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ORIGINAL CONTRIBUTION

Integrity of erythrocytes of hypercholesterolemic and normocholesterolemic rats during ingestion of different structured lipids

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Abstract
Purpose To assess the effect of medium-chain fatty acid (MCFA)-rich mustard oil and polyunsaturated fatty acid (PUFA)-rich mustard oil on erythrocyte membrane composition and osmotic fragility in normal and hypercholesterolemic rats.
Method Membrane composition was analyzed using standard kits. Osmotic fragility was determined using method described by Dacie and Lewis. Fatty acid composition of membrane was analyzed using gas chromatographic methods. Membrane shape analysis was performed using scanning electron microscope.
Results Osmotic fragility data suggested that the erythrocyte membrane of hypercholesterolemic rats were relatively more fragile than that of the normal rat's membrane, which could be reversed with the addition of MCFA- and PUFA-rich oil in the diet. The increased plasma cholesterol in hypercholesterolemic rats could also be lowered by the experimental oils. There was also marked changes in the fatty acid composition of the plasma and erythrocyte membrane phospholipids. Polyunsaturated fatty acids decreased in the plasma of the hypercholesterolemic subjects were increased with the treatment of the experimental oils. Shape changes of the membrane holes were observed in the hypercholesterolemic condition, which was brought to normal shape with the administration of the experimental oils.
Conclusion In conclusion, rat erythrocytes appear to be deformed and became more fragile in cholesterol-rich blood. This deformity and fragility was partially reversed by experimental oils by virtue of their ability to lower the extent of hypercholesterolemia.

Keywords Hypercholesterolemia · Erythrocyte membrane · Membrane fragility · Membrane lipid profile · Scanning electron microscopy

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Introduction

Hypercholesterolemia has consistently been shown to be the risk factor for cardiovascular diseases [1]. High dietary cholesterol may affect lipid metabolism in various organs. Changes in cholesterol content as well as changes in the fatty acid composition of erythrocyte membrane affect fluidity and enzyme activities and may change the deformability of erythrocytes through the capillary vessels. As there is a permanent exchange of lipids between plasma lipoproteins and the erythrocyte membrane, severe hypercholesterolemia is shown to be a risk factor leading to alterations in erythrocyte membrane lipid composition and fluidity [2]. Consequently, hypercholesterolemia adversely affects the rheological properties of the erythrocyte [3–5] and imposes additional burden on the compromised circulation.

Triacylglycerol containing medium-chain fatty acid (MCT) is a special-purpose food that could be used as a supportive nutritional therapy and may be used to increase the calorie value, improve the palatability, digestibility, and absorption. MCTs have a number of properties that may be beneficial in preventing atherosclerosis. Among these are that MCTs have anti-coagulating effects and have been shown to lower serum cholesterol. In addition, MCTs reduce levels of cholesterol in the liver and other tissues [6].

Polyunsaturated fatty acids (PUFA) are remarkably diverse molecules, both structurally and functionally. Human physiology depends in various ways on PUFA, either as components of membrane phospholipids in specific tissues or as precursors of eicosanoids (e.g. prostaglandins) [7], which are vital for biological processes in the human body. They have a number of nutraceutical and pharmaceutical applications [8, 9]. Eicosapentaenoic acid (EPA, C 20:5 *w*3) and docosahexaenoic acid (DHA, C 22:6 ω-3) are the important ω-3 PUFA. EPA and DHA are important in treatment of arthrosclerosis, cancer, rheumatoid arthritis, psoriasis, and diseases of old age, such as Alzheimer's and age-related macular degeneration [10, 11]. A beneficial effect of marine ω-3 PUFA on atherogenesis is supported from the majority of animal studies, showing that feeding with fish oil decreases atherosclerosis [12]. ω-3 PUFA may also reduce thrombogenesis, thereby decreases the risk of thrombotic complications to plaque rupture/fissure.

In this study, we used high-cholesterol diets supplemented either with MCT or with PUFA enriched mustard oil, and we observed changes in erythrocyte lipid content, erythrocyte lipid composition, changes in fatty acid composition of phospholipids, and the changes in osmotic fragility and compared it with normal control. Literature shows that dietary fatty acids may induce extensive modification in the fatty acid composition of erythrocyte membranes [13–16].

