2 c	5.7 ± 0.1 c	5.1 ± 0.1 b
triacyglycerols	18.5 ± 1.1 a	13.5 ± 0.8 b	15.1 ± 0.5 c	12.4 ± 0.8 b	15.4 ± 0.5 c	16.5 ± 0.6 c	13.7 ± 0.7 b
phospholipids	18.1 ± 1.5 a	16.5 ± 1.7 a	17.1 ± 1.2 a	18.1 ± 1.4 a	17.6 ± 0.9 a	17.9 ± 0.2 a	17.5 ± 0.9 a

[a] Values show the mean ± SD ($n = 4$ rats); values in the same row with common letters are not significantly different at $P < 0.01$; B, blended oils; I, interesterified oils.

Table 6. Fatty Acid Composition (wt %) of Serum in Rats Fed Native, Blend, and Interesterified Oils[a]

	CNO	RBO	CNO+RBO(B)	CNO+RBO(I)	SESO	CNO+SESO(B)	CNO+SESO(I)
12:0	0.9 ± 0.2 a	nd	nd	nd	nd	nd	nd
14:0	6.2 ± 0.6 a	1.7 ± 0.4 b	0.9 ± 0.03 b	0.81 ± 0.02 b	2.4 ± 0.7 b	2.4 ± 0.2 b	1.3 ± 0.01 b
16:0	28.8 ± 1.4 a	24.8 ± 1.8 a	27.0 ± 2.0 a	27.0 ± 1.8 a	25.2 ± 1.1 a	27.9 ± 1.8 a	27.1 ± 2.1 a
16:1	5.6 ± 0.2 a	1.9 ± 0.3 a	2.8 ± 0.01 a	2.4 ± 0.1 a	1.3 ± 0.08 a	3.2 ± 0.04 a	2.5 ± 1.1 a
18:0	10.4 ± 0.7 a	12.3 ± 1.6 a	12.8 ± 0.2 a	10.3 ± 0.3 a	11.3 ± 0.3 a	8.9 ± 0.6 a	9.9 ± 0.4 a
18:1	34.9 ± 3.0 a	32.0 ± 2.6 a	28.4 ± 2.1 a	32.7 ± 1.2 a	29.4 ± 1.8 a	27.6 ± 1.7 a	27.6 ± 2.0 a
18:2	6.8 ± 0.1 a	16.1 ± 0.5 b	13.2 ± 0.3 b	15.5 ± 0.3 b	19.0 ± 1.2 b	17.5 ± 1.6 b	20.2 ± 0.9 b
20:4	6.4 ± 0.2 a	11.1 ± 0.6 b	14.3 ± 0.9 b	11.2 ± 0.5 b	11.3 ± 0.6 b	13.1 ± 0.8 b	10.9 ± 0.7 b
P/S ratio	0.29	0.71	0.68	0.70	0.78	0.73	0.81

[a] nd, not detected; values show the mean ± SD ($n = 4$ rats); values in the same row with common letters are not significantly different at $P < 0.01$; B, blended oils; I, interesterified oils.

Effect of Blended Oils on Lipid Parameters in Serum. Rats fed blended oil consisting of CNO+RBO had a serum cholesterol concentration of 50.4 mg/dL, which was 23.8% lower than in rats given CNO (**Table 4**). When CNO was blended with SESO to get a balanced fatty acid composition and fed to rats, the serum cholesterol was found to be 52.6 mg/dL, which was at 20.5% lower concentration as compared to rats fed CNO. LDL-C and TAG concentration in serum also showed decreases in rats fed blended oils as compared to rats given CNO. Rats fed blended oils of CNO+RBO showed a 32.4 and 13.9% decrease, respectively, in LDL-C and TAG in serum compared to those fed CNO. LDL-C and TAG concentrations in rats fed blended oil consisting of CNO+SESO showed 34 and 12.9% decrease, respectively, compared to the rats given CNO (**Table 4**).

