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Hyperlipidemia Induced *In Vitro* Hypoosmotic Fragility of Rat Erythrocytes

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ABSTRACT

The erythrocyte membrane fluidity is due to arrangement and interaction of different lipid components of lipid bilayer. Feeding of rats with high fat diets is known to alter lipid composition and fluidity of plasma membranes of erythrocytes that in turn reduce the osmotic resistance of erythrocytes. The present study was conducted to investigate the effect of feeding High Fat Diet (HFD) on osmofragility of erythrocytes in vitro in Wistar albino rats. For the present study, 12 male Wistar albino rats of uniform weight and age were selected and were divided in to two groups, viz., control group and HFD fed group, with six animals in each group. All the animals received basal diet in the form of pellet for a period of four weeks. HFD group animals received HFD comprising of coconut oil and vanaspati in the ratio of 2:3 w/w @ 10 ml kg body weight in addition to basal diet during the four weeks of study period. At the end of the study period, blood collected in heparinized vacutainer from each animal was subjected for osmofragility test using decreasing concentrations of phosphate buffer saline solutions. Absorbance of supernatant fluid obtained from each of the graded phosphate buffer saline and erythrocyte mixture was read at 540 nm (Spectronic-200) and absorbance values were utilized to determine the per cent hemolysis. The percentage of hemolysis was significantly (P<0.05) higher in HFD fed group animals compared to the control group rats at each concentration of phosphate buffer saline solution. It was concluded that the hyperlipidemic diet would alter the erythrocyte membrane fluidity and increases their fragility for hypoosmotic stress.
Key Words: Hyperlipidemia, Osmofragility, Hemolysis and Lipid Peroxidation.

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Introduction

The erythrocyte membrane though accounts for only 1 per cent of the total weight of the red cell it plays an important role in the maintenance of erythrocyte integrity. The RBC membrane and its skeleton provide the erythrocyte the flexibility, durability and tensile strength to undergo large deformations during repeated passages through narrow microcirculatory channels. Basic knowledge of the normal physiology of the erythrocytic membrane provides an insight into the mechanisms underlying hemolysis [1]. Plasma membranes are fluid structures and their fluidity is a prerequisite for cellular viability, growth, reproduction and function [2]. Any factor that alters the structure and fluidity of erythrocyte membrane predisposes them to phagocytosis, lysis and fragmentation, resulting in their reduced lifespan [3]. Membrane fluidity is influenced by several extrinsic factors like temperature, pH and blood storage [4] and intrinsic factors like free cholesterol content (rigid sterol ring of cholesterol decreases lipid bilayer fluidity), fatty acid composition, degree of phospholipid fatty acid saturation and protein matrix. As the lipid composition is crucial for maintaining the structure and fluidity of erythrocyte membrane, any change in the membrane lipid composition of erythrocytes due to changes in plasma lipids make them exquisitely sensitive hemolysis [5]. Cholesterol Oxidation Products (COPs) also affect vital functions such as cell growth, proliferation, membrane structure and functions [6]. Osmotic resistance, an inherent capacity of the erythrocyte to resist the osmotic variation, is influenced by membrane fluidity, which gets altered during hyperlipidemia. The erythrocyte osmotic fragility test is a measure of erythrocyte resistance to osmotic stress. Hence, the present study was undertaken to ascertain the influence of hyperlipidemia on membrane stability and osmotic resistance of erythrocyte in Wistar albino rats

