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Rats fed blended oils containing coconut oil with groundnut oil or olive oil showed an enhanced activity of hepatic antioxidant enzymes and a reduction in LDL oxidation

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Abstract

The effects of feeding Wistar rats with blended and interesterified oils of coconut (CO):groundnut (GNO) or CO:olive (OLO) on liver antioxidant enzyme activities and susceptibility of LDL to oxidation were studied. The hepatic lipid peroxidation (LPO) levels in the rats fed CO:GNO blend and interesterified oils were increased by 31% and 21%, when compared to the rats given CO. The superoxide dismutase activity was increased by 31% and 28%, and catalase (CAT) activity was increased by 37% and 39%, respectively, in rats given blends and interesterified oils of CO:GNO, as compared to those given CO. The activities of glutathione peroxidase (GPx) were increased by 17% and 20% in rats given CO:GNO blend and interesterified oils. Glutathione transferase (GST) level was also found to be increased by 26% and 31% after feeding blended and interesterified oils of CO:GNO, compared to those given CO. The LDL oxidation, which was elevated by feeding GNO, was found to be reduced by 10% and 14%, respectively, in the groups fed blended and interesterified oils of CO:GNO. Similarly, CO:OLO blended oils enhanced SOD, CAT, GPx and GST activities by 34%, 43%, 27% and 23%, respectively, compared to the rats given CO-containing diets. The corresponding increases in theses antioxidant enzyme activities when CO:OLO interesterified oils were fed to rats were 38%, 50%, 28% and 26%, respectively, when compared to rats given CO-containing diets. There was a significant reduction in hepatic LPO as well as oxidation of LDL, when blended and interesterified oils of CO:OLO were fed to rats. The results demonstrated that blended and interesterified oils of CO with GNO or OLO enhance hepatic antioxidant enzymes, decreased lipid peroxidation in liver and reduced the susceptibility of LDL to oxidation.

Keywords: Coconut oil; Groundnut oil; Olive oil; Fatty acids; Lipid peroxidation; Anti-oxidant enzymes; LDL oxidation

1. Introduction

Dietary fat affects the fatty acid composition, lipid peroxidation and antioxidant defence systems of the body (Pulla Reddy & Lokesh, 1994). Lipid peroxidation has been implicated in the modification of DNA, protein and membrane structure, formation of age pigment and in the deposition of plaques associated with low-density lipoprotein modification. Many of these effects are initiated by reactive oxygen species (ROS), such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\cdot}$). These are the products of normal metabolism in the body but can become deleterious, if produced in uncontrolled fashion. Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) defend the host against the damaging effects of these free radical species.

Studies have shown that polyunsaturated fatty acid (PUFA) is vulnerable to lipid peroxidation and the ratio

Abbreviations: CO, coconut oil; GNO, groundnut oil; GPx, glutathione peroxidase; GST, glutathione transferase; LPO, lipid peroxidation; MUFA, mono unsaturated fatty acid; OLO, olive oil; PUFA, poly unsaturated fatty acid; ROS, reactive oxygen species; SFA, saturated fatty acid; SOD, superoxide dismutase.

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of PUFA to saturated fatty acid (SFA) in the diet determines the susceptibility to lipid of low-density lipoprotein (LDL) peroxidation (Buckingham, 1992). The fatty acid composition and antioxidant levels influence the susceptibility of LDL to oxidation, which is a crucial event in atherosclerosis (Thomas, Thornburg, Manning, Hooper, & Rudel, 1994). Hence, the fatty acid composition of dietary lipids needs to be balanced. According to the Indian Council of Medical Research, the desirable proportions of saturated, oleic and polyunsaturated fatty acids in dietary fats should be about 1:1:1 (Ghafoorunissa, 1998). The World Health Organization recommends the intake of fat with a PUFA/SFA ratio of 0.8–1.0 (FAO/WHO, 1994).

Keeping these recommendations in focus, we recently prepared blended oils containing coconut oil (CO) with groundnut oil (GNO) (Anitha & Lokesh, 2007). Furthermore, we also prepared blended oils containing CO with olive oil (OLO) to provide a oil containing SFA and oleic acid in equal proportions. This combination had the high stability needed for Indian cooking practices. These blended oils were further subjected to lipase catalysed interesterification reactions, interchanging the fatty acids in the triglyceride molecules of oils. When these blended or interesterified oils were fed to rats, a significant decrease in the serum lipids were observed, compared to rats fed CO alone (Anitha & Lokesh, 2007). Since the blending of oils altered the proportion of PUFA to SFA, it was of interest to study the effect of these modified oils on lipid peroxidation and the antioxidant defence mechanism in rats.