Effect of Interesterified Oils on Lipid Parameters in Serum. The rats fed interesterified oil of CNO+RBO had significantly lower concentration of serum cholesterol by 14.6 and 35% compared to rats fed blended oil of CNO+RBO and CNO alone, respectively (**Table 4**). The cholesterol concentrations in rats fed interesterified oil of CNO and RBO were comparable to those given RBO alone (**Table 4**). Rats given interesterified oils of CNO+SESO showed a decrease in the serum cholesterol concentrations by 16% compared to those given blended oil of CNO+SESO. LDL-C and TAG concentrations in serum of rats fed interesterified oils of CNO+RBO showed a 49.2 and 23% reduction, respectively, compared to those fed CNO and a reduction of 24.7 and 10.7% compared to those fed its blend CNO+RBO. The rats given interesterified oils of CNO+SESO showed a reduction of 47.0 and 19.8% in LDL-C and TAG concentration, respectively, compared to the rats given CNO and a reduction of 19.6 and 7.8%, respectively, compared to rats fed blended oil of CNO+SESO. The HDL and phospholipids concentrations however remained unaltered in all the groups (**Table 4**).

Effect of Pure Oils on Lipid Parameters in Liver. The liver is an important site for lipid metabolism. Total cholesterol in liver of rats fed CNO was 6.2 mg/g tissue while those fed RBO had liver cholesterol concentration of 4.6 mg/g (**Table 4**). Thus, a 25.8% decrease in liver cholesterol was observed in rats given RBO compared to those fed CNO. Liver cholesterol concentration of rats fed SESO was 5.5 mg/g tissue, which was 11.2% lower than that observed in rats fed CNO. The liver TAG concentration was also altered by the type of fat given to the rats. There was a decrease in the TAG concentration in liver by 27% and 16.7% in rats fed RBO and SESO, respectively, compared to rats fed CNO (**Table 5**). There was no difference in hepatic phospholipids of rats given different dietary lipids.

Effect of Blended Oils on Lipid Parameters in Liver. Rats fed blended oil consisting of CNO+RBO showed a significant decrease in TC and TAG by 16% and 13.4%, respectively, compared to the rats given CNO (**Table 5**). Feeding rats with blended oil of CNO+SESO showed a decrease in cholesterol and TAG concentration by 8.1 and 10.8%, respectively, compared to rats given CNO alone (**Table 5**).

Effect of Interesterified Oils on Lipid Parameters in Liver. The rats fed interesterified oils of the CNO+RBO combinations showed a decrease in TC and TAGs by 27.4 and 32.9% compared to the rats given CNO alone and by 13.5 and 17.8% compared to the rats fed blended oils of CNO+RBO (**Table 5**). Similarly rats given interesterified oils of CNO+SESO showed a decrease in cholesterol and TAGs by 17.7 and 25.9%, respectively, as compared to rats given CNO alone and by 10.5 and 16.7% compared to rats fed blended oils of CNO+SESO (**Table 5**). The phospholipid composition in liver remained unaltered irrespective of the type of fat fed to the rats.

Fatty Acid Composition of Serum and Liver Lipids. Fatty acid analysis of serum lipids reflected on the type of fat fed to rats. Linoleic acid (18:2) and arachidonic acid (20:4) content of rats fed CNO was significantly lower than that in rats fed RBO or SESO (**Table 6**). When rats were fed blended and interesterified oils of CNO with RBO or SESO, there was a significant increase in linoleic and arachidonic content in serum fatty acids compared to rats fed CNO alone (**Table 6**).

The linoleic acid concentration in serum lipids was enhanced by 1.94-fold and 2.28-fold in rats given CNO+RBO blend and CNO+RBO interesterified oils, respectively, as compared to those given CNO alone. Similarly, the linoleic acid concentrations were enhanced by 2.52-fold and 2.91-fold in rats given CNO+SESO blended and interesterified oils, respectively, compared to those given CNO alone (**Table 6**). The arachidonic acid concentrations were also enhanced by 1.7–2.2-fold in rats given blended or interesterified oils as compared to those given CNO alone. This indicated the improved essential fatty acid (EFA) status in rats given blended or interesterified oils.

