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Original Research

Comparative Studies on the Effect of High Fish and Corn Oil Diet on Lipid Profiles, PPAR-α and FAS mRNA in Swiss Mice

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Abstract

Background: The hypolipidemic actions of oils reflect their quality and safety for human health. PUFA include omega 3 and omega 6, considered to be essential fatty acids. Eicosapentaenoic acid (EPA, 20: 5 n-3) and docosahexaenoic acid (DHA, 22: 6 n-3) are two important long-chain unsaturated FA in essential omega 3 family and are found mainly in fish oil, while arachidonic acid (20 : 4 n-6) is a member of omega 6 family found mainly in plant oils.
Objective: The current study was conducted to test the lipidemic effects of two oil supplements and genes expression related to lipid metabolism.
Methodology: Experimental mice were fed on normal diet and foods enriched in corn oil or fish oil for one month. Lipid profiles were measured and gene expression of PPAR-α and fatty acid synthase were semi-quantitated using RT-PCR analysis.
Results: The current study revealed a significant increase in lipid profiles in corn oil fed group. LDL-C and blood glucose were decreased while HDL-C was significantly increased ($p < 0.005$) in the group of mice fed with fish oil compared to the mice fed with normal diet. FAS mRNA expression was higher in corn oil fed group while PPAR-α was high in fish oil fed group.
Conclusion: dietary supplementation of fish oil alters lipid profiles and expression of lipid metabolizing enzymes and is better for health than corn oil.

INTRODUCTION

Human diets have been changed greatly in the past 50 - 100 years especially in the types of fat and fatty acids ingested [1]. Polyunsaturated fatty acid (PUFA), are long-chain fatty acids (FA) containing two or more double bonds [2, 3]. PUFA include omega 3 and omega 6, they considered to be essential FA [4]. Eicosapentaenoic acid (EPA, 20: 5 n-3) and docosahexaenoic acid (DHA, 22: 6 n-3) are two important long-chain unsaturated FA in essential omega 3 family and are found mainly in fish oil, while arachidonic acid (20 : 4 n-6) is a member of omega 6 family found mainly in plant oils(5). Under normal PUFA supplementation, it has been reported that n-3 and n-6 PUFA may improve lipid metabolism [2, 5]. However, there is no available data on the physiological effects of these FA under high fat diet regimen.

Peroxisome proliferator-activated receptors (PPAR) are a class of ligand-activated nuclear receptors that modulate gene expression [6]. PPAR-α activates genes involved primarily in fatty acid and lipoprotein metabolism [7]. Polyunsaturated fatty acids and eicosanoids produced from arachidonic acid by the cyclo-oxygenase and lipoxygenase pathways are ligands that activate PPAR-α and PPAR-γ [8]. Fatty acid synthase (FAS) plays a central role in lipogenesis by catalyzing the synthesis of saturated long chain fatty

acids from acetyl CoA, malonyl CoA, and NADPH [9]. FAS may also be involved in the production of an endogenous ligand for the nuclear receptor PPAR-α that help to treat hyperlipidemia [10]. Usage of oil in food cooking is a common practice all around the world. The good oil for human health is the one that has a hypolipidemic action. More saturated fatty acids (n-6 fatty acids and trans fatty acids) are being consumed with a decrease in n-3 fatty acids intake. This is the cause of the increase in nutrition-related chronic diseases such as cardiovascular diseases, obesity, cancer and immune-related diseases. The incidence of these diseases is low in populations consuming a diet rich in marine lipid [11].

The aim of the present study is to examine and compare the effects of fish and corn oils (under high supplementation) on plasma lipid profiles (total blood cholesterol, triacylglycerol, phospholipid, free fatty acids, low and high density lipoproteins) and FAS and PPAR-α gene expression under different feed regimen in mice.

MATERIALS AND METHODS

Animals

Twenty four Swiss male mice (8 weeks old, 23.4 ± 0.2 g) were obtained from the breeding unit of the Egyptian Organization for Biological Products and Vaccine (Helwan, Egypt) and were used throughout this study. The animals were acclimated for 2 weeks in steel mesh cages (8 animals / cage) before starting the experiment at the animal house of the Physiology Department, Faculty of Veterinary Medicine, Zagazig University (Egypt). During the experimental period, the animals were kept under standard temperature (20 – 25ºC) and humidity (55±15 %) on a 12 hr light–dark cycle. Animals were given 12.5 g/animal/week (100 g/group/week) of freshly prepared diets (Table 1) and filtered tap water. Routine hygienic procedures throughout the experimental period were followed.

