The role of Omega 3 on some biochemical, Histological and mRNA expression of PPAR α in rats

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Abstract: This experiment was conducted to illustrate the effect of EPA Omega-3 on lipogram, glucose, histology and mRNA expression of PPARα in the liver of rats. Two groups of albino rats (n = 24) were equally divided to control and treated. Blood and liver samples were collected before and after the injection of fish oil. Blood and liver samples were prepared and stored after sacrificing the rats. The results indicated a significant decrease in cholesterol, TG, LDL and glucose, while HDL was significantly increased. Fish oil injection revealed no change in histological picture of the liver and the mRNA expression of PPARα increased significantly. In conclusion, the biochemical data was found that EPA possess a hypolipidemic and hypoglycemic effect, also EPA has no harmful effect on the liver, the increased expression of PPARα in liver after EPA injection may explain the role of omega-3 as hypolipidemic effect and suppression the lipid synthesis in the liver.

1. Introduction

Cold water fish oil contain long chain polyunsaturated fatty acids of the omega-3 type that have considerable beneficial effect on the human body. The main omega-3 fatty acids responsible for these benefits are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). EPA can be either converted into natural anti-inflammatory molecules DHA or can also integrate itself into cell membranes. DHA can also modify cell membrane and therefore enhance their function (Janet, 2005).

Omega-3 fatty acids have many biological function. They are required for the transport and metabolism of both cholesterol and triglycerides. They are able to lower high cholesterol lipid levels up to 25% and high triglycerides up to 65% (Kesavalu et al., 2002). Omega-3 fatty acids and sulphur amino acids together are involved in oxygen transport across the cell membranes, effectively improve blood circulation and therefore decrease the risk of diabetes (Rotstein et al., 2003).

Nakatami et al., (2003) reported that the liver plays an important role in lipid homeostasis and might be influenced by disturbance in W-6 : W-3 PUFA ratio. In mice, W-3 PUFAs alleviated liver inflammation. Moreover pretreatment with W-3 PUFAs significantly decreased the extent of microcirculatory failure which followed ischemia. Perfusion injury and protect against hepatocellular damage in the macrosteatotic mice liver.

The metabolic changes caused by dietary fat are regulated at the level of gene expression because lipid regulated transcription factors such as PPARs, sterol regulatory element binding protein SREBP-1C (Shimomura et al., 1990). PPARs are fatty acid regulated nuclear hormone receptors that could control lipid oxidation (Desvergne and Wahli, 1999).

Adipocyte differentiation, glucose and lipid storage, and inflammation (Rosen and Spiegelman, 2001). The demonstration of the fatty acid regulation of genes coded for lipid metabolism through PPARs and SREBP provides a rational basis for the early findings that diets high in unsaturated fat are less adipogenic than diets high in saturated fat (Pan et al., 1994). A high (n-3) PUFA rich fish oil diet is less adipogenic (Cunnane et al., 1986) due to a coordinated induction of fatty acid oxidation genes through PPARα (Ren et al., 1997) and suppression of lipogenic genes through SREBP-1C (Nakatani et al., 2003).

Peroxisome proliferation – activated receptor alpha (PPARα) is a nuclear receptor deeply involved in the maintenance of lipid and glucose homeostasis (Smith, 1996) and is mainly expressed in liver, muscle and heart (Staels et al., 1998). Activation of PPARα in liver, muscle and heart increases the expression and activity of lipoprotein lipase (LPL), an enzyme involved in lipolysis (Schoonjans et al., 2006), and therefore increases the clearance of TG-rich lipoproteins, and circulating TG levels. Paola et al., (2004) reported that ingestion of PUFA will lead to their distribution to virtually every cell in the body with effects on membrane composition and function, eicosanoid synthesis, cellular signaling and regulation of gene expression.

In a trial to discover the beneficial potential of W-3 fatty acids, the aim of the present study was to examine the effect of fish oil on lipogram, glucose level, histopathology of the liver and mRNA expression of PPARα in the liver.

2. Material and Methods
Animals:

Twenty four male albino rats with average body weights of 130-150 gm, were acclimated for 2 weeks prior to the experiment. They were housed in group of 6 each in wire cages at room temperature (22-25°C). animals were provided with a commercial balanced diet. They were housed with 12 hr light and 12 hr dark cycle.