The fatty acid composition of liver lipids also followed a pattern similar to that of the serum. Rats fed CNO had

Table 7. Fatty Acid Composition (wt %) of Liver in Rats Fed Native, Blend, and Interesterified Oils[a]

	CNO	RBO	CNO+RBO(B)	CNO+RBO(I)	SESO	CNO+SESO(B)	CNO+SESO(I)
12:0	0.4 ± 0.01 a	nd	nd	nd	nd	nd	nd
14:0	1.7 ± 0.25 a	nd	0.9 ± 0.15 a	1.2 ± 0.02 a	0.4 ± 0.01 a	0.3 ± 0.02 a	0.7 ± 0.2 a
16:0	29.7 ± 1.7 a	24.3 ± 1.9 a	26.7 ± 1.5 a	26.5 ± 1.4 a	22.1 ± 1.3 a	25.3 ± 1.0 a	24.9 ± 2.3 a
16:1	4.7 ± 0.1 a	2.7 ± 0.8 a	2.4 ± 0.04 a	2.2 ± 0.7 a	1.3 ± 0.02 a	1.5 ± 0.03 a	1.6 ± 0.04 a
18:0	17.6 ± 1.2 a	12.0 ± 1.4 a	15.3 ± 1.5 a	10.5 ± 0.8 a	14.3 ± 1.1 a	12.8 ± 0.5 a	11.7 ± 0.6 a
18:1	33.2 ± 1.2 a	37.0 ± 1.7 a	29.0 ± 2.1 a	37.2 ± 3.3 a	33.2 ± 2.1 a	30.5 ± 3.0 a	33.6 ± 2.9 a
18:2	5.5 ± 0.3 a	13.6 ± 1.2 b	13.0 ± 0.5 b	11.5 ± 0.1 b	14.9 ± 0.8 b	15.7 ± 0.9 b	16.0 ± 0.3 b
20:4	6.8 ± 0.4 a	10.3 ± 0.14 b	12.5 ± 0.2 b	10.7 ± 0.7 b	13.8 ± 1.0 b	13.7 ± 1.8 b	11.1 ± 1.8 b
P/S ratio	0.25	0.64	0.59	0.58	0.78	0.76	0.72

[a] nd, not detected; values show the mean ± SD ($n = 4$ rats); values in the same row with common letters are not significantly different at $P < 0.01$; B, blended oils; I, interesterified oils.

significantly lower linoleic acid and arachidonic acid content in liver lipids compared to rats fed RBO and SESO. Feeding rats with blended and interesterified oils of CNO+RBO and CNO+SESO significantly enhanced linoleic and arachidonic acid compared with rats given CNO alone. Rats fed blended oils of CNO+RBO or CNO+SESO showed a 2.36- and 2.85-fold increase in linoleic acid concentration compared to rats given CNO alone. The rats given interesterified oils of CNO+RBO or CNO+SESO had 2- and 2.9-fold more linoleic acid content compared to those given CNO. Rats fed blended or interesterified oils of CNO+RBO and CNO+SESO had a 1.57–2-fold increase in arachidonic acid content compared to rats fed CNO alone (**Table 7**).

DISCUSSION

The present study was undertaken to examine the effect of blended oils that contained approximately equal amounts of SFA/MUFA/PUFA. These oils were also enriched with nutraceuticals, which were not found in CNO, and used in the diet as control fat. CNO is a rich source of medium chain SFAs. Medium chain fatty acids (MCFAs) per se have many desirable characteristics such as high oxidative stability, low viscosity, low melting point, and high solubility in water, which makes CNO useful for Indian cooking. In India, 80% of oil is used for frying dishes where they are subjected to high temperatures of 180 °C and above. Hence, stability of oil under frying conditions is a desirable quality in Indian cooking. Oils, in addition, should also provide good nutritional qualities. Even though CNO contains MCFAs, concerns have been expressed as it also contains high amounts of myristic acid and palmitic acid, which elevate serum cholesterol (*8, 56, 57*). CNO is limiting in EFAs.

Lesser known oils, like RBO and SESO, that are used in India are rich in unsaturated fatty acids, and they have hypocholesterolemic effect (*38, 40*). To keep the stability of a saturated fat for cooking purposes and to provide good nutritional qualities, one needs to balance different types of fatty acids in the oils. The Indian Council of Medical Research has advocated that the edible oil should be well balanced in terms of different fatty acids. They further recommend for equal proportions of SFA/MUFA/PUFA in oils. Keeping these guidelines in mind, we have prepared blends of CNO with RBO or SESO to achieve a balance in different fatty acids because no single oil used in India can provide such a balanced oil.