Diets and experimental design

Mice were divided randomly into three groups (n = 8/group). The first group was fed on normal diet as a control (ND), while the 2nd group was fed on diet rich in fish oil (n-3 PUFA) and the 3rd group was fed on diet rich in corn oil (n-6 PUFA) for 1 month (Sigma Aldrich Co. USA). Omega-3 was purchased from Everett (Canada). All diet components were purchased from Delta Food Industry Company (Egypt). The diet was prepared fresh weekly in order to protect and preserve fatty acids from harm of oxidation process. All ingredients expressed in weight and percentage of total volume as represented in Table (1).

Table 1. Composition of normal diet, fish oil and corn oil fed groups

Ingredient	Normal diet	Corn oil fed group	Fish oil fed group
Casein purified high nitrogen	295 g (29.5%)		
Sucrose	348 g (34.5%)		
Corn starch	250 g (28%)	125 g (12.5%)	
AA (n-6) (92% purity)	10 ml (1%)	155 ml (15.5%)	-
EPA and DHA (n-3) (89% purity)	10 ml (1%)	–	155 ml (15.5%) (46.5 g EPA and 23.25 g DHA)
Soybean oil *	10 ml (1%)	-	-
Mixed vitamins (B, E, A, D-3, K-1, folic acid, biotin, nicotinic acid)	22 g (2.2%)		
Mixed minerals (Ca, K, Fe, Zn, Mn, Na, Cl, S)	51 g (5.1%)		
DL-methionin	4 g (0.4%)	4 g (0.4%)	4 g (0.4%)
Total energy (kcal/g)	2.6	3.8	3.8

All ingredients expressed in weight and percentage of total volume. AA (arachidonic acid), EPA (eicosapentaenoic acid), DHA (docosahexaenoic acid), * soybean oil was added to supply the minimum requirement of essential FAs to control group according to AIN-93 diet formula

Table 2. Primers and PCR conditions used in this study

Gene	Accession No.	Primer Sequence (5'→3')	Ann. Temp.	Product size (bp)
PPAR-α	NM 013196	For: GAGGTCCGATTCTTCCACTG Rev: ATCCCTGCTCTCCTGTATGG	60 Cº for 1 min	680
FAS	NM 017332	For: CCTGATAGTGAGCGGGAAAG Rev: CGTGAGGTTGCTGTTGTCTG	60 Cº for 1 min	462
GAPDH	NM 500506	For: TAAAGGGCATCCTGGGCTACACT	59 Cº for 30 sec	200

Sequence of primers and PCR conditions used in this study. PPAR-α (Peroxisome Proliferator Activated Receptor-□), FAS (Fatty acid synthase) G3PDH (glyceraldehyde-3-phosphate dehydrogenase), For. (forward primer), Rev. (reverse primer) and Ann.temp. (Annealing temperature).

Sampling

After 1 month of fish and corn oil supplementation in diet, mice were fasted overnight and anaesthetized. Blood samples were collected before decapitation via heart puncture using heparinized syringe. Collected blood was centrifuged at 3000 rpm for 15 minutes at 4 ^{0}C to remove the cells. The obtained plasma was divided into aliquots and stored at – 20 ºC until analysis. Liver and epididymal fat (white adipose tissue) were dissected and snap frozen at –80 °C for RT-PCR analysis.

Plasma biochemical measurements

Plasma total cholesterol (TC), triacylglycerols (TG), phospholipid and free fatty acids were measured spectro-photmetrically [12, 13]. Low density lipoproteins (LDL) and High density lipoprotein-cholesterol (HDL-C) were measured using enzymatic and calorimetric kits [14-16], respectively. Low density lipoprotein cholesterol (LDL-C) was calculated according to study of *Friedewald et al*. [17]. Glucose was quantified using glucose oxidase method [18].

RT-PCR analysis and gene expression

Livers and epididymal adipose tissue were collected from mice, flash frozen in liquid nitrogen and stored at -70°C. Frozen samples (approximately 100 mg of tissue per sample) were immediately added to 1 ml of TriZol reagent (Invitrogen, Carlsbad, CA) and homogenized using a Polytron 300 D homogenizer (Brinkman Instruments, Westbury, NY). One milliliter of the tissue homogenate was transferred to a microfuge tube, and total RNA was extracted via chloroform extraction followed by nucleic acid precipitation with isopropanol. The pellet was washed with 75% ethanol and re-suspended in molecular biology grade water. Nucleic acid concentration was determined spectro-photmetrically at 260 nm (Smart-Spec; Bio-Rad Laboratories, Hercules, CA), and RNA integrity was evaluated using an Agilent bioanalyzer (model 2100; Agilent Technologies, Foster City, CA).

