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Enhanced Hypocholesterolemic Effects of Interesterified Oils Are Mediated by Upregulating LDL Receptor and Cholesterol 7-α- Hydroxylase Gene Expression in Rats[1–3]

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Abstract

The concentration of LDL cholesterol in plasma is strongly influenced by the amount and type of lipid in the diet. Our studies have shown that positional changes in the fatty acids in blended oil introduced using lipase-catalyzed interesterification differentially modulate circulating LDL levels in rats compared with those observed in rats given a physical blend of oils. To investigate the molecular basis of these differences, transcriptional profiling of genes involved in cholesterol homeostasis was studied after feeding rats with a semipurified diet containing 10% fat from native oils; coconut oil (CNO), rice bran oil (RBO), or sesame oil (SESO); blended (B); CNO+RBO(B) or CNO+SESO(B) and interesterified oil (I); CNO+RBO(I) or CNO+SESO(I) for 60 d. Hepatic LDL receptor (LDL-R) expression significantly increased in rats fed interesterified oils by 100–200% compared with rats fed blended oils and by 400–500% compared with rats fed CNO. Positional alteration in fatty acids of oils used in the diet induced changes in LDL-R expression, which was accompanied by parallel changes in cholesterol-7α-hydroxylase (CYP7A1) and SREBP-2 genes. This suggested that not only the fatty acid type but also its position in the TG of dietary lipids play an important role in maintaining plasma cholesterol levels by suitably modulating gene expression for LDL-R in rat liver. J. Nutr. 141: 24–30, 2011.

Introduction

Dietary fat plays an important role in modulating risk factors for cardiovascular diseases (1). High concentrations of serum cholesterol, especially LDL cholesterol, are considered a high risk factor of cardiovascular diseases. The deposition of oxidized LDL leads to plaque formation and thickening of the arteries (2). Serum cholesterol concentration therefore needs rigorous control. Fats rich in long-chain SFA increase total and LDL cholesterol in serum (3), whereas unsaturated fatty acids decrease these serum lipids (4). Excessive consumption of unsaturated fatty acids leads to higher peroxidation in lipoproteins (5). A balance in the fatty acid composition of dietary lipids is necessary for deriving the optimal health benefits of oils. The Indian Council of Medical Research and the AHA (6,7) have recommended intakes of dietary oils that contain approximately equal amounts of SFA, MUFA, and PUFA for improved health benefits.

In India, dietary habits, particularly fat consumption, vary from region to region. Coconut oil (CNO)[6] is the predominant dietary fat used by populations from Kerala and coastal regions of Karnataka in South India. About 90% of fatty acids present in CNO are SFA. The other oils used include sesame oil (SESO) and rice bran oil (RBO). Oils used in India do not possess the desired fatty acid composition recommended by the Indian Council of Medical Research. This underscores the need for modifying oils and fats used in Indian cooking.

One feasible approach for obtaining oil with a balanced fatty acid composition is to blend 2 or more oils in appropriate amounts. Although one can achieve the desirable proportions of fatty acids by blending selected oils, this may not provide the anticipated physicochemical and nutritional properties. This is because the individual TG molecules of each oil used for blending retain their original structure and physical characteristics (8). The fatty acids in TG molecules of oils can be rearranged using either an enzymatic or chemical interesterification process. This rearrangement of the fatty acid can occur within and between TG molecules (9), which alters the compo-

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[6] Abbreviations used: B, blended oil; CNO, coconut oil; CYP7A1, cholesterol 7-α-hydroxylase; HMG-CoA, 3-hydroxy-3-methylglutaryl CoA; I, interesterified oil; LDL-R, LDL receptor; RBO, rice bran oil; SESO, sesame oil; SREBP, sterol regulatory element binding protein; TC, total cholesterol.

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sition of existing TG species in oils (10). Interesterification is therefore a useful tool for the production of designer lipids with improved physicochemical properties (11).The properties of blended oils, which contain nearly equal proportions of SFA, MUFA, and PUFA, were reported by us (12). These oils were subjected to interesterification reactions using an immobilized lipase. Rats fed interesterified oils had an improved hypocholesterolemic effect compared with the blended oil-fed group (12,13).