Experimental design :

Two groups of rats (12 each) were randomly assigned as : group I served as control and injected with saline (I/P) group 2 was injected with fish oil. The experimental group was injected intraperitoneally a daily dose fish oil (0.5 ml = 0.05 g/kg body weight). Fish oil contents are Eicosapente acid 1.25-2.82g. α Tocopherol 0.015-0.0296 kg and Lectithin 1.2 g, fish oil was (purchase from fresenius AG, Bad Hamburg, Germany.

Tissue preparation and assays :
Preparation of the liver :

Rats were fasted 24 hrs prior to each designated time point and then sacrified. The liver was removed, washed with cold 0.1 M phosphate buffer, Ph 7.4, and chilled on ice. All the procedures were carried out in cold conditions. Specimens from liver were kept after sacrification at –80°C until analysis.

Histopathological technique :

Samples from the liver were processed by standard paraffin methods, dehydrated in a series or graded concentration of ethyl alcohol, cleared in xylene, embedded in melted paraffin at 60°C and then sectioned at 4 μ thickness. The sections were routinely stained with hematoxyline and eosin stain.

The blood was collected by cardiac punture. Glucose was measured in fresh blood according to Hoffmister and Junge (1970). Separated serum was assayed for Cholesterol, triglycerides, HDL and LDL according to Allain et al., (1978).

Gene Expression procedure :

1- Total RNA extration from liver according to Chomczynski and Sacchi (1987). RNA concentration was quantified spectro-photometrically at absorbance 260 (nm). The isolated RNA was stored at –80°C until used.

2- Reverse Transcription – RT (cDNA). cDNA was synthesized using reverse transcriptase from RNA. RT mixture was reported, then incubated at 25°C for 10 minutes, then at 42°C for 50 minutes then the reaction was stopped by heating at 95°C for 3 minutes. The cDNA was stored at –20°C until used.

3- Semi-Quantitative Polymerase Chain Reaction (PCR). The reverse transcriptional products were subjected to PCR amplification using specific forward and reverse primers for PPARα designed according to the published nucleotide sequence of cDNA available in the Gen Bank Database.

Amplification was started with 5 minutes of denaturation at 94°C and 30 cycles at 60°C as annealing temperature were used in PPARα and housekeeping gene (GAPDH) glyceraldehydes –3– phosphate dehydrogenese. After amplification, 5 μl of each PCR reaction product was added to 1 μl from loading dye, then electrophoresed for 30 minutes at 100 V through 1% agarose gel containing ethidium bromide (0.5 mg/ml). The PCR products were visualized by (UV) illumination after electrophoresis, PCR band intensities were expressed as relative intensity and normalized for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression, to allow semi-quantitative comparison of PCR product. The data are presented as the ratio with the respective controls.

The primer sequences for Rat PPARα mRNA amplification were constructed from rat PPARα gene sequence available in the gene-bank product size (bp) 680.

Forward primers
gaggtccgattcttccactg
Reverse primers :
atccctgctctcctgtatgg
Primer sequences for GAPDH rat product size (bP) 200
Forward primers
taaagggcatcctgggctacact
Reverse primers :
ttactccttggaggccatgtagg
Statistical analysis : all data were expressed as means ±SD. An unpaired t-test was used to evaluate the comparisons between two means of two groups. P value less than 0.05 were considered to be statistically significant.

3. Results

The effect of short term omega-3 G.P. injections on serum biochemical analysis in male albino rats was recorded in table (1). The result revealed a significant decrease in cholesterol, TG, LDL and glucose while HDL revealed a significant (P<0.05) elevation.

Table (2) indicated the correlation coefficient between different variables in oil injected rats.

Histopathological examination of liver of control and experiment group showed normal apparance of liver cells without any damage due to fish oil injections (Fig 1, 2).

Table (3), relative mRNA expression of PPARα in liver of control and EPA treated albino rats as compared with GAPDH mRNA relative expression revealed an elevated expression in the experimental group.

Fig. (3, 4) showed electrophoretic picture of mRNA gene expression of GAPDH in liver (200bp) and expression of PPARα in liver (680 bp).

