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Efficiency of dietary fish oil for regulation of hyperlipidemia and hyperglycemia in diabetic rats

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ABSTRACT

The efficacy of fish oil (FO) on serum biochemical parameters and performance was investigated in streptozotocin (STZ)-diabetic rats. Four weeks before the start of FO treatment, diabetes was induced in rats by a single intraperitoneal injection of streptozotocin (STZ = 60 mg/kg bodyweight). Then, male Wistar rats (n = 32) were randomly divided into 4 groups of non-diabetic (T1, control), treated non-diabetic (T2), diabetic (T3), and treated diabetic (T4). Standard rat non-purified diet was fed either alone or supplemented with an additional 2.5% fish oil. Diabetic rats had significantly increased serum levels of cholesterol, triglycerides, glucose and low density lipoprotein cholesterol (LDL-C) and decreased serum high DL-C concentration. The fish oil treatment of diabetic rats resulted in significant recovery in bodyweight, heart weight: bodyweight ratio and blood glucose levels. The administration of FO reduced cholesterol, triglyceride and LDL-C levels, and increased HDL-C levels, in the serum of diabetic rats. In conclusion, fish oil can reduce the risk of cardiovascular disease in diabetes with a significant improvement and regulation in lipid metabolism and also can moderate glucose level of diabetic rats and regulate its secretion that may be reduce of type 1 diabetes mellitus.

Key words: Fish oil, Diabetic rats, Serum glucose and lipids.

INTRODUCTION

The importance of omega-3 fatty acids in health promotion and disease prevention cannot be overstated. When every man and in every study, article and in anywhere, the topic is omega-3, the three most nutritionally important thereof, alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are deputies in response to reasons [1]. One of the key reason as to why omega 3 fish oil has such a powerful effect on fat and carbohydrate metabolism

is that the insulin levels secretion can be changed to FO or the omega-3 PUFAs thereof [2]. Fish oil have been administered to poultry to improve the body's ability to respond to insulin by stimulating the secretion of leptin, a hormone that helps regulate food intake, body weight and metabolism, and is expressed primarily by adipocytes or fat cells [3] and also, to decrease the amount of lipids (fats such as cholesterol and triglycerides) circulating in the bloodstream [4].

On the one hand, insulin resistance is associated with type 2 diabetes mellitus (T2DM), cardiovascular diseases (CVD), both independently and in association with the insulin resistance syndrome [5]. Decreased insulin sensitivity has been documented in those known to be at risk for T2DM [6]. In most people with T2DM, insulin resistance is generally present for many years before the diagnosis [7, 8].

An impressive body of evidence has established the link between dietary lipids, membrane lipids and insulin resistance in animal studies. Storlein *et al.* [9] showed that replacement of as little as 6 percent n-6 PUFAs (safflower oil) with long chain (LC) n-3 PUFAs (fish oil) is capable to prevent the development of insulin resistance. Further, n-6 PUFAs rich oil feeding intensifies insulin resistance when compared with feeding LC n-3 PUFAs [10, 11]. LC n-3 PUFAs supplementation can improve insulin action, reversing the adverse effects of saturated fatty acids (SFAs) and sucrose [12]. However in human, studies have not shown strong evidence as in rodents [13].

Protective effect of fish intake on the development of insulin resistance has been reported in prospective epidemiological studies [14]. The impact of LC n-3 PUFAs supplementation on metabolic parameters in healthy volunteers has not been investigated adequately. Some researchers have reported an improvement in insulin sensitivity in response to n-3 PUFAs supplementation [15], while others have observed no change [16, 17].

