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Fenoterol Alters Lipid Composition and Fatty Acids Profile Of Small Intestine In Swiss Albino Male Mice

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ABSTRACT

The goal of this study was to evaluate the influence of fenoterol a beta agonist on lipid composition and fatty acid profile in the small intestinal mucosa of swiss albino male mice. Fenoterol was orally administered with 1.5mg/kg body wt. for 28 days. Fenoterol treated mice had significantly lower conc. of cholesterol, phospholipid and triglycerides in the duodenum, jejunum and ileum compared with normal. Fatty acid composition of the intestinal mucosa was severely affected by fenoterol treatment. Increase in the relative percentage of long chain poly unsaturated fatty acids in drug treated mice paralleled to lower fatty acids proportions in normal mice duodenum, jejunum and ileum. The structure of small intestine was severely affected as assessed by histopathological studies. Our results suggest that oral administration of fenoterol at 28 days stage dramatically modifies intestinal membrane lipid composition. Changes in the lipid composition of the small intestinal mucosa and in phospholipid distribution as well as in the fatty acid profile may alter membrane fluidity and organization. These alterations appear to affect the activity of membrane-bound hydrolytic enzymes.

INTRODUCTION

Gastrointestinal tract undergo biochemical, ultrastructural and morphological changes with age, leading to development of mature mucosa (Henning.,1999). Changes in the mucosal lipid composition have been shown to occur during differentiation and migration of cells from crypt's base to the villus tip in the small intestine .The major compositional changes in the small intestine are increased phospholipid , cholesterol content along the crypt villus axis. These modifications, together with the changes in the distribution of phospholipid species and fatty acid composition, are associated with reduction in plasma membrane fluidity and decreased luminal membrane permeability to macromolecules. Fatty acids binding proteins are expressed in mammalian enterocytes. Most modern studies of the fat content of the intestinal mucosa during fat absorption have been concerned with the changes in the quantity and quality of the phospholipids. Though, lipids are important structural and functional components of muscles, they have been ignored as far as the effects of beta- agonists on the lipid status in muscles are concerned. As is clear from the previous findings, β-agonists may have therapeutic potential for pathologies where muscle wasting is indicated, such as cancer, muscular dystrophy and age related muscle wasting. But before these drugs can be used as legitimate therapies, some safety concerns especially, their side effects on muscles and vital organs like intestine need to be considered. Although several investigators have tried to highlight the side effects of many β-adrenergic agonists on skeletal muscles and vital organs but still lesser is known about the effect of fenoterol which is a full agonist of β_1 and β_2- adrenoceptors (Bremner *et al*., 1996) on the important vital organ small intestine. Fenoterol, may directly affect the epithelial cells counteracting any villus alterations or degeneration of fibers. Based upon anabolic properties, ß-agonists have been proposed as valuable adjunct to the treatment of muscle wasting conditions. The potential clinical benefit of treatment by this agonist is to reduce loss of mass and forces in atrophied muscles. Fenoterol treatment causes a time and concentration dependent development of constitutive β_2- adrenoceptor activity, which can be reversed by various antagonists. Beta - agonist induced changes could represent a novel regulation mechanism of β_2 - adrenoceptor activity. Alterations in lipds and fatty acids levels were studied from 7-28 days in normal and drug treated mice. IN recent years several workers have studied quantitative methods the various metabolites and enzymes of the fat body of the mice. However, no information is available on in this tissue. Here, we report the results of a biochemical study under taken to demonstrate lipid with fatty acids in the fat body of the mice. We interpret the decrease in phospholipid and increase in fatty acid activity as an adaptation of the enteric mucosa to maintain the absorptive function of small intestine.

MATERIALS AND METHODS

Adult Swiss albino male mice of Balb- C strain weighing 25-30g were procured from Central Research Institute (CRI), Kasauli, Himachal Pradesh. They were housed in polypropylene cages under controlled conditions of temperature and light (24 ± 20C; 16 hr day light) and fed upon Hindustan lever pellet diet and water *ad libitum*. All experimental procedures were conducted after the approval of Institutional Animal ethics committee, Himachal Pradesh University (IAEC /BIO/4-2006), Shimla. Mice were randomly assigned into two independent groups: One group containing normal mice served as control and the other group included mice as treated groups. Animals of second group were given daily oral administration of fenoterol (1.5 mg/ kg body wt) for 28 days).