Materials and methods

Animals

Male albino rats of Charles Foster strain were housed in individual cages. The work was done under the supervision of the Animal Ethical Committee of the Department of Chemical Technology (University of Calcutta). The rats were acclimatized for 2 weeks while receiving free access to water and were fed a standard laboratory diet ad libitum. After the 2-week period, animals were switched to the experimental diets. For the duration of the study, the rats were exposed to a 12-h light–dark cycle. The animals were divided into six groups (average body weight 130–160 g), each consisting of six animals. The weekly body weight of the rats was monitored. Diets were prepared weekly and stored at−20 °C. Three groups were made hypercholesterolemic by feeding added cholesterol (1% of total lipid), and three groups were kept normal. Dietary composition of the diet given is shown in Table 1. After 4 weeks of feeding of experimental diets, the rats were anaesthetized by chloroform and 5 mL of blood was taken from the heart and before that the subjects were kept in fasting for 12 h.

Table 1 Proximate composition of the control and experimental diet

Diet	Control diet	MCT diet	PUFA diet
Starch	55%	55%	55%
Fat-free casein	18%	18%	18%
Mineral mixture	4%	4%	4%
Husk	3%	3%	3%
Mustard oil	2%	–	–
Capric acid-rich mustard oil	–	2%	–
PUFA-rich mustard oil	–	–	2%

Food of 10 g/day/rat was given

Multivitamin capsule was also added to the food as 200 mg/100 g basis

Two multivitamin capsules (Pfizer, India) used per kg of diet possessed the following composition: Vitamin A I.P. 10,000 units, Thiamine mononitrate I.P. 5 mg, Riboflavin I.P. 5 mg, Pyridoxine hydrochloride I.P. 1.5 mg, Vitamin B_{12} I.P. 5 mg, Calcium Pantothenate USP 5 mg, Niacinamide I.P. 50 mg, Ascorbic acid I.P. 400 units, Cholecalciferol USP 15 units, Menadione I.P. 0.1 mg, Folic acid I.P. 1 mg, Vitamin E. USP 0.1 mg

Preparation of oils

MCT-rich mustard oil, i.e. capric acid-rich mustard oil was prepared by the reaction between capric acid and mustard oil in a packed bed bio-reactor. The second experimental oil, EPA- and DHA-rich mustard oil, was prepared by blending fish oil and mustard oil. The final fatty acid compositions of both the oils were determined by GC.

Osmotic fragility determination

The method described by Dacie and Lewis [17] employed here provided different concentrations of sodium chloride 0.1–0.9% in a series of tubes made from appropriate dilutions of 1% sodium chloride phosphate buffer, pH 7.4 to a final volume of 5 mL. Freshly heparinized blood (20 μL) was pipetted into these tubes containing varying sodium chloride concentration. The contents were gently mixed and allowed to stand for 30 min at room temperature. At the end, the contents of the tubes were mixed again and centrifuged at 500 × g for 10 min. Absorbance of the supernatant was measured at 540 nm against water blank. The degree of hemolysis was expressed as a percentage, where 100% represents full hemolysis.

Preparation of erythrocyte membranes

All procedures were done at 0–5 °C (typically on ice), and all centrifugations were performed in a Sorvall SS-34 rotor at 15,000 rpm unless specified. Rat red cells and hemoglobin-free ghosts were prepared as described in the literature [18], except that the hemolysis buffer was 5 mM ∼ NaPi (pH 8), 0.01 mM MgSO4, and the membranes were

suspended for 10 min in this buffer before each centrifugation to allow hemoglobin to exit fully.

Extraction of lipids from erythrocytes and plasma

Blood samples for laboratory analyses were taken into EDTA-containing vacuum tubes after 12-h fasting. Plasma was separated by centrifugation and stored at −70 °C until analyzed. In the analysis of fatty acid composition of erythrocyte membrane and plasma lipids, 0.5 g of samples were homogenized with 1 mL of 0.74% potassium chloride and 2 mL of chloroform and methanol in the proportion of 1:1 v/v (Chloroform:Methanol) followed by 2 mL of chloroform and methanol in the proportion of 2:1 v/v (Chloroform:Methanol) for 2 min and then centrifuged. Then, the chloroform layer was filtered through a Whatman filter paper (No. 1). The chloroform layer was dried, the lipid contents of the erythrocyte membrane and plasma were measured, and the lipid was used for lipid analysis [19].

Lipid analysis

The lipid components such as total cholesterol were analyzed using enzyme kits supplied by Merck India Ltd. (Catlog No. 117679), following the standard methods. Phospholipid was estimated using the method of Chen et al. [20]. After obtaining the total cholesterol and phospholipid values, their ratios were also calculated.