The role of these fatty acids in decreasing cholesterol and triglyceride levels has been proven [18, 19]. Fish oil was shown to favorably modify the balance of apolipoproteins [20]. Apolipoprotein B is the principle protein of LDL, comprising nearly 90% of total protein mass, and fish oil was shown to reduce apolipoprotein B. Earlier studies reported higher concentrations of LDL-C and lower concentrations of HDL-C in diabetic patients [21]. The administration of fish oil to diabetic and non-diabetic rats significantly decreased HDL-C and not significantly increased HDL-C compared with control animals. Further, increased dietary LC n-3 PUFAs may alter the binding affinity of the insulin receptor and improve glucose transport into cells via glucose transporters [22]. VLDL-triglycerides are important energy source in peripheral tissues, a mechanism based on fuel switching with reduced fatty acids and increased glucose utilization (the glucose-fatty acid cycle of Randle) cannot be ignored.

Postulated mechanisms for the putative beneficial effects of LC n-3 PUFAs on insulin action include beneficial alterations in the physical properties of the cellular membranes, such as increased fluidity [23, 24]

The present study was conducted in streptozotocin (STZ)-induced diabetic rats to determine whether fish oil could normalize irregular serum glucose and lipid levels, which are believed to play an important role in decline type 1 diabetes mellitus, regulates type 2 diabetes and atheroschlorosis resulting from heart disturbed with coronary disease.

MATERIALS AND METHODS

Animals, diets and housing

A total of 32 4-wk-old Male Wistar rats were assigned to rodent cages. The water and modified commercial rodent diet fed to rats free access during a 2-wk acclimation period as followed an environmentally controlled atmosphere (temperature 23°C, 45% relative humidity) with 15 air changes of 100% fresh HEPA filtered air per hour and a reverse 10 h:14 h light: dark cycle. The health status of the rats was monitored daily. After the acclimation period, the rats were weighed; tail painted, and randomly assigned to diet groups. The rats were randomly divided into 4 groups of non-diabetic (T1, control), treated non-diabetic (T2), diabetic (T3), and treated diabetic (T4) and fed the experimental diets for 6 wk. All male rats had same contents of serum glucose under 250 mg/dl in non-fasting conditions, before initiating the controlled feeding. Before the start of fish treatment (trail), diabetes was induced in rats by a single intra-peritoneal injection of STZ (60 mg/kg bodyweight) dissolved in 0.2 mL of 0.1 mol/L citrate buffer, pH 4.5. Control rats were injected with the vehicle (0.2 mL of 0.1 mol/L citrate buffer, pH 4.5) alone. Three days after STZ injection, development of diabetes was confirmed by analyzing tail vein blood glucose levels. Rats with blood glucose levels of 200–300 mg/dL and glycosuria were considered to be diabetic and prepared to a six weeks fish oil treatment. Standard rat non-purified diet (Mouse/Rat sterilized Diet, Pars Co., Karaj) was fed either alone (T1 or T3) or supplemented with additional 2.5% fish oil (T2 and T4, Table 2). Fish oil was obtained from Iranian sources (Mehregan Khazer Co., Bander Abbass, Iran). Body weights were recorded every 2 wk, beginning before the initiation of the experimental diet period.

In diabetic rats, the treatment was started 4 weeks after the onset of diabetes because, in many studies, the duration of diabetes required to induce cardiac dysfunction in experimental rats has been found to be 4 until 6 weeks.

Serum lipid and glucose analysis

Tail blood samples for measurement of glucose and low density lipoprotein cholesterol (LDLC) and high-density lipoprotein–cholesterol (HDL-C) levels 15-µl blood samples were collected after 16–18 h of food deprivation at the start of the experiment and 6 wk thereafter. Serum was separated from RBC by centrifugation at 1100 *g* at 4°C and assayed for total, cholesterol, and triglyceride concentrations on a ALCYON-300, automated analyzer (American) using fully enzymatic methods and were determined using diagnostic kits from commercial kit (Kone kit, Japan). Blood glucose was monitored regularly once a week and blood glucose concentrations were measured using an Ames glucometer (Bayer Diagnostics, France).