Cholesterol homeostasis is tightly controlled by coordinated changes in the concentrations of mRNA encoding multiple enzymes involved in cholesterol biosynthesis, uptake, and efflux pathway (14). 3-Hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase is the rate-limiting enzyme in cholesterol biosynthesis. Cholesterol oxidation by cholesterol-7α-hydroxylase (CYP7A1) is the rate-limiting enzyme in bile acid biosynthesis, a major efflux pathway for the elimination of cholesterol from the body (15). LDL receptors (LDL-R) play a vital role in the hepatic uptake and clearance of plasma cholesterol (16). Sterol regulatory element binding protein-2 (SREBP-2) is a transcription factor that regulates cholesterol homeostasis by virtue of its ability to bind and activate the promoters of the genes encoding for LDL-R and HMG-CoA reductase (17). Both the LDL-R and HMG-CoA reductase genes have a sterol regulatory element in their promoter regions and are therefore commonly regulated by SREBP-2 (18).

Most earlier studies have focused on the effect of different dietary fatty acids on total and LDL cholesterol concentrations and their effects on the expression of genes involved in cholesterol metabolism (19). However, the superior cholesterol-lowering property of interesterified oil, compared with blended oil with a similar fatty acid composition, has not been addressed. In this investigation, we studied the effect of blended and interesterified oils with similar fatty acid compositions on the mRNA abundance of genes involved in cholesterol homeostasis in rat liver.

Materials and Methods

Reagents

CNO and SESO were purchased from a local supermarket. Physically refined RBO was provided by A. P. Solvex Limited. Lipozyme IM RM was a gift from NOVO Nordisk Bioindustrial. Boron trifluoride in methanol, DTT, 5,5'-dithiobis (2-nitrobenzoic acid), NADPH, HMG-CoA, TRI reagent, and deoxy nucleoside triphosphate mixtures were purchased from Sigma Chemical. All solvents used were analytical grade and distilled before use.

Preparation of blended and interesterified oils

Blended and interesterified oils were prepared as described earlier (12). The blending was carried out by mixing predetermined amounts of oils with stirring for 1 h at 40°C on a magnetic stirrer and continuous flushing with nitrogen. The mixing efficiency was monitored by estimating the fatty acid composition of the blended oils periodically and comparing it with the theoretical value.

Interesterified oil was prepared by incubating the blended oils with an immobilized lipase (*Rhizomucor miehei* lipozyme IM RM) at 1% (wt:wt) and agitating at a speed of 160 rpm in a shaking water bath (BS-31) for 72 h at 37°C. The oil was decanted, and the recovered enzyme was washed with hexane for reuse. The quality of interesterified oil was then evaluated by measuring peroxide values and FFA content. Interesterification did not significantly affect the quality, nutraceutical contents, or total fatty acid composition of the oils compared with blended oils (12).

Fatty acid composition of oils

The fatty acid compositions of native, blended, and interesterified oils were analyzed as methyl esters by GC (20). The FAME were prepared by saponifying oils with 0.5 mol/L KOH and methylating FFA with 40% boron trifluoride in methanol. The FAME were separated using a fused silica capillary column (25 m $\times$ 0.25 mm, Parma bond FFAP-DF-0.25: Machery-Negal) connected to a GC (Shimadzu 14B fitted with FID). The operating conditions were: 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 acids were identified by comparing them with the retention times of standards (Nuchek Prep) and quantified using Clarity Lite software.

Rat experiments

All the experiments involving rats were approved by the animal ethical committee at the Central Food Technological Research Institute, Mysore, India, which in turn was approved by the Government of India. Male Wistar rats were bred and supplied by the CFTRI Animal House [OUTB-Wistar, IND-cft (2c)]. Rats weighing 40–45 g were grouped (n = 6) by random distribution and housed in individual cages under a 12-h-light/-dark cycle in an approved animal house facility at the Central Food Technological Research Institute, Mysore, India. Rats were fed daily and leftover feed was weighed and discarded. The gain in body weight of rats was monitored at regular intervals. The rats had free access to food and water throughout the study. Each group of rats were fed with an AIN-76 diet containing 10% fat from either CNO, RBO, SESO, CNO+RBO(B), CNO+SESO(B), CNO+RBO(I), or CNO+SESO (I); sucrose, 60%; casein, 20%; cellulose, 5%; mineral mix, 3.5%; vitamin mix, 1.0%; choline chloride, 0.2%; and DL-methionine, 0.3% (21). The effect of feeding RBO or SESO or their blended oils, CNO+RBO(B) or CNO+SESO(B) or interesterified oils, CNO+RBO(I) or CNO+SESO(I) were compared with native CNO. The effects of interesterified oils were also compared with their respective blended oils. After 60 d of feeding, rats were deprived of food and killed under diethyl ether anesthesia. Blood was collected by cardiac puncture. The collected blood was left at ambient temperature for 4 h. The serum was obtained after centrifuging the clotted blood at 750 $\times$ g for 15 min. No hemolysis occurred in any of the samples. The liver was removed and immediately frozen in liquid nitrogen for storage at -80°C.