Determination of fatty acid composition of plasma lipid

Plasma lipid extracts were saponified using methanolic KOH, and methyl esters of corresponding fatty acids were made by the standard method [21]. Fatty acid methyl esters were analyzed using gas chromatography. The GC (make: Agilent, model: 6,890 N) instrument used was equipped with FID detector and capillary DB-Wax column (30 mL, 0.32 mm I.D, 0.25 μm FT). N_2, H_2, and airflow rate was maintained at 1 mL/min, 30 mL/min, and 300 mL/min, respectively. Inlet and detector temperature was kept at 250 °C, and the oven temperature was programmed as 150–190–230 °C with increase rate of 15 °C/min and 5 min hold up to 150 °C and 4 °C/min with 10 min hold up to 230 °C. The percentage proportions of fatty acids were calculated.

Determination of fatty acid composition of erythrocyte membrane phospholipids

The presence of phospholipids were first confirmed by thin layer chromatography (TLC) by spotting the lipid mixture after extraction on a Silica Gel G plate (0.2 mm thick) using hexane–diethyl ether–acetic acid (90:10:1) as a developing solvent system. The lipid spots were identified by iodine absorption [22–24]. The phospholipid was then extracted from the TLC plate using chloroform. The fatty acid compositions of the phospholipids were again determined by GC following the same method.

Scanning electron microscopy

Erythrocyte membrane ghost suspension was made in a cold buffered saline (10 mM NaH2PO4, pH 7.4) containing 1% glutaraldehyde. After 1 h, the ghosts were washed three times with phosphate buffer and finally fixed with 1% osmic acid in the same buffer. Fixed cells were dehydrated sequentially with ethanol and propylene oxide, spread over a cover glass and air dried. After coating with gold, cells were examined in a scanning electron microscope.

Statistical analysis

All the data were presented as mean ± S.D. Significance was calculated using two-way ANOVA. Differences were considered significant at $p < 0.05$.

Results

Fatty acid composition of experimental oils

Analysis of the lipids in the diet showed that MCT-rich mustard oil contained 19.87% capric acid (C_{10}) and PUFA-rich mustard oil contained 18.83% eicosapentaenoic acid ($C_{20:5}$) and docosahexaenoic acid ($C_{22:6}$). The fatty acid composition of the control oil (mustard oil) and experimental oils, MCT-rich mustard oil and PUFA-rich mustard oil, are given in Table 2.

Changes in body weight

The fat level was kept constant at 20% in all the dietary groups. The amount of diet consumed in the different groups was comparable. The effect of feeding dietary lipids on body weight gain of normal and hypercholesterolemic rats is shown in Fig. 1. There was no significant difference in the food intake of the different groups both in the normal and hypercholesterolemic rats. However, there was significant difference between the body weight gain of normal and hypercholesterolemic group ($p < 0.01$). There was no significant difference between the body weight gain of the group fed with mustard oil and the group fed with MCT-rich mustard oil in the normal case. On the other hand, the body weight gain decreased on feeding the rats with MCT-rich mustard oil in hypercholesterolemic condition. The body

Table 2 Fatty acid composition of control and experimental oils

Fatty acids (%w/w)	Mustard oil (control)	MCT-rich mustard oil	PUFA-rich mustard oil
$C_{10:0}$	–	19.87	–
$C_{16:0}$	2.24	1.75	1.33
$C_{18:0}$	1.15	1.02	2.33
$C_{18:1}$	9.42	11.06	9.31
$C_{18:2}$	17.94	10.55	17.52
$C_{18:3}$	10.75	7.22	8.18
$C_{20:0}$	0.80	0.60	0.20
$C_{20:1}$	5.15	3.57	4.87
$C_{20:4}$	–	–	1.68
$C_{20:5}$	–	–	10.35
$C_{22:0}$	2.00	1.92	1.59
$C_{22:1}$	48.55	40.35	33.00
$C_{24:0}$	1.30	2.09	1.19
$C_{22:6}$	–	–	8.48
ΣPUFA	**28.69**	**17.77**	**46.21**

