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Effect of Fish Oil Supplementation on Cholesterol and Blood Glucose Levels of Diabetic Rats

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Abstract: An experiment was conducted to study the effect of fish oil supplementation on blood glucose and lipids levels in non-diabetic or streptozotocin (STZ) induced diabetic rats. Male *Wistar* rats (n = 32) were randomly assigned into 4 groups of non-diabetic (T1, control), treated non-diabetic (T2), diabetic (T3) and treated diabetic (T4). Standard rat nonpurified diet was fed either alone or supplemented with an additional 2.5% fish oil (treated rats). Serum glucose and low and high density lipoprotein cholesterol (LDL-C and HDL-C, respectively) levels were determined at the start of the experiment and 6 wk thereafter. The administration of FO significantly reduced LDL-C levels and increased HDL-C levels, in the serum of diabetic rats. At the end of trial, diabetic rats had increased blood glucose values compared with control and non-diabetic rats. Fish oil treatment not only, reduced blood glucose levels in diabetic rats, but also, increased the level in non-diabetic rats as compared with control animals. The administration of fish oil to diabetic and non-diabetic rats significantly decreased HDL-C and not significantly increased HDL-C as compared with control animals. Hence, these results suggested that supplementation of FO effectively moderate glucose level and reduce bad-cholesterol level of blood in diabetic rats that may prevent type 1 *diabete mellitus*.

Key words: Fish oil · Diabetic rats · Blood Glucose and Cholesterol

INTRODUCTION

The importance of omega-3 fatty acids in health promotion and disease prevention cannot be overstated. The three most nutritionally important omega-3 fatty acids are alpha-linolenic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). 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 [1]. Protective effect of fish intake on the development of insulin resistance has been reported in prospective epidemiological studies [2]. The impact of LC n-3 PUFAs supplementation on metabolic parameters in healthy volunteers has not been adequately investigated. Some researchers have reported an improvement in insulin sensitivity in response to n-3 PUFAs supplementation [3, 4], while others have observed no change [5, 6]. 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 improves 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

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important strategy to improve health in diabetes. On the other hand, the role of omega-3 fatty acids in decreasing cholesterol and triglyceride levels has been proven [7, 8]. Fish oil was shown to favourably modify the balance of apolipoproteins [9]. 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 [10]. However, studies on the effect of fish oil on lipid metabolism in diabetes are varied. But, further researches are needed to understand regulative effect of fish oil on blood glucose, especially in diabetic animals. Therefore, an experiment was conducted to determine the effect of fish oil supplementation on blood glucose and lipids level in diabetic or non-diabetic rats.

MATERIALS AND METHODS

Animals and Diets: Male *Wistar* rats (n = 32; 4 wk old) were housed rodent cages with free access to a modified rodent diet (Pars Co. Karaj) and water during a 2-wk acclimation period. 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 a non-fasting conditions, before initiating the controlled feeding. Before the start of fish treatment (trail), diabetes was induced in rats by a single intraperitoneal injection of streptozotocin (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 analysing tail vein blood glucose levels. Rats with blood glucose levels of 200-300 mg/dL and glycosuria were considered to be diabetic [11] and prepared to a six weeks fish oil treatment. Standard rat nonpurified diet (Mouse/Rat Sterilizable Diet, Pars Co. Karaj) was fed either alone (T1 and T3) or supplemented with an additional 2.5% fish oil (T2 and T4, Table 2).

Blood Sampling: Tail blood samples for measurement of glucose and LDL-C and HDL-C levels, were collected at the start of the experiment and 6 wk thereafter. Serum after centrifugation at 1100 *g* at 4°C assayed for biochemical analysis by ALCYON-300, that were determined using diagnostic kits (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: Data were 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

Blood Glucose and Lipids: Diabetic rats showed consistent fasting hyperglycaemia throughout the study. At the end of trial, diabetic rats had increased blood glucose values compared with control and non-diabetic rats, as indicated in Table 1. Fish oil treatment not significantly ($p<0.5$) reduced blood glucose levels in diabetic rats but significantly ($p<0.5$) increased in non-diabetic rats compared with control animals.

