

EFFECT OF FISH OIL AND ANIMAL FAT SUPPLEMENTATION ON LIPID FRACTIONS AND THYROID HORMONES IN ADULT MALE RATS

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ABSTRACT

Epidemiological studies have demonstrated that n-3 polyunsaturated fatty acid (PUFA) consumption is associated with a reduced risk of atherosclerosis and hyperlipidemia. It is well known that lipid metabolism is also influenced by thyroid hormones. The aim of the current study was to test whether fish oil versus beef fat supplementation would affect lipid metabolism in correlation with thyroid hormones in adult male rats. In this investigation, dietary lipids (fish oil) and (beef fat) were fed to adult male albino rats. Both dietary lipids were fed in concentration of 27% and 45 % of the diet's dry matter, for 14 days. At the end of the experiment, the animals were sacrificed; the plasma and liver fractions including cholesterol, triglycerides, high and low density lipoprotein were measured biochemically; and the plasma levels of T₃ and T₄ were determined using RIA. The results revealed the following: (a) the fish oil diets decreased the plasma lipid fractions, except the high density lipoprotein which was increased, while the beef fat diets increased the plasma lipid fractions except the high density lipoprotein which was decreased. (b) the beef fat diets increased the liver lipid fractions more than the fish oil diets. (c) the T₄ levels showed non significant change . (d) the plasma T₃ levels revealed a significant increase in the animal groups fed the diets containing 27% of either fish oil or beef fat. These results suggested that fish oil has a hypolipidemic effect while beef fat has a hyperlipidemic effect. Furthermore, the influence of lipid calories on the plasma T₃ levels may be more pronounced than that of carbohydrate when a normal amount of carbohydrate was ingested.

INTRODUCTION

Evidence from multiple research paradigms, including in vitro studies, animal experiments, observational studies, and clinical trials, supported the cardiovascular benefits of long-chain poly unsaturated fatty acids (ω -3 PUFAs), especially for fatal cardiovascular disease (CVD) events [1]. Of particular note are the n-3 PUFA: eicosapentaenoic acid [EPA, 20:5 n-3] and docosahexaenoic acid (DHA, 22:6 n-3) [2]. Fish are the major food sources of DHA and EPA and are carried in the circulation as triglycerides, especially phospholipids [3]. There are several experimental studies showed that the n-3 PUFA perform several functions in relation to the structure and function of the membrane, tissue metabolism, and gene regulation [4]. These fatty acids play important roles in reducing hypertriglyceridaemia [5], low density lipoprotein cholesterol (LDLc), very low density lipoprotein cholesterol (VLDLc), and increasing high density lipoprotein cholesterol (HDLc) concentrations [6] as well as various components of these molecules e.g. ApoA1 and ApoB100 of HDL and LDL/VLDL respectively. EPA and DHA improve hypertension [7], insulin sensitivity and glycaemia [8].

Moreover, it is well established that dyslipidaemia is one of the major risk factors for atherosclerosis, in particular CAD, cerebrovascular disease and peripheral vascular disease [9]. In particular, it is

well established that there is a causal link between atherosclerosis and elevated plasma concentrations of total cholesterol and elevated plasma LDL concentration [10]

Nakatani et al [11] reported that the liver plays an important role in lipid homeostasis and might be influenced by disturbance in ω -6 : ω -3 PUFA ratio. In mice, ω -3 PUFAs alleviated liver inflammation. Moreover pretreatment with ω -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 [12]. PPARs are fatty acid regulated nuclear hormone receptors that could control lipid oxidation [13].

Thyroid hormones affect reactions in almost all pathways of lipid metabolism (14). ω -3 PUFAs present in fish oil potently decrease serum lipids, which is also an effect of thyroid hormones [15]. Both PUFAs and thyroid hormones affect hepatic lipid metabolism, and it has been hypothesized that a long-term diet rich in ω -3 PUFAs would enhance thyroid hormone action in the liver [16]. However, it has been reported that high consumption of fish carries a lower risk of CVD as a consequence of

dietary ω -3 PUFA; especially (EPA) and (DHA) content. A controversy exists about the component/s responsible of these beneficial effects and, in consequence, which is the best proportion between both fatty acids [2]. Clearly therefore, the aim of the present study was: firstly, to examine the effect of dietary fish oil (polyunsaturated fat) and beef fat (saturated fat) on some lipid fractions in both plasma and liver; secondly, to examine the effect of caloric content of the diets containing these lipids on the plasma levels of T_3 and T_4 in adult male rats.