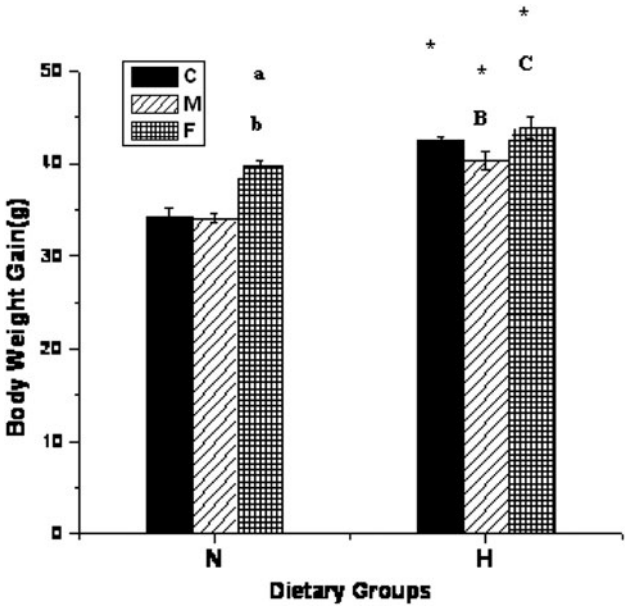

Fig. 1 Effect of feeding dietary lipids on body weight gain of normal and hypercholesterolemic rats (*N* normal, *H* hypercholesterolemic, *C* control, *M* MCT-rich mustard oil, *F* PUFA-rich mustard oil) Values are mean ± SD of 6 rats per group. [a]Comparison between normal C and normal F ($p < 0.05$); [b]Comparison between normal M and normal F ($p < 0.05$); *Comparison between normal and hypercholesterolemic groups ($p < 0.05$); [B]Comparison between hypercholesterolemic C and hypercholesterolemic M ($p < 0.05$); [C]Comparison between hypercholesterolemic C and hypercholesterolemic F ($p < 0.05$)

weight gain was increased significantly by feeding the rats with PUFA-rich mustard oil both in normal and hypercholesterolemic group.

Osmotic fragility of erythrocytes

The effect of dietary intake of different oils on the osmotic fragility of erythrocytes of normal and hypercholesterolemic rats is presented in Table 3. In normal control rats, hemolysis began at 0.8% NaCl concentration and completed at 0.15% NaCl concentration, with mean cell fragility(50% hemolysis) being evident at 0.54% NaCl concentration. In hypercholesterolemic control rats, hemolysis of red blood cells began in hypotonic saline at 0.85% NaCl concentration and completed at 0.35% NaCl concentration, with a mean cell fragility (50% hemolysis) at 0.61% NaCl concentration. Thus, the osmotic fragility data suggested that the red blood cells of high cholesterol diet fed animals were relatively fragile.

The increased osmotic fragility of red blood cells evidenced in hypercholesterolemic rats was partially reversed by dietary oils MCT-rich mustard oil and PUFA-rich mustard oil as shown in Table 3. The mean cell fragility of red blood cells was at 0.59 and 0.48% NaCl concentration under MCT-rich oil and PUFA-rich oil, respectively.

Changes in total lipid content of membrane and plasma

The changes occurred in lipid content are shown in Table 4. The results show that the lipid content was maximum in the case of normal control rats in normal condition and the content decreased due to the administration of different experimental oils. Similarly, in the hypercholesterolemic condition, the content was maximal in untreated controls and was diminished by treatment with both experimental oils. Similar results were seen both for erythrocyte membrane lipid (Table 4) and plasma lipid (Table 5).

Changes in erythrocyte membrane lipid profile

Lipid profiles of erythrocyte membranes of normal and hypercholesterolemic rats are presented in Table 4. The amount of membrane cholesterol and phospholipid was higher in the normal control rats fed mustard oil than in the normal experimental rats fed with the two experimental oils. Enrichment of mustard oil with MCT and PUFA resulted in a decrease in membrane cholesterol and increase in membrane phospholipid levels and thus lowered the cholesterol:phospholipid (C:P) ratio also. Consistent with hypercholesterolemia, as expected, erythrocyte membranes were enriched with cholesterol in cholesterol fed animals. Erythrocyte membrane phospholipid decreased in hypercholesterolemia. This resulted in an elevated cholesterol:phospholipid ratio in the membrane red blood cells in hypercholesterolemic rats. All the two experimental oils significantly lowered the alteration in cholesterol and

Table 3 Osmotic fragility of erythrocytes in normal and hypercholesterolemic rats maintained on different oils

