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Blood lipid levels and aryl esterase activity of alloxan-induced diabetic rats: effects of 2 weeks omega-3 fatty acid supplementation

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ABSTRACT

Diabetes mellitus is a complex metabolic disorder associated with chronic hyperglycaemia and hyperglucosuria arising from insulin deficiency. It is accompanied by dyslipidemia, a major risk factor contributing to cardiovascular diseases. Therefore, the effects of omega-3 fatty acid (O3FA) supplement on plasma, erythrocyte and lipoproteins lipid profiles as well as aryl esterase activities in plasma and the lipoproteins of alloxan-induced (150 mg/kg body weight (b.wt), intraperitoneally) diabetic male rats were investigated. O3FA (0.4 g/kg b.wt/day) was administered as pre- and post- treatment for 2 weeks. The blood parameters were measured spectrophotometrically. The lipid levels were significantly increased ($p<0.05$) in the various compartments of the diabetic rats. The increase was from 30% - 107% for cholesterol, 20% - 52% for phospholipid and 38% - 111% for triacylglycerol. Lipid peroxidation was also increased from 67% in extracellular fluid to 164% in the high density lipoprotein (HDL) compartment. O3FA administration significantly reduced ($p<0.05$) the levels of cholesterol, phospholipid and triacylglycerol by varying extents in the various compartments while increasing cholesterol and phospholipid levels in HDL. Lipid peroxidation was significantly reduced ($p<0.05$) by O3FA suggesting that it has protective effect against oxidative damage. Aryl esterase activities in the lipoproteins were also activated by supplementation with O3FA (except in HDL pre-treatment). This study indicates that O3FA supplement attenuates dyslipidemia associated with diabetes mellitus and should be included in diets for prevention and treatment of diabetes mellitus.

Keywords: Diabetes mellitus, omega-3 fatty acid, dyslipidemia, aryl esterase

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INTRODUCTION

Diabetes mellitus, often simply referred to as diabetes, is a group of metabolic diseases in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced [1].

The complications which may result from diabetes mellitus include chronic and acute complications such as diabetic retinopathy, diabetic nephropathy, diabetic foot ulcers, cardiovascular diseases and macrovascular diseases such as ischemic heart diseases (angina and myocardial infarction), stroke and peripheral vascular disease [2]. A prominent

biochemical mechanism of the pathogenesis of these complications is disorder of lipid metabolism. While it was estimated that 150 million people suffer from diabetes mellitus, and the number might double in 2025 by WHO [3], the IDF [4] estimated that 382 million people had diabetes and that by 2030, this number will almost double. Diabetes affects approximately 6.4% of the world's population with the highest prevalence in North America and Caribbean (10.2%) followed by middle East and North Africa (9.3%) [4]. Most of the cases of diabetes in developing countries occur due to population growth, unhealthy diets, ageing, obesity and sedentary lifestyle [3]. Hyperlipidemia is an associated complication of diabetes mellitus [5]. The ability of a hypolipidemic agent to lower blood glucose levels is a significant advantage in the management of diabetes mellitus. However, control of blood glucose levels is only one goal of a healthy eating plan for people with diabetes. A diet for those with diabetes should also help achieve and maintain a normal body weight as well as prevent heart and vascular disease, which are frequent complications of diabetes [6].

Aryl esterase, also known as paraoxonase (PON1), is a high density lipoprotein (HDL) linked enzyme which hydrolyzes phenyl acetate to phenol and acetate. It is a calcium-dependent esterase [7]. The human PON gene family consists of three members, PON1, PON2, and PON3, aligned next to each other on chromosome 7 [8]. One natural physiological function of PON1 appears to be the metabolism of toxicoxidized lipids of both low density lipoprotein (LDL) particles as well as HDL particles [9]. Studies in the last 2 decades have demonstrated PON1's ability to protect against atherosclerosis by hydrolyzing specific derivatives of oxidized cholesterol and/or phospholipids in oxidized low-density lipoprotein and in atherosclerotic lesions. It may also be an important modulator of cardiovascular disease risk [10, 11 and 12].

Omega-3 fatty acids (O3FA) from seafood and plant sources can reduce coronary heart disease [13]. There is evidence that rheumatoid arthritis sufferers taking O3FA have reduced pain compared to those receiving standard non-steroidal antiinflamatory drugs. The possible mechanism of anti-inflammatory effect of O3FA is the decreased levels of arachidonic acid inflammatory pathway in the cells [14]. Administration of O3FA to a group of 14 patients with familial combined hyperlipidemia for eight weeks resulted in a modest (10%) but significant increase in plasma PON1 concentration [15].