In the present study we examined the effect of feeding blended and interesterified oils, consisting of CO and GNO, in which SFA:MUFA:PUFA levels were balanced, to give approximately 1:1:1 proportion, on lipid peroxidation, hepatic antioxidant enzymes and on LDL oxidation in rats. We prepared blended and interesterified oils containing CO and OLO to give an SFA to oleic acid ratio of approximately 1:1. Both SFA and oleic acid are relatively stable to oxidation. OLO in addition provide phenolic antioxidants (Baldioli, Sevili, Perretti, & Montedoro, 1996). Hence the effect of these combinations on parameters affecting lipid peroxidation was studied.

2. Materials and methods

2.1. Animals

Male Wistar rats (weaning), weighing 45 ± 3 g [OUTB-wistar, IND-cft (2c)] were grouped by random distribution ($n = 5$ animals per group). They were placed in individual cages. The temperature of the room was maintained at 22 °C with 12 h light-dark cycle in an approved animal house facility at Central Food Technological Research Institute, India. Animals were fed a fresh diet daily and left over food was weighed and discarded. The animals had free access to food and water throughout the study. The experimental protocol was approved by the institutional animal ethical committee.

2.2. Diet composition

The ingredients used in the basal diets were (g/100 g): casein 20, cellulose 5, sucrose 60, AIN 76 mineral mix 3.5, AIN 76 Vitamin mix 1, methionine 0.3, choline chloride 0.2 and fat 10 (Anonymous, 1977). Native oils, blend and interesterified oils as indicated were used in the diet.

2.3. Chemicals

Thiobarbituric acid and ferrous sulfate ($FeSO_4 \cdot 7H_2O$) were obtained from Qualigen Fine Chemicals Ltd., Mumbai, India. Glutathione, glutathione reductase, hydrogen peroxide, NADPH, epinephrine, 1-chloro2,4-dinitrobenzene (CDNB) and 1,1,3,3-tetramethoxypropane were purchased from Sigma Chemical Co. (St. Louis, MO). Refined groundnut oil, coconut oil and extra virgin olive oil were purchased from a supermarket in Mysore, India. Immobilised lipase IM-60 from *Mucor meihei* was a gift from Novo Nordisk Bioindustrial Inc. (Danbury, CT). All solvents used were of analytical grade and were distilled prior to use.

2.4. Preparation of blended oils

After determining the fatty acid composition of the native oils, CO and GNO were blended in appropriate amounts so that the SFA:MUFA:PUFA ratio in the oil was approximately 1:1:1. In another combination CO was blended with OLO to provide SFA:oleic acid in a ratio approximately 1:1. The oils were mixed by stirring at 40 °C for 1 h after flushing with nitrogen, and the mixing efficiency was followed by periodically withdrawing the aliquots and determining the fatty acid composition of the resulting blend.

2.5. Interesterification reaction

The blended oils were subjected to an interesterification reaction with 0.5% immobilized lipase lipozyme IM-60 from M*ucor meihei* (specific activity of lipase is 6.2 ± 0.2 μmol/mg protein) at 37 °C with constant shaking in a water bath for 96 h (Anitha & Lokesh, 2007). The oil was decanted, the immobilised enzyme was washed using hexane to collect the traces of oil entrapped, and hexane was removed under vacuum.

2.6. Fatty acid composition

The fatty acid composition of the native, blended and interesterified oils were determined as fatty acid methyl esters (Morrison & Smith, 1963) using gas chromatography (a Fison GC fitted with FID). The oils were saponified with 0.5 M KOH and methylated with 40% BF_3 in methanol. The individual fatty acid methyl esters were separated using a fused silica capillary column (25 m × 0.25 mm; Permabond, film thickness 0.25 mm: Machery–Nagel

GmbH, Duren, Germany). The operating conditions were: initial column temperature 120 °C, raised by 15 °C per 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 their retention times with those of standards (Nuchek Prep, Elysian, MN) and was quantified by an online Chromatopac CR-6A integrator.

2.7. Tocopherols

The tocopherols were extracted in heptane after saponification of oil and reacted with a mixture of ferric chloride and 2,2′-bipyridyl in ethanolic solution, which gives a characteristic red colour and was read at 520 nm (Joshi & Desai, 1952). The tocopherols were quantitated using α-tocopherol from Merck Company as a reference standard.