Animal groups	NaCl concentration (%) causing 50% hemolysis	
	Normal	Hypercholesterolemic
Control	0.54 ± 0.01	0.61 ± 0.01^{a}
MCT-rich mustard oil	0.47 ± 0.01^{b}	$0.59 \pm 0.01^{a,b}$
PUFA-rich mustard oil	$0.39 \pm 0.03^{b,c}$	$0.48 \pm 0.04^{a,c}$

Values are expressed as mean ± SEM of 6 animals per group. The superscript letters represent statistical significance at $p < 0.05$

[a] Comparisons are made between normal and hypercholesterolemic group

[b] Comparisons are made between control and experimental group

[c] Comparisons are made between MCT-rich mustard oil and PUFA-rich mustard oil group

Table 4 The effect of dietary oils on lipid profile of erythrocyte membrane in normal and hypercholesterolemic rats and fatty acid composition of erythrocyte membrane phospholipids

Parameters		Normal group			Hypercholesterolemic group		
		Control	MCT-rich mustard oil	PUFA-rich mustard oil	Control	MCT-rich mustard oil	PUFA-rich mustard oil
Total cholesterol (g/l)		2.38 ± 0.09	1.67 ± 0.02^{b}	$0.99 \pm 0.08^{b,c}$	6.34 ± 0.11^{a}	$5.34 \pm 0.09^{a,b}$	$3.64 \pm 0.12^{a,b,c}$
Phospholipid (g/l)	Total	7.49 ± 0.12^{a}	$7.79 \pm 0.08^{a,b}$	$7.95 \pm 0.16^{a,b,c}$	3.09 ± 0.11	3.36 ± 0.09^{b}	$3.55 \pm 0.12^{b,c}$
	SFA (% w/w)	34.40 ± 0.54	27.65 ± 1.22^{b}	$17.78 \pm 1.09^{b,c}$	40.14 ± 0.76^{a}	$37.21 \pm 0.98^{a,b}$	$36.12 \pm 0.12^{a,b}$
	MUFA (% w/w)	33.06 ± 1.34	31.65 ± 0.89^{b}	$27.79 \pm 1.12^{b,c}$	37.10 ± 1.00^{a}	35.00 ± 0.78^{a}	$33.21 \pm 0.67^{a,b,c}$
	PUFA (% w/w)	32.54 ± 0.45	40.70 ± 2.34^{b}	$44.83 \pm 0.46^{b,c}$	22.76 ± 0.78^{a}	$27.79 \pm 1.11^{a,b}$	$30.67 \pm 0.99^{a,b,c}$
C/P ratio		0.32 ± 0.01	0.22 ± 0.03^{b}	$0.13 \pm 0.02^{b,c}$	2.05 ± 0.07^{a}	$1.58 \pm 0.22^{a,b}$	$1.03 \pm 0.09^{a,b,c}$
Total lipid content (% w/w)		7.91 ± 0.23	3.63 ± 0.09^{b}	$2.67 \pm 0.12^{b,c}$	11.38 ± 0.54^{a}	$7.34 \pm 0.24^{a,b}$	$5.35 \pm 0.19^{a,b,c}$

Values are expressed as mean ± SEM of 6 animals per group. The superscript letters represent statistical significance at $p < 0.05$

[a] Comparisons are made between normal and hypercholesterolemic group

[b] Comparisons are made between control and experimental group

[c] Comparisons are made between MCT-rich mustard oil and PUFA-rich mustard oil group

phospholipid content in hypercholesterolemic condition. Therefore, the experimental oils also decreased the C:P ratio to a significant level ($p < 0.05$).

Changes in fatty acid composition of erythrocyte membrane phospholipids

The phospholipids present in the membranes were identified by TLC, and its fatty acid composition was determined GC. The fatty acid compositions of phospholipids of different dietary groups are presented in Table 4. The table depicts that the normal control rats showed 34.4% SFA, 33.06% MUFA, and 32.54% PUFA in membrane phospholipids. Four weeks of feeding with both the experimental oils showed a significant decrease in the SFA and MUFA level and significant increase in the PUFA level. On the other hand, the hypercholesterolemic control rats showed 40.14% SFA, 37.10% MUFA, and 22.76% PUFA in their membrane phospholipids. Here, also a significant decrease in SFA and MUFA levels and significant increase in PUFA levels were observed by feeding the hypercholesterolemic rats with experimental oils. The increase in PUFA was much more in the rats fed with PUFA-rich mustard oil in comparison with the rats fed with MCT-rich mustard oil.