Table (1) : Effect of intraperitoneal injection of (10%) fish oil on lipograms and glucose in adult male albino rats.

| Parameters \ Condition		*Before injection*	After injection
Glucose mg/dl	Cont.	150.4±2.4	148.8±3.2
	Exp.	158.5±3.1	143.3±2.2*
Cholesterol mg/dl	Cont.	123.6±1.9	125.4±.3.2
	Exp.	126.2±1.7	101.2±3.4*
TG mg/dl	Cont.	282.1±3.1	291.3±4.6
	Exp.	278.3±2.8	213.6±3.2*
LDL mg/dl	Cont.	125.4±1.8	124.3±1.5
	Exp.	121.3±1.6	104.4±1.3*
HDL mg/dl	Cont.	38.3±1.4	39.1±2.1
	Exp.	39.2±1.3	51.7±1.3*

The results indicated a significant ($P<0.05$) decrease in glucose, cholesterol, TG, and LDL concentration and a significant ($P<0.05$) increase in HDL concentration.

**Table (2) : Correlation coefficient between different variables in oil injected rats.**

	HDL	**LDL**	**Chol.**	**TG**	**Glucose**
HDL		-0.87**	-0.72**	-0.83**	0.88**
LDL			0.71**	0.82**	-0.81**
Chol.				0.81**	-0.75**
TG					-0.83**
Glucose					

Table (2) showed a high correlation coefficient between glucose and HDL, while a negative correlation between HDL, TG, and LDL.

Histopathology examination showed no different change in case of liver following fish oil (10%) injections in adult male rats.
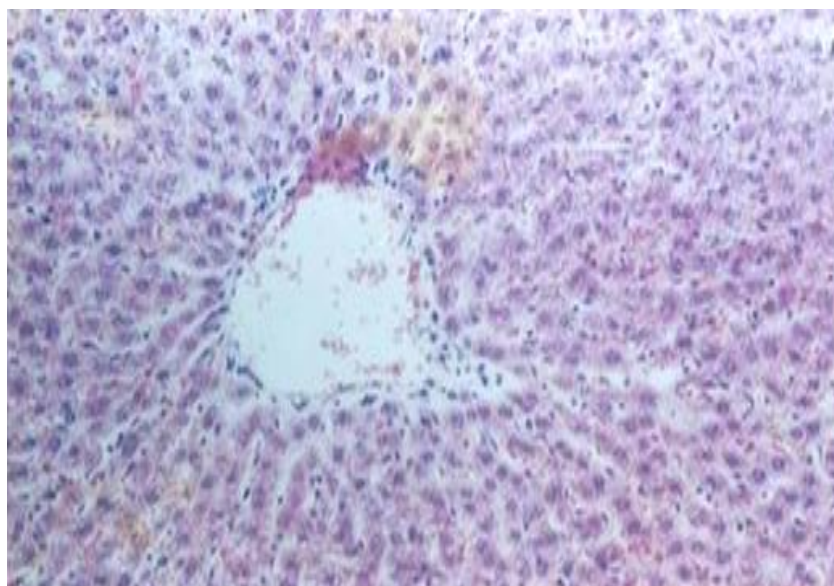
Fig. (1) : Liver of control rats showing radial arranged hepatic cord and central vein

(H & E, X 300)
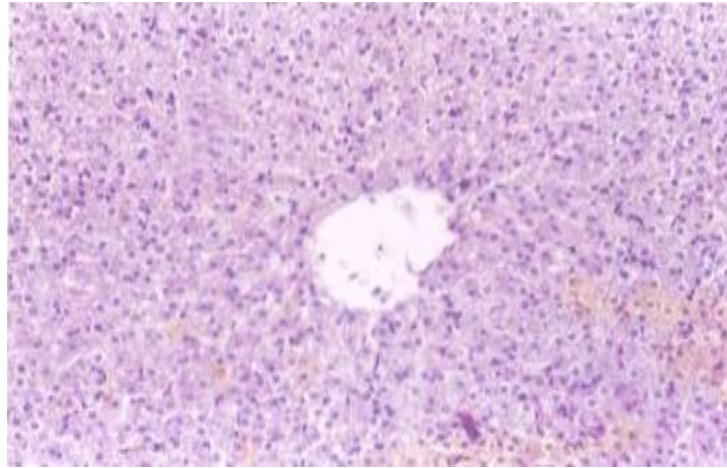
Fig. (2) : Liver of treated rats with fish oil showing normal structure of liver alls and central vein (H & E, X 300)

