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Influence of dietary partially hydrogenated fat high in *trans* fatty acids on lipid composition and function of intestinal brush border membrane in rats

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Abstract

The effect of dietary hydrogenated fat (Indian vanaspati) high in *trans* fatty acids (6 en%) on lipid composition, fluidity and function of rat intestinal brush border membrane was studied at 2 and 8 en% of linoleic acid. Three groups of weanling rats were fed rice-pulse based diet containing 10% fat over a ten week period: Group I (groundnut oil), Group II (vanaspati), Group III (vanaspati + safflower oil). The functionality of the brush border membrane was assessed by the activity of membrane bound enzymes and transport of D-glucose and L-leucine. The levels of total cholesterol and phospholipids were similar in all groups. The data on fatty acid composition of membrane phospholipids showed that, at 2 en% of linoleic acid in the diet, *trans* fatty acids lowered arachidonic acid and increased linoleic acid contents indicating altered polyunsaturated fatty acid metabolism. Alkaline phosphatase activity was increased while the activities of sucrase, γ-glutamyl transpeptidase and transport of D-glucose and L-leucine were not altered by dietary *trans* fatty acids. However at higher intake of linoleic acid in the diet, *trans* fatty acids have no effect on polyunsaturated fatty acid composition and alkaline phosphatase activity of intestinal brush border membrane. These data suggest that feeding dietary fat high in *trans* fatty acids is associated with alteration in intestinal brush border membrane polyunsaturated fatty acid composition and alkaline phosphatase activity only when the dietary linoleic acid is low.

Keywords: Intestine; Brush border membrane; *trans* fatty acids; Lipid composition; Membrane function; Rats

1. Introduction

A number of studies have demonstrated that dietary lipids can influence the lipid composition and functional activities of the biological membrane [1,2]. *Trans* fatty acids are formed during partial hydrogenation of vegetable oils and form part of the human diet. Substitution of *trans* fatty acids for *cis* fatty acids in diet of animals has been known to alter membrane fatty acid composition, membrane fluidity and functional properties like enzyme activities and receptor function [3–5]. Moreover *trans* fatty acids have been shown to influence polyunsaturated fatty acid (PUFA) metabolism resulting in decreased formation of eicosanoid precursors [6,7].

The cells lining the small intestine have well defined brush border and basolateral membrane. The intestinal brush border membrane (BBM) is rich in large number of functional proteins including bound enzymes and transport system that participate in digestion and absorption of nutrients. Indian vanaspati (hydrogenated vegetable oil) contains high level of *trans* fatty acids (~25%). In recent years due to increased intake of bakery products and fast foods which are usually prepared in vanaspati, there has been an increase in the intake of *trans* fatty acids. Modification of lipid composition of intestinal BBM by dietary fat are known to alter its functional properties [8–10]. The objective of the present study was to investigate the effect of dietary *trans* fatty acids present in Indian vanaspati on lipid composition, fluidity, enzyme activities and transport of nutrients of intestinal BBM at different levels linoleic acid (18:2n-6).

2. Materials and methods

TRIS, HEPES and DPH were purchased from Sigma Chemical Company USA. ^{14}C glucose was obtained from

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BARC, Bombay, India. H^3L-Leucine was obtained from Dupont, USA. All other chemicals used were of analytical grade.

Twenty four male weanling Wistar NIN rats were divided equally into three groups. The rats were housed individually in an animal house maintained at 23°C with 12 h light and dark cycles. Indian vanaspati was used as the source for *trans* fatty acids. Each group was fed rice-pulse based diet containing 10% fat of either groundnut oil (Group I), vanaspati (Group II) or a combination of vanaspati and safflower oil to equalize 18:2n-6 content to that of groundnut oil (Group III). The diet composition (g/100 g diet) is as follows: rice - 69; pulse - 13, casein - 2.9, fat - 10, vitamin mix - 1, salt mix - 4, and choline chloride - 0.1. The mineral and vitamin mixtures were prepared according to the AIN-93 [11]. The rats had free access to water and food. The food intake of individual rats were recorded daily. Body weight of the rats was recorded weekly.

