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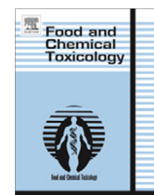

Effect of feeding blended and interesterified vegetable oils on antioxidant enzymes in rats

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ABSTRACT

The present study was undertaken to evaluate the effect of feeding blended and interesterified oils prepared using coconut oil (CNO) with rice bran oil (RBO) or sesame oil (SESO), with a polyunsaturated/saturated (P/S) ratio of 0.8–1.0, on oxidative stress and endogenous antioxidant system. Feeding blended oils resulted in significantly increased hepatic lipid peroxide levels in rats given blended oil CNO + RBO or CNO + SESO by 1.3 and 1.6-fold, respectively compared to rats fed diet containing CNO. The lipid peroxide level in erythrocyte membrane also increased in rats fed blended oil compared to rats fed with CNO. Rats fed interesterified oils prepared from these blended oils also showed increased lipid peroxide level compared to rats given CNO diet, however it was not significantly different from rats fed with their respective blends. There was a significant increase in the activity of endogenous antioxidant enzymes super oxide dismutase, catalase, glutathione peroxidase and glutathione-s-transferase after feeding blended and interesterified oils. The activities of Na^{+}/K^{+}-ATPase and Ca^{2+}/Mg^{2+}-ATPase were increased in rats fed blended and interesterified oils. These results indicated that the P/S ratio of dietary fat is an important factor in determining the oxidative stress, activity of endogenous antioxidant enzymes and activity of membrane bound enzymes.

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1. Introduction

One of the suggested mechanisms for atherosclerosis is the increased generation of oxidized low density lipoprotein (LDL) (Steinberg, 1997) which leads to the development of foam cells from macrophages in blood vessels (Lusis, 2000). Decreasing the consumption of dietary cholesterol and saturated fatty acid (SFA)s was found to be helpful in reducing risk factors for cardiovascular diseases (Kang et al., 2005). Consumption of monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acid (PUFA)s has been shown to decrease plasma lipids, exhibit anti-inflammatory effects on the endothelium resulting in improvements in vascular function (De Caterina et al., 2000; Christon, 2003). Studies have also shown that PUFA containing lipids are prone to lipid peroxidation. The susceptibility of fatty acid to lipid peroxidation increases in proportion to its degree of unsaturation (Pamplona et al., 1999). The ratio of PUFA (P) to SFA (S) in the diet determines the susceptibility of LDL to peroxidation (Esterbauer et al., 1992). Therefore there should be a balance in the P/S ratio of lipids in the diet.

Oxidative stress is one of the causative factors that link hypercholesterolemia with atherogenesis (Halliwell, 1996; Dimitrova-Shumkovska et al., 2010). Erythrocytes are constantly exposed to both extracellular and intracellular sources of reactive oxygen species (ROS). Hypercholesterolemia leads to increased cholesterol accumulation in the erythrocytes (Kempaiah and Srinivasan, 2002). Studies by Yang et al. (2008) have shown that hyperlipidemia increases the oxidative stress in human. Thus, erythrocytes are extremely vulnerable to these oxidative challenges and hypercholesterolemia (Vijayakumar and Nalini, 2006).

The generation of ROS such as super oxide anion ($O_2^{\cdot -}$) and hydrogen peroxide (H_2O_2) during metabolism is an essential and normal process (Linnane and Eastwood, 2006). Generation of ROS in high doses and/or its inadequate removal result in oxidative stress, cause severe metabolic malfunctions and damage biological macromolecules (Mates et al., 1999). Oxidative stress has been shown to be involved in the pathophysiology of a number of chronic diseases including cardiovascular diseases (Willcox et al., 2004). The biological effects of free radicals are controlled by various cellular defense mechanisms consisting of enzymatic and nonenzymatic scavenger components (Mates et al., 1999). The antioxidant enzymes in animal cell that prevent deleterious effect of ROS include superoxide

Abbreviations: CNO, coconut oil; RBO, rice bran oil; SESO, sesame oil; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; MDA, malondialdehyde; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; GST, glutathione-s-transferase; ROS, reactive oxygen species; (B), blended oil; (I), interesterified oil.

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dismutase, catalase, glutathione peroxidase, glutathione transferase and glutathione reductase (Valko et al., 2007).