MATERIALS AND METHODS

Twelve male Wistar albino rats of three months age with an average body weight of 240-260 g were procured from laboratory animal house, Indian Institute of Science, Bangalore, for the study. They were divided into two groups, *i.e.,* control group and high fat diet (HFD) fed group with six animals in each group. Control group animals were fed with basal diet in the form of pellets and HFD group animals were fed with HFD comprising of coconut oil and vanaspati in the ratio of 2:3 w/w @10 ml/kg body weight in addition to basal diet for four weeks to induce hyperlipidemia. At the end of study period about 2 ml (1 ml in heparinized vacutainer + 1 ml in clot activator coated vacutainer) of blood was collected from each animal of both the groups. Blood samples collected in clot activator coated vacutainer was left undisturbed for about half an hour and then centrifuged at 3000 rpm for 30 minutes to separate the serum. The serum was stored at – 20 ºC and later utilized for the determination of lipid profile. The blood samples collected in heparinized vacutainer were immediately subjected for centrifugation at 3000 rpm for 30 minutes to separate the erythrocyte form the plasma. Then the red blood cells of each blood sample were washed three times in isotonic saline solution. The separated erythrocytes were subjected to osmofragility test within 1 hour after the blood collection using phosphate buffered saline solution. In a series of 19 test tubes about 5 ml of decreasing concentration of phosphate buffer saline solution (0.90% - 0.00% with an interval of 0.05%) was taken. About 20 µl of washed red blood cells was added to each of 5 ml of graded concentration phosphate buffered saline solutions. The blood and the phosphate buffer saline was gently mixed and incubated at 37ºC for 30 min. The contents of each test tube were subjected for centrifugation @ 3000 rpm for 5 minutes and the supernatants were separated. The absorbance of the supernatant fluid of each tube was measured at 540 nm (Spectronic-200) and these absorbance values were utilized for the determination of per cent hemolysis of erythrocyte.

Per cent hemolysis is calculated using the formula

$$\text{Percentage of Hemolysis} = \frac{\text{OD of the blank}}{\text{OD of the test}} \times 100$$

The data obtained in the study were statistically analyzed using computerized Student's t-test.

Results and Discussion

The serum total cholesterol and triglyceride concentrations were significantly ($P<0.05$) higher at the end of the experimental period (4 weeks) in HFD group however, their levels did not show any significant ($P>0.05$) changes in control group rats (data published elsewhere).

The mean percentage of hemolysis observed in control and high fat diet (HFD) fed group / hyperlipidemic rat's blood is depicted in Table 1. In the present study, the 0.70% phosphate buffered saline initiated hemolysis (point of minimum resistance) and 0.45% phosphate buffered saline resulted in complete hemolysis (point of maximum resistance) both in control and HFD group rats. However, percentage of hemolysis varied significantly ($P<0.05$) between the control group and hyperlipidemic group at each phosphate buffered saline concentration (Table 1). The blood of hyperlipidemic rats showed significantly ($P<0.05$) higher percentage of hemolysis compared to control group rats in all the buffered saline concentrations. At point of minimum resistance the percentage of hemolysis was significantly ($P<0.05$) higher in hyperlipidemic rats (HFD) blood (03.11 ± 1.43) compared to the blood of normal rats (00.89 ± 1.21). At the point of maximum resistance also the percentage of hemolysis was significantly ($P<0.05$) higher in the blood of hyperlipidemic rats (95.47 ± 2.80) compared to the blood of normal rats (93.00 ± 3.26).

Table. 1. Total cholesterol and serum triglyceride concentration (Mean ± SE) of control and HFD group on 0 week and after 4th fourth of experimentation

Sl. No.	Lipid Profile	Control Group		HFD Group	
		0 Week	4th Week	0 Week	4th Week
1	Total Cholesterol	41.65 ± 1.06	46.93 ± 0.99	41.73 ± 1.26	101.26 ± 01.74
2	Serum Triglyceride	29.17 ± 0.80	32.90 ± 2.08	27.42 ± 0.63	175.68 ± 16.34

The significantly higher percentage of hemolysis observed in hyperlipidemic rats compared to control rats in the present study was in agreement with the findings of Dimeski *et al*. [7] and Sengupta and Ghosh in [8] in rats and Kanakaraj and Singh [9] and Abdelhalim and Moussa [10] in rabbits. All these researchers also observed that the erythrocyte membrane of hypercholesterolemic rats and rabbits were relatively more fragile than that of the normal rat's / rabbit's erythrocyte membrane. The study of Kempaiah and Srinivasan [11] in rats also indicated that the erythrocytes of hypercholesterolemic rats remain significantly fragile compared to normal controls. Abdelhalim and Moussa [10] attributed the

increase in the fragility of the erythrocytes of hyperlipidemic status to disturbances in ionic motion through the membrane and change in molecular properties of the membrane macromolecules.