After ten weeks on experimental diet animals were sacrificed by decapitation after overnight fasting. The proximal small intestine was excised and rinsed with the cold saline. BBM vesicles were prepared from mucosal scraping by calcium precipitation technique [12]. The purity of BBM relative to the homogenate was assessed by estimating the activity of marker enzyme alkaline phosphatase [13]. Protein was determined by the method of Lowry et al using bovine serum albumin as a standard [14]. Activities of sucrase and γ-glutamyltranspeptidase were estimated by established procedures [15,16].

Total lipids from BBM were extracted according to the method of Bligh and Dyer [17]. Cholesterol and total phospholipids were assayed according to the methods of Zak et al. [18] and Bartlett [19] respectively. Total lipids were subjected to TLC on silica gel G plates and developed using petroleum ether: diethylether: acetic acid (87:17:0.5, by vol). The fatty acid profile of the membrane phospholipids and diets were determined after methylation. Fatty acid methyl esters (FAME) were analyzed by GC using a SP-2330 capillary column (30 m × 0.32 mm i.d, Supelco, USA) in a Perkin Elmer gas chromatograph equipped with flame ionization detector [21]. For better resolution and separation of *trans* isomers, SP-2560 capillary column (100 m × 0.32 mm i.d, Supelco, USA) was used. The initial oven temperature of 120°C was held for 1 min and raised to 160°C at 10°C/min and then raised to 220°C at 1.5°C/min. The injector and detector temperatures were maintained at 235°C. Individual fatty acids were identified using authentic standards (NuChek, USA). Heptadecanoic acid was used as internal standard. Steady state fluorescence polarization with 1,6-diphenyl-1,3,5-hexatriene (DPH) was used to estimate relative membrane fluidity [22]. Sodium dependent D-glucose and L-leucine uptake was done by rapid filtration technique using millipore filter of 0.45 μm pore size [20].

Table 1
Fatty acid composition of diet: (g/100 g diet)

Fatty acid[a]	Group I	Group II	Group III
Total SFA	1.6	2.8	2.0
Total MUFA	4.7	5.7	4.6
18:1 cis	4.5	3.1	2.3
18:1 *trans*	—	2.6 (6 en%)	2.1 (6 en%)
Total PUFA	3.5 (8 en%)	1.0 (2 en%)	3.3 (8 en%)
18:2 n-6	3.3	0.9	3.2

[a] SFA, saturated fatty acids, MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

2.1. Statistical analysis

Results are expressed as means ± standard deviation (n = 8). Statistical analysis of the data was done by one way ANOVA. A *p*-value of less than 0.05 was considered significant.

3. Results

The fatty acid composition of the diet is summarized in Table 1. In group I and III, 18:2n-6 furnished 8% of total fat calories (energy %) and in group II it was 2 en%. The 18:1 *trans* was the major geometric isomer present in Indian vanaspati and accounted for 6 en%.

There was no significant difference in either food intake or body weight gain among three groups during the 10 week feeding period (data not shown). The isolated BBM showed about a ten fold enrichment of the marker enzyme, alkaline phosphatase as compared to the homogenate. The marker enzyme enrichment was almost identical in all three groups. BBM total cholesterol, total phospholipid and membrane fluidity were similar in all the groups (Table 2). The phospholipid fatty acid composition of the BBM is shown in Table 3. Feeding *trans* fatty acids (Group II) led to the incorporation of 18:1 *trans* in BBM phospholipids, increased 18:2n-6, decreased 20:4n-6 and 20:4n-6/18:2n-6 ratio. Equalizing 18:2 n-6 content of the diet (Group III) to that of group I diminished the incorporation of 18:1 *trans*,

Table 2
Brush border membrane lipid composition and fluorescence anisotropy of rats fed different dietary fats

	Group I	Group II	Group III
Cholesterol[a]	147 ± 12	141 ± 12	143 ± 10
Phospholipid[a]	223 ± 12	240 ± 10	230 ± 15
Cholesterol/ phospholipid ratio	0.66 ± 0.06	0.59 ± 0.06	0.62 ± 0.03
Fluorescence anisotropy of DPH	0.249 ± 0.010	0.253 ± 0.014	0.251 ± 0.007

Values are Mean ± SD/n-8.
[a], nmole/mg protein.