RNA (1µg) was reverse transcribed using 100 units of moloney murine leukemia virus (MMLV) reverse transcriptase (Gibco), 50 pmol of poly (dT) primer and 20 nmol of dNTPs in a total volume of 10 µl at 37°C for 1 h. After heating at 94°C for 5 min, PCR amplification was performed with 2.5 units Taq polymerase (Perkin-Elmer, Foster City, CA, USA), 3 mM MgCl2 and 50 pmol of forward and reverse primers specific for respective genes in a total volume of 50 µl**.** The PCR conditions for different tested genes (FAS, PPAR-□) are listed in Table (2). A primer pair for glyceraldehyde-3-phosphate dehydrogenase (G3PDH) was used as an internal positive control in RT-PCR. Amplification was started with 5 minutes of denaturation at 94°C followed by 20 cycles for G3PDH**.** PCRs cycles for PPAR-α and FAS genes were 30 cycles with final extension for 7 minutes. PCR products were electrophoresed in 1.5% agarose gel, photographed by Alfa-GelfoxTM 2D v.3.0 gel documentation unit (UK). The Integrated Density Value (IDV) was estimated by the Alpha Ease software for windows v.4.0.0 and band intensities were corrected to those of G3PDH as follows:

IDV of a sample gene = IDV of gene / IDV of G3PDH

Statistical analysis

The data were expressed as mean ± standard deviation (mean± SD) and statistical significance was evaluated by one way Analysis of Variance (ANOVA) using SPSS (version 16.0) program. Values were considered statistically significant when $P \leq 0.05$.

Table 3. Plasma lipid profiles in normal and experimental groups

Groups	Parameters (mg/dl)				
	TC	LDL-C	HDL-C	VLDL	Triacylglycerols
Normal diet	34.27±2.5	6.38±0.58	16.33±1.53	11.56±1.81	57.81±5.68
Corn oil	48.52±3.93*	15.01±1.98 *	12.13±1.93*	21.38±2.7*	109.92±11. 02*
Fish oil	28.35±2.25*#	3.34±1.53*#	21.49±2.65*#	3.53±1.01*#	16.43±1.98*#

TC (total cholesterol), HDL-C (high density lipoprotein-cholesterol), LDL-C (low density lipoprotein-cholesterol), VLDL (very low density lipoprotein-cholesterol). Values are means ± SD obtained from 8 different rats for each group. *p ≤ 0. vs. compared to control (normal diet) and . # P ≤ 0.05 vs. corn oil group (Duncan's test).

Table 4. Levels of plasma phospholipids, free fatty acids and glucose in normal and experimental groups

Groups	Parameters		
	Phospholipids (mg/dl)	Free fatty acids(FFA) µEq/L	Glucose (mg/dl)
Normal diet	18.49±2.03	214.33±19.2	53.15±4.73
Corn oil	23.33±3.21*	261.17±21.59*	43.32±4.04*
Fish oil	12.67±1.83*#	131.42±12.53*#	29.87±3.51*#

All values were expressed as means ± SD. They are obtained from 8 different rats for each group. *$p \leq 0$. vs. compared to control (normal diet) and # P ≤ 0.05 vs. corn oil group (Duncan's test).

RESULTS AND DISCUSSION

Plasma lipids showed different patterns within each experimental group as showed in Table (3). TC, VLDL and TG were significantly increased (P≤ 0.05) in corn oil fed group compared to normal diet group and significantly decreased (P≤ 0.05) in fish oil fed group compared to normal diet fed group. The levels HDL-c showed significant increase in fish oil fed group and significant decrease in corn oil fed group compared to normal diet group. The findings for LDL-c were the opposite for that of HDL-c and significant effects for LDL-c and HDL-c were seen between corn and fish oil diet groups. These findings were in agreement with other studies [19, 20], who reported that plasma TG concentration were reduced as compared to control on supplementation of hypercholesterolemic diet with fish oil and pure fish oil, respectively. *Roche and Gibney* [21] reported that n-3 PUFA decreased plasma TG concentration through reducing the endogenous VLDL production, which may be mediated through alteration in particle size, structure and chemical composition or alteration in lipoprotein lipase (LPL) activity [22]. Stimulation of LPL activity was induced by supplementation with fish oil - rich in DHA that resulted in the decrease in Apo CIII and consequently the increase in TG clearance (22). n-3 PUFA can decrease TG concentration through the inhibition of hepatic VLDL-TG synthesis due to the decrease in the expression of hepatic gene transcription factor, sterol responsible element binding protein-1c (SREBP-1c), key factor in controlling lipogenesis [23]. Moreover, n-3 PUFA also regulates Apo CIII expression as PPAR-α, down-regulates Apo CIII expression while NF-κb up-regulates Apo CIII expression [24].