Therefore, the effects of omega-3 fatty acid (O3FA) supplement on plasma and erythrocyte lipid profiles and arylesterase activities in plasma and the lipoproteins of alloxan-induced diabetic male rats were investigated.

MATERIALS AND METHODS

Experimental Animals

Thirty-five male albino rats, weighing 145-220g were used for this study. They were housed in a quiet room with 12:12-hr light-dark cycle room temperature, 25^{o}C. Acclimatizing was for two weeks, after which they were divided into seven groups of five animals each. The normal control rats were given distilled water and paraffin oil; control diabetic rats: alloxan and paraffin oil; control omega-3 fatty acid rats: distilled water and omega-3 fatty acids; post-treatment rats: alloxan followed by omega-3 fatty acid; pre-treatment rats: omega-3 fatty acid followed by alloxan; Control baseline and diabetic baseline rats were respectively given distilled water and alloxan only. Water and rat chow were provided *ad libitum*. The rats were checked daily and their body weights were recorded accordingly till the end of the experiment.

Induction of Diabetes

Diabetogenesis in the rats was by intraperitoneal injection of alloxan monohydrate following the methods of Al-Haj Baddar et al [16] and Rajasekaran et al [17] after an 8 hr fasting with modification. The rats were not allowed to eat but had free access to water over 2 days. They were then given 5% glucose solution over 24 hours after injection to overcome drug-induced hypoglycemia. The blood glucose level of the rats was determined before and after induction of diabetes at two days interval for seven days using an on-call glucometer and test strips manufactured by CAN laboratory, Inc., USA. Rats with fasting blood glucose greater than 200 mg/dl were considered diabetic and used in the study.

Administration of Omega-3 fatty acid supplement

Omega-3 fatty acid (containing 1000 mg Pure Salmon oil of 320 mg EPA and 180 mg DHA) was administered for 2 weeks to the rats orally at the dosage of 0.4 g/kg body weight of rats/day. Omega-3 fatty acid capsules were obtained from NOW FOODS, Bloomingdale, IL, USA.

Samples collection
After nineteen days of treatment, the rats were weighed and sacrificed after overnight fast under light ether anaesthesia. Blood samples were collected by cardiac puncture using heparinised needle and syringes into heparinised tubes. The blood samples were centrifuged at 4,000rpm for 10minutes to separate the plasma from the red blood cells (RBC).

Isolation of HDL
The procedure of Gidez et al [18] was used to isolate HDL from plasma. Heparim-$MnCl_2$ (20µl) was added to 200µl of plasma in a test tube and the resultant mixture was thoroughly mixed and allowed to stand for 10 min. This was centrifuged at 4000 rpm for 20 min. and the supernatant decanted into Eppendorf tubes. The supernatant (HDL) and residue very low density lipoprotein + low density lipoprotein (VLDL+LDL) were stored at -20 ºC for analysis.

Biochemical analyses
Plasma and HDL lipid profiles
The plasma and HDL concentrations of total cholesterol, triacylglycerol and phospholipid were determined spectrophotometrically using Cypress diagnostic kits.

RBC lipid profile
Lipids were extracted from the RBC using chloroform - isopropanol (7:11, v/v) as described by Rose and Oklander [19]. For cholesterol determination, 0.1 ml of RBC extract was evaporated to dryness at 60^{o}C and 20 µl of Triton X-100/chloroform mixture (1:1, v/v) was added to the dried extract for resolution. This was evaporated again and then 1 ml of the cholesterol kit reagent was added, mixed and incubated for 30 min. before reading the absorbance. Triacylglycerol concentration was determined by evaporating to dryness 0.1 ml of the extract and then adding 0.1 ml of 97% ethanol to re-suspend the dried lipid. To this suspension, 1 ml of the triacylglycerol kit reagent was added, mixed and incubated for 30 min. before the absorbance reading was taken. For phospholipids determination, 0.1 ml of the extract was evaporated and 1 ml of the phospholipid kit reagent was added, mixed and incubated for 30 min. before taking absorbance reading.

Determination of aryl esterase activity in plasma, HDL and VLDL
Paraoxonase (PON1) activity as aryl esterase using phenylacetate as substrate was determined in plasma and HDL in 100 mM Tris-acetate buffer containing 10 mM Calcium chloride as described by Junge and Klees [20]. The rate of phenol generation was monitored at 270 nm and a molar extinction coefficient of 148 was used to calculate the enzyme activity.