Analysis of lipids

Serum lipid extraction. Serum lipids were extracted according to the method of Bligh and Dyer (22). Methanol and chloroform (2 mL each) were added to 0.8 mL serum and mixed well. The extract was filtered using Whatman no. 1 filter paper. The filtrate was allowed to settle and the chloroform layer was separated and used for further analysis.

Total cholesterol extraction. The total cholesterol in the serum was quantified by the method of Searcy and Bergquist (23). An aliquot of the chloroform extract was dried under a stream of nitrogen followed by the addition of 1.5 mL of ferric chloride-acetic acid reagent, mixed thoroughly, and kept in the dark at 25 $\pm$ 2°C for 15 min. Concentrated sulphuric acid (1 mL) was added, mixed, and left at 25 $\pm$ 2°C in the dark for 45 min. The color intensity was measured at 540 nm by using a UV-VIS spectrophotometer (Shimadzu 1601). The cholesterol in serum samples was quantified by using a calibration curve generated with cholesterol (30–150 μg of 99.5% purity).

Lipoprotein estimation

HDL cholesterol was estimated after precipitating apoB-containing lipoproteins (LDL+VLDL) with heparin (5 MU/L)-manganese chloride reagent (2 mol/L) (24). The solution was mixed well with serum and kept at 4°C overnight. This was then centrifuged at 3500 $\times$ g for 20 min. After the removal of VLDL and LDL by precipitation, HDL cholesterol was measured using the same method as for total cholesterol (23).

HMG-CoA reductase activity

Liver microsomes were isolated as described (25). HMG-CoA reductase activity in liver microsomes was measured as described (26). The protein concentration in microsomes was measured by the Lowry method using BSA as a reference standard (27).

RNA extraction

Frozen liver (100 mg) was pulverized in liquid nitrogen and homogenized using TRI Reagent (Sigma-Aldrich Chemical). Total RNA was

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extracted into bromochloropropane and precipitated in isopropyl alcohol. RNA concentration and purity (A_{260}:A_{280}) were determined using a UV-VIS spectrophotometer (Shimadzu 1601). The acceptable ratio of A_{260}:A_{280} was 1.8–2.1. The integrity of the RNA was assessed by using 1.5% agarose gel electrophoresis and visualized under UV.

cDNA synthesis
Total RNA (10 μg) was used as a template for RT-PCR to generate cDNA using a High Capacity cDNA Archive kit following the manufacturer's protocol (Applied Biosystems).

Gene-specific primers were designed using Primer Express software package version 1.5a (Applied Biosystems). The gene sequences used were retrieved from Gene Bank (http://www.ncbi.nlm.nih.gov/pubmed/, http://blast.ncbi.nlm.nih.gov/Blast.cgi). These primers (**Supplemental Table 1**) synthesized by Sigma-Genosys (Sigma-Aldrich Chemical) were diluted to a final concentration of 1 mmol/L with nuclease free water and stored at −20°C. This stock solution was diluted to 10 μmol/L just before use.

qPCR
cDNA (50 ng in 4 μL) was transferred to a 96-well PCR plate to which 5 μL of a cocktail of SYBR Green PCR Core reagents (PE Biosystems), forward and reverse primers (1 μL from 10 μmol/L) were applied and PCR was carried out in an ABI PRISM 7700 sequence detection system (Applied Biosystems) with 40 cycles of 95°C for 30 s and 60°C for 30 s each. The reaction mixture with no cDNA was considered as the negative control to confirm the absence of primer dimerization. The cycle threshold was determined by Sequence Detection System software version 1.7a. β-Actin was used as an internal control. The differences in the gene expression of HMG-CoA reductase, LDL-R, CYP7A1, and SREBP-2 were determined by normalizing the mRNA concentrations of the gene of interest to that of the housekeeping gene, β-actin. Qualitative PCR was performed to confirm formation of a single product in each reaction before quantitation.