Table 3
Fatty acid composition (% total) of brush border membrane phospholipids of rats fed different fats

	Group I	Group II	Group III
16:0	25.5 ± 2.7	27.4 ± 2.9	28.6 ± 1.4
16:1	1.7 ± 0.5	1.6 ± 0.5	2.0 ± 0.3
18:0	30.2 ± 3.3	28.3 ± 3.4	28.2 ± 2.1
18:1 *cis*	12.4 ± 2.3	10.1 ± 1.3*	10.3 ± 1.3*
18:1 *trans*	—	1.9 ± 0.28	—
18:2 n-6	15.5 ± 1.6	18.5 ± 1.3**	16.1 ± 1.9
20:1	2.6 ± 0.6	1.5 ± 0.4	2.3 ± 0.3
20:4 n-6	12.0 ± 1.1	10.4 ± 0.4**	11.9 ± 1.2
20:4 n-6/18:2 n-6	0.77 ± 0.01	0.56** ± 0.03	0.72 ± 0.01
Saturated	55.7 ± 2.1	55.7 ± 2.4	56.8 ± 1.4
Monoene	16.7 ± 1.4	15.1 ± 1.2*	14.6 ± 1.0*
Polyene	27.5 ± 1.1	28.9 ± 1.5	28.0 ± 2.1
Double bond index‖	0.96 ± 0.02	0.94 ± 0.04	0.94 ± 0.03

Values are Mean ± SD/n-8.

Values with superscript * are significantly different, $p < 0.05$ compared to group I.

Values with superscript ** are significantly different, $p < 0.05$ compared to group I and group III.

‖ Double bond index is the sum of each unsaturated fatty acid chain multiplied by number of double bonds/100.

increased 20:4n-6 and 20:4n-6/18:2n-6 ratio. The double bond index of the total fatty acids in the membrane phospholipids was not significantly different among the three groups.

Among BBM enzymes, the activity of alkaline phosphatase was increased by *trans* fatty acid feeding compared to groundnut oil feeding (Table 4). However equalizing 18:2 n-6 content of the diet (Group III) to that of group I lowered alkaline phosphatase activity.

Uptake of D-glucose and L-leucine across BBM in the presence of sodium gradient is shown in Fig. 1. D-glucose and L-leucine uptake showed an overshoot phenomenon with a peak uptake at 20 sec. There was no significant change in transport of D-glucose and L-leucine among three groups.

Table 4
Brush border membrane enzyme activities of rats fed different dietary fats.

Enzyme (μ mole/min/mg protein)	Group I	Group II	Group III
Alkaline phosphatase	2.92 ± 0.19	3.96 ± 0.31*	2.85 ± 0.14
Sucrase	0.69 ± 0.11	0.63 ± 0.07	0.68 ± 0.06
γ-glutamyltranspeptidase	0.36 ± 0.02	0.39 ± 0.04	0.35 ± 0.07

Data are Mean ± SD/n-8.

Values with superscript * are significantly different, $p < 0.05$ compared to group I and group III.