MATERIALS AND METHODS

Mature male albino rats weighing 200 g were used in this study. They were divided into five groups,

ten animals each which were housed in five cages. The 1st group fed on a control diet; the 2nd and the 3rd groups fed on diets containing two levels of beef fat, and the 4th and 5th groups fed on diets containing two levels of fish oil (**table 1**). The experimental diets were prepared from natural ingredients as fish meal and wheat bran and were analyzed to calculate the protein, lipid and carbohydrate percentages in each diet. Then, the caloric content in each diet was calculated according to the physiological fuel of 4.0, 9.0 and 4.0 for protein, fat and carbohydrate respectively. The experimental animals were fed on experimental diets for two weeks.

Table (1): Diets formulation and composition

Dry diet ingredients (g)	G I (control)	G II	G III	GIV	G V
Fish meal	24	27	30	27	30
Wheat bran	65	42	20	2	20
Fish oil	-	-	-	20	20
Beef fat	4	24	43	-	-
Vitamins and mineral mixtures	7	7	7	7	7
Total grams	100	100	100	100	100
Calculated protein	21.45	21.3	21.3	21.3	21.3
Calculated lipids	7.2	27	45.8	27	45.8
Calculated carbohydrate %	64.35	44.7	25.9	44.7	25.3
Calculated caloric content	408	507	601	507	601

At the end of the experiment, the animals were sacrificed and the plasma obtained from the collected blood were stored at -20 °C till analyzed. The lipids were extracted from the livers according to **Kates [17]** and stored at -20 °C till analyzed. The total cholesterol (TC) concentrations were determined according to **Allain et al [18]**, triglycerides (TG) according to **Wieland [19]**, high density lipoprotein-cholesterol (HDL-C) according to **Finaley [20]**, and low density lipoprotein cholesterol (LDL-C) according to **Friedewald et al [21]**. The T_3 and T_4 were determined using RIA technique according to **Abraham [22]**. The results were statistically analyzed using analysis of variance (ANOVA), F-test according to **Snedecor and Cochran [23]** and Duncan's multiple-range test according to **Duncan [24]**.

RESULTS

The mean plasma concentrations of cholesterol (mg/dl), triglycerides (mg/dl), HDL-cholesterol (mg/dl), LDL-cholesterol (mg/dl), T_3 (ng/dl) and T_4 (mg/dl) are presented in table 2. The hepatic lipid fractions concentration are presented in table 3.

1. Plasma lipid fractions:

The cholesterol concentration showed significant increase of 64% in the 3rd group while the 5th group revealed a decrease of 40% less than the control group. The triglycerides indicated an increase of 33% and 51% in the 2nd and 3rd groups respectively and showed a decrease of 24% and 28% in the 4th and 5th groups orderly. HDL-cholesterol indicated a decrease of 32% in the 3rd group but showed 46% and 10.7% increase in the 4th and 5th groups respectively. The LDL-cholesterol indicated an increase of 52% in the 3rd group, while showed a decrease of 32% in the 5th group.

2. Plasma T_3 and T_4 :

The plasma T_3 showed an increase of 43% and 55% in the 2nd and 4th groups, while plasma T_4 levels did not show a significant changes.

3. The hepatic lipid fractions:

There was a proportional increase in the hepatic lipid fractions with the increase of the dietary lipids intakes. The cholesterol concentrations revealed a significant increase of 25%, 51%, 11% and 20% in the 2nd, 3rd, 4th and 5th groups respectively. The triglycerides showed, in the same respective groups, an increase of 11%, 28%, 14% and 31%. HDL-

cholesterol indicated 33%, 63%, 75% and 78% increase in the same consecutive groups. LDL-

cholesterol showed in the same previous orderly groups and increase of 10%, 24%, 18% and 33%.