The TG lowering effect of fish oil may be due to simultaneous increase in mitochondrial and/or peroxisomal β-oxidation, which may be a direct result of PPARα-induced increase in acyl-CoA oxidase gene expression and therefore lead to reduced FA substrate for TG synthesis [4, 25-26]. This is in accordance with our results that showed a decreased level of free fatty acids in mice supplemented with high fish oil in comparison to corn oil fed ones. Plasma total cholesterol levels in this study were comparable to another result [27]. Moreover, fish oil diet decreased plasma TC and this might be attributable in part to a higher binding affinity of LDL to liver plasma membranes in comparison with saturated diets [27]. Also, supplements of purified n-3PUFA significantly lowered TC levels in normal and hypercholesterolemic rats [28].

The fall in plasma total cholesterol and LDL-C levels in the high fish oil fed group was confirmed [29] as EPA and DHA present in fish oil lowered plasma LDL cholesterol levels by more than 50%. The decrease in LDL cholesterol levels was predominantly due to a decrease in the number of LDL particles [30]. Thus, a decrease in the rate of LDL entry into plasma may be due to a reduction in the number of VLDL particles secreted by the liver or due to a decrease in the

proportion of VLDL that is metabolized to LDL [26].

The affinity of EPA and DHA (or their metabolites) as a ligand to PPAR was confirmed and was comparable to arachidonic acid or its metabolite [3-4]. Therefore, it is acceptable to postulate that n-3 PUFA is more potent as a lipolytic factor than n-6 PUFA for improving lipid profiles due to the difference in location and number of double bonds and carbon atoms within each FA(5). Results of serum phospholipids and FFA (Table 4) were in accordance with *Ide et al*. [31] as fish oil significantly decreased serum phospholipids and FFAs concentration. This reduction is probably due to the increase in hepatic concentration of phospholipids due to a decrease in phospholipase activity or to an increase in phospholipids turnover [32].

The blood glucose level of fish oil fed group was observed to be significantly lower when compared with corn oil fed mice and the control group. It has been shown that rats fed on 200 mg conjugated linoleic acid -rich in DHA- for 4 weeks exhibited lower plasma blood glucose and FFA levels [21]. The improvement in glucose metabolism in the face of accelerated fatty acid oxidation would appear to be in conflict with the idea that higher rates of fatty acid oxidation actually impair glucose utilization [33]. An explanation for this paradox may lie in the role that dietary fatty acids play in determining membrane fluidity [34-35]. Further studies are needed to confirm the hypoglycemic effect of fish oil, and correlations with insulin secretion must be outlined.

PCR amplification and image analysis of FAS and PPAR-α mRNAs (Fig.1) showed that FAS mRNA was significantly increased (P≤ 0.05) in liver tissues of corn-oil fed group as compared to fish-oil fed group. However, it was decreased in adipose tissue of fish oil-fed group as compared to corn oil-fed group. PPAR-α mRNA exhibited significant increase (P≤ 0.05) in liver of corn oil-fed group than in fish oil-fed group. It was not-significantly increased, however, in the adipose tissues of fish oil fed group relative to the control and corn oil fed groups. Changes in mRNA expression for both genes were almost linear in adipose tissues of all the groups, i.e. linear increase of PPAR-α (decrease of FAS) from control to corn oil-fed group to fish oil-fed group. As known, fish oil, rich in EPA and DHA, suppressed the hepatic activity of lipogenic enzyme effectively [36]. The mRNA expression levels of FAS and PPAR α genes in liver showed that FAS gene expression was markedly elevated in corn oil fed group but not in control and fish oil fed groups. It has been shown that supplementation of rats with corn oil resulted in marked elevation of FAS mRNA and FAS activity when compared with fish oil fed groups [37]. Rats fed on 15% fish oil exhibited down regulation of FAS mRNA relative to palm oil fed group [31].