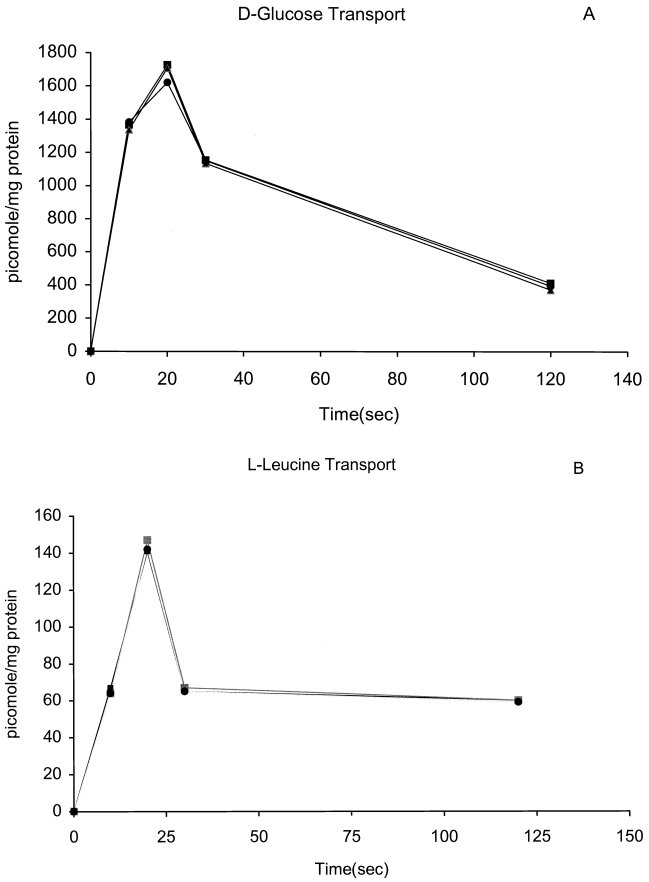

Fig. 1. Effect of dietary fats on sodium dependent D-Glucose (A) and L-Leucine (B) transport of intestinal BBM of rats. Diet groups were: Groundnut oil (. . . . .), Indian vanaspati (——) and Vanspathi + safflower oil (. . . . .). Values are mean + SD.

4. Discussion

Several studies have demonstrated that dietary fat manipulation markedly affects intestinal BBM lipid composition which in turn alters fluidity, membrane bound enzyme activities and transport of nutrients [8–10,23]. The present study was conducted to evaluate the effects of *trans* fatty acids from vanaspati on structural and functional properties of intestinal BBM. Since dietary *trans* fatty acids increase 20:3 n-9 (biochemical marker of essential fatty acid deficiency) and aggravates the symptoms of essential fatty acid deficiency [24], the study was done at two levels of 18:2 n-6 (2 and 8 en%). The 18:2n-6 content of vanaspati is low (0.9 en%) and its use in a semisynthetic diet would produce essential fatty acid deficiency. The rice-pulse based diet provides ~1.1 en% 18:2 n-6 which is biologically available [25] and therefore this study was done with rice-pulse based diets. The observation that 20:3 n-9 was not detected in BBM further suggests that 2 en% 18:2n-6 (rice and pulse 1.1 en% and vanaspati 0.9 en%) meets the 18:2n-6 requirement. The present study shows that feeding 6 en% *trans* fatty acids to rats alter intestinal BBM PUFA composition

when the dietary 18:2 n-6 levels are low (2 en%). The observed lower 20:4 (n-6)/18:2 (n-6) ratio suggests that *trans* fatty acids inhibit the conversion of 18:2 n-6 to 20:4 n-6. Several animal studies have shown that *trans* fatty acids inhibit the desaturase activity resulting in impaired conversion of 18:2n-6 to the biologically important metabolite arachidonic acid (20:4 n-6) [27–30]. Despite alterations in the BBM PUFA composition, fluidity was not altered. This could be because the major determinants of membrane fluidity [31] namely the degree of unsaturation and the molar ratio of cholesterol to phospholipids were not altered. At higher level of 18:2n-6 in the diet, *trans* fatty acids did not alter the intestinal BBM PUFA composition. Our findings agree with other reports which show that, *trans* fatty acids may not alter PUFA metabolism if adequate 18:2n-6 is provided in the diet [6,26].

Intestinal BBM proteins are classified structurally and functionally as intrinsic and extrinsic to the membrane [32, 33]. The extrinsic enzymes (disaccharidases, leucine aminopeptidase and γ glutamyl transpeptidase) are not influenced by membrane lipids. However, the activities of intrinsic proteins (alkaline phosphatase and glucose and aminoacid transporter) can be altered by changes in membrane lipid composition. In the present study despite changes in BBM PUFA composition, the activities of extrinsic (sucrase, γ-glutamyltranspeptides) and intrinsic proteins (D-glucose and L-leucine) except alkaline phosphatase, were not altered. Compared to other BBM proteins, alkaline phosphatase activity is more sensitive to changes in membrane lipid composition [32]. The observed increase in alkaline phosphatase activity was not due to change in membrane fluidity. Studies have shown that alteration in lipid composition of BBM may be more important in the regulation of alkaline phosphatase activity than membrane fluidity per se [10,34,35]. The increase in alkaline phosphatase activity without alteration in membrane fluidity could be due to possible compositional change in the lipid microenvironment in the vicinity of the enzyme due to the dietary *trans* fatty acids. Such changes could alter the lipid fluidity in these microdomaines rather than the overall fluidity thereby altering the activity of alkaline phosphatase. The stimulatory effect of trans fatty acids on alkaline phosphatase activity was diminished at high intake of 18:2 n-6 in the diet. The requirement of 18:2n-6 to prevent the undesirable effect of high amounts of dietary *trans* fatty acid was investigated by Zevenbergen et al [6] and found that 2 en% of 18:2n-6 was sufficient to prevent the undesirable effect of high *trans* fatty acids on liver mitochondrial PUFA composition and function in rats. In the present study we observed that 2 en% of 18:2n-6 was not sufficient to prevent the alterations in intestinal BBM PUFA composition and alkaline phosphatase activity. It is likely that intestinal BBM is more sensitive to lipid modification because of fast turnover of intestinal epithelial cells.