Table (2): Plasma lipid fraction concentrations and T₃ and T₄ levels in rats fed beef fat and fish oil for two weeks.

	G I (control)	G II	G III	G IV	G V
TC (mg/dl)	85.8 ^a ±6.4	110.3 ^{ab} ±9.3	140.6 ^b ±12.7	72.6 ^{ac} ±5.3	51.8 ^c ±3.8
TG (mg/dl)	190.7 ^a ±10.8	260.3 ^b ±15.3	290.7 ^b ±20.8	160.3 ^c ±8.8	151.6 ^c ±10.3
HDL-C (mg/dl)	27.6 ^a ±1.8	25.3 ^{ab} ±2.3	18.8 ^b ±1.5	40.2 ^c ±2.4	57.2 ^c ±4.3
LDL-C (mg/dl)	46.9 ^a ±2.3	58.1 ^{ab} ±3.6	71.3 ^b ±5.3	38.6 ^{ac} ±2.1	31.7 ^c ±1.9
T3 (ng/dl)	23.8 ^a ±1.6	34.0 ^b ±2.3	20.1 ^a ±1.8	37.0 ^b ±2.6	20.0 ^a ±1.9
T4 (µg/dl)	11.3 ^a ±1.4	11.1 ^a ±1.2	10.9 ^a ±1.3	11.3 ^a ±1.1	11.0 ^a ±1.5

Means ± S.E.; a,b,c = Means in the same rows sharing the same superscript are not significantly different from each other at P < 0.05; and Choles. refers to total cholesterol

Table (3): Hepatic lipid fraction concentrations in male rats fed animal fat and fish oil for two weeks.

	G I (control)	G II	G III	G IV	G V
TC (mg/100 g)	252.6 ^a ±10.1	314.8 ^b ±15.3	380.7 ^c ±20.3	280.4 ^{ab} ±20.1	302.0 ^b ±17.8
TG (mg/100 g)	290.3 ^a ±17.3	320.8 ^a ±25.6	370.8 ^b ±30.4	330.6 ^a ±21.6	380.6 ^{cb} ±20.3
HDL-C (mg/100 g)	80.3 ^a ±7.8	106.7 ^{ab} ±8.1	130.8 ^{bc} ±7.6	140.8 ^{bc} ±8.8	142.8 ^{bc} ±7.1
LDL-C (mg/100 g)	163.8 ^a ±10.1	180.7 ^{ab} ±10.3	203.6 ^b ±10.8	193.6 ^{ab} ±11.8	218.6 ^b ±15.3

Means ± S.E.; a,b,c = Means in the same rows sharing the same superscript are not significantly different from each other at P < 0.05.

Figure 1: Plasma lipid fraction concentrations levels in rats fed beef fat and fish oil for two weeks.