Statistical analysis
Data were analyzed by using SPSS statistical software (version 10.0 for Windows). The values are presented as means ± SD. Statistical analysis was performed using 1-way ANOVA followed by Duncan's multiple range test. Pearson correlation coefficients were calculated. Differences were considered significant at $P < 0.05$.

Results

Fatty acid composition of dietary lipids. The fatty acid composition of the dietary lipids indicated that CNO contains ~91% SFA (**Table 1**). The total unsaturated fatty acids present in RBO and SESO were 78 and 84%, respectively. The SFA:MUFA: PUFA ratio of CNO was 1:0.08:0.02, RBO was 1:1.95:1.63, and SESO was 1:2.67:2.61. Blending CNO with RBO or SESO resulted in SFA:MUFA:PUFA ratios of 1:0.98:0.81 and 1:0.96:0.91, respectively. This is as close to the value of an oil with an equal proportion of SFA:MUFA:PUFA that we could achieve. The fatty acid composition of the lipase catalyzed interesterified oils did not differ significantly from their respective blended oils (Table 1).

Dietary fat and serum lipids. The type of fat fed to rats influenced the serum cholesterol concentration (**Table 2**). Rats fed the CNO diet had significantly higher serum total and VLDL+LDL cholesterol concentrations compared with the rats fed either RBO or SESO. The rats fed CNO+RBO(B) and CNO+SESO(B) had significantly lower total cholesterol by 26 and 31%, respectively, compared with those fed CNO alone. Rats fed CNO+RBO(I) or CNO+SESO(I) had a further decrease in serum total cholesterol concentrations by 36 and 43%, respectively, compared with rats fed CNO (Table 2). It is interesting to note that the rats fed interesterified oil had significantly lower concentrations of cholesterol compared with those fed the blended oil of similar fatty acid composition. Rats fed CNO+RBO(B) or CNO+SESO(B) had lower VLDL+LDL cholesterol concentrations than the CNO group ($P < 0.001$). Rats fed CNO+RBO(I) or CNO+SESO(I) had a lower VLDL+LDL cholesterol ($P < 0.001$) concentration compared with the CNO-fed group and it was also lower ($P < 0.001$) compared with their respective blended oil-fed groups (Table 2). The serum HDL cholesterol concentration did not differ among the groups.

HMG-CoA reductase activity. Rats fed the CNO diet had lower hepatic HMG-CoA reductase activity than those fed RBO (23%) or SESO (18%) (**Table 3**). Rats fed CNO+RBO(I) and CNO+SESO(I) had higher activity ($P < 0.01$) than the CNO group. HMG-CoA reductase activity did not differ between rats fed blended oils and those fed CNO.

Hepatic HMG-CoA reductase, CYP7A1, LDL-R, and SREBP-2 mRNA abundance. Dietary fatty acids did not significantly affect the mRNA expression of HMG-CoA reductase (**Fig. 1*A***).

The mRNA abundance of CYP7A1 was 2.8- and 2.4-fold greater in the rats fed RBO and SESO, respectively, compared with the CNO group (Fig. 1*B*). Rats fed CNO+RBO(B) and CNO+SESO(B) had an higher mRNA concentration ($P < 0.05$)

TABLE 1 Fatty acid composition of the dietary fats[1]