Statistical analysis

Results are expressed as the mean ± SD for six animals in each group. Statistical analysis was performed using one-way analysis of variance (anova) followed by the post hoc least significant difference (LSD) test. Statistically significant variations were compared as follows: control versus fish oil-treated control rats, control versus diabetic rats and diabetic rats versus fish oil-treated diabetic rats. Results were considered significantly different if $P < 0.05$.

RESULTS AND DISCUSSION

Bodyweight, heart weight and heart weight: bodyweight ratio

Table 2 gives the body weight, heart weight and heart weight: bodyweight ratio in experimental animals. There was a significant decrease in bodyweight with a significant increase in heart weight: body weight ratio in diabetic rats compared with control rats (Figure 1). However, fish

oil treatment of diabetic rats increased body weight and decreased the heart weight: body weight ratio.

In diabetic rats, there was a significant decrease in bodyweight, with an increase in the heart weight: body weight ratio, as reported in past studies [2]. The increase in the heart weight: bodyweight ratio is indicative of cardiac hypertrophy, which is due to accumulation of cholesterol, triglycerides, phospholipids and glycated protein in the myocardium [25]. Administration of fish oil resulted in a significant increase in bodyweight and a reduction in the heart weight: bodyweight ratio in diabetic rats (Figure 1). This could be due to improved glycaemic control and hypolipidaemic activity produced by fish oil in diabetic rats.

Table 1. Bodyweight, heart weight and heart weight: bodyweight ratio in experimental animals

	Control rats	Control rats + fish oil	Diabetic rats	Diabetic rats + fish oil
At the start of trial				
BW (g)	507 ± 11	499 ± 8	275 ± 10*	391 ± 14*†
HW (mg)	1118 ± 42	1202 ± 3.3	920 ± 13*	855 ± 8*†
HW : BW ratio (mg/g)	2.16 ± 0.24	2.17 ± 4.7	3.22 ± 0.12*	2.25 ± 0.08*†

*Values are expressed as the mean±SD for six animals in each group. *P < 0.05 compared with control; †P < 0.05 compared with diabetic rats. BW= bodyweight; HW= heart weight.*

Blood glucose and lipids

Streptozotocin-induced diabetic rats showed consistent fasting hyperglycaemia throughout the study, with signs of polyuria, polydipsia and polyphagia. Also, a significant increase in serum levels of cholesterol, triglycerides, and LDL-C, with a concise decrease in HDL-C, in diabetic rats, as indicated in Tables 2 and 3. At the end of trial, diabetic rats had increased blood glucose values compared with control and non-diabetic rats. Fish oil treatment not significantly reduced blood glucose levels in diabetic rats but significantly increased in non-diabetic rats compared with control animals. Also, the administration of fish oil to diabetic and non-diabetic rats significantly decreased serum cholesterol, triglycerides and not significantly increased HDL-C compared with control animals.

The STZ-induced diabetic rats showed consistent fasting hyperglycaemia throughout the study and the administration of fish oil to diabetic rats regulate elevated blood glucose levels, while increased in non diabetic rats in compared with control groups. Fish oil have been shown to reduce elevated blood glucose levels in both type 1 and type 2 diabetic animals [26, 27]. Fish oil improved glucose tolerance in normal rats [28].

Insulin is a hormone that reduces the use of fat for fuel. While promote fat storage in the presence of excess calories. It inhibits the action of hormone sensitive lipase, which is responsible for beraking down stored fat and preparing in for use as energy. In addition, insulin activites an enzyme, which, along with fatty acid synthesis, is responsible for converting carbohydrate into fat [29]. Therefore, the drop in insulin levels when diet supplemented with fish oil, would have allowed more fat to be used for energy accompanying raise blood glucose. Researchers previously have been reported that diet rich in polyunsaturated fatty acid increase the amount of serum glucose because of decline insulin secretion [30, 31]. Mori *et al.*, [32] were reported that with feeding Dietary fish and fish oil / meal to human and animals, decreased blood pressure, glucose content were higher ($P<0.05$). Long chain n-3 enrichment of a high-saturated fat diet exerts a rapid effect to lower insulin secretion from the islets of langerhans and raising the plasma glucose concentration [2, 33]. The presented findings did showed regulative

effect of fish oil on raised glucose level of blood in diabetic rats (Figure 1) and while increased serum glucose content in non-diabetic rats because of the drop insulin level.