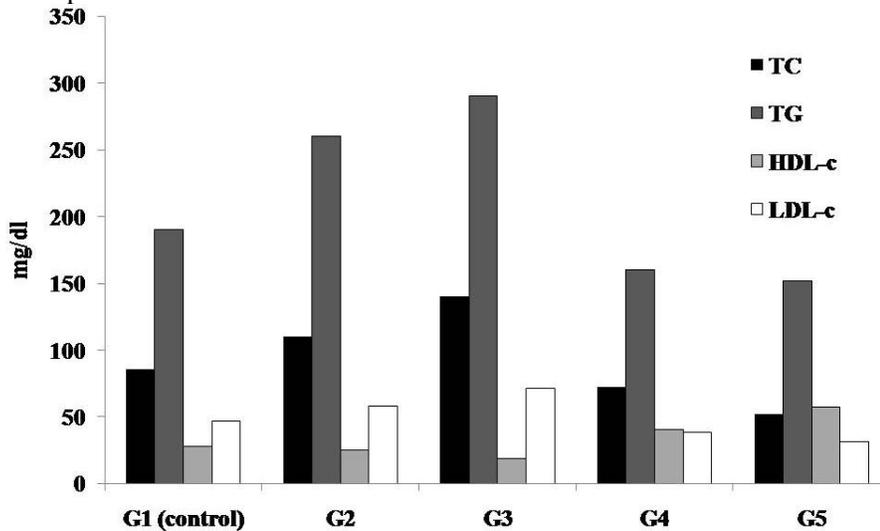
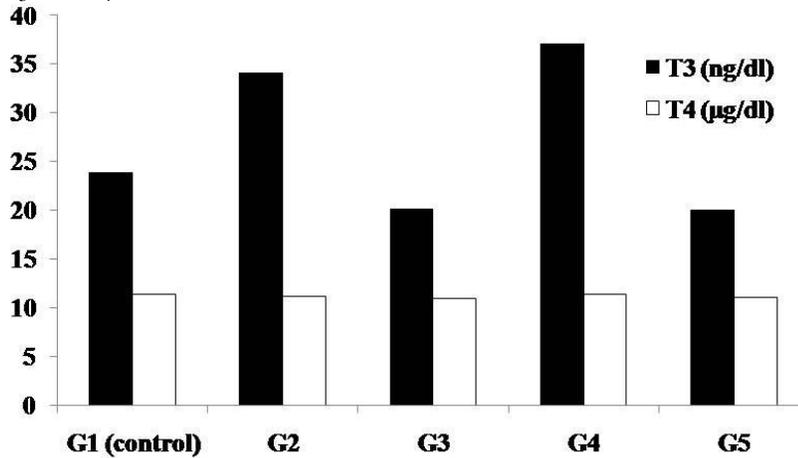
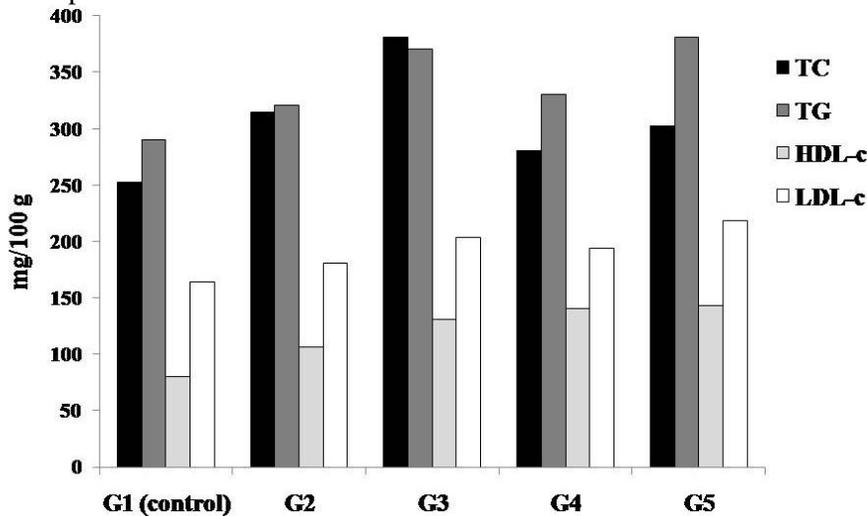


Figure 2: Plasma T₃ and T₄ levels in rats fed beef fat and fish oil for two weeks.**Figure 3:** Hepatic lipid fraction concentrations in male rats fed animal fat and fish oil for two weeks.

DISCUSSION

Cardiovascular disease is one of the major health problems in the world. It is dramatically increasing in the last 10 years [25]. Blood lipid profile determines the risk of cardiac disease. Lipid profile includes TC, HDL-C (often called good cholesterol), LDL-C (often called bad cholesterol) and TG [26]. Hyperlipidemia remains the strongest risk factor for CVD. Prevention of CVD risk factors such as obesity and dyslipidemia has been an important challenge in developing countries [26]. CVD is a major health problem in the world resulting in premature morbidity and mortality since it has been reported that high intake of cholesterol induces multiple cardiac complications including coronary heart disease and stroke [27].