2.8. Determination of phenols

The total phenolic content in OLO and its blends was determined by colorimetric methods using the Folin–Ciocalteau reagent (Carmen Raminez-Tortosa et al., 1999).

2.9. Biochemical analysis

After 60 days on specified diets, rats were fasted overnight and sacrificed under diethyl ether anaesthesia. Blood was drawn by cardiac puncture and serum was separated by centrifugation at 700*g* for 10 min.

2.9.1. Isolation of low-density liproprotein (LDL)

LDL was isolated by discontinuous-density gradient ultracentrifugation, as described by Chung, Wilkinson, Geer, and Segrest (1980). The LDL fractions separated in the density range of 1.02–1.08 g/ml were exhaustively dialyzed overnight against water at 4 °C.

2.9.2. LDL susceptibility to oxidation

LDL (200 μg protein) was suspended in 4 ml of 50 mM phosphate buffer saline (pH 7.4). The oxidation of LDL was initiated with the addition of 10 μM $CuSO_4$; 0.5 ml of aliquot was drawn at 6 , after incubation at 37 °C. Oxidation was arrested by adding 10 mmol EDTA. Lipid peroxides in oxidised LDL were determined as thiobarbituric-acid-reactive substances (TBARS), using 1,1,3,3-tetramethoxypropane as reference standard (Yagi, 1984). Appropriate blanks were included in the experiments. The oxidation of LDL was expressed as nmol TBARS/mg LDL protein.

2.9.3. Hepatic enzyme activities

Liver was removed and rinsed in ice-cold saline, blotted, weighed and homogenised (1 g/10 ml) in 20 mM phosphate buffer, pH 7.4, using a glass homogeniser. The homogenate was centrifuged at 600*g* for 15 min and used for the analysis of enzyme activities.

SOD activity was measured by the inhibition of epinephrine and monitored at 430 nm (Misra & Irwin Fridovich, 1972). One unit of SOD was defined as the amount required to inhibit epinephrine oxidation by 50%. CAT activity was assayed according to the method of Abei (1984), by following the decomposition of hydrogen peroxide, measured at 240 nm. GPx activity was determined by NADPH oxidation in a coupled reaction system, consisting of oxidised glutathione (Tappel, 1978). GST was measured with 1-chloro 2,4-dinitrobenzene as the substrate, and the enzyme activity was expressed as μmoles of CDNB–GSH conjugates formed per minute per mg protein (Hoilg, Pabst, & Jakoly, 1974). All spectrophotometric measurements were carried out in a Shimadzu ultraviolet 160 A spectrophotometer (Shimadzu, Kyoto).

Protein was estimated by the method of Lowry, Rosebrough, Farr, and Randall (1951), using bovine serum albumin as the reference standard.

2.9.4. Statistical analysis

The results were analysed by analysis of variance (Fisher, 1970). Data were expressed as means ± S.D. Analysis of variance was employed to evaluate the differences between the groups. A difference of $p < 0.05$ was considered to be significantly different.

3. Results

3.1. Dietary fatty acids

The fatty acid compositions of dietary fats used in this study are shown in Table 1. CO contains 87% saturated fatty acids. The proportion of SFA:MUFA:PUFA in this oil is 87:10:3. GNO contains 40% oleic acid and 33% linoleic acid but the SFA:MUFA:PUFA proportion in this oil was found to be 27:40:33. The proportion of SFA:MUFA:PUFA in OLO is 20:73:7, respectively. Thus none of these oils has the ideal combination of SFA:MUFA:PUFA, as recommended by nutritionists for a balanced fatty acid composition. One of the objectives of this study is to have oils containing approximately equal proportions of SFA:MUFA:PUFA or an equal proportion of SFA:total unsaturated fatty acids. This was achieved by blending appropriate proportions of CO with GNO or OLO (Table 1), and these oils were fed to rats for 60 days.

3.2. Tocopherol and polyphenol content of oils

The total tocopherol content of CO was 56 mg/kg oil, 592 mg/kg oil in GNO and 215 mg/kg oil in OLO. The CO:GNO and CO:OLO blended oils had 431 mg/kg and 145 mg/kg of tocopherols in the oil, respectively. There was no significant difference in the tocopherol content of blended and interesterified oils.