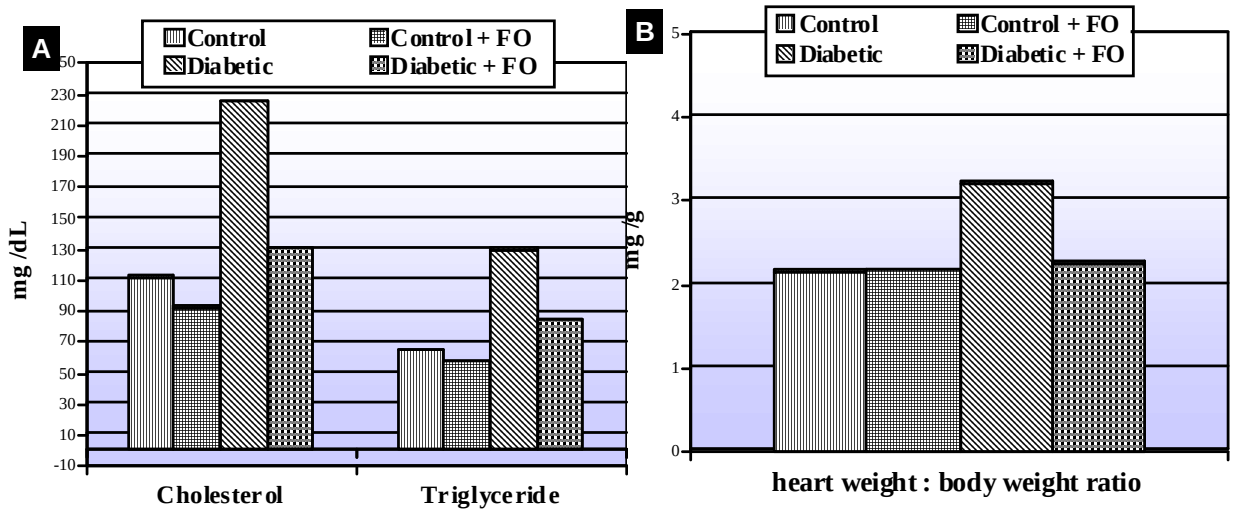

Figure 1: Serum cholesterol and triglyceride (A) and heart weight : bodyweight ratio (B) of non-diabetic and diabetic rats. Diabetes was induced in rats by a single intraperitoneal injection of streptozotocin (STZ = 60 mg/kg bodyweight). Values of figures are correspondent to those shown in Table 1 and 3. FO= fish oil

Diabetes is a disease of carbohydrate metabolism whose hallmark is high blood sugar. Type 1 diabetes reflects lack of insulin, the hormone that controls blood sugar. Type 1 diabetes is excluded from this discussion. In type 2 diabetes, which is much more common than type 1, insulin is present, but it functions improperly. As a result, blood sugar levels rise. In diabetes, VLDL levels are markedly increased, thereby increasing the likelihood of heart disease. Because, as a result both sugar and fatty acid levels in blood, rise. The pancreas makes more insulin in an effort to clear the blood sugar. The liver takes up the fatty acids and returns them to the blood as fat hitched to proteins. As diabetes progresses, the pancreas loses its ability to produce insulin. This leads to deterioration in other tissues and the development of circulatory problems, hypertension, kidney disease, impaired regulation of blood clotting, retinopathy, and above all heart disease. Treatment with drugs, diet, weight loss, and exercise can retard and possibly halt this chain of events. Diet enrichment, especially by omega-3 fatty acids is a frontline strategy for controlling diabetes. Likewise, restricting the consumption of saturated and Trans fats can improve blood lipid levels and may slow the progression of diabetes. While “Omega-3s” from fish are highly polyunsaturated fatty acids that lower triglycerides, reduce abnormal heart rhythms, reduce blood pressure by small but significant amounts, and improve blood clotting regulation. Therefore, eating fatty fish regularly is an important strategy to improve health in diabetes.