Changes in plasma lipid profile

Plasma lipid profile in normal and hypercholesterolemic rats maintained on different oils was presented in Table 5. Compared to the normal control group, the two normal experimental groups showed a decrease in total cholesterol, non-HDL cholesterol, and triglyceride levels and an increase in HDL cholesterol level. On the other hand, the plasma total cholesterol, non-HDL cholesterol, and triacylglyceride levels of hypercholesterolemic control were much higher than the normal control, and the HDL cholesterol was much lower. However, the plasma total cholesterol, non-HDL cholesterol, and triglyceride levels of hypercholesterolemic rats were significantly lowered by dietary oil treatment, and the level of HDL cholesterol was effectively increased by experimental oils. Results showed

Table 5 Plasma lipid profile (g/l) in normal and hypercholesterolemic rats The effect of dietary oils on fatty acid composition of plasma

Parameters		Normal group			Hypercholesterolemic group		
		Control	MCT-rich mustard oil	PUFA-rich mustard oil	Control	MCT-rich mustard oil	PUFA-rich mustard oil
Cholesterol (g/l of plasma)	Total	8.72 ± 0.11	6.80 ± 0.22[b]	7.67 ± 0.19[b,c]	9.33 ± 0.19[a]	6.08 ± 0.22[a,b]	7.24 ± 0.28[a,b,c]
	HDL	23.00 ± 2.21	30.62 ± 1.82[b]	41.67 ± 2.32[b,c]	16.92 ± 2.11[a]	24.00 ± 1.85[a,b]	32.44 ± 1.78[a,b,c]
	Non-HDL	39.17 ± 3.40	13.46 ± 0.9[b]	3.32 ± 2.30[b,c]	48.41 ± 2.30[a]	27.53 ± 1.20[a,b]	16.65 ± 0.40[a,b,c]
Triacylglycerol	Total (g/l)	125.16 ± 0.22	119.64 ± 0.23[b]	112.34 ± 0.89[b,c]	139.77 ± 2.29[a]	125.88 ± 2.11[a,b]	116.66 ± 0.67[a,b,c]
	SFA (% w/w)	30.03 ± 1.34	21.95 ± 0.98[b]	19.05 ± 0.34[b,c]	35.28 ± 1.09[a]	32.88 ± 1.08[a,b]	31.05 ± 0.12[a,b]
	MUFA (% w/w)	32.03 ± 0.99	30.74 ± 0.12[b]	28.18 ± 0.67[b,c]	39.92 ± 1.24[a]	35.34 ± 0.34[a]	33.12 ± 1.45[a,b,c]
	PUFA (% w/w)	37.94 ± 1.22	47.31 ± 2.34[b]	52.77 ± 1.56[b,c]	24.80 ± 1.56[a]	31.78 ± 0.21[a]	35.83 ± 0.99[a,b,c]
Total lipid content (%)		5.53 ± 0.17	2.03 ± 0.35[b]	0.94 ± 0.06[b,c]	7.84 ± 0.12[a]	6.46 ± 0.08[a]	3.56 ± 0.25[a,b,c]

Values are expressed as mean ± SEM of 6 animals per group. The superscript letters represent statistical significance at $p < 0.05$

[a] Comparisons are made between normal and hypercholesterolemic group

[b] Comparisons are made between control and experimental group

[c] Comparisons are made between MCT-rich mustard oil and PUFA-rich mustard oil group

that compared to MCT enriched mustard oil, PUFA enriched mustard oil produced better outfalls.

Changes in fatty acid composition of plasma lipids

The fatty acid compositions of plasma lipids of normal and hypercholesterolemic rats are presented in Table 5. The lipid of plasma of normal rats fed with experimental oils showed a significant decrease in SFA and MUFA levels and a significant increase in the PUFA levels. The plasma membrane lipid of hypercholesterolemic subjects showed a significant rise in SFA and MUFA levels and a significant lowering of PUFA levels in comparison with the normal subjects. The changes were reversed in the case of administration with experimental oils to hypercholesterolemic subjects. The table depicts that the changes were more pronounced in the case of PUFA enriched mustard oil in both normal and hypercholesterolemic subjects.