The total phenolic contents of OLO, CO:OLO blend and CO:OLO interesterified oil were 190, 112 and 115 mg/kg oil, respectively. The major phenolic fractions

Table 1
Fatty acid composition (%) of the dietary lipids

Fatty acid (%)	CO	GNO	OLO	CO–GNO (B)	CO–GNO (I)	CO–OLO (B)	CO–OLO (I)
8:0	5.1	nd	nd	nd	nd	nd	nd
10:0	5.0	nd	nd	nd	nd	nd	nd
12:0	49.2	nd	nd	9.5	10.3	17.6	19.3
14:0	18.0	nd	nd	5.1	5.5	8.5	8.3
16:0	10.0	21.7	15.5	16.5	15.7	14.2	13.1
18:0	nd	4.0	4.0	4.4	3.9	3.9	2.9
18:1	10.0	40.0	72.6	38.3	37.4	48.0	49.0
18:2	2.4	32.5	7.7	25.9	26.8	6.8	6.6
20:0	nd	1.5	nd	nd	nd	nd	nd
SFA (S)	87.3	27.2	19.5	35.5	35.4	44.2	43.6
MUFA (M)	10.0	40.0	72.6	38.3	37.4	48.0	49.0
PUFA (P)	2.4	32.5	7.7	25.9	26.8	6.8	6.6
S:M:P	87:10:3	27:40:33	20:73:7	36:38:26	36:37:27	44:48:8	44:49:7
P/S	0.03	1.19	0.39	0.72	0.76	0.15	0.15

B: blended oils; I: interesterified oils; MUFA: monounsaturated fatty acids; nd: not detected; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids.

of olive oil, as determined by HPLC, were hydroxytyrosol (3,4-dihydroxyphenylethanol) and tyrosol (4-hydroxyphenylethanol) (Data not shown).

3.3. Effect of dietary lipids on growth parameters

The amount of food consumed by rats fed different dietary lipids was comparable (Table 2). There was no significant change in the weight of the liver, food efficiency ratio, body weight gained by rats and weight of the liver in the rats fed different diets containing blended and interesterified oils. These studies indicated that both blended and interesterified oils were readily accepted by rats and supported their growth adequately.

3.4. Fatty acid composition of liver lipids

The fatty acid composition of liver lipids reflected the nature of the dietary fats fed to the animals (Table 3). The GNO diet enhanced linoleic acid content in the liver lipids by 2.5-fold and arachidonic acid by 2.4-fold, compared to levels found in the rats given the CO-enriched diet. The PUFA/SFA ratio in liver lipids of GNO-fed animals were also enhanced 2.8-fold. When rats were given a diet containing CO:GNO blends or CO:GNO interesterified oils, the linoleic acid and arachidonic acid levels in liver lipids were enhanced by 2.5-fold and 2-fold, respectively, compared to rats given CO-containing diets. Similar changes in the fatty acid composition were observed in rats given a diet containing CO blended with GNO or in rats given CO with GNO, after subjecting to interesterification reactions. In OLO fed animals the oleic acid levels were increased by 1.2-fold and the P/S ratio of the liver lipids in these groups were similar to those given CO in the diet. Linoleic acid and arachidonic acid levels were enhanced by 20–25% in rats given CO:OLO blends or CO:OLO interesterified oils, as compared to those given CO-containing diets (Table 3).

3.5. Hepatic lipid peroxide and antioxidant enzymes

The lipid peroxides in GNO fed animals were found to be 38% higher than that in rats given CO-enriched diets. The rats given CO:GNO blended oils had 31% higher levels of lipid peroxides, while those given CO:GNO oil blends, after subjecting them to interesterification reactions, had 21% higher levels of lipid peroxides, compared to the rats given CO-enriched diets. The animals fed OLO

Table 2
Growth and liver weight of rats fed blended and interesterified oils

Parameters	CO	GNO	OLO	CO:GNO (B)	CO:GNO (I)	CO:OLO (B)	CO:OLO (I)
Food intake (g/d/rat)	12.5 ± 0.7^a	11.9 ± 0.7^a	13.5 ± 1.3^a	12.6 ± 0.6^a	12.8 ± 0.7^a	12.6 ± 0.4^a	12.4 ± 1.0^a
Final b.w. at the time of sacrifice (g)	234 ± 17.8^a	221 ± 22.3^a	255 ± 35.1^a	227 ± 17.5^a	244 ± 19.7^a	226 ± 22.2^a	246 ± 26.2^a
FER	0.32 ± 0.01^a	0.34 ± 0.02^a	0.31 ± 0.30^a	0.30 ± 0.01^a	0.32 ± 0.01^a	0.36 ± 0.02^a	0.31 ± 0.01^a
Liver weight*	3.20 ± 0.3^a	3.35 ± 0.3^a	3.20 ± 0.2^a	3.10 ± 0.3^a	3.20 ± 0.35^a	3.20 ± 0.2^a	3.20 ± 0.2^a