a) **Phospholipids**: Ammonium molybdate method of Ames (1966) was undertaken for the quantitative estimation of phospholipids. 2 ml. of lipid extract was taken in each test tube and evaporated to dryness at 70^0C in an oven. 0.2 ml of 10% Mg $(NO_3)_2$. $6H_2O$ was added and heated on flame till the brown fumes disappeared and then cooled to room temperature. 1.5 ml of 0.5N HCl was added to each tube and was kept in preheated oven at 95^0C for 15 minutes to hydrolyse any pyrophosphates. Test tubes were cooled and 4 ml. of colour developing reagent was added to each test tube. These were further incubated at 60^0C for 30 minutes and absorbance was noted at 800 nm. Standard calibration curve was drawn using various concentrations of KH_2PO_4.
b) **Free fatty acids:** It was done according to the method of Itaya (1977)**.** 40µl of homogenate was mixed with 0.7 ml of 0.89 % NaCl solution and centrifuged at 3000 rpm for 5-10 minutes. Supernatant so obtained was taken in test tube containing 4 ml. of choloroform and 0.5 ml. of 0.5M phosphate buffer (pH 6-7). It was shaken and then allowed to stand for 15- 30 minutes. Upper layer was then pipetted out with the help of fine tipped pipette. To the residual layer, 2 ml of copper reagent was added. Solution was thoroughly mixed and absorbance was noted at 550 nm. Standard calibration curve was drawn using various concentrations of palmitic acid.

RESULTS

PHOSPHOLIPIDS (Table and Fig-I)

Several authors have suggested that the phospholipids may participate in the absorption of fats. Mucosal phospholipids rapidly take up free fatty acids from the lumen of the small intestine. Alterations in the phospholipid level in small intestine were studied from 7 to 28 days in normal and drug treated mice.

Duodenum

Phospholipids concentration in control mice duodenum was found to be 0.27 ± 0.01 mg/g of fresh tissue weight. Phospholipids were estimated to be 0.24 ± 0.01 mg/g of fresh tissue weight after 7 days of drug treatment whereas after 14 days of fenoterol administration the phospholipids were found to be 0.15 ± 0.09 mg/g of fresh tissue weight. Further decline in the phospholipid level in the duodenum part of small intestine was found to be 0.10 ± 0.08 mg/g of fresh tissue weight after 21 days, the phospholipids were found to be 0.04 ± 0.02 mg/g of fresh tissue weight after 28 days of the fenoterol administration.

Jejunum

Control jejunum mice phospholipids were 0.62 ± 0.02 mg/g of fresh tissue weight at 7 days stage. Phospholipids declined to 0.55 ± 0.02 mg/g of fresh tissue weight after 7 days of drug administration. Similar trend was observed at 14 days and 21 days stages after fenoterol treatment. At 14 days stage the phospholipids were found to be 0.42 ± 0.01 mg/g of fresh tissue weight and at 21 days stage, it further declined to 0.32 ± 0.02 mg/g of fresh tissue weight. After drug treatment at 28 days stage the phospholipids were 0.22 ± 0.01 mg/g of fresh tissue weight. Regular decrease in the phospholipid concentration was observed from 7 to 28 days of fenoterol administration.

Ileum

Ileum is the last part of small intestine and the level of phospholipids were observed from 7 to 28 days stage in control and drug treated mice. In the normal mice, the ileum has 0.89 ± 0.01 mg/g of fresh tissue weight phospholipid concentration. After 7 days of fenoterol administration the phospholipids decreased to the value 0.72 ± 0.008 mg/g of fresh tissue weight whereas at 14 days and 21 days stages the level of phospholipids were further decreased to 0.68 ± 0.006 mg/g and 0.56 ± 0.002 mg/g of fresh tissue weight respectively. At 28 days stage, the phospholipids were recorded to be 0.27 ± 0.001 mg/g of fresh tissue weight. The values are statistically significant at all the stages in treated mice in comparision to control ($P^* < 0.05$).

FATTY ACIDS (Table and Fig-II)

Duodenum

A lot of interest has been focused on the uptake of fatty acids in to the mucosal cells as well as on their esterification to complex lipids and further transport. Information concerning oxidation and remodeling of fatty acids within this organ is, however, sparse. Fatty acids were found to be 1.33 ± 0.03 mg/g of fresh tissue weight in control mice duodenum. After fenoterol treatment at 7 days stage, the concentration of fatty acids increases from the normal value to 2.13 ± 0.05 mg/g of fresh tissue weight. Further increase in the level of fatty acids (2.43 ± 0.08 mg/g of fresh tissue weight was observed at 14 days stage after drug treatment. At 21 days stage, further increase in the level of fatty acid and was found to be 3.86 ± 0.10 mg/g of fresh tissue wt. After drug treatment at 28 days stage, the fatty acids get increased to 4.41 ± 0.12 mg/g of fresh tissue weight.