Table 2. Blood glucose, low-density lipoprotein–cholesterol and high-density lipoprotein–cholesterol in the serum of experimental animals

	Control rats	Control rats + fish oil	Diabetic rats	Diabetic rats + fish oil
Blood glucose (mg/dL)	132 ± 3.4	159 ± 4.7	484 ± 4.1*	259 ± 1.9*†
Serum TC (mg/dL)	112 ± 7	92 ± 4*	225 ± 6*	131 ± 11*†
Serum TG (mg/dL)	65.1± 4.8	57.7 ± 2.1*	129.8 ± 4.4*	84.8 ± 1.8*†

*Values are expressed as the mean±SD for six animals in each group. *P < 0.05 compared with control; †P < 0.05 compared with diabetic rats. TC= total cholesterol; TG= triglycerides;*

Table 3. Blood glucose, low-density lipoprotein–cholesterol and high-density lipoprotein–cholesterol in the serum of experimental animals

	Control rats	Control rats + fish oil	Diabetic rats	Diabetic rats + fish oil
LDL-C (mg/dL)	74.41 ± 1.61	70.23 ± 2.07*	123.2 ± 2.45*	80.18 ± 2.41*†
HDL-C (mg/dL)	41.14 ± 1.73	45.11 ± 1.44*	32.44 ± 1.81*	35.15 ± 2.01*†

Values are expressed as the mean±SD for six animals in each group. *$P < 0.05$ compared with control; †$P < 0.05$ compared with diabetic rats. LDL-C= low-density lipoprotein–cholesterol; HDL-C= high-density lipoprotein–cholesterol.

Serum lipoproteins

Fish oil treatment of diabetic rats improves hyperlipidemia with regulate serum lipids. Christopher *et al.* [2] were reported that omega-3 fatty acids reduce the blood VLDL levels, acting to lower the circulating free LDL concentration and also, reduce the rate of triglyceride synthesis in the liver. Researchers were showed that low HDL and high LDL are values associated with atherosclerosis and coronary heart disease. Changes in the plasma lipoprotein profile resulting in an increased risk of coronary heart disease have been reported in diabetes [34]. In diabetes, the VLDL undergoes changes in structure and composition such that they may become poorer substrates for lipoprotein lipase. Lipoproteins of diabetic origin are hydrolysed by lipoprotein lipase at a significantly slower rate than lipoprotein from normal rats [34]. Thus, in diabetes, there is a decrease in the clearance of VLDL [35]. Earlier studies reported higher concentrations of LDL-C and lower concentrations of HDL-C in diabetic patients [21]. The administration of fish oil to diabetic and non-diabetic rats significantly decreased HDL-C and not significantly increased HDL-C compared with control animals.

In the present study, the serum levels of lipoproteins were altered in diabetic rats and administration of fish oil to diabetic rats ameliorated these modifications. It has been reported that FO suppresses LDL-C and not significantly decreased HDL-C in rats fed omega-3 enriched diet [18]. Apolipoprotein B is the principle protein of LDL, comprising nearly 90% of total protein mass, and fish oil was shown to reduce apolipoprotein B [20]. Therefore FO was shown to favorably modify the balance of apolipoproteins.

CONCLUSION

In conclusion, the dietary administration of fish oil to streptozotocin-induced diabetic rats was regulated the elevated blood glucose and lipids levels and also increased body weight and decreased the heart weight: body weight ratio. However, studies on the effect of fish oil on lipid metabolism in diabetes are varied. But further research need carried out to understand regulative effect of fish oil on blood glucose especially in diabetic animals.

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