Changes in erythrocyte membrane holes as seen by scanning electron microscope

The holes in hemoglobin-free ghosts generated by osmotic lysis of erythrocytes of normal and hypercholesterolemic rats were characterized. The pore formation in the erythrocyte membrane was confirmed by scanning electron microscopy as shown in Fig. 2. The control rats showed an uneven surface texture of the hole, whereas the experimental rats showed a smooth surface. It can be also seen from the figures that the texture of the surface of hypercholesterolemic control was more uneven than normal control and the texture was smoothened by administration of different experimental oils. The texture of the surface of the membrane ghost holes of rats treated with PUFA-rich mustard oil was smoother in comparison with rats treated with MCT-rich mustard oil.

Discussion

The fluidity of the erythrocyte membrane is determined by a number of factors among which cholesterol content and fatty acid composition have significant influences [25, 26]. The interactions of these factors seem to affect the physiological properties of the membranes to a varying degree. In this study, we have shown that erythrocyte membrane composition can be altered by different types of dietary PUFAs. The basic features of the fatty acid composition were largely dependent on the diet, particularly on the ratio of saturated and unsaturated fatty acids in the diet. In a number of investigations, there have been attempts to correlate the osmotic fragility of erythrocytes to their lipid and fatty acid composition. As a result, the stabilization of erythrocyte membrane has been generally attributed to increased cholesterol/phospholipid ratio and increased unsaturated fatty acids. The rate of osmotic hemolysis measures the cell rupture process that follows the cell swelling [27–29].

In the present study, the membrane composition was altered by feeding a hypercholesterolemic diet to rats. As a result, the cholesterol content of plasma was increased, the concomitantly higher cholesterol to phospholipids (C/P) ratio in the blood would have a direct influence on cholesterol transfer from plasma to erythrocytes, resulting in the accumulation of cholesterol in the erythrocyte membrane.

Where there was an alteration in membrane lipid composition, changes in membrane properties would be

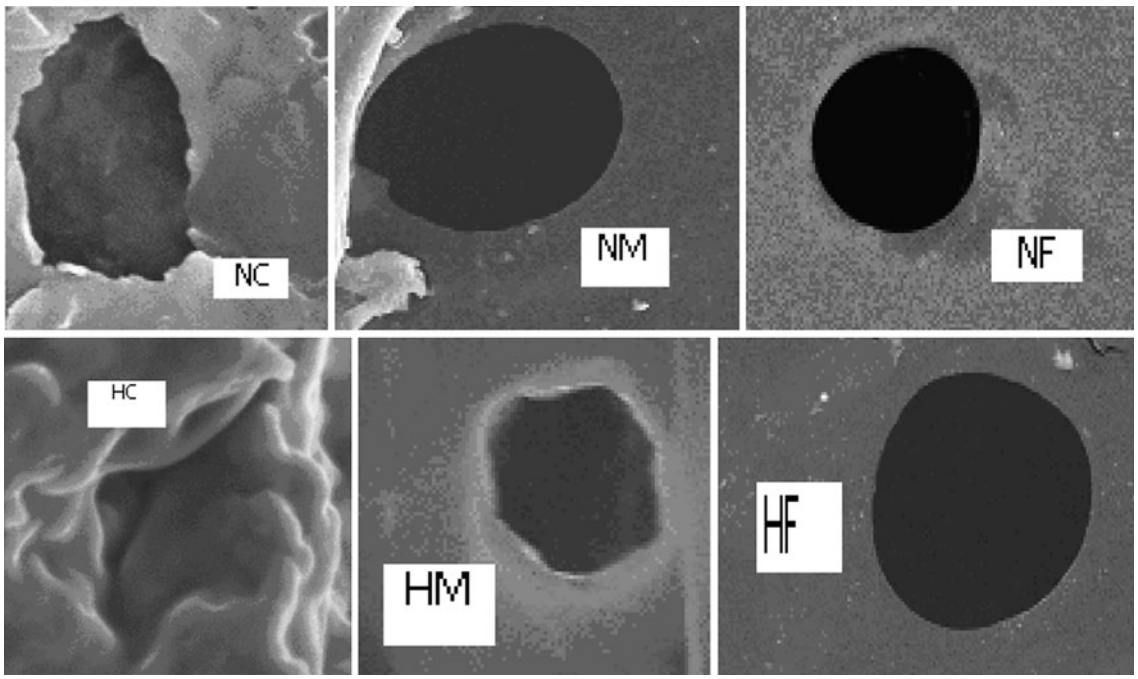

Fig. 2 Erythrocyte membrane ghost hole of normal rats (*NC* normal control, *NM* normal fed with MCT-rich oil, *NF* normal fed with PUFA-rich oil) and hypercholesterolemic rats (*HC* hypercholesterolemic control, *HM* hypercholesterolemic fed with MCT-rich oil, *HF* fed hypercholesterolemic with PUFA-rich oil)

expected, and this study confirms that in erythrocytes from rats under alimentary hyperlipidemia/hypercholesterolemia, membrane fragility was slightly increased in line with a higher C/P ratio. Inclusion of different experimental oils in the diet improved the erythrocyte fluidity, concomitant with the partial restoration of the altered C/P ratio in the membrane.