The results of the current study demonstrated the effect of dietary fat saturation on both plasma and liver lipid fractions and lipoprotein. It would appear that the lipid fractions decreased in the group of animals fed fish oil (rich in ω -PUFA) while

increased in the groups of animals fed animal fat (rich in saturated fatty acids). These results are in agreement with a similar study of **Foxall and Shwaery** [28], who found that adhesion of platelets and lipid profile from swine fed fish oil was significantly lower than those fed butter fat. Moreover, **Kawasaki et al** [29] indicated that dietary fish oil and sulfur amino acid, L-methionine and L-cystine, have hypolipidemic effects in cancer-related hyperlipidemia, and that the effects of these two factors on the decrease in these serum lipid concentrations are additive; these two factors may affect the lipid metabolism via different pathways and mechanisms. However, several mechanisms of action for the hypocholesterolemic effect of polyunsaturated fats and plant proteins has been suggested by **Jackson et al** [30] including: (1) increase fecal excretion of bile acids and/or neutral steroids, (2) decrease cholesterol absorption, (3) decrease cholesterol synthesis, (4) increase cholesterol deposition in body tissues and (5)

altered lipoprotein structure and metabolism. Furthermore, **Wong et al [31]** concluded that the hypotriglyceridemia induced by fish oil feeding reflects; diminished lipogenesis; increase ketogenesis and fatty acid oxidation; as well as reduction in triglyceride secretion by the liver. However, the findings of **Bravo et al [32]** indicated that feeding rats mono- or n-6 polyunsaturated as compared to saturated fat in the diet promotes the storage of cholesteryl ester in the liver and leads to increased bile acid synthesis, resulting in the more rapid excretion of cholesterol originating from the diet via the bile. Moreover, the results of **Wong et al [33]** suggested that the TG-lowering effect of ω -3 FAEEs is associated with the decreased VLDL-TG secretion rate and hence lower plasma VLDL-TG concentration in obesity. Acute dietary n-3 PUFA dietary supplementation can improve fasting as well as postprandial lipid metabolism and components of the associated inflammatory response in the JCR:LA-cp rat. Further, moderate dose n-3 PUFA supplementation may reduce corresponding body weight during conditions of hypercholesterolaemia and/or modulate inflammation associated with obesity and the metabolic syndrome [34]

It would appear from the present study that the plasma T_3 levels increased in groups of animals fed diets containing 27% lipids, 44.7% carbohydrates and 507 calories. However, these results partially contraindicated the results of **Danforth et al [35]** who indicated that under caloric-conditions in human subjects, replacement of dietary carbohydrate with fat for 3 weeks, was associated with decreased T_3 and increased reverse T_3 levels. When additional calories were added as carbohydrates, the T_3 levels increased and the reverse T_3 decreased. If the additional calories were given as a fat, the T_3 and reverse T_3 levels did not change. The present study and those of **Davidson and Chopra [36]**, however, may not be directly comparable because of the different period of feeding the diet and the different proportion of carbohydrate, protein and lipids in the diets. Although increased lipid intake did not alter T_3 levels as reported by **Danforth et al [35]**, the T_3 production rate were enhanced by dietary fat [37]. Moreover, **Glade and Rimmers [38]** found a decrease in T_4 and an increase of T_3 levels in horse when fed diets containing 130% of their energy requirement, suggesting increased conversion of T_4 to T_3 . In addition **Gavin and Moeler [39]** showed that the increase in T_3 levels after high carbohydrate meals, have been associated with accelerated

peripheral conversion of T_4 to T_3 in rats. However, our results are consistent with the results of **Davidson and Chopra [36]** who suggested that non-carbohydrate as well as carbohydrate calorie sources are the important modulators of plasma T_3 levels in man, moreover, the influence of non-carbohydrate calories may be more pronounced than that of carbohydrate when at least a normal amount of carbohydrate is ingested.

With respect the correlation between fish oil and thyroid function, the data of **Souza et al [15]** suggested that the increase in thyroid hormone signaling pathways in the liver may be one of the mechanisms by which ω -3 PUFAs exert part of their effects on lipid metabolism. In addition, oral administration of EPA inhibited reduction of thyroid hormone levels and the change of thyroid follicles in induced hypothyroid rats. These findings suggested that FFA may affect thyroid functions and EPA may prevent induced hypothyroidism [14]

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