The composition and organization of lipids in biological membranes are important factors that determine the fluidity. Fatty acid composition of membrane phospholipids are known to affect a wide variety of membrane properties (Stillwell et al., 1993; Hulbert et al., 2005). This influences the activity of membrane bound proteins such as enzymes, receptors, carriers or ion channels. ROS mediated reactions can significantly damage the polyunsaturated fatty acids and proteins in the membranes (Girotti, 1998). Thus peroxidation of lipids alters membrane fluidity and also affects the function of integral proteins associated with the membrane (Knowles and Donaldson, 1996). The control of lipid peroxidation is an essential process in aerobic organisms, as lipid peroxidation products can damage DNA. Lipid peroxidation can also directly inhibit activities of enzymes such as Na^+/K^+-ATPases (Da Silva et al., 1998).

In our earlier studies we have shown that the atherogenic potentials of coconut oil (CNO) can be significantly decreased when blended with an unsaturated oil like rice bran or sesame oil to give a P/S ratio of 0.8–1.0 (Reena and Lokesh, 2007). During this process however the P/S ratio of CNO + RBO and CNO + SESO was significantly increased as compared to that found in CNO. This in turn may alter the susceptibility of rats to oxidative stress. To study this, investigation was undertaken to evaluate the susceptibility of liver and erythrocyte lipids to oxidative stress measured in terms of lipid peroxidation and enzymes involved in scavenging of free radicals. Since unsaturated lipids and lipid peroxides also affect the membrane associated enzyme activities, the influence of feeding diet containing CNO and its blends with RBO or SESO were also studied on the activities of Na^+/K^+-ATPase and Ca^{2+}/Mg^{2+}-ATPase, two membrane bound enzymes. We have also observed that interesterified fats have higher capacity to lower serum cholesterol levels even though its fatty acid composition is similar to that of blended oils (Reena and Lokesh, 2007). Hence the effects of feeding interesterified fats on oxidative stress in rats were also monitored.

2. Materials and methods

Edible coconut oil (Parachute brand Bangalore, India) and sesame oil (Ganesh-gingelli brand, Kangayam, India) were purchased from a local super market. Physically refined rice bran oil was provided by A.P. Solvex Limited, Dhuri, India. Lipozyme IM-60 was a gift from NOVO Nordisk Bioindustrial Inc., (Danbury, CT, USA). BF_3 in methanol, xanthine, xanthine oxidase, cytochrome C, glutathione, NADPH, glutathione disulfide, thiobarbituric acid, hydrogen peroxide were purchased from Sigma Chemical Co., St. Louis, MO, USA. Ascorbic acid and trichloroacetic acid were purchased from SISCO Laboratory Pvt. Ltd., Mumbai, India. All the solvents used were of analytical grade and distilled prior to use.

2.1. Preparation of blended and interesterified oils

Blended and interesterified oils containing CNO with RBO or SESO were prepared as described earlier (Reena and Lokesh, 2007). The blended oils contained SFA: MUFA: PUFA in the proportion of approximately 1:1:1 and P/S in the ratio of 0.8–1.0. The blending of oils were carried out by mixing the predetermined amounts of oils and stirring for 1 h at 40 °C on a magnetic stirrer after flushing with nitrogen. The mixing efficiency was monitored by estimating fatty acid composition of the blended oils periodically.

Interesterified oil was prepared by incubating the blended oils with immobilized lipase (*Rhizomucor miehei* lipozyme IM-60) at 1% (w/w) and agitating at a speed of 160 rpm in a shaking water bath (BS-31, Tokyo, Japan) for 72 h at 37 °C. The oil was decanted, and enzyme was washed with hexane for reuse. The quality of interesterified oil was then evaluated by measuring peroxide value and free fatty acid content. Interesterification process did not affect the quality, nutraceutical contents and total fatty acid composition of the oils (Reena and Lokesh, 2007).

2.2. Fatty acid composition of oils

Fatty acid composition of native, blend and interesterified oils were analyzed as methyl esters by gas chromatography (Morrison and Smith, 1964). The fatty acid methyl esters were prepared by saponification of oils with 0.5 M KOH and later methylating fatty acids with 40% BF_3 in methanol. The fatty acid methyl esters were separated by GC using a fused silica capillary column 25 m × 0.25 mm (Parma bond FFAP-DF-0.25: Machery-Negal GmBH Co., Duren, Germany) connected to GC (Shimadzu 14B fitted with FID) 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 with the retention times of standards (Nuchek Prep, Elysin, MN, USA) and quantified using Clarity Lite software.