Jejunum

Fatty acids in control jejunum were found to be 0.973 ± 0.004 mg/g of fresh tissue wt. After fenoterol treatment at 7 days stage the level of fatty acids increased from the normal value to 2.45 ± 0.02 mg/g of fresh tissue weight. After drug treatment at 14 days stage, the fatty acids further increased to 3.08 ± 0.12 mg/g of fresh tissue weight. At 21 and 28 days stages of drug treatment, the fatty acids concentration reached to 3.73 ± 0.20 and 4.25 ± 0.24 mg/g of fresh tissue weight respectively. Overall considerable increase in the level of fatty acids takes place from 7 to 28 days of drug treatment.

Ileum

The fatty acids were found to be 1.673 ± 0.01 mg/g of fresh tissue weight in control ileum. After drug treatment at 7 days, the level get increased to 2.52 ± 0.02 mg/g of fresh tissue weight. Slight increase was observed at 14 days stage after fenoterol treatment and was found to be 2.89 ± 0.06 mg/g of fresh tissue weight. At 21 days stage, its level get further increased to 3.87 ± 0.14 mg/g of fresh tissue weight and reached to 4.53 ± 0.16 mg/g of fresh tissue weight at 28 days stage after fenoterol administration. The values are statistically significant at all the stages in treated mice comparison to control (P*<0.05).

Table and Fig. I

		Days			
Tissues		7	14	21	28
Duodenum	**Normal**	0.27 ± 0.01	0.26 ± 0.02	0.27 ± 0.01	0.28 ± 0.02
	Treated	0.24 ± 0.01*	0.15 ± 0.09	0.10 ± 0.08*	0.04 ± 0.02*
Jejunum	**Normal**	0.62 ± 0.02	0.61 ± 0.01	0.63 ± 0.02	0.62 ± 0.01
	Treated	0.55 ± 0.02	0.42 ± 0.01*	0.32 ± 0.02*	0.22 ± 0.01*
Ileum	**Normal**	0.89 ± 0.01	0.88 ± 0.01	0.87 ± 0.03	0.88 ± 0.02
	Treated	0.72 ± 0.08*	0.68 ± 0.06*	0.56 ± 0.002*	0.27 ± 0.001

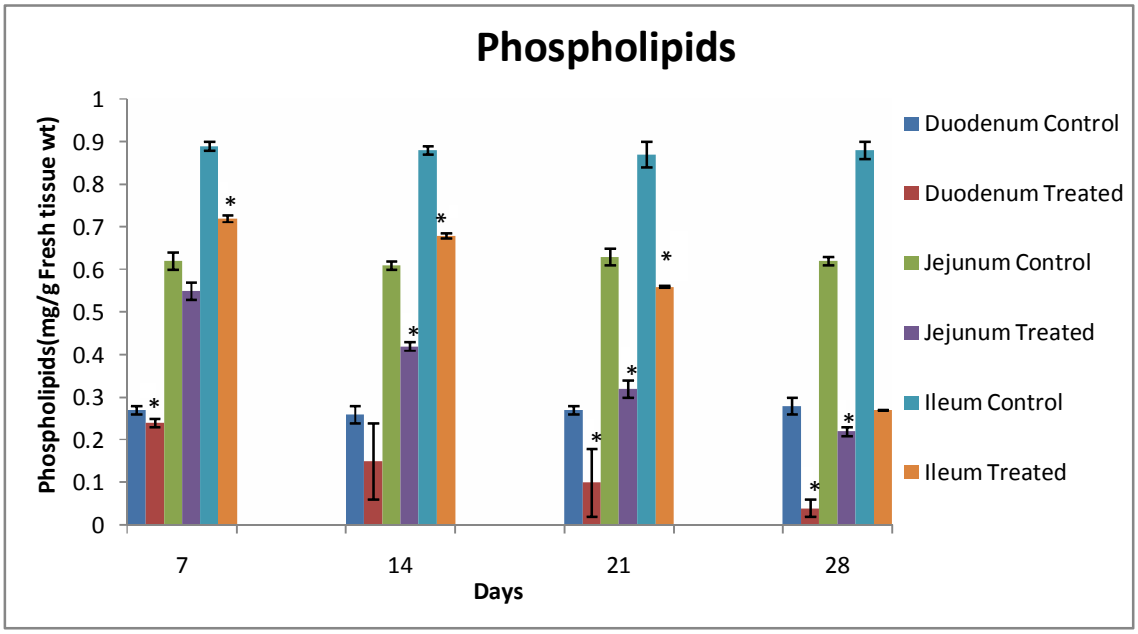

Table & Fig. VII: Phospholipids (mg/g of fresh tissue wt) in duodenum, jejunum and ileum of normal and drug treated mice during 7- 28 days period. Values are mean ± SEM; n = 6 (P*< 0.05).

