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Energy metabolism in albino rat when fed dietary formulations of n-3 polyunsaturated fatty acids (PUFA)

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Consumption of fish especially that of marine, which is a rich source of n-3 polyunsaturated fatty acids (PUFA) prevent the occurrence of coronary heart diseases. Besides, n-3 PUFA are essential in growth and development of humans. Levels of ATP and ADP were more with higher significance ($P<0.001$) when compared to AMP in both liver and muscle of male and female albino rats fed dietary formulations of high n-3 PUFA (Control<Low n-3 PUFA diet<Medium n-3 PUFA diet<High n-3 PUFA diet). Energy charge (EC) represents the levels of ATP and ADP in both sexes of rats fed dietary formulations of n-3 PUFA, whereas the levels of AMP were more in rats fed low n-3 PUFA diet (Control>Low n-3 PUFA diet>Medium n-3 PUFA diet>High n-3 PUFA diet) and low energy charge is a reflection of higher level of AMP.

Key words : n-3 PUFA, Energy charge, ATP, Metabolism, Albino rat.

INTRODUCTION

The metabolism of individual polyunsaturated fatty acids (PUFA) may be influenced differently by nutritional status and nutritional intake (Nilsson *et al*., 1996). The energy liberated by catabolism is not used directly by cells but is applied instead to the formation of certain high-energy compounds, which serve as carriers of energy. Structurally these compounds are anhydride, amidine or thioester derivatives of a phosphoric acid or a carboxylic acid (Ramesh Chand, 1994). Adenosine triphosphate (ATP), Adenosine diphosphate (ADP) and Adenosine monophosphate (AMP) are the most important adenylate nucleotides in the animal body. Knowledge on fish oils especially PUFA is limited and so in the role of PUFAs in human nutrition and health, hence this also substantially contributes knowledge on fish physiology, biochemistry and aquaculture and its applied studies and ultimately to the field of medicine and health education. Besides, marine fish *Rastrelliger kanagurta* which showed higher levels of PUFA in the muscle and liver, the freshwater fishes also have PUFA in the form of alphalinolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) can also be recommended as sources of PUFA and we can include these for nutritional requirements (Nagabhushan Reddy and Basha Mohideen, 2005).

Most of the vital activities of the animal cell such as secretion or contraction, rely on the conversion of ATP-ADP phosphate transfer system and for some biosynthetic processes, the energy is derived from the hydrolysis of ADP to AMP (Hoar, 1976). As a primary energy source, these adenylate compounds serve as allosteric modulators in many important metabolic reactions. Therefore, an attempt was made in this study to estimate the energy metabolism in albino rat when fed dietary formulations of n-3 PUFA.

MATERIALS AND METHODS

The more commonly used rats and mice; they have blood lipid profiles and responses to dietary fatty acids that resemble those of humans (Fernandez, 2001). Thus, the albino rat, *Rattus norvegicus albinus* of Wistar strain was selected for the experiment.

Experimental design:

The animals were divided in to 4 groups of 12 rats each (6 males and 6 females). Males weighing 180±10g (aged about 120 days) and females weighing 160±10g (aged about 110 days) were bred in central animal house. All the rats were housed in plastic cages under controlled conditions of 12h light and 12h dark cycle, 50% humidity and at 30±2°C and were maintained in accordance with the guidelines of National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India.

These experimental rats of both the sexes were fed different formulations of dietary n-3 PUFA (300-400g) (prepared by mixing different amount of muscle of a marine fish mackerel, *Rastrelliger kanagurta* which is a rich source of n-3 PUFA with the standard pellet diet) at regular intervals of 5-6h and water was given *ad libitum* for a period of 6 weeks.

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Group-1 (Control):

Rats were given standard pellet diet (Hindustan Lever Limited, Bangalore, India)

Group-2 (Low n-3 PUFA diet):

Rats were given mixed diet (1g muscle of Rastrelliger kanagurta / 25g standard pellet diet)

Group-3 (Medium n-3 PUFA diet):

Rats were given mixed diet (3g muscle of Rastrelliger kanagurta / 25g standard pellet diet)

Group-4 (High n-3 PUFA diet):

Rats were given mixed diet (5g muscle of Rastrelliger kanagurta / 25g standard pellet diet)

*1g muscle of *Rastrelliger kanagurta* composed of proportions of n-3 PUFA (ALA: 25-50mg, EPA: 75-125mg and DHA: 150-250mg). These mixed diets were sealed and stored at -20°C until they were thawed for 2h prior to being given to the rats.

Prior to the estimation of levels of adenylate compounds like ATP, ADP and AMP the rats were anaesthetized with methane sulphonate (MS 222) in order to make the movements of the rat stop instantaneously, thereby keeping the stationary concentration of the nucleotides and were estimated by the method of Bergmeyer (1974).

Energy charge was calculated by the formula given by Atkinson and Walton (1967).

$$EC = \frac{ATP + (½\ ADP)}{ATP + ADP + AMP}$$

The experimental data was subjected to students 't'-test.

RESULTS AND DISCUSSION

Liver of males and females has more levels of ATP, ADP and EC with higher significance (P<0.001) in high n-3 PUFA fed rats and lower levels in control and with intermediate levels both in low n-3 PUFA (P<0.05) and medium n-3 PUFA (P<0.001) diet fed rats in the order: Control<Low n-3 PUFA diet<Medium n-3 PUFA diet<High n-3 PUFA diet fed rats. Muscle of both males and females has also shown similar changes as in liver but with lower levels. The variations of AMP content were quite opposite to that of ATP or ADP or EC exhibiting inverse relationship both in liver and muscle of male and female albino rats when fed dietary formulations of n-3 PUFA (Table 1 and 2).