Values are mean SD ($n = 5$ rats). Values not sharing a common superscript within a row are statistically significant ($p < 0.05$). FER: Food efficiency ratio, b.w.: body weight.
* g/100g body weight.

Table 3
Fatty acid composition (%) of the liver lipids in rats fed with native, blended and interesterified oils

Fatty acid	CO	GNO	OLO	CO:GNO (B)	CO:GNO (I)	CO:OLO (B)	CO:OLO (I)
12:0	2.6 ± 1.1^{a}	nd	0.6 ± 0.1^{a}	1.1 ± 1.8^{a}	1.6 ± 0.8^{a}	1.4 ± 0.3^{a}	1.6 ± 0.02^{a}
14:0	2.0 ± 0.4	nd	nd	nd	nd	nd	nd
16:0	29.3 ± 0.4^{a}	25.2 ± 0.4^{b}	29.4 ± 0.5^{a}	25.9 ± 0.9^{b}	27.2 ± 0.9^{a}	26.2 ± 0.6^{b}	24.5 ± 0.6^{b}
16:1	5.8 ± 1.1^{a}	1.8 ± 0.1^{b}	1.9 ± 0.5^{b}	1.8 ± 0.4^{b}	1.9 ± 0.3^{b}	2.7 ± 0.1^{c}	1.9 ± 0.3^{b}
18:0	9.8 ± 0.3^{a}	11.3 ± 0.1^{b}	8.8 ± 0.3^{c}	10.8 ± 0.8^{b}	10.4 ± 0.4^{b}	10.0 ± 0.2^{a}	11.1 ± 0.2^{b}
18:1	33.2 ± 0.2^{a}	22.6 ± 1.3^{b}	43.9 ± 2.2^{c}	24.2 ± 2.6^{b}	23.1 ± 1.3^{b}	39.2 ± 0.1^{d}	40.7 ± 1.1^{c}
18:2	6.2 ± 0.5^{a}	15.6 ± 0.7^{b}	6.6 ± 0.3^{a}	16.0 ± 1.6^{b}	15.2 ± 0.1^{b}	7.9 ± 0.5^{c}	7.8 ± 0.2^{c}
20:4	10.2 ± 2.1^{ac}	23.7 ± 1.9^{b}	9.2 ± 2.1^{a}	20.2 ± 2.3^{b}	21.1 ± 1.7^{b}	12.2 ± 0.1^{c}	12.8 ± 2.8^{c}
P/S	0.38	1.1	0.41	0.96	0.93	0.54	0.56

Values show the means ± S.D. of 5 rats. Values in the same row with common superscript letters are not significantly different at $p < 0.05$. B: blended oils; I: interesterified oils; nd: not detected.

had 21% higher levels of lipid peroxides, compared to those given CO. Animals given CO:OLO blend and CO:OLO interesterified fat showed 17% and 10% higher levels of lipid peroxides, when compared to those given CO (Table 4).

The specific activities of antioxidant enzymes SOD, CAT, GPx and GST were found to be higher by 20%, 16%, 4% and 20%, respectively, in GNO-fed animals, as compared to those given CO enriched diets. The corresponding increases in the specific activities of these antioxidant enzymes were 31%, 37%, 17% and 26%, respectively, in animals given CO:GNO blends, compared to those given the CO diet alone. When rats were given a diet containing interesterified fats of CO:GNO, the SOD, CAT, GPx and GST activities were enhanced by 28%, 39%, 20% and 31%, respectively, compared to rats given the CO diet (Table 4).

The antioxidant enzymes SOD, CAT, GPx and GST were elevated by 15%, 29%, 7% and 6%, respectively, in rats given OLO, compared to those given CO. The elevated levels of these enzyme activities were observed in rats given blended or interesterified oils of CO–OLO. The corresponding increase of the antioxidant enzymes in the rats given blended oils of CO:OLO were 34%, 43%, 27% and 23%, respectively, while those given interesterified fat showed an increase in the activities of these enzymes by 38%, 50%, 28% and 26%, respectively (Table 4).