Table (3) : Relative mRNA expression of PPARα in liver of control and treated albino rats with EPA as compared with GAPDH mRNA relative expression.
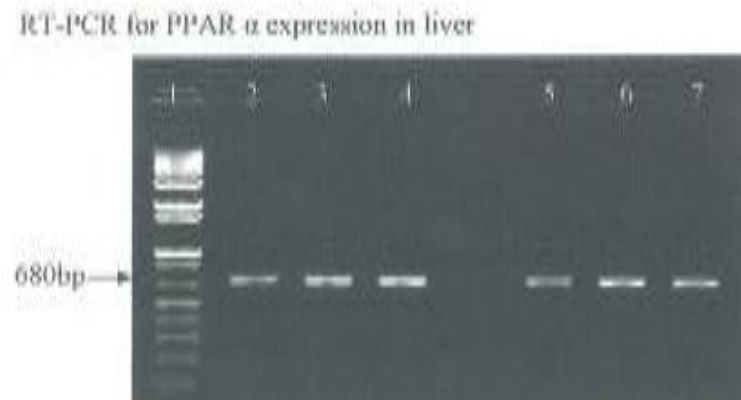
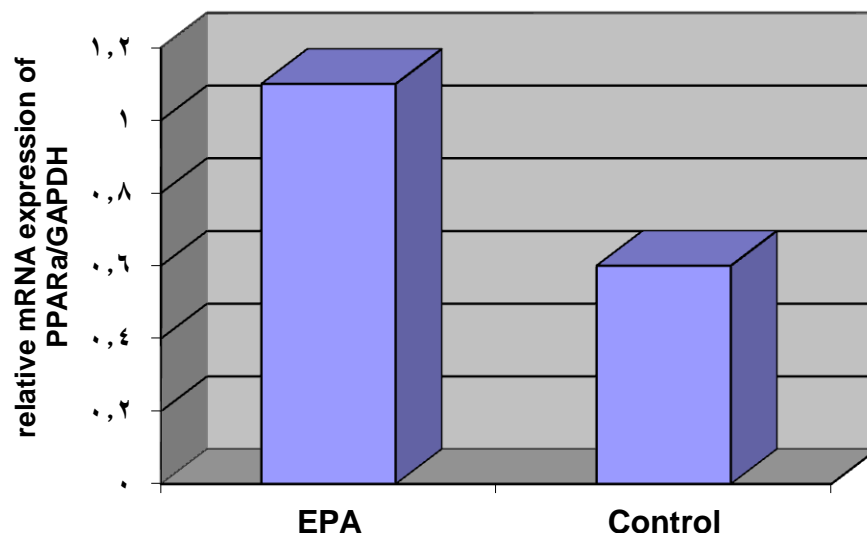| Parameter	EPA	Control
PPAR-α*	1.10±0.04 *	0.60±0.07

Values are expressed as Means ±SE

* significant differences at (P<0.05)
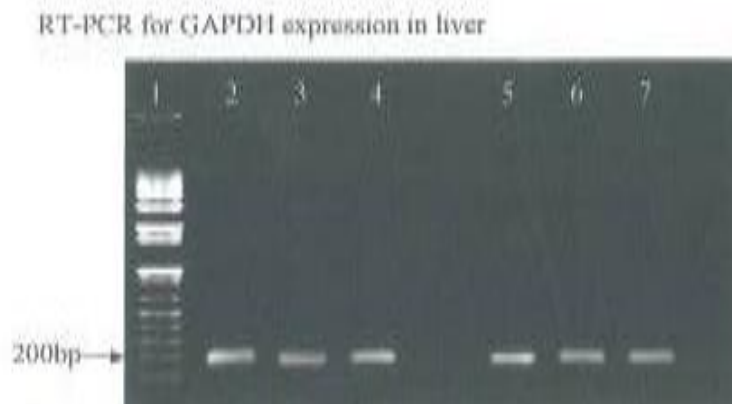
Fig. (3) : Electrophoretic picture of mRNA gene expression of GAPDH in liver.