	Dietary fat						
Fatty acids	CNO	RBO	CNO+RBO(B)	CNO+RBO(I)	SESO	CNO+SESO(B)	CNO+SESO(I)
				g/100g			
8:0	2.9 ± 0.4^a	nd[2]	0.6 ± 0.1^b	0.5 ± 0.1^b	nd	0.8 ± 0.1^b	1.0 ± 0.2^b
10:0	5.1 ± 0.3^a	nd	1.2 ± 0.1^b	1.2 ± 0.2^b	nd	1.5 ± 0.1^b	1.4 ± 0.1^b
12:0	50.1 ± 1.4^a	nd	10.2 ± 0.3^c	10.5 ± 0.3^c	nd	13.3 ± 0.3^b	11.7 ± 0.4^{bc}
14:0	21.2 ± 0.9^a	nd	4.3 ± 0.2^b	4.7 ± 0.3^b	nd	4.9 ± 0.2^b	4.3 ± 0.2^b
16:0	9.0 ± 0.3^c	20.2 ± 1.2^a	17.7 ± 0.4^b	17.3 ± 0.5^b	10.3 ± 0.3^c	9.5 ± 0.4^c	9.3 ± 0.2^c
18:0	2.7 ± 0.2^c	1.6 ± 0.2^d	1.7 ± 0.1^d	1.7 ± 0.2^d	5.6 ± 0.3^a	4.6 ± 0.2^b	4.7 ± 0.3^b
18:1	7.2 ± 0.4^c	42.6 ± 2.3^a	35.1 ± 1.1^b	34.3 ± 1.3^b	42.6 ± 1.5^a	33.5 ± 0.6^b	34.5 ± 1.2^b
18:2	1.8 ± 0.1^d	35.1 ± 1.8^b	28.8 ± 0.9^c	29.4 ± 0.7^c	41.1 ± 1.0^a	31.6 ± 0.9^c	32.8 ± 0.8^c
18:3	nd	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
SFA:MUFA:PUFA	1:0.08:0.02	1:1.95:1.63	1:0.98:0.81	1:0.96:0.83	1:2.68:2.61	1:0.96:0.91	1:1.06:1.02

[1] Values are mean ± SD, n = 3 different oil preparations. Means in a row with superscripts without a common letter differ, $P < 0.01$.
[2] nd, Not detected, < 0.3%.

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TABLE 2 Serum cholesterol concentrations in rats fed native, blended, or interesterified oils for 8 wk[1]

Dietary fat	Total cholesterol	HDL cholesterol	LDL+VLDL cholesterol
		mmol/L	
CNO	1.98 ± 0.08[a]	0.57 ± 0.05	1.41 ± 0.06[a]
RBO	1.19 ± 0.04[e]	0.52 ± 0.03	0.73 ± 0.08[d]
CNO+RBO(B)	1.46 ± 0.05[b]	0.53 ± 0.04	0.92 ± 0.03[b]
CNO+RBO(I)	1.26 ± 0.06[d]	0.51 ± 0.02	0.68 ± 0.05[e]
SESO	1.29 ± 0.04[d]	0.53 ± 0.03	0.76 ± 0.03[d]
CNO+SESO(B)	1.36 ± 0.07[c]	0.54 ± 0.03	0.82 ± 0.05[c]
CNO+SESO(I)	1.13 ± 0.06[e]	0.51 ± 0.03	0.62 ± 0.04[e]

[1] Values are the mean ± SD, $n = 6$. Means in a column with superscripts without a common letter differ, $P < 0.01$.

than the CNO group. Rats fed CNO+RBO(I) or CNO+SESO(I) had increased mRNA expression ($P < 0.001$) of CYP7A1 compared with rats fed CNO. The elevated expression of CYP7A1 in CNO+RBO(I) and CNO+SESO(I) was higher ($P < 0.01$) than their respective blended oils.

Rats fed the CNO diet had lower hepatic LDL-R expression than those fed RBO (80%) or SESO (77%) (Fig. 1*C*). Rats fed either CNO+RBO(B) or CNO+SESO(B) had a higher ($P < 0.001$) mRNA abundance for LDL-R compared with rats fed the CNO diet. Rats fed CNO+RBO(I) or CNO+SESO(I) had further enhanced mRNA expression ($P < 0.001$) of LDL-R compared with the CNO group. Rats fed CNO+RBO(I) or CNO+SESO(I) had a higher mRNA abundance for LDL-R ($P < 0.001$) compared with their respective blended oils (Fig. 1*C*).

The response of SREBP-2 gene expression to different dietary fats was similar to that observed for LDL-R and CYP7A1 expression. Rats fed the CNO diet had lower SREBP-2 expression than those fed RBO (73%) or SESO (70%) (Fig. 1*D*). Rats fed CNO+RBO(B) and CNO+SESO(B) had a higher ($P < 0.05$) mRNA expression compared with the CNO group. Rats fed CNO+RBO(I) or CNO+SESO(I) further enhanced ($P < 0.001$) the mRNA expression of SREBP-2 compared with the CNO group. Rats fed CNO+RBO(I) had a higher ($P < 0.05$) mRNA abundance for SREBP-2 compared with rats given the respective blended oil, CNO+RBO(B). Rats fed CNO+SESO(I) also had a higher ($P < 0.001$) mRNA abundance for SREBP-2 compared with those given blended oil CNO+SESO(B) (Fig. 1*D*).