The energy status of an animal is a reflection of the available concentration of the energy rich nucleotides like ATP, ADP and AMP (Ramesh Chand, 1994). Higher levels of ATP represents the greater energy charge and low energy charge is a reflection of higher amount of AMP, which was evident from the present study both in liver and muscle of male and female albino rats fed high n-3 PUFA diet. In this context, it is necessary to mention the SDH, a vital enzyme of TCA cycle which converts succinate to fumarate, while cytochrome oxidase (cyt a_3) is the key enzyme of terminal oxidation, reflecting the activity of mitochondrial ETS and hence ATP turnover. Plasma glucose increased as n-3 fatty acid concentrations increased (Dewailly *et al.*, 2001). Therefore, these variations in the energy rich nucleotides like ATP are ultimately correlated to the energy demands of the animal (Caldwell, 1967) was evident from the present study also.

Table 1 : Levels of ATP, ADP, AMP (µM/g wet wt. of tissue) and EC in male albino rats when fed dietary formulations of n-3 PUFA. Each Mean±S.D. is an average of six individual estimations (n=6). Level of significance is in relation to control

Sr. No.	Parameter	Males			
		Dietary formulations of n-3 PUFA			
		Control	LPD	MPD	HPD
Liver					
1.	ATP	1.15±0.047	1.23±0.051#	1.36±0.049*	1.52±0.055*
2.	ADP	0.85±0.035	0.89±0.018$	0.98±0.037@	1.10±0.039*
3.	AMP	0.31±0.011	0.29±0.009#	0.25±0.008@	0.20±0.007*
4.	EC	0.681±0.029	0.695±0.030#	0.714±0.015*	0.734±0.016*
Muscle					
1.	ATP	0.80±0.030	0.85±0.033#	0.94±0.038@	1.05±0.041*
2.	ADP	0.67±0.029	0.70±0.030$	0.76±0.032@	0.87±0.037*
3.	AMP	0.43±0.011	0.40±0.014$	0.37±0.012@	0.34±0.010*
4.	EC	0.597±0.036	0.615±0.036$	0.637±0.016@	0.657±0.020*

* denotes level of significance (P<0.001) @denotes level of significance (P<0.01)
#denotes level of significance (P<0.05) $ denotes Non-significant LPD = Low n-3 PUFA diet
MPD = Medium n-3 PUFA diet HPD = High n-3 PUFA diet

Table 2 : Levels of ATP, ADP, AMP (µM/g wet wt. of tissue) and EC in female albino rats when fed dietary formulations of n-3 PUFA. Each Mean±S.D. is an average of six individual estimations (n=6). Level of significance is in relation to control

Sr. No.	Parameter	Females			
		Dietary formulations of n-3 PUFA			
		Control	LPD	MPD	HPD
Liver					
1	ATP	1.01±0.039	1.08±0.042#	1.19±0.049*	1.33±0.052*
2	ADP	0.76±0.031	0.79±0.033$	0.86±0.036@	0.95±0.037*
3	AMP	0.36±0.012	0.35±0.011#	0.33±0.009*	0.30±0.009*
4	EC	0.652±0.028	0.664±0.028#	0.680±0.029*	0.699±0.015*
Muscle					
1	ATP	0.66±0.028	0.70±0.031$	0.77±0.030@	0.86±0.034*
2	ADP	0.58±0.026	0.60±0.027$	0.65±0.029@	0.74±0.032*
3	AMP	0.51±0.015	0.49±0.013$	0.47±0.011@	0.46±0.012*
4	EC	0.542±0.043	0.552±0.032#	0.578±0.025@	0.595±0.027*

* denotes level of significance (P<0.001)
@denotes level of significance (P<0.01)
#denotes level of significance (P<0.05)
$ denotes Non-significant
LPD = Low n-3 PUFA diet
MPD = Medium n-3 PUFA diet
HPD = High n-3 PUFA diet

Even a slight decrease in 'EC' leads to many significant changes in the concentration of many metabolites. If this system is 'filled' or fully charged with high-energy phosphate, then all the adenosine nucleotides are in the form of ATP and the energy charge is '1'. On the other hand, if all the nucleotides are in the form of AMP, the system is 'empty' and the energy charge is '0'. At an average charge of 0.5, all the adenosine compounds will be in the form of ADP or an equimolar mix of ATP and AMP. In majority cases, the energy charge lies in between 0.75 and 0.98 (Atkinson, 1968).

Although there have been numerous studies on the influence of dietary fats on lipid metabolites, the current study is to focus on the variations in energy metabolism in an animal when fed fish muscle mixed with the standard pellet diet and not by supplementing the fish oils. Present findings were consistent with a correlation between polyunsaturation in the diet and increased disorder with in energy rich nucleotides and energy charge. An explanation of these observations will likely involve factors such as the molecular conformation of PUFA in particular an appreciation of the consequences of the position of double bond. Thus, whatever may be the tissue and whatever may be the level of increase in these nucleotides and energy charge, the elevation was conspicuous in ATPs and energy charge obviously reflects improvement of oxidative metabolism at the mitochondrial level.

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