Table and Fig. II

Days					
Tissues		7	14	21	28
Duodenum	**Normal**	1.33 ± 0.03	1.32 ± 0.02	1.34 ± 0.04	1.32 ± 0.02
	Treated	2.13 ± 0.05	2.43 ± 0.08*	3.86 ± 0.10*	4.41 ± 0.12*
Jejunum	**Normal**	0.97 ± 0.004	0.98 ± 0.002	0.97 ± 0.006	0.98 ± 0.004
	Treated	2.45 ± 0.02*	3.08 ± 0.12*	3.73 ± 0.20	4.25 ± 0.24*
Ileum	**Normal**	1.67 ± 0.01	1.68 ± 0.02	1.67 ± 0.02	1.67 ± 0.06
	Treated	2.52 ± 0.02*	2.89 ± 0.06*	3.87 ± 0.14*	4.53 ±0.16

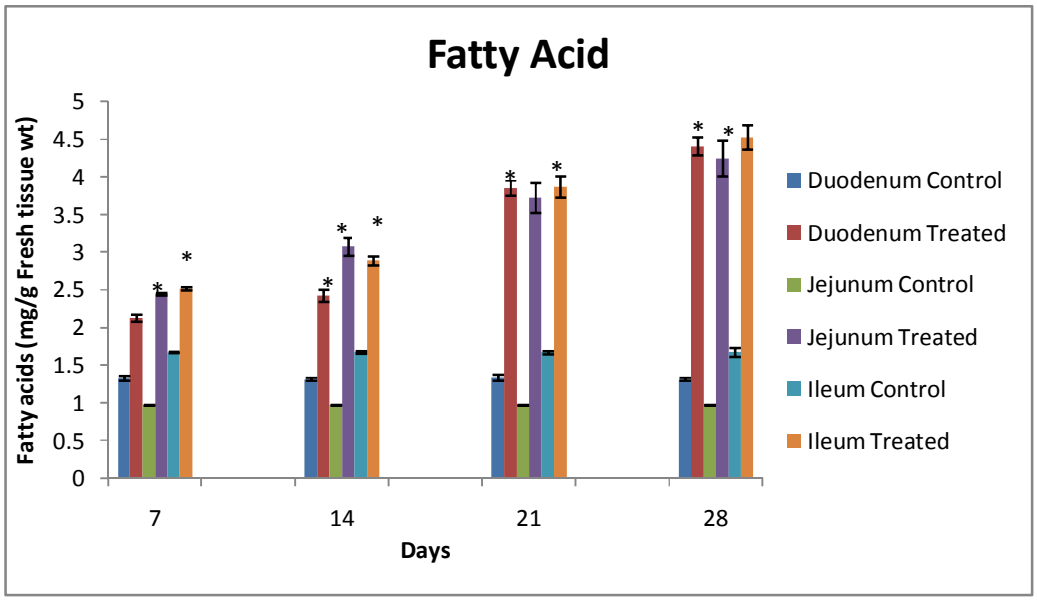

Table & Fig.VI: Fatty acid (mg/g of fresh tissue wt) in duodenum, jejunum and ileum of normal and drug treated mice during 7-28 days period. Values are mean ± SEM; n = 6 (P*< 0.05)

DISCUSSION

Phospholipids in the gut lumen may affect the membrane characteristics of enterocytes or have a direct effect on cellular cholesterol transporters thus affecting intestinal cholesterol uptake. Although there is limited data to directly support this mechanism, it is well known that the biological function of cell membranes is very dependent on component PLs and their interaction with intramembrane sterols (Quinn *et al.,* 2009).Furthermore, the activity of membrane proteins is directly affected by their interaction with membrane phospholipids (Dowhan *et al.*, 2009). In present study the phospholipid concenteration was found to be decreased in duodenum, jejunum and ileum from 7 to 28 days of investigation. The %age decrease in duodenum at 7 days stage was found to be 11.2%. Similar decrease in phospholipids concenteration was observed in the previous study of Eriyamerimu et al., 2009 and it was 24.2% in the duodenum**.** Several authors have suggested that the phospholipids may participate in the absorption of fat. The mucosal phospholipids rapidly take up the free fatty acid from the lumen of the small intestine (Raghwan *et al.,* 1965). The %age decrease at 28 days stage was found to be 57% in duodenum. In jejunum at 7 days stage the %age decrease was calculated to be 11.3%. At 21 days stage the percentage decrease was 50% and at 28 days stage it was found to be 64.6%. Phospholipids are the main component of the lipoprotein surface, and hence a reduction in the content of