Correlation analysis. Differences in the mRNA abundance of major proteins involved in cholesterol homeostasis were correlated with differences in serum total and LDL cholesterol concentrations in the 7 groups. The serum total cholesterol concentration was negatively correlated with the mRNA abundance of HMG-CoA reductase ($r = -0.77$), CYP7A1 ($r = -0.85$), and LDL-R ($r = -0.81$) ($P < 0.05$). The LDL cholesterol concentration also was negatively correlated with the mRNA abundance of CYP7A1 ($r = -0.86$) and LDL-R ($r = -0.82$) ($P < 0.05$). HMG-CoA reductase activity was negatively correlated with HMG-CoA reductase mRNA abundance ($r = -0.87$; $P < 0.01$), indicating that the lower activity was due to a decrease in protein synthesis.

TABLE 3 Liver HMG-CoA reductase activity in rats fed native, blended, or interesterified oils for 8 wk[1]

Dietary fat	HMG-CoA reductase activity, *pmol·mg protein^{-1}·min^{-1}*
CNO	220 ± 12[c]
RBO	285 ± 20[a]
CNO+RBO(B)	231 ± 14[b]
CNO+RBO(I)	258 ± 18[b]
SESO	267 ± 21[b]
CNO+SESO(B)	244 ± 18[b]
CNO+SESO(I)	260 ± 17[b]

[1] Values are the mean ± SD, $n = 6$. Means without a common letter differ, $P < 0.01$.

Discussion

The objective of this study was to understand the molecular mechanism(s) whereby oils with different degrees of unsaturation, as well as those containing different TG molecular species but similar fatty acid composition, affected cholesterol homeostasis. The expression patterns of enzymes or proteins involved in cholesterol metabolism, i.e. synthesis, uptake, and efflux pathways, were then evaluated following administration to rats of CNO, RBO, SESO, or blended oils; CNO+RBO(B) or CNO+SESO(B) and interesterified oils; CNO+RBO(I) or CNO+SESO(I).

A diet rich in SFA, such as that containing CNO, elevated the concentration of plasma LDL cholesterol. A diet containing RBO or SESO resulted in a significantly lower serum cholesterol concentration in rats. Blending of CNO with either RBO or SESO provided an opportunity to reduce the hypercholesterolemic effects of CNO. Rearrangement of fatty acids in TG molecular species of blended oils through lipase-mediated interesterification further enhanced the hypocholesterolemic effects. These observations are in agreement with our earlier results (12,13).

Interesterification brings about the exchange of fatty acid molecules within and between the TG molecules, resulting in altered molecular species of TG. These changes also alter its physicochemical properties (10). Such changes were reported to influence digestion and absorption of fat (28) and cause significant changes in their nutritional properties (29). Kritchevsky et al. (30,31) showed that randomization of fatty acids in peanut oil and lard reduced the atherogenicity by 37 and 10%, respectively. Feeding of randomized butter fat to humans reduced serum cholesterol by 211% (32). Thus, all these studies show that the TG structure of oils influences their ability to alter cholesterol levels and atherogenicity (33). Structured TG-containing medium-chain fatty acids prevented body fat accumulation in healthy individuals (34). However, the molecular mechanisms underlying the anti-atherogenic properties of modified oils was not known. In this investigation we studied molecular mechanisms involved in the hypocholesterolemic effect of interesterified oils compared with their blended oils.

Rats fed CNO showed a series of changes, including lowered HMG-CoA reductase activity. The decreased activity could be due to a feedback inhibition resulting from the high concentrations of serum cholesterol in CNO-fed rats. Although serum cholesterol concentrations were reduced by 23–46% in rats given RBO, SESO, blends, or the corresponding interesterified oils, the changes in the mRNA levels for HMG-CoA reductase were not significant compared with rats fed CNO. These results suggest that the fatty acid composition had a marginal effect on HMG-CoA reductase gene transcription. Interestingly, hamsters fed SFA had a reduced mRNA abundance for the HMG-CoA reductase gene compared with those fed a diet containing high concentrations of MUFA and PUFA (35).

