Dietary Fat Sources with Varying Degree of Un-saturation Modify Osmotic Fragility of Plasma Membrane of Red Blood Cells

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Introduction

Plasma membranes (PM) are dynamic structures and maintain basic architectural features that govern their function and behavior (Mcmurchie, E.J., 1988). The membrane fluidity is the nature, arrangement and interactions of different molecular species of lipids present in the membrane. The PM of the red blood cell (RBC), similar to the other PM mainly consists of a lipid bilayer and proteins. Fluidity of the PM is determined by the proportion of unsaturated fatty acids in the tail part of the phopholipid molecules of the lipid bilayer. Thus, with an increase in degree of unsaturation, the fluidity of the membrane increases and then the fragility is also increased (Octavio T *et al.*, 1990). Membrane fluidity is maintained by its fatty acid and cholesterol content. Long fatty acid chains are with ability to form stronger intermolecular interactions which restrict fluidity. Bends and kinks in the fatty acid chains are formed as a result of unsaturated *cis* double bonds that may interfere with intermolecular interactions which promote fluidity. Membrane fluidity can therefore be controlled by varying the number of double bonds and the length of fatty acid chains.

Since phospholipids are important components of cell membranes, the changes in their fatty acid composition might be expected to have an impact on various membrane properties such as membrane fluidity (Guffy, M.M *et al.,* 1982). Previous studies have shown that the relationship between the fatty acid composition of phospholipids and membrane fluidity (Burns, C.P *et al* 1979, King, M.E *et al.*, 1978). It is also found that there is an increase in the percentage of polyenoic fatty acids with a resultant increase in the mean number of double bonds per fatty acid molecule that exerts a membrane fluidizing effect (Guffy, M.M *et al.,* 1982). Thus, the degree of fragility can be used as an indicator of the fluidity of the membrane. Fluidity of plasma membrane greatly influences its functional properties.

It has also been reported that mechanisms underlying the anti-tumour effects of fatty acids include lipid peroxidation, modulation of eicosanoid production by changes in fatty acid composition and changes in membrane fluidity (Tsuzuki, T., 2004).

Anwar *et al* showed that RBC membrane fragility has decreased in the membrane flexibility (increased fragility) of hyperlipidemic rabbits, which may be due to the disturbance of ionic motion through the membrane and/or the change in the molecular properties of the macromolecules forming the membrane (Anwar, M *et al.*, 2010).

The lipid composition of erythrocyte membrane (EM) can alter *invivo* by dietary fat (Fransworth, P *et al* 1965 and March, B.E *et al* 1966). It is also found out that feeding of various oils not only induced changes in the fatty acid composition, but also resulted in altered osmotic fragility of the EM (Vajreshwari, A and Narayanareddy, K , 1992).

In the present experiment, the osmotic fragility of RBC membrane was evaluated to determine the fluidity of the RBC membrane in mice fed with two different oil sources; *Linum usitatissimum* (Linseed) and *Cocos nucifera* (Coconut) with varying degrees of unsaturation. Extent of release of hemoglobin (Hb) from the RBC to the media upon a challenge to distilled water and varying concentrations of NaCl solutions estimated to access the intensity of RBC damage due to osmotic fragility.

In the current experiment, it was hypothesized that feeding the mice with two different oil sources with varying degrees of unsaturation would influence the composition of RBC membrane and then change the osmotic fragility of the membrane. Thereby, influences the withstanding capacity RBC membrane against different osmotic challenges.

Materials and Methods

A group of mice (n=15) was fed with a normal broiler starter diet during the three weeks acclimatizing period. Then the animals were randomly assigned into three groups (n=5 per group). Mice in two separate experimental groups were given either linseed or coconut oil by using a feeding needle (0.5g of oil /15g of diet were given to each mouse). Out of three groups, one was kept as a control. After 10 days of regular feeding, the blood samples were collected to heparinized tubes and blood was used for the estimation of osmotic fragility of RBC. The blood samples were diluted 1:2 with 0.9% NaCl solution before the fragility test was performed.

A series of seven plastic centrifuge tubes were prepared separately by adding 5ml of distilled water, 0.2%, 0.4%, 0.6%, 0.9%, 1.2% and 1.8% NaCl solutions. 200µl of blood was added to each centrifuge tube, mixed gently and was allowed to stand undisturbed in a rack for 10 min. Then the tubes were centrifuged for 2min. at 2000rpm. The absorption (OD) values of released Hb were read at 580nm to assess the degree of fragility of the membrane as it has been performed previously (Anwar, M *et al.*, 2010 and Azeez, O.I *et al* 2009) using UV spectrophotometer.