intestinal phospholipids could lead to alterations of lipoprotein conformational structure and secretion. Tso *et al*., (1984) reported that the infusion of esterified fatty acids in the form of phospholipids instead of triglycerides enhances the formation and secretion of very low density lipoprotein (VLDL) into lymph as the major vehicle for transporting lipids. Feldman *et al*., (1983) demonstrated that cholesterol and triglycerides differentially affect particle size of intestinal lymph lipoproteins. With greater cholesterol absorption, more lipids were carried by VLDL; this contrasts with the preferential rise in chylomicroms in which more triglycerides were absorbed.

At 7 days stage the percentage decrease in ileum was calculated to be 20%. At 14 days stage the % age decrease was 22.4%. Incidence of developing gastroduodenal ulcers and/or gastrointestinal (GI) bleeding is significantly increased in subjects taking NSAIDs. It has been recently reported in the previous study that the ability of NSAIDs to induce GI bleeding and ulceration in rats can be reduced if the drugs are chemically associated with zwitterionic phospholipids (Kurrata *et al.,*1990). The % age decrease in the level of phospholipids in ileum part of small intestine was found to be 35.7% at 21 days stage and 69.4% at 28 days stage. This work was similar with the work of Billington *et al*., (1978**)** who found 33% phospholipids in the ileum of mouse. The decrease in phospholipid content was 69.32% at 28 days stage.

Fatty acids that are water-soluble form triglycerides in the absorptive cells and combine with cholesterol,phospholipids, and similar substances with a protein coat as chylomicrons. These pass through the lymphatic system before entering the blood stream. Absorption process gives background knowledge and a rationale for nutritional therapy for disorders associated with malabsorptive syndrome and reasons for enterostomies (tube placement for enteral feeding). Free fatty acids are approximately doubled in amount 7 days after drug treatment. The % age increase is found to be 60% in duodenum after 7 days of drug administration. It is calculated to be 84% after 14 days of fenoterol administration. % age is 188% after 21 days and 234% after drug treatment. There is regular increase in the % age of fatty acids in drug treated mice as compared to control mice. The free fatty acids also showed increased % age in jejunum of drug treated mice from 7 -28 days of investigation. At 7 days stage, the % age increase was found to be 54.4% and at 14 days stage the %age increase was 215%. The increase in the fatty acids content was found to be 282% atb21 days stage. The increase in the fatty acids content was calculated to be 335% in fenoterol treated mice at 28 days stage.This work was similar with the work of Alexander leaf, (2004**)** who observed elevated level of fatty acids (21- 39%) were observed. Free fatty acids, are highly hydrophobic, shunning the aqueous medium of body fluids. A high free fatty acid level, which is related to abdominal fat, diabetes and insulin resistance (Groop *et al*., 1993**)**, may also stimulate gluconeogenesis, reduce the action of insulin to suppress hepatic glucose production. Saloranta *et al*., (1993) worsen the glucose tolerance, and have a direct toxic effect on beta cells (Nuutila *et al*., 1992). The percentage of fatty acids at 7 days stage after drug treatment was found to be 50% in ileum. At the end of the investigation the percentage get increased to 170%. High free fatty acids may have a direct role on hypertension. No other epidemiological study has examined the role of free fatty acids on hypertension incidence but clinical studies have suggested such a direct effect and gave possible explanations for a causal relationship (Stepniakowski *et al*., 1995). Free fatty acids may increase the neurovascular tone by enhancing α_1 – adrenoreceptor sensitivity and raising sympathetic drive, and may inhibit endothelium-dependent vasodilation, as recently reviewed (Chamber *et al.,* 1992). Fenoterol stimulates lipolysis by increasing nor- adrenaline (NA) release from sympathetic nerve terminals. This increase in nor- adrenaline activates adrenergic receptors which increases cAMP levels in fat cells and muscle cells. This has the effect of increasing lipolysis in fat cells and increasing protein synthesis in muscle tissue. Luminal fatty acids are either oxidized or esterified to form triglycerides, Plasma derived free fatty acids are exclusively allowed to provide an energy source or a substrate for membrane phospholipids. It is also recognized that fenoterol induces mobilization of free fatty acids from adipose tissue resulting in an increased plasma concentration of free fatty acids.

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