3.6. LDL oxidation

Table 5 shows that native LDL of rats fed CO and OLO had lower TBARS values than rats fed diets containing GNO. Extensive oxidation of LDL was observed when it was incubated with Cu^{2+} (10 μM) at 37 °C for 6 h. The copper-induced LDL oxidation in rats fed GNO, CO:GNO blend and interesterified oils was found to be higher by 22%, 9% and 4%, in comparison to rats fed CO. In rats given OLO, CO:OLO blend and interesterified oils, the LDL oxidation was reduced by 11%, 26% and 29%, respectively, compared to those given CO-containing diets. Therefore, while GNO in the diet enhances the susceptibility of LDL to oxidation, OLO or its blend with CO in the diet decreased susceptibility of LDL to oxidation.

Table 4
Effect of dietary fat on hepatic lipid peroxides and antioxidant enzymes in rats fed native, blended and interesterified oils

Parameters	CO	GNO	OLO	CO–GNO (B)	CO–GNO (I)	CO–OLO(B)	CO–OLO(I)
LPO (nmoles TBARS/mg protein)	2.9 ± 0.2^{a}	$4.0 \pm 0.3b^{c}$	3.5 ± 0.3^{b}	3.8 ± 0.3^{b}	3.5 ± 0.3^{b}	3.4 ± 0.2^{c}	3.2 ± 0.3^{c}
SOD (units/min/mg protein)	37.2 ± 0.2^{a}	44.5 ± 2.0^{b}	42.7 ± 1.7^{b}	48.7 ± 3.9^{b}	47.5 ± 1.7^{b}	50.0 ± 1.8^{c}	51.2 ± 2.2^{c}
CAT (μmol/min/mg protein)	49.6 ± 2.3^{a}	57.7 ± 2.3^{b}	63.8 ± 1.8^{c}	68.0 ± 2.0^{c}	$69.1 \pm \pm 1.9^{c}$	71.0 ± 2.1^{d}	74.2 ± 2.6^{d}
GPx (μmol/min/mg protein)	69.5 ± 1.6^{a}	72.5 ± 1.1^{b}	74.0 ± 0.6^{b}	81.0 ± 0.3^{c}	83.1 ± 1.5^{c}	88.5 ± 1.3^{d}	89.2 ± 1.2^{d}
GST (μmol/min/mg protein)	47 ± 2.5^{a}	56.5 ± 2.4^{b}	49.7 ± 1.3^{a}	59.0 ± 2.5^{b}	61.5 ± 1.2^{bc}	58.0 ± 2.8^{b}	59.0 ± 3.6^{b}

Values show the means ± S.D. of 5 rats. Values in the same row with common superscript letters are not significantly different at $p < 0.05$. B: blended oils; CAT: catalse; GPx: glutathione peroxidase; GST: glutathione reductase; I: interesterified oils; LPO: lipid peroxidation; SOD: superoxide dismutase.

Table 5
Thiobarbituric acid-reactive substances (TBARS; nmoles/mg protein) formed during oxidation of LDL in rats fed different dietary lipids

	CO	GNO	OLO	CO–GNO (B)	CO–GNO (I)	CO–OLO (B)	CO–OLO (I)
LDL	8.1 ± 1.2^{a}	9.5 ± 2.0^{b}	7.4 ± 1.5^{c}	8.8 ± 2.2^{b}	8.3 ± 3.1^{b}	7.0 ± 1.1^{c}	7.2 ± 1.4^{c}
LDL + Cu^{2+}	25.8 ± 1.7^{a}	31.4 ± 3.1^{b}	23.0 ± 2.0^{a}	28.2 ± 2.0^{c}	26.9 ± 3.7^{ab}	19.0 ± 2.2^{d}	18.2 ± 3.2^{d}

Values show the means ± S.D. of 5 rats. Values in the same row with common superscript letters are not significantly different at $p < 0.05$. B: blended oils; I: interesterified oils.