Statistical analysis

Statistical analysis was carried out using the GraphPad Prism 5 Software. One way ANOVA was performed at confidence level 95%. A statistical significance was considered if the $P < 0.05$. Mean OD values of each experimental group were compared at each concentrations of NaCl and distilled water.

Results

The mean OD values of each experimental group (n=5 mice per group) due to released Hb from d RBC membrane due to exposure varying osmotic challenges are shown in Fig.1.

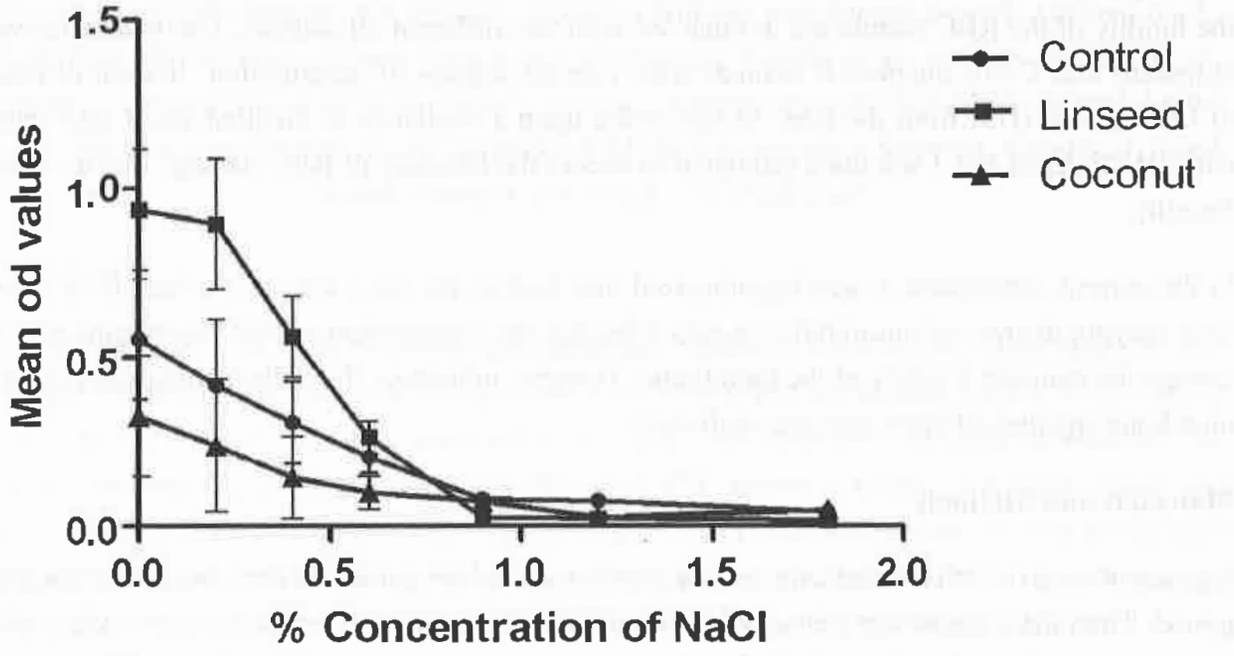

Fig.1 Fragility of RBC (as indicated by the OD due to released hemoglobin from broken RBC) against distilled water and different concentrations of NaCl in mice fed with different oils. The values are expressed as Mean ± SEM of n=5 animals.

Results revealed that the highest level of fragility of RBC was recorded in linseed oil fed group followed by control and then the coconut oil fed group at the concentrations of 0.2% NaCl and distilled water. There were significant differences among three groups ($p < 0.05$; Fig.1). No significant effects were observed at other concentrations of NaCl.

Discussion

According to Kirchgesser *et al* 1994 the osmotic fragility of erythrocytes was also influenced by dietary oil, respectively fatty acid pattern of the erythrocytes. In this study the degrees of fragility of RBC (as indicated by OD values of liberated Hb due to damaged RBC) against different concentration gradients are shown in Fig 1. These results indicate that there was a significantly higher level of fragility of RBC in linseed oil fed mice as compared with other two groups by osmotic challenges exerted by distilled water and 0.2% NaCl solution. The lowest fragility was observed in coconut

oil fed mice. These results clearly indicate the fact that the polyunsaturated fatty acids present in linseed oil has increased the fluidity of the RBC membrane, whereas the saturated fatty acids present in coconut oil acted in a opposite way. It is a well known fact that increases in the fluidity of the membrane, affect the functional properties of the membrane in a favorable manner.

Conclusion

The fragility of RBC membrane increases with the feeding of oil sources rich in unsaturated fatty acids. This results indicates the fact that dietary unsaturated fatty acids increase the membrane fluidity, thereby reducing the ability to withstand the osmotic challenges exerted by solutions of varying concentrations of NaCl.

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