Fig. (4) : Electrophoretic picture of mRNA gene expression of PPARα in liver.

Fig. (5) : Relative mRNA expression of PPAR-α in liver of control and EPA treated rats.

4. Discussion

The present study revealed significant lower levels of cholesterol, TG and LDL while an increased HDL level after fish oil administration for 2 weeks (Table 1).

These results are in agreement with previous studies by Nogi et al., (2007), Dasgupta and Bhattacharyya (2007) and Kelley et al., (2007). As for hypotriglyceridemic effect, there are various biochemical mechanisms can be related to that, Shirouchi et al., (2007) concluded them in two ways, suppression of fatty synthesis and the enhancement of fatty acid B-oxidation. Fish oil may elicit their effect by contributing PPAR-α activation in liver and skeletal muscle (Clarke, 2001).

The decrease of cholesterol level observed in the present study may be attributed to the inhibition of hepatic hydroxyl-3 methylglutaryl-CoA reductase activity (Hromadova et al., 1994).

The lowering in LDL level may be explained by alteration in the membrane lipid composition which enhanced hepatic LDL receptors function and clearance (Kuo et al., 1990).

As for the increase in HDL following fish oil administration. HDL exerts several potentially anti-atherogenic effects including reverse cholesterol transport from peripheral cells to liver and W3 PUFA had increased HDL in experimental investigations (Hersberger and Von Echardstein, 2005). A shift toward fish oil diet has been shown to be beneficial in the protection against heart disease due to its W-3 content. Schulz et al., (2001) reported that W-3 (1 gm daily), Vit. E (300 mg daily) reduced by 10% the combined end point of stroke, myocardial infarction

and mortality rate as well as reduces atherosclerosis in animal model.

The data presented in table (1) revealed a significant decreased glucose levels after fish oil administrations, this decreased glucose levels might be caused by the elevated insulin concentrations, which increased glucose uptake by tissues, helps to enhance muscle glycogen and supply extra energy. Erasmus (1999) noted that W-3 improve muscle glucose uptake by improving insulin sensitivity. Syrah et al., (2002) cited that fish oil supplements have shown to correct many of the squeal associated with insulin resistance.

Nagla and Madiha (2010) could explain the observed dyslipidaemia by two significant enzymatic defects, reduced lipoprotein lipase and increased hepatic triglyceride lipase activity. Mahmoud and Amer (2004) noted that injection of fish oil decreased concentration of lipoprotein lipase enzyme, this result supported the view that insulin inhibits the activity of lipoprotein lipase. Because elevated lipoprotein constitute an independent risk factor for development of atherosclerosis (Tomonage et al., 2002). Fish oil fatty acid administration is effective in counter-acting the proatherogenic lipid profile of lipoprotein (Anne et al., 2000).

The histopathological examination showed no harmful effect occurring in liver following fish oil injections (Fig. 1-2) in adult male rats.

The present study revealed that EPA administration in rats produced up regulation in mRNA expression of PPAR-α in liver (Table 3). This result is in parallel to the studies of Larter et al., (2007) and Shan-Ching and Ching-Jang, 2006) and Pawar and Jump (2003).

This result was also in agreement with the previous findings of Holness et al., (2007) and Neschen et al., (2007) who observed that EPA administration produced activation of hepatic PPAR-α and reduced hepatic lipid accumulation and counteract hepatic insulin resistance. (Clarke, 2001) reported hypolipidemic effect of W-3 PUFA. This can be explained through interaction of PPAR-α with PUFA leading to the induction of genes encoding proteins involved in lipid transport, oxidation, thermogenesis resulted in suppressing lipid synthesis in the liver and up regulating fatty acid oxidation in liver.

In conclusion, this study suggested that fish oil injection can provide a useful mean to the antiatherogenic potential and diabetes mellitus, and affect the liver positively by suppression lipid synthesis and improve health and obesity which may open a new dietary approach to diseases.

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