In this study, rats fed with either RBO or SESO had significantly higher levels of mRNA transcripts for CYP7A1

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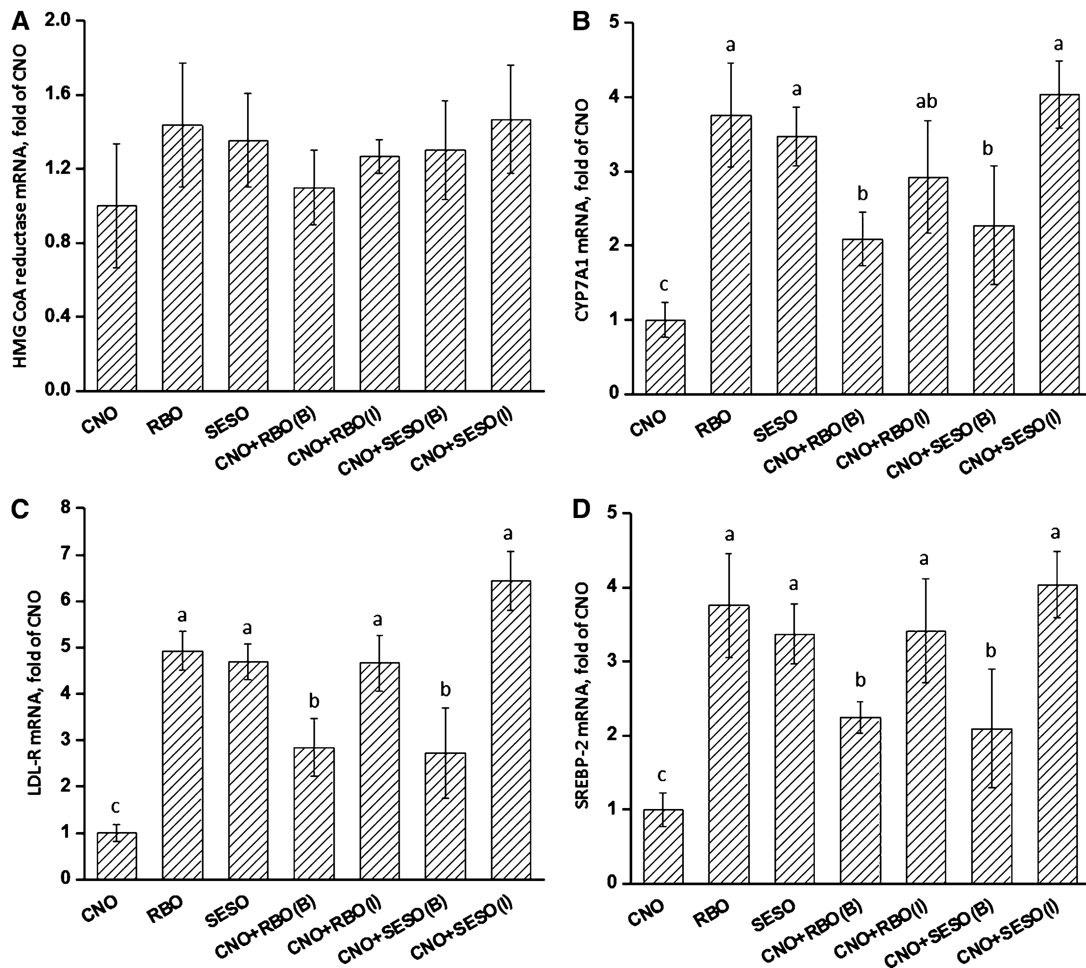

FIGURE 1 Relative abundance of HMG-CoA reductase (*A*), CYP7A-1 (*B*), LDL-R (*C*), and SREBP-2 (*D*) in rats fed native, blended, or interesterified oils for 8 wk. Data are the mean ± SD, $n = 6$. Labeled bars without a common letter differ, $P < 0.05$. The relative abundance of the CNO fed group was set at 1.