4. Discussion

The objective of this study is to evaluate the effect of dietary lipids which contain approximately equal amounts of SFA, MUFA and PUFA or equal amounts of SFA and total unsaturated fatty acids on lipid peroxidation and antioxidant enzyme activities in rat liver. This was compared against the values obtained in rats given CO which contains 87% saturated fatty acids or those in rats given GNO, which contained 73% unsaturated fatty acids, or those in rats given OLO, which contained 80% unsaturated fatty acids. GNO contained 33% PUFA while OLO contained only 7% PUFA. The PUFA-containing lipids are known to be more prone to lipid peroxidation. The susceptibility of fatty acids to lipid peroxidation increases in proportion to its degree of unsaturation (Cortinas et al., 2005). However, the extent of lipid peroxidation may also be regulated by antioxidant molecules, such as tocopherols, as well as antioxidant enzymes, such as SOD, CAT, GPx and GST. In the present investigation effects of dietary fatty acids with different degrees of unsaturation on tissue lipid peroxidation and the levels of protective antioxidant enzyme activities were monitored.

An increase in the liver lipid peroxides was observed in the rats fed GNO, CO:GNO blend and CO:GNO interesterified oils, compared to rats fed CO. A marginal increase in the LPO levels were also observed in rats given OLO, CO:OLO blend or CO:OLO interesterified oils. GNO and OLO and their blends with CO also enhanced PUFA levels in hepatic tissues. There was a significant correlation between the P/S ratio of dietary lipids and levels of LPO (Fig 1a) and a weaker correlation between P/S ratio of liver lipids and LPO in hepatic tissues (Fig 1b).

The change in the dietary lipids also influenced the activities of the antioxidant enzymes involved in scavenging the oxygen free radicals, which initiate lipid peroxidation. These antioxidant enzymes protect tissues from oxidative injury by lowering lipid peroxidation. The activity of SOD was increased significantly in the rats fed GNO and the blended oils containing CO:GNO. Similar results were obtained when the blended oils of CO:GNO were subjected

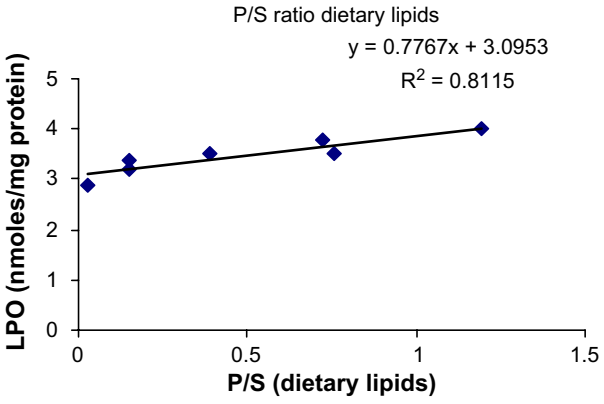

Fig. 1a. Effect of dietary P/S ratio on LPO of rats fed native, blended and interesterified oils.

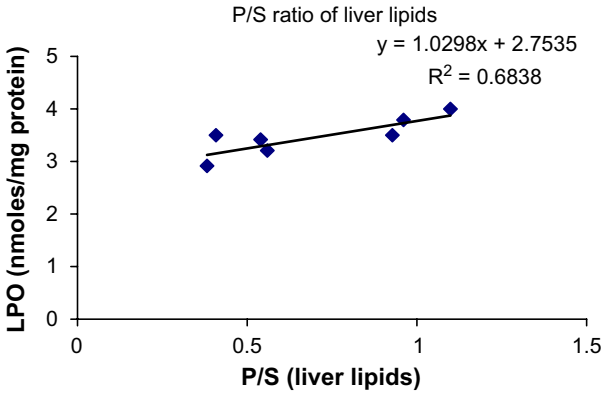

Fig. 1b. Effect of liver P/S ratio on LPO of rats fed native, blended and interesterified oils.

to interesterification and fed to rats. Similar changes were also observed when the rats were given a diet containing blended or interesterified oils containing CO with OLO. These studies indicated that though the blending of CO with GNO or OLO enhances the unsaturation index of the resulting oil and thereby increases LPO in rats, there is a simultaneous increase in antioxidant enzyme activities, which may control to a certain extent the LPO levels, which were increased by a higher degree of unsaturation of the lipids in the diet. Therefore, the antioxidant enzymes seem to adjust their levels to protect against the higher unsaturation of the lipids provided in the diet.