Membrane cholesterol and phospholipid was measured from the membrane lipid extract. The diet supplemented with 1% cholesterol induced an increase in the erythrocyte membrane cholesterol and phospholipid level compared to the basal or control diet. However, the administration of different experimental diets produced hypocholesterolemic effect in hypercholesterolemic subjects and thus reduced the membrane cholesterol and phospholipid levels due to an increase in the unsaturated fatty acids in the membrane lipids of experimental animals. Erythrocyte membrane cholesterol decreased both in normal and hypercholesterolemic group (Table 4). Most mammalian somatic cells, including erythrocytes, are unable to catabolize cholesterol and need to export cholesterol in order to maintain cholesterol homeostasis [30]. Reportedly, at least two independent mechanisms have been identified to account for cholesterol homeostasis. One is a non-specific diffusion-mediated cholesterol efflux from the surface of cell. Cholesterol molecule desorbed from the cell can be trapped by various extracellular acceptor, including lipoprotein and albumin, and extracellular cholesterol esterification mainly on HDL may be a driving force for the net removal of cell cholesterol by maintaining a cholesterol gradient between lipoprotein surface and cell membrane. The other mechanism is an apo-lipoprotein-mediated process to generate HDL by removing cellular cholesterol and phospholipids.

In this study, the diet containing experimental oils induced an decrease in total and LDL cholesterol and triglyceride levels and a increase in HDL cholesterol level in comparison with diet containing control oil. The experimental oils also had a hypolipidemic effect in hypercholesterolemic rats. This suggests that both capric acid and n-3 fatty acids have hypolipidemic effect.

The hypercholesterolemic situation achieved in these animals by maintaining rats on cholesterol enriched diet also resulted in a significant enrichment of erythrocyte membranes with cholesterol. Although membrane phospholipid concentration was altered to a lesser extent, the ratio of erythrocyte membrane cholesterol: phospholipid was increased in cholesterol enriched diets. This increased C:P ratio was enough to produce changes in osmotic sensitivity of red blood cells. It is said that cholesterol leads to an increase in the anisotropic nature of the lipid bilayer of the membrane due to the effects of its rigid sterol structure on lipid components on the membrane [31]. Thus, an increase in C:P ratio of the erythrocytes from hyperlipidemic rats should promote an important reduction of membrane fluidity, which in the present study was reflected in a significant decrease in the membrane osmotic fragility.

Dietary oils containing MCT and PUFA have significantly reversed this alteration in C:P ratio of the erythrocytes by reducing the cholesterol enrichment in their membranes. It is probable that the decreased membrane fluidity of hypercholesterolemic rats would also be reversed by these dietary oils, as suggested by the osmotic fragility data.

The present data on osmotic fragility confirmed the relatively higher fragility of erythrocytes in hypercholesterolemic animals. Possibly, because of the increased C:P ratio, the cells have become more fragile, which contributed to the decreased deformability of these cells as was depicted by the scanning electron micrographs of membrane holes. The ability of the erythrocyte to deform, one of the most important determinants for their survival in the circulation involves several factors, including the deformability of the membrane itself, the surface per volume ratio, and the internal viscosity [32]. In this study, experimental oils were shown to effectively counter the increased mean cell fragility of erythrocytes by lowering the membrane cholesterol level, thereby decreasing the C:P ratio of the membrane.

It has also been reported that cholesterol-rich erythrocytes from patients with atherosclerotic disease developed compensatory changes in phospholipid and fatty acid composition in order to minimize the adverse effect of the excess cholesterol in the erythrocyte membrane [33]. Phospholipid and fatty acid composition of erythrocyte membrane needs to be examined in order to have an insight into whether hypolipidemic oils involve any changes in phospholipid asymmetry and fatty acid composition.

In conclusion, rat erythrocytes appear to be deformed and become more fragile in cholesterol-rich blood. This deformity and fragility were partially reversed by experimental oils by virtue of their ability to lower the extent of hypercholesterolemia.

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