compared with those fed CNO. Blending CNO with either RBO or SESO, which increased the PUFA and MUFA, resulted in a significantly increased expression of CYP7A1 compared with the CNO group. This indicates that either the MUFA or PUFA content played a key role in the observed hypocholesterolemic property of dietary fat. Feeding rats with a diet containing interesterified oils further increased mRNA transcripts for CYP7A1 compared with the blended oil-fed group. Both the blended and interesterified oils have the same SFA:MUFA:PUFA composition, yet differential effects on CYP7A1 expression were observed. Therefore, these studies suggest that the position of the fatty acid on the TG molecule can influence the lowering of cholesterol through effects on the cholesterol oxidation pathway. These results are similar to those reported by Cheema et al. (15), who compared the effects of SFA-, MUFA-, and PUFA-rich diets on CYP7A1 in mice. They observed that a diet rich in PUFA significantly increased CYP7A1 activity in conjunction with significantly increased levels of mRNA transcripts compared with mice fed with diets containing SFA or MUFA.

The mRNA abundance for LDL-R in different dietary groups followed the same pattern as that of CYP7A1. Thus, dietary MUFA and PUFA significantly increased expression of the LDL-R. Bennet et al. (36) previously showed that hamsters fed diets containing tripalmitin and trimyristin had significant decreases in LDL-R mRNA compared with triolein-fed hamsters. Horton et al. (37) reported that hamsters fed CNO had decreased mRNA abundance for LDL-R compared with those fed sunflower oil. It is plausible that this enhancement was due to the unsaturated fatty acid content in the oils. The different effects of the blended and interesterified oils with the same fatty acid content can be attributed to their distribution on the TG. These positional effects of fatty acids in TG of oils that result in upregulation of LDL-R mRNA and a concurrent increased hypocholesterolemic effect need further investigation.

The expression pattern for SREBP-2 in this study followed the same pattern as that of LDL-R, indicating that the lower serum cholesterol in rats fed RBO, SESO, or their blended oils and interesterified oils with CNO activates SREBP-2 expression. This in turn is reflected by the increased expression of LDL-R. SREBP-2 mRNA expression also increased in rats fed oils that contained higher concentrations of PUFA compared with rats fed CNO. Studies by Dorfman and Litchenstein (35) showed that dietary PUFA increases the mRNA transcripts of SREBP-2 gene.

However, minor components present in oils are also reported to have an effect on cholesterol metabolism at the transcriptional level. Studies by Chen and Cheng (38) have shown that in diabetic rats, a RBO diet containing 0.4% (wt percent of diet) γ-oryzanol, and 1.5 mg/d γ-tocotrienols significantly increased the hepatic LDL-R, CYP7A1, and HMG-CoA reductase mRNA expression. In the present study, rats ingested 0.13% (weight percent of diet) γ-oryzanol, and 0.77 mg/d γ-tocotrienol in the RBO-fed group. Rats fed blended and interesterified oil of

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CNO+RBO ingested 0.104% (weight percent of diet) γ-oryzanol and 0.66 mg/d γ-tocotrienol, suggesting that some of the observed effects could be due to γ-oryzanol. Sesamin has also been shown to have a hypocholesterolemic effect. In this study, the diet of rats fed on SESO contained 98 mg/kg sesamin and 0.166 mg/kg sesamolin, which could have contributed to the observed effects on expression of genes involved in cholesterol homeostasis. Lim et al. (39) have shown that sesamin (2 g/kg) and sesamolin (0.6 g/kg) present in SESO are capable of modulating the expression of genes involved in the fatty acid oxidation. Increased fatty acid oxidation is reflected by the decreased lipid concentration in serum. Also, a diet containing 0.5% sesamin significantly decreased the cholesterol absorption in rats by 30% (40). Therefore, it is possible that in addition to the fatty acid composition, the minor components present in RBO or SESO could also have exerted their effect on gene expression, resulting in a reduction of the cholesterol concentration observed in rats fed blended and interesterified oils. However, this possibility was not addressed in the present study.

In conclusion, the present study provides some insight into the molecular mechanisms involved in the cholesterol-lowering properties of blended and interesterified oils. The hypocholesterolemic effect observed in rats fed interesterified oils can be mainly attributed to increased uptake of LDL cholesterol by liver through the upregulation of LDL-R and by enhancing the catabolic pathway via activation of CYP7A1 gene expression. Both interesterified and blended oils lowered serum cholesterol in rats by upregulating cholesterol clearance pathways. However, the effectiveness of interesterified oils to lower serum cholesterol is significantly higher than that observed with blended oil. The reasons for these differential effects of blended and interesterified oils are currently under further investigation.

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