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References

[1] Murphy, M.G, Dietary Fatty Acids and Membrane Protein Function, J Nutr Biochem, 1, 68–79 (1990).
[2] Stubbs, C.D. and Smith, A.D, The Modification of Mammalian Membrane Polyunsaturated Fatty Acids Composition in Relation to Membrane Fluidity, Biochim Biophys Acta, 779, 89–137 (1984).
[3] Alam, S.G., Ren, Y.F. and Alam, B.S, Effect of Dietary *Trans* Fatty Acids on Some Membrane Associated Enzymes and Receptors in Rat Heart, Lipids, 24, 39–44 (1989).
[4] Ren, Y.F., Alam, S.Q., Alam, B.S. and Keifer, L.M, Adenylate Cyclase and β-Receptors in Salivary Glands of Rats Fed Diets Containing *Trans* Fatty Acids, Lipids, 23, 304–308 (1988).
[5] Barnard, D.E., Sampugna, J., Berlin, E., Bhathena, S.J. and Knapka, J.J, Dietary *Trans* Fatty Acids Modulate Erythrocyte Membrane Fatty Acyl Composition and Insulin Binding in Monkeys, J Nutr Biochem, 1, 190–195 (1989).
[6] Zevenbergen, J.L. and Haddenman, E, Lack of Effect of *Trans* Fatty Acids on Eicosanoid Biosynthesis with Adequate Intake of Linoleic Acid, Lipids, 24, 555–563 (1989).
[7] Bysted, A., Holmer, G. and Lund, P, Influence of Moderate Amounts of *Trans* Fatty Acids on the Formation of Polyunsaturated Fatty Acids, J Am Oil Chem Soc, 75, 225–234 (1998).
[8] Brasitus, T.A., Dudeja, P.K., Bolt, J.G., Sitrin, M.D. and Baum, C, Dietary Triacylglycerol Modulates Sodium Dependent D-Glucose Transport, Fluidity and Fatty Acid Composition of Rat Small Intestinal Brush Border Membrane, Biochim Biophys Acta, 979, 177–186 (1989).
[9] Kaur, M., Kaur, J., Ojha, S. and Mahmood, A, Dietary Fat Effects on Brush Border Membrane Composition and Enzyme Activities in Rat Intestine, Ann Nutr Metab, 40, 269–276 (1996).
[10] Wahnon, R., Cogan, U. and Mokady, S, Dietary Fish Oil Modulates the Alkaline Phosphatase Activity and Not the Fluidity of Rat Intestinal Microvillus Membrane, J Nutr, 122, 1077–1084 (1992).
[11] Reeves, P.G., Nielsen, F.H. and Fahey, G.C, AIN-93 Purified Diets for Laboratory Rodents, J Nutr, 123, 1939–1951 (1993).
[12] Kessler, M., Acuto, O, Storelli, C., Murer, H, Miller, M., and Semenza, G, A Modified Procedure for the Rapid Preparation of Efficiently Transporting Vesicles from Small Intestinal Brush Border Membrane: Their Use in Investigating Some Properties of D-Glucose and Choline Transport Systems, Biochim Biophys Acta, 506, 136–154 (1978).
[13] Thambidorai, D. and Bachhawat, B.K, Purification and Properties of Brain Alkaline Phosphatase, J Neurochem, 29, 503–512 (1977).
[14] Lowry, O.H., Rosebrough, W.J., Farr, A.L. and Randall, R.J, Protein Measurement with the Folin Phenol Reagent, J Biol Chem, 193, 265–275 (1951).
[15] Dahlqvist, A, Assay of Intestinal Disaccharidases, Anal Biochem, 22, 99–107 (1968).
[16] Rosalki, S.B. and Tarlow, D, Optimized Determination of γ-Glutamyltransferase by Reaction-Rate Analysis, Clin Chem, 20, 1121–1124 (1974).
[17] Bligh, E.G. and Dyer, W.J, A Rapid Method of Total Lipid Extraction and Purification, Can J Biochem Physiol, 37, 911–917 (1959).
[18] Zak, B., Moss, N., Boyle, A.J. and Zlatkis, A, Reactions of Certain Unsaturated Steroids with Acid Iron, Anal Chem, 26, 776–777 (1954).
[19] Bartlett, G.R, Phosphorus Assay in Column Chromatography, J Biol Chem, 234, 466–468 (1959).