The fluidity of erythrocyte membrane gets altered during hyperlipidemia and hypercholesterolemia [2]. In hypercholesterolemia, the damage to the plasma membrane of erythrocytes occurs due to higher concentration of cholesterol in plasma and cell membranes, lipids peroxidation and changes in protein confirmation [12]. Membrane fluidity of erythrocytes in rats diminishes during hyperlipidemia-hypercholesterolemia despite the fact that in these cells the cholesterol/phospholipid molar ratio is nearly 45% higher than in erythrocytes from control animals [13]. Increased fragility of erythrocytes of hyperlipidemic rats compared to erythrocytes of normal rats observed in the present study could be due to increased production of superoxide free radicals (hydroperoxyls) and resultant peroxidation of the membrane lipids leading to damage of the RBCs membrane in the hyperlipidemic rats.

Table. 2. Mean ± SE values of percent hemolysis in normal and hyperlipidemic Wister albino rat's blood in decreasing phosphate buffered saline concentrations (n = 6).

Test Tube No.	Phosphate Buffered Saline (%)	Percent Hemolysis	
		Control Group Rats Blood	High Fat Diet Group Rats Blood
1	0.90	00.00 ± 0.00	00.00 ± 0.00
2	0.85	00.00 ± 0.00	00.00 ± 0.00
3	0.80	00.00 ± 0.00	00.00 ± 0.00
4	0.75	00.00 ± 0.00	00.00 ± 0.00
5	0.70	00.89 ± 1.21	03.11 ± 1.43*
6	0.65	03.75 ± 1.49	13.54 ± 5.39**
7	0.60	14.65 ± 4.34	35.98 ± 4.66***
8	0.55	56.08 ± 4.91	70.28 ± 4.49**
9	0.50	76.88 ± 4.87	82.73 ± 4.16*
10	0.45	93.00 ± 3.26	95.47 ± 2.80*
11	0.40	100.00 ±0.00	100.00 ± 0.00
12	0.35	100.00 ± 0.00	100.00 ± 0.00
13	0.30	100.00 ± 0.00	100.00 ± 0.00
14	0.25	100.00 ± 0.00	100.00 ± 0.00
15	0.20	100.00 ± 0.00	100.00 ± 0.00
16	0.15	100.00 ± 0.00	100.00 ± 0.00
17	0.10	100.00 ± 0.00	100.00 ± 0.00
18	0.05	100.00 ± 0.00	100.00 ± 0.00
19	0.00	100.00 ± 0.00	100.00 ± 0.00

* P < 0.05 significant as compared to control group rats

** P<0.01 significant as compared to control group rats

*** P < 0.001significant as compared to control group rats

Increased saturated fatty acids content of the RBCs membrane decrease the fluidity and the increased unsaturated fatty acids content increases the fluidity of the RBCs membrane lipid bilayer (Gallagher and Forget, 2011). Significantly increased osmofragility of erythrocytes of hyperlipidemic rats could also be attributed to marked changes in fatty acid composition of erythrocyte membrane phospholipids as a result of HFD feeding resulting in increased saturated fatty acid concentration and decreased polyunsaturated fatty acids in the membrane. It was concluded that the fatty acid composition of the dietary fats affects serum total cholesterol and triglyceride concentrations in rats leading to altered fatty acid composition of erythrocyte membranes and their fluidity resulting in significantly increased susceptibility of hyperlipidemic rats erythrocytes to osmofragility.

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