Effect of n-3 Polyunsaturated Fatty Acids on the Fatty Acid Profile in Rats

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Effect of a polyunsaturated fatty acid (PUFA) concentrate containing about 80% free acids at a level of 1% of the diet, on the fatty acid profile of the lipids of liver and heart of rats fed on it for a period of three months was investigated. Low levels of dietary PUFA influence fatty acid composition of the lipids in liver and heart. The pattern of changes was different in the two organs, indicating tissue specificity to n-3 PUFA.

Key words: PUFA, feeding, lipid profile

Fish oil is rich in long chain n-3 polyunsaturated fatty acids (n-3 PUFA) which are strong hypolipidemic and antithrombotic agents (Bang *et. al.*, 1976; Dolocek and Granditis, 1991; Philipson *et. al.*, 1985; Ide *et. al.*, 1996). Flier *et. al.* (1985) reported that dietary n-3 PUFA influence rat liver plasma membrane and associated enzymes. Dietary supplementation of 20:5 n-3 (eicosapentaenoic acid, EPA) and 22:6 n-3 (docosahexaenoic acid, DHA) results in increased levels of these acids in the tissue (Swanson and Kinsella, 1986; Gudbjarnason and Oskarsdottir, 1975; Bruckner *et. al.*, 1984; Innis *et. al.*, 1995). The role of dietary fatty acids in influencing the fatty acids profile of tissue lipids and consequent alterations in metabolic processes are of great significance. Kroft *et. al.* (1964) reported that diet induced changes in fatty acid composition are dose related and the magnitude and nature of the changes vary according to the type of PUFA supplement and the type of tissues. Most of the above studies were made using fish oils at levels of 5% of the diet or more. Effect of a fatty acid concentrate containing EPA and DHA enriched diet on the fatty acid composition of heart and liver lipids of rats was studied.

Materials and Methods

Six-week-old male albino rats (Wistar strain) weighing about 100g each were used in the study. The feed contained 15% casein, 4% salt mixture, 1% vitamin mixture, 5% cellulose, 1% cholesterol, 0.1% methionine and corn starch to make up to 90%. This basal diet with 10% coconut oil served as control. Experimental diet contained basal diet, 9% coconut oil and 1% PUFA concentrate containing 80% PUFA of which 60-65% was EPA and DHA. Feed and water were given *ad libitum*. After one month's feeding, five rats from control and experimental group were sacrificed and liver and heart were removed. Feeding of remaining rats was continued for two more months and the animals were sacrificed for liver and heart. Lipid was extracted using the method of Bligh and Dyer (1959). Methyl esters of fatty acids were prepared by using

boron trifluoride-methanol reagent (AOAC, 1990) and analysed by gas chromatography using 0.53 mm x 30 m CP Sil 88 column and flame ionisation detector and nitrogen as carrier gas. Identification and quantification of the fatty acids were done using Sigma/Altech standards.

Results and Discussion

Fatty acid composition of the liver and heart tissue lipids of rats fed on control and experimental diets is given in Table 1. Weight of liver was slightly lower in the PUFA fed group compared to the control group, whereas the weight of heart was same in both. Lipid content of the liver of experimental group was almost double that of the control group.

Table 1. Fatty acid composition of liver and heart lipids of rats fed on PUFA diet for 1 and 3 months

Fatty acids	Liver				Heart			
	Control		Experimental		Control		Experimental	
	1	3	1	3	1	3	1	3
C12:0	1.4	1.6	1.0	1.4	4.1	7.5	4.0	9.4
C14:0	3.8	4.9	2.3	4.1	3.8	6.2	3.7	7.3
C15:0	0.2	0.8	0.2	0.3	-	-	-	-
C16:0	21.0	22.0	20.2	16.9	17.8	16.5	17.4	18.7
C17:0	0.3	0.4	-	0.2	-	0.2	-	0.2
C18:0	6.3	4.4	7.4	4.1	10.4	9.0	5.3	7.7
Total saturated	33.3	34.0	30.9	27.1	36.2	39.3	30.4	43.2
C14:1n7	0.8	1.1	0.3	0.6	-	0.5	0.5	0.7
C16:1n7	10.5	17.0	7.4	8.9	6.5	7.6	8.4	7.0
C18:1n9	31.4	33.4	20.9	23.5	24.5	19.3	23.4	18.8
C20:1n9	0.7	-	-	-	-	-	-	-
Total monounsaturated	43.4	51.4	28.6	33.0	31.0	27.4	32.3	26.5
C18:2n6	7.4	3.5	7.3	6.1	13.7	15.6	17.4	7.8
C18:3n3	0.3	-	-	0.5	-	-	-	-
C18:4n3	0.4	-	0.4	0.9	-	-	-	0.4
C20:2n9	0.5	0.4	-	-	0.2	0.6	0.4	-
C20:4n6	10.3	5.2	9.2	4.7	12.7	13.8	10.1	6.9
C20:5n3	0.4	0.4	5.3	5.2	-	-	2.6	1.3
C22:4n3	-	-	0.3	-	-	-	0.3	-
C22:5n3	-	-	1.9	-	-	-	-	-
C22:6n3	1.6	3.2	14.6	13.9	1.6	1.0	6.0	8.0
Total PUFA	21.0	12.6	38.9	31.3	28.4	31.0	36.8	24.4
Others and unidentified	2.4	2.0	1.7	8.7	4.6	2.2	0.8	5.9