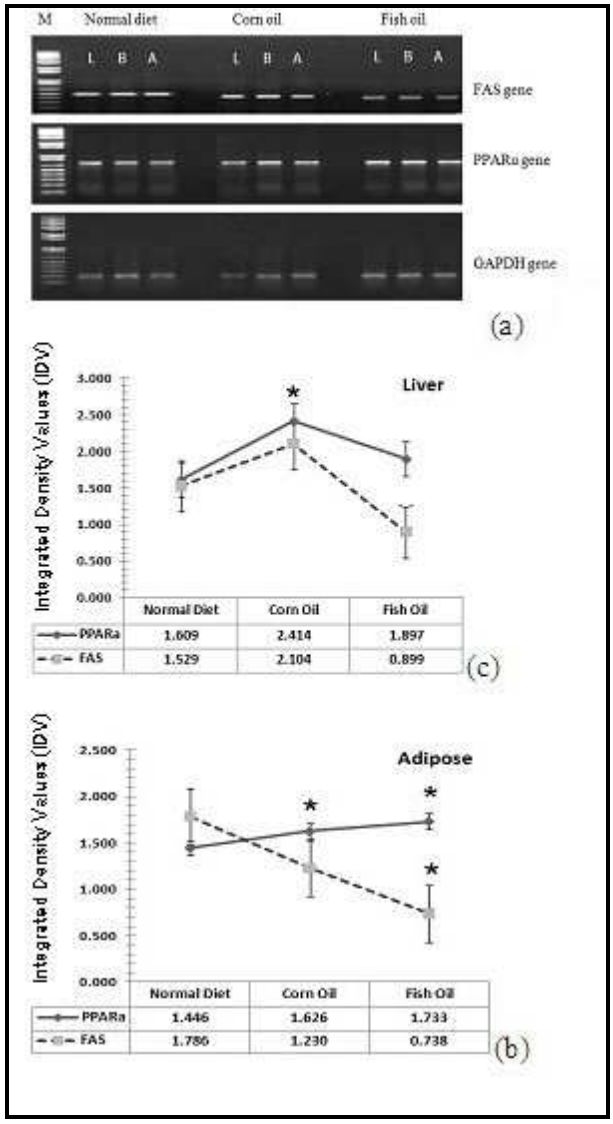

Fig.1. RT-PCR analysis of FAS and PPAR-□ expression in epididymal and liver of Swiss mice.

(A) PCR products for mRNAs of epididymal fat for genes of FAS, PPARα- and G3PDH. L: B: A: M: DNA ladder marker. (B) and (C) Gene expression levels represented in mean Integrated Density Values (IDV) by AlfaEase FC software (ver.4.4) and correlated to G3PDH. *P ≤ 0.05 relative to normal diet

Plasma TG concentration and hepatic FAS activity in rats administered α linolenic acid were correlated with a low amount of FAS mRNA [38]. As known, PUFAs regulate hepatic lipogenesis through suppressing SREBP-1c nuclear abundance with a comparable decrease in the transcription rate of hepatic FAS [31]. Hypolipidemia induced by fish oil can be explained by increased fatty acid oxidation [38], inhibition of de novo fatty acid synthesis secondary to decreased fatty acid synthase gene expression [37] and the induction of carnitine palmitoyl transferase which regulates hepatic

fatty acid oxidation [39-40]. The upregulation of PPAR-α expression can be explained by the effect of n-3 PUFA. As n-3 PUFA can activate PPAR-α, thereby increasing expression of fatty acid oxidation genes and decreasing hepatic and plasma TG concentrations [24, 43]. This effect is beneficial as cardio-protective for hyper-triglyceridemic patients [41].

In conclusion, n-3 polyunsaturated fatty acids (PUFA) content in food can affect lipid metabolism in different tissues and in different ways. The control of hepatic gene expression by n-3 PUFA might be regulated by three pathways. Firstly, n-3 PUFA induces mono- and ß-oxidation that requires PPAR-α [42]. Secondly, n-3 PUFA suppresses *de novo* lipogenesis and mono-unsaturated fatty acid synthesis that requires SREBP-1c and apolipoproteins [19, 32]. Thirdly, n-3 PUFA interferes with the glucose-stimulated hepatic L-type pyruvate kinase gene transcription. The carbohydrate regulatory element-binding protein (ChREBP), MAX-like factor X (MLX), and hepatic nuclear factor-4α (HNF-4α) are involved in the glucose-mediated induction of hepatic L-type pyruvate kinase (L-PK) gene transcription [42-44]. Further studies are needed to investigate the anti-obesity effect and safety of these PUFA under high supplementation condition.

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