Table 1: Blood glucose, low-density lipoprotein-cholesterol and high-density lipoprotein-cholesterol in the serum of experimental animals

	T1	T2	T3	T4
At the start of trialBlood glucose (mg/dL)	134 ± 4.2	133 ± 3.3	480 ± 6.4*	484 ± 2.8*†
At the end of trialBlood glucose (mg/dL)	132 ± 3.4	159 ± 4.7	484 ± 4.1*	259 ± 1.9*†

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. T1= non-diabetic, control, T2= treated non-diabetic, T3= diabetic and T4= treated diabetic.

Table 2: LDL-cholesterol and HDL-cholesterol in the serum of experimental animals

	T1	T2	T3	T4
At the start of trial LDL-C (mg/dL)	72.62 ± 1.17	74.11 ± 2.07*	122.3 ± 2.6*	124.12±2.29*†
HDL-C (mg/dL) At the end of trial	42.14 ± 1.73	42.31 ± 1.33*	30.86 ± 1.81*	31.15±2.01*†
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. T1= non-diabetic, control, T2= treated non-diabetic, T3= diabetic and T4= treated diabetic.

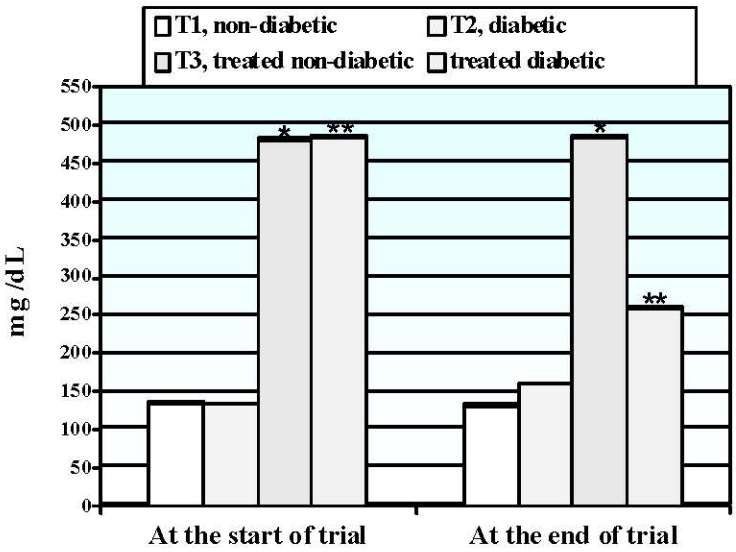

Fig. 1: Blood glucose, in the serum of experimental rats

The administration of fish oil to diabetic and non-diabetic rats significantly ($p<0.5$) decreased HDL-cholesterol and not significantly ($p<0.5$) increased HDL-C compared with control animals.

DISCUSSION

Streptozotocin-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 as compared with the control groups. Fish oil have been shown to reduce elevated blood glucose levels in both type 1 and type 2 diabetic animals [12]. Fish oil improved glucose tolerance in normal rats [4, 13]. Researchers previously has been reported that diet rich in polyunsaturated fatty acid increase the amount of serum glucose because of decline insulin secretion [14, 15]. Mori *et al.* [16] reported that with feeding dietary fish and fish oil / meal to human and animals, decreased blood pressure, glucose content were higher . 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 [1, 6]. The current findings did showed regulative effect of fish oil on raised glucose level of blood in diabetic rats and while increased serum glucose content in non-diabetic rats because of the drop insulin level.

Diet enrichment, especially by omega-3 fatty acids is a frontline strategy for controlling diabetes. Likewise, restricting the consumption of saturated and trans fats improves 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.

Figure 1. Serum glucose 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. $^{*}P < 0.05$ compared with non-diabetic rats; $^{**}P < 0.05$ compared with diabetic rats

Serum Lipoproteins: Dietary fish oil declined bad lipoprotein (LDL-cholesterol) streptozotocin-induced diabetic rats and regulate serum lipids in treated rats. The role of these fatty acids in decreasing cholesterol and triglyceride levels has been proven [1, 7, 8].

Christopher *et al.* [1] 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. 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 [7]. Apolipoprotein B is the principle protein of LDL, comprising nearly 90% of total protein mass and fish oil was shown to reduce apolipoprotein B [9]. Therefore FO was shown to favourably modify the balance of apolipoproteins.

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