Phenylacetate solution (1.0 ml) was added to 1.0 ml of Tris-acetate buffer and allowed to stand for 10 min. at room temperature. The mixture was poured into a quartz cuvette and used to zero the spectrophotometer; then 20 µl of HDL or 10 µl of plasma was added and mixed thoroughly. The absorbances of the different samples were read at 270nm and at 30 sec interval for 3 min.

For VLDL aryl esterase activity, the precipitated VLDL+LDL was first brought to original plasma volume by adding 100 mM Tris-acetate buffer after which the PON activity associated with the fraction was determined as above.

Estimation of lipid peroxidation
Lipid peroxidation was determined according to the procedure of Bouge and Aust [21] spectrophotometrically by measuring the thiobarbituric acid reactive substances (TBARS) in the various compartments. An aliquot (0.1 ml) of the supernatant was treated with 2 ml of the TBA reagent (thiobarbituric acid, hydrochloric acid, Trichloroacetic acid in 1:1:1 v/v/v). It was incubated at 95 ^{o}C for 15 min, placed on ice and then centrifuged. The clear supernatant was then read at 535 nm.

Statistical analyses
Results are expressed as mean ± standard deviation (S.D) and the level of homogeneity among the results of groups was tested using analysis of variance (ANOVA) with $p < 0.05$ considered as significant. Where heterogeneity occurred, the groups were separated using Duncan Multiple Range Test (DMRT). All analyses were done using Statistical Package for Social Sciences (SPSS) version 16.

RESULTS AND DISCUSSION

The effects of O3FA supplementation on cholesterol levels in the plasma, RBC and the lipoproteins was shown in table 1. Cholesterol levels were significantly ($p< 0.05$) increased in the diabetic groups compared to the control groups in plasma and RBC. The percentage increase in the cholesterol level in the plasma and RBC of the diabetic control were 30 and 107 compared with the normal control. The reverse was observed for the HDL cholesterol. As observed in the fluids, the cholesterol levels of LDL and VLDL were elevated in the diabetic groups in comparison to the control groups. The O3FA supplementation significantly ($p< 0.05$) reduced the cholesterol levels in the plasma (18%, 19%) and RBC (56%, 61%), LDL (44%, 38%) and VLDL (40%, 22%) in the diabetic omega-3 and omega-3 diabetic groups respectively except HDL where an increase (102%, 69%), was observed when compared with diabetic control group.

Table 1. Effects of O3FA on cholesterol levels (mg/dL) in plasma, RBC and lipoproteins

Group	Plasma	RBC	HDL	LDL	VLDL
Normal control	61.97±4.06^{a}	40.74±1.09^{a}	25.20±7.02^{a}	29.09±2.00^{a}	9.68±0.18^{a}
Diabetic control	80.75±7.02^{c}	84.53±4.61^{d}	18.65±1.65^{b}	39.45±3.75^{c}	18.86±2.75^{c}
Omega-3 control	54.97±4.79^{b}	39.06±1.26^{a}	38.18±8.65^{c}	8.52±2.75^{b}	8.14±0.08^{a}
Diabetic omega-3	66.25±9.01^{a}	37.46±0.41^{a}	33.69±8.47^{c}	22.25±1.25^{a}	11.28±1.57^{a}
Omega-3 diabetic	65.22±9.17^{a}	32.84±1.23^{b}	28.19±4.01^{a}	24.30±1.50^{a}	14.72±1.09^{b}
Control baseline	64.02±9.63^{a}	31.35±1.75^{b}	26.49±2.64^{a}	28.58±2.50^{a}	10.95±1.18^{a}
Diabetic baseline	110.87±10.49^{d}	54.38±6.75^{c}	20.59±5.28^{b}	66.40±3.50^{d}	20.53±2.02^{d}

Values are mean ±S.D (n=5). Values with different superscript are significantly different from one another (p<0.05)

In table 2, the effects of O3FA on triacylglycerol levels in HDL, plasma and RBC were summarized. The elevated triacylglycerol level was sustained throughout the experiment as shown by the values obtained for the diabetic baseline and the diabetic control. The triacylglycerol level of the diabetic groups was significantly ($p< 0.05$) higher than their control groups and the increase was 38%, 111% and 77% in the HDL, RBC and plasma respectively in the diabetic control compared with normal control. O3FA supplementation reduced the elevated triacylglycerol level in the plasma of the diabetic control by 37% and 23% in the diabetic omega-3 and omega-3 diabetic groups respectively. The reduction of the triacylglycerol in the HDL of the diabetic control was 34% and 37% in the diabetic omega 3 and omega 3 diabetic accordingly.