In the present study, we prepared the oils with SFA/MUFA/PUFA in equal proportions and in this process also achieved a desirable P/S ratio of 0.8–1.0. Earlier workers have also noticed that blending of oils improves the physicochemical (*25, 58, 59*) and nutritional properties of the oil. Koba et al. have shown that feeding rats with a diet containing blended oils comprising RBO and safflower oil in the ratio of 70:30 increased the HDL concentrations and increased the HDL/TC ratio significantly (*60*). Sugano and Tsuji (*61*) explained the specific cholesterol lowering effect of this blended oil based on the combined effect of the linoleic acid from sunflower oil and the unsaponifiable matter present in the RBO. Our present studies have similarly shown a significant cholesterol lowering effect of blended oils consisting of CNO+RBO and CNO+SESO in comparison with rats given CNO alone.

Even though increasing the dietary PUFA/SFA ratio has been recommended by earlier investigators for reducing risk factors for CVD, a high PUFA/SFA ratio of diet may enhance oxidative stress because PUFAs are highly susceptible to oxidation. Feeding rats with fats having different concentrations of PUFA/SFA, Kang et al. (*62*) showed that a PUFA/SFA ratio of 1.0–1.5 is a desirable range to reduce risk factors for CVD. Chang et al. (*63*) found that not only the P/S ratio but also the P+M/S ratio is important in controlling the serum and hepatic lipids in rats. They fed rats with oils having different concentrations of P+M/S ratios, but in all cases, the P/S ratio was kept nearer to 1.0. The oils with P+M/S ratio of around 2 showed beneficial effects on serum and tissue lipids, but oils with P+M/S ratio of greater than 4 increased the serum TC, LDL-C, and VLDL-cholesterol. Therefore, there should be a balance among SFAs, MUFAs, and PUFAs of dietary lipids. The blended oils we have prepared had a P/S ratio of 0.8–1.0 and a P+M/S ratio of 1.92–2.42. The step one dietary management of CHD promoted by the Indian Council of Medical Research (*16*) recommended that dietary fat should have 1:1:1 ratio for SFA/MUFA/PUFA and that the diet should provide 20–25% energy from fat. The blended oils we have used in this investigation adhere to these requirements.

It is also interesting to note that when CNO with a P/S ratio of 0.02 was fed to rats, the P/S ratio of serum fatty acids was 0.25. However, when this P/S ratio was enhanced to 0.8–1.0 by blending CNO with RBO or with SESO, the P/S ratio in serum fatty acids was increased to 0.68–0.81. RBO with a P/S ratio 1.5 and SESO with P/S ratio of 2.8 could increase the P/S ratio of serum lipids to 0.71 and 0.78, respectively, indicating that higher concentrations of P/S ratio of oils have limitations to the extent that the serum fatty acid composition can be altered. Hence, a P/S ratio of 0.8–1.0 in the oil may suffice to maintain EFA status in the animals.

It is well established that both the quality and the quantity of dietary TAGs in the oils can influence plasma cholesterol concentrations in humans and in a range of animal species (*64*). In a blended oil, the individual TAGs from the parent oils retain their native structure. However interesterification reaction rearranges the fatty acids in the TAG molecules and produces fats with altered properties. Interesterification using lipases randomizes the fatty acid distribution in the TAGs leading to modifica-

tions in the chemical composition and, hence, may also change nutritional properties of the oil(s) (*65*, *25*, *31*). Studies by Otero et al. (*66*) showed that transesterification of 80% SESO with 20% fully hydrogenated soybean oil resulted in decrease in fully liquid TAG species in SESO of trilinolein, 1,3-dilinoleoyl 2-oleate, and 1,3-dioleoyl 2-linoleate from 15.97 to 4.84%, 31.5 to 17.46%, and 21.15 to 13.93%, respectively. The TAG responsible for solid fat in fully hydrogenated fat, tristearin, and 1,3-distearoyl 2-palmitate decreased from 73.74 to 0.07% and 26.3 to 0.43%, respectively, in interesterified fat. Interesterification resulted in the production of a semi liquid 21.27% TAG, 1,2dioleoyl 3-palmitate, which was only 1.86% in SESO. Christensen and Hoelmer (*67*) synthesized a human milk fat substitute containing omega-3 fatty acids by an interesterification reaction catalyzed by *R. miehei*. Enzymatic interesterification lowered the solid fat content of butter fat above 20 °C when it is interesterified with 40 and 30% canola oil (*68*). Oba and Witholt (*69*) showed the incorporation of about 50% oleic acid when milk fat is interesterified with oleic acid using immobilized *Rhizopus oryzae* lipase. Randomized structured TAGs improved the digestion, absorption, and lymphatic transport of lipids and fat-soluble vitamins (*70*). Structured TAGs that contain one or two MCFAs may provide a vehicle for rapid hydrolysis and absorption because of their smaller molecular size and greater water solubility in comparison to long chain TAGs (*71*). Feeding rats with structured lipid significantly reduced the liver cholesterol concentration by 27% compared to the blend of coconut and safflower oil (1:0.7 ratio) with similar fatty acid composition (*72*).