Lipid peroxidation is also involved in the oxidative modification of low-density lipoprotein (Esterbauer, Gerbicki, Puhl, & Jurgens, 1992). Oxidised LDL plays an important role in atherosclerosis. Diets enriched in linoleate increase the content of linoleic acid in LDL lipids, which increases the susceptibility of LDL to oxidation. Studies have also shown that a MUFA-rich diet renders LDL less prone to oxidation (Bonanome et al., 1992). Earlier studies have implicated that the phenolic compounds in OLO as one of the factors responsible for reducing the susceptibility of LDL to oxidation. (Covas et al., 2000). LDL in hypercholesterolaemic patients fed a diet containing oleic acid-rich olive oil showed increased resistance to oxidation (Baroni, Amelio, Sangiorgi, Gaddi, & Battino, 1999). Our present study has shown that LDL oxidation catalysed by Cu^{2+} was reduced in rats fed OLO or CO:OLO blend or CO:OLO interesterified oil. This is in agreement with the protective effects of OLO or its phenolics on LDL oxidation (Terner et al., 2005).

OLO is a rich source of phenolics such as hydroxytyrosol, tyrosol, oleuropein aglycone and decarboxymethyl oleuropein aglycone. It has been shown that hydroxytyrosol is the most active antioxidant compound in virgin olive oil (Papadopoulos & Boskou, 1991). Laponite, Goulet, Couillard, Lamarche, and Lemieux (2005) investigated the effect of Mediterranean foods (rich in phenolics) on circulating oxidised-LDL levels in healthy women. The Mediterranean diets significantly decreased oxidised-LDL levels by 11.3% after 12 weeks of nutritional intervention.

Ochoa-Herrera, Huertas, Quites and Matix (2001) compared the effects of virgin olive oil and high oleic sunflower oil on antioxidant status in rabbits. Despite similarity in the fatty acid compositions of the oils, the animals fed high oleic sunflower oil showed higher peroxidative stress, measured in terms of hydroperoxide content and TBARS. The groups fed olive oil showed lower TBARS and hydroperoxides, higher tocopherols and ubiquinone content. The olive oil fed groups showed highest activity of glutathione peroxidase. These studies indicated that in addition to the fatty acid composition of the dietary lipids, the minor constituents such as polyphenols present in olive oil can greatly influence antioxidant status in animals. Our results are in agreement with these findings.

In addition to polyphenols, Vitamin E also play a beneficial role in reducing lipid peroxidation levels. α-Tocopherol is the active form of Vitamin E. Supplementation of diets with α-tocopherol has been found to reduce the susceptibility of LDL particles to oxidation in healthy, diabetic and dislipidaemic subjects (Lonn et al., 2002; Wen, Killalea, Norris, Cooke, & Feely, 1999). α-Tocopherol can inhibit highly reactive lipid peroxyl and alkoxyl radicals, which promote lipid peroxidation (Abudu, Miller, & Levinson, 2004). α-Tocopherol may affect the assembly of NADPH-oxidase, responsible for reactive oxygen species production (Munteanu, Zing, & Azzi, 2004).

Recently, Nevin and Rajmohan (2006) studied the effect of virgin coconut oil (VCO) on the antioxidant enzyme activities and lipid peroxidation level in rats. VCO had higher levels of unsaponifiable components, containing Vitamin E and polyphenols, than refined CO. The LPO levels and the conjugated dienes were significantly lower in the heart, liver and kidney of rats fed VCO, compared to the rats fed refined CO. Refined CO had a similar fatty acid composition to that of VCO. Virgin coconut oil also significantly increased the antioxidant enzyme activities and reduced the lipid peroxide content (Nevin & Rajmohan, 2006). VCO with higer polyphenol content also rendered LDL less prone to oxidation (Nevin & Rajmohan, 2004). These studies again indicated that the minor constituents in an oil may have greater influence on antioxidant systems in the body. Blending of oils thus provides an opportunity to enrich the native oils with useful minor constituents.

In conclusion, our study has shown that the blended and interesterified oils of CO:GNO and CO:OLO enhanced antioxidant enzyme activities, lowered hepatic lipid peroxidation and reduced the susceptibility of LDL to oxidation. Hence, oils with balanced fatty acid composition and also containing minor components may beneficially modulate antioxidant status in the body. OLO is extensively used by people in the Mediterranean region and has been attributed as the main reason for the low incidence of cardiovascular diseases reported for the population in these regions. OLO is not used as a mainstream vegetable oil in India. The high cost of OLO in India restricts its usage. Blended oils containing OLO in smaller amounts may reduce its cost without compromising the overall health benefits of OLO. This may help in introducing OLO to the Indian subcontinent.

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