2.3. Animal treatment

All the experiments involving animals were approved by the ethical committee. Male Wistar rats [OUTB-Wistar, IND-cft (2c)] (*Rattus norvegicus*) weighing 40–45 g were grouped (6 rats in each group) 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 with fresh diet daily, and left over diets were weighed and discarded. The gain in body weight of animals was monitored at regular intervals. The rats had free access to food and water throughout the study. Each group of rats were fed with, AIN-76 diet containing 10% fat from either CNO, RBO, SESO, blends of CNO + RBO(B), CNO + SESO(B), or interesterified oils 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 methionine 0.3% (Anonymous, 1977). Animals were fed on test diets for 60 days and following an overnight fast on day 60, were anesthetized and blood was collected in heparinized tubes (17 units/mL blood) by cardiac puncture. The liver was removed, washed in saline and stored at −80 °C until the analysis was completed.

2.4. Lipid peroxides in liver homogenates

One gram of liver was homogenized in 10 mL of 0.15 M potassium chloride, in a Teflon homogenizer. The homogenate was filtered through cheesecloth and used for assay. Liver homogenates (4 mg protein) in 0.15 M potassium chloride, 0.025 M Tris-hydrochloride buffer pH 7.5, 2 mM adenosine diphosphate and 10 μM ferrous sulphate were incubated at 37 °C for 5 min. The reaction was initiated by adding 0.1 mM ascorbic acid and incubated at 37 °C for 30 min. The final volume of the reaction mixture was 1 mL. The reaction was terminated using 2 mL of thiobarbituric acid (0.375% thiobarbituric acid, 15% trichloroacetic acid in 0.2 N hydrochloric acid) containing 10 μM butylated hydroxyl anisole. Samples were heated for 15 min in a boiling water bath. Malondialdehyde (MDA) formed was measured at 535 nm and quantitated using an extinction coefficient of $1.56 \times 10^{-5}\ cm^{-1}$. The lipid peroxides were expressed as nmol of MDA/mg protein. Appropriate blank samples were included for measurements (Buege and Aust, 1989).

2.5. Antioxidant enzyme activities

Superoxide dismutase (SOD) in the hepatic tissue homogenates were measured by the inhibition of cytochrome C reduction mediated via superoxide anions generated by the xanthine/xanthine oxidase system and monitored at 550 nm. Activities are expressed as unit/mg protein, where one unit of SOD was defined as the amount required to cause half-maximal inhibition of cytochrome C reduction (Flohe and Otting, 1984). Catalase was determined according to Aebi (1984) by following the decomposition of hydrogen peroxide at 240 nm. Glutathione peroxidase activity was determined by NADPH oxidation in a coupled reaction system containing oxidized glutathione (Tappel, 1978). Glutathione transferase was measured with 1-chloro 2,4-dinitrobenzene as the substrate (Jensson et al., 1985). The enzyme activity is expressed as μmol of CDNB-GSH conjugates formed per minute per mg protein. All spectrophotometric measurements were carried out in a Shimadzu ultraviolet spectrophotometer (Shimadzu UV–VIS 1601) with 1.0 mL quartz cuvettes.

2.6. Preparation of erythrocytes membranes

Erythrocyte membranes were prepared according to the method of Fairbanks et al. (1971). After removing plasma from freshly drawn heparinised blood, blood cells were diluted with saline (1:1, v/v), mixed gently and centrifuged at 700×g for 10 min at 4 °C. Sedimented cells were washed three times and the supernatant became colorless. The cells (0.5 mL) were lysed by forcing them through 19.5 mL of phosphate buffer (5 mM, pH 8.0) in a centrifuge cup. The contents were centrifuged at 12,000×g for 20 min at 4 °C to sediment erythrocyte ghosts. Ghosts membranes were washed with phosphate buffer (5 mM, pH 8.0) four times and the colour became almost white or pale pink. Finally, the pellet was washed with Tris buffer (10 mM, pH 7.0) and suspended in the same buffer. The samples were used for measuring Na^+/K^+- and Ca^{2+}/Mg^{2+}-ATPase activity and for fatty acid analysis.

2.7. Na^+/K^+-ATPase activity

The activity of Na^+/K^+-ATPase in erythrocyte membrane was assayed spectrophotometrically as described earlier by Savitha and Panneerselvam (2006) by measuring the phosphate released from ATP and quantitated using KH_2PO_4

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