In the present investigation, we studied the effect of feeding the interesterified oils on serum and liver lipid parameters. Rats fed interesterified oils of CNO+RBO(I) showed a decreased cholesterol concentration compared to rats fed the blend of CNO+RBO(B). The effect of interesterified oil was comparable to that observed in rats fed RBO. Similarly, rats fed interesterified fats containing CNO+SESO(I) showed a significant decrease in serum and hepatic cholesterol concentrations compared to rats given the blend of CNO+SESO(B) of similar composition. Therefore, interesterified fats showed higher hypolipidemic effect compared to blended oils. This is in agreement with earlier observations on the efficacy of interesterified oil over blended oil in beneficially modifying many metabolic effects cited.

The ability of a fat to alter serum lipids depends not only on the type of fatty acid but also on the minor constituent present in the oil. Studies on humans (*73*) have shown that the cholesterol reducing property of RBO is due to the unsaponifiable compounds present in the oil. Oryzanol lowers the cholesterol concentration by suppressing cholesterol absorption and enhancing the fecal sterol excretion (*74*, *75*). RBO is also rich in β- and γ-tocotrienols (*76*). Studies on humans have shown that tocotrienols lower cholesterol through the inhibition of HMG–CoA reductase, the rate limiting enzyme in cholesterol biosynthesis (*77*). The tocotrienol rich fraction of RBO is also reported to lower serum TC and LDL-C concentrations in hypercholesterolmic individuals (*37*, *74*). Feeding oryzanol at 0.5% concentration showed cholesterol lowering effect in hamsters (*78*), and a 0.2% concentration of oryzanol was effective in reducing the cholesterol concentration in rats (*79*). In the present study, rats consumed a diet containing 0.125% oryzanol in the groups given RBO alone and 0.102% by rats given blended and interesterified oils of CNO+RBO. This may also have contributed to the cholesterol lowering effect of CNO+RBO combinations.

Sesamin from SESO has been proven to be hypocholesterolemic. Feeding rats with 0.5% sesamin showed significant decrease in cholesterol absorption by 30% compared with control rats (*80*). Studies by Chen et al. have shown that consumption of 0.6% of sesamin and sesamolin exhibit hypolipidemic effect in humans (*40*). In the present study, rats consumed a diet containing 0.2% and 0.33% sesamin and sesamolin daily when fed with SESO alone and 0.16 and 0.26% sesamin and sesamolin when rats were given blended and interesterified oils of CNO+SESO. Hence, sufficient lignans were provided to rats given blended and interesterified oils containing SESO. Sesamin is the precursor of enterolactone (*41*), which reduces the risk of acute coronary events (*42*)—it is unique to SESO. This unique compound, which improves cardiovascular health, can be provided using blends of SESO .

In conclusion, the present study was undertaken to provide an oil with balanced fatty acid composition of SFA, MUFA, and PUFA in a 1:1:1 ratio by blending CNO with RBO or SESO. This also resulted in an oil with a P/S ratio of 0.8–1.0. Feeding rats with these blended oils resulted in lowering the hypercholesterolemic effect of CNO. When the blended oils were subjected to interesterification and fed to rats, it further reduced serum and liver cholesterol. The higher hypolipidemic effect of interesterified oils was observed even though both blended and interesterified oils had similar fatty acid compositions. Work is in progress to understand the reasons for higher hypolipidemic activity of interesterified oils compared to that of blended oils.

ABBREVIATIONS USED

CNO, coconut oil; RBO, rice bran oil; SESO, sesame oil; MUFA, monounsaturated fatty acid; SFA, saturated fatty acid; PUFA, polyunsaturated fatty acid; CVD, cardiovascular disease; P/S, polyunsaturated/saturated; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol; TC, total cholesterol; CHD, coronary heart disease; P+M/S, polyunsaturated and monounsaturated/saturated; TAG, triacylglycerol; MCFAs, medium chain fatty acids; (B), blend; (I), interesterified; FER, food efficiency ratio.

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