Fatty acid profile of heart and liver lipid shows that PUFA supplemented diet altered the fatty acid composition in a major way. After one month on PUFA diet, the proportion of monounsaturated acids decreased significantly while that of 20:5 n-3 and 22:6 n-3 increased in liver lipids. Concentration of n-6 fatty acids viz. 18:2 n-6 and 20:4 n-6, did not undergo appreciable change. The pattern was somewhat

similar after three months. However, a difference in the 18:2 n-6 levels was noticed in control (3.5%) and the experimental group (6.1%). Levels of 20:4 n-6 were not affected.

At the end of one month's feeding with PUFA diet, lipids 18:2 n-6 increased in the heart muscle while 20:4 n-6 decreased and levels of 20:5 n-3 and 22:6 n-3 increased. After three months, proportions of the major n-6 acids, 18:2 and 20:4, decreased by about 50% and 22:6 n-3 increased. The differences in the fatty acid composition by feeding PUFA supplemented diet show that diet-induced changes did not stabilise in one month, and continued feeding resulted in further modifications.

Bruckner *et. al.* (1984) observed that fatty acid composition of lipids of lungs, liver, aorta and platelets in rats fed on diets enriched with DHA and EPA was altered and increase in the proportions of n-3 PUFA was at the expense of arachidonic acid. Dietary n-3 PUFA competes with 20:4 n-6 for the C-2 position of phospholipids in liver, plasma and heart in rats (Iritani and Fujikawa, 1982). Levels of 20:4 n-6 were significantly reduced when 25% fish oil was included in the diet (Huang *et. al.*, 1982). They found that dietary fish oil at concentrations of 25% or higher significantly reduced the ratios of 20:4 n-6/18:2 n-6 in all the tissues examined. In the present study concentration of n-3 PUFA increased significantly both in liver and heart lipids. In liver lipids, this increase was not at the expense of the n-6 acids. PUFA of cardiac muscle are more susceptible to dietary manipulation than those of other tissues and the increased availability of 22:6 n-3 fatty acid results in increase in the concentration of this fatty acid in the phospholipids at the expense of 18:2 n-6 and 20:4 n-6 (Gudbjarnason and Oskarsdottir, 1977).

The quantity of PUFA available to the animal was about 0.6 % of the diet in the presence of a very high complement of saturated acids (Table 2). Even at this relatively low level these acids were effective in altering fatty acid composition of the lipids of liver and heart. The biological availability of n-3 PUFA from fish oil depends on the positional distribution of the fatty acids in the glyceride molecule and when free acids are included in the diet they are available to the organ sites or even the walls of vascular system in a format different from those originating from fish oils (Ackman, 1992).

Table 2. Fatty acid composition of the PUFA supplemented feed

Saturated (total)	Monounsaturated (total)	18:2 n-6	20:4 n-6	20:5 n-3	22:6 n-3	PUFA (total)
83.8	9.3	1.3	0.3	1.4	2.6	6.1

The monounsaturated acids also were affected as a result of feeding PUFA supplemented diets. After one month on PUFA diet, total monounsaturated acids decreased in the liver and the pattern remained the same even after 3 months. In the liver, 16:1 n-7 and 18:1 n-9 decreased and in heart they did not show any appreciable change. Whether or not these changes are directly related to the changes in the levels of PUFA is not clear.

It can be concluded that relatively small amounts of n-3 PUFA in the free acid form in the diet results in alterations in the fatty acid composition of the liver and heart lipids of rats. The extent to which the n-3 PUFA such as 20:5 n-3 and 22:6 n-3 get accumulated in liver suggests that either these acids are selectively absorbed and retained in the tissue or synthesized preferentially as a result of supplementation with PUFA. The increased lipid content of the liver did not affect the health of the animals.

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