[20] Tiruppathi, C., Miyamoto, Y., Ganapathy, V. and Leibach, F.H, Fatty Acid-Induced Alterations in Transport Systems of the Small Intestinal Brush Border Membrane, Biochem Pharmacol, 37, 1399–1405 (1988).
[21] Ghafoorunissa, Reddy, V. and Sesikaran, B, Palmolein and Groundnut Oil have Comparable Effects on Blood Lipids and Platelet Aggregation in Healthy Indian Subjects, Lipids, 30, 1163–1169 (1995).
[22] Schachter, D. and Shinitzky, M, Fluorescence Polarization Studies of Rat Intestinal Microvillus Membranes, J Clin Invest, 59, 536–547 (1977).
[23] Brasitus, T.A., Davidson, N.O. and Schachter, D, Variations of Dietary Triacylglycerol Saturation Alter the Lipid Composition and Fluidity of Rat Intestinal Plasma Membranes, Biochim Biophys Acta, 812, 460–472 (1985).
[24] Privett, O.S., Phillips, F., Shimasaki, T., Nozawa, T. and Nickel, E, Studies of Effects of *Trans*-Fatty Acids in the Diet on Lipid Metabolism in Essential Fatty Acid Deficient Rats, Am J Clin Nutr, 30, 1009–1017 (1977).
[25] Ghafoorunissa, Availability of Linoleic Acid from Cereal-Pulse Diets, Lipids, 25, 763–766 (1990).
[26] Zevenbergen, J.L., Houtsmuller U.M.T. and Gotrenbos, J.J, Linoleic Acid Requirement of Rats Fed, Trans Fatty Acids, Lipids, 23, 178–186 (1988).
[27] Anderson, R.L., Fullmer, C.S. and Hollenbach, E.J, Effects of *Trans* Isomers of Linoleic Acid on the Metabolism of Linoleic Acid in Rats, J Nutr, 105, 393–406 (1975).
[28] Lawson, L.D., Hill, E.G. and Holman, R.T, Suppression of Arachidonic Acid in Lipids of Rat Tissues by Dietary Mixed Isomeric *Cis* and, Trans Octadecenoates, J Nutr, 113, 1827–1835 (1983).
[29] Mahfouz, M.M., Smith, T.L. and Kummerow, F.A, Effects of Dietary Fats on Desaturase Activities and the Biosynthesis of Fatty Acids in Rat-Liver Microsomes, Lipids, 19, 214–222 (1984).
[30] Rosenthal, M.D. and Doloresco, M.A, The Effects of *Trans* Fatty Acids on Fattyacyl Delta 5 Desaturation by Human Skin Fibroblasts, Lipids, 19, 869–874 (1984).
[31] Schachter, D, Fluidity and Function of Hepatocyte Plasma Membranes, Hepatology, 4, 140–151 (1984).
[32] Brasitus, T.A., Schachter, D, and Mamouneas, T, Functional Interactions of Lipids and Proteins in Rat Intestinal Microvillus Membranes, Biochemistry, 18, 4136–4144 (1979).
[33] Brasitus, T.A. and Schachter, D, Lipid Dynamics and Lipid-Protein Interactions in Rat Enterocyte Basolateral and Microvillus Membranes, Biochemistry, 19, 2763–2769 (1980).
[34] Brasitus, T.A., Dahija, R., Dudeja, P.K. and Birronnette, B.M, Cholesterol Modulates Alkaline Phosphatase Activity of Rat Intestinal Microvillus Membranes, J Biol Chem, 263, 8592–8596 (1988).
[35] Stenson, W.F., Seetharam, B., Talkad, V., Pickett, W., Dudgeja, P, and Brasitus, T.A, Effects of Dietary Fish Oil Supplementation on Membrane Fluidity and Enzyme Activity In Rat Small Intestine, Biochem J, 263, 41–45 (1989).