Table 3 depicts the effects of O3FA on phospholipid levels in the HDL, plasma and RBC. There were elevations in phospholipid levels in plasma and RBC of the diabetic animals and the reverse was the case for HDL. The phospholipid levels in the plasma and RBC of the diabetic groups were significantly ($p<0.05$) higher than that of the normal control. The administration of O3FA reduced the phospholipid levels in the RBC of the diabetic control by 36% and 30% in the diabetic omega-3 and omega-3 diabetic groups respectively. The reduction in the phospholipids' level of HDL was 32% in the diabetic control compared with normal control. O3FA increased the phospholipids' level of the HDL by 42% and 55% in the diabetic omega 3 and omega 3 diabetic groups compared with diabetic control respectively.

Table 2. Effects of O3FA on triacylglycerol levels (mg/dL) in plasma, RBC and HDL

Group	Plasma	RBC	HDL
Normal control	46.45±6.26^{a}	20.34±1.89^{a}	20.69±6.97^{a}
Diabetic control	82.26±4.56^{d}	42.90±3.52^{c}	28.65±1.65^{c}
Omega-3 control	39.72±6.36^{b}	17.36±1.40^{a}	15.09±4.11^{b}
Diabetic omega-3	51.45±7.77^{a}	30.69±1.79^{b}	18.81±2.47^{c}
Omega-3 diabetic	62.99±4.10^{c}	28.40±2.07^{b}	18.19±4.01^{c}
Control baseline	51.07±7.27^{a}	21.32±3.05^{a}	20.93±5.94^{a}
Diabetic baseline	109.07±5.94^{e}	43.85±2.48^{c}	30.09±1.48^{c}

Values are mean ±S.D (n=5). Values with different superscript are significantly different from one another (p<0.05)

Table 3. Effects of O3FA on phospholipid levels (mg/dL) in plasma, RBC and HDL

Group	Plasma	RBC	HDL
Normal control	110.85±26.51^{a}	160.01±9.98^{a}	102.58±16.97^{a}
Diabetic control	133.26±22.34^{c}	242.90±13.75^{d}	69.68±9.45^{b}
Omega-3 control	92.72±17.97^{b}	91.16±11.22^{b}	110.64±14.11^{a}
Diabetic omega-3	111.62±21.90^{a}	155.86±12.54^{a}	98.93±12.47^{a}
Omega-3 diabetic	111.13±20.62^{a}	187.11±17.18^{c}	108.09±14.01^{a}
Control baseline	94.73±16.18^{b}	153.98±13.86^{a}	92.13±11.33^{a}
Diabetic baseline	145.93±39.47^{d}	243.88±13.95^{e}	80.68±11.48^{c}

Values are mean ±S.D (n=5). Values with different superscript are significantly different from one another (p<0.05)

Table 4 is the summary of the effect of O3FA on the atherogenic and coronary risk indices. There was a significant (p< 0.05) elevation of the atherogenic and coronary risk indices of the diabetic groups compared with the normal control. The increase in the atherogenic and coronary risk indices was 27% and 19% in diabetic control compared with normal control. O3FA supplementation reduced the atherogenic and coronary risk indices respectively in the diabetic omega-3 group by 48% and 37% while the reduction amounted to 36% and 24% in the omega-3 diabetic group.

Table 4. Effect of O3FA on atherogenic and coronary risk indices (AI and CRI)

Group	AI	CRI
Normal control	1.21±0.06^{a}	2.81±0.08^{a}
Diabetic control	1.54±0.12^{d}	3.35±0.12^{d}
Omega-3 control	0.23±0.07^{b}	1.43±0.05^{b}
Diabetic omega-3	0.80±0.04^{c}	2.12±0.12^{a}
Omega-3 diabetic	0.98±0.02^{c}	2.56±0.18^{c}
Control baseline	1.20±0.18^{a}	2.65±0.06^{a}
Diabetic baseline	3.01±0.17^{e}	5.02±0.17^{e}

Values are mean ±S.D (n=5). Values with different superscript are significantly different from one another (p<0.05)

The effects of O3FA on lipid peroxidation in HDL, plasma and RBC are shown in table 5. Lipid peroxidation was elevated in the diabetic baseline and sustained during the experiment. There was a significant (p<0.05) increase in lipid peroxidation of the diabetic control compared with normal control. The increase was 67%, 68% and 164% in the plasma, RBC and HDL accordingly. O3FA supplementation reduced the lipid peroxidation of plasma, RBC and HDL respectively by 30%, 33% and 55% in diabetic omega-3 and 28%, 23% and 39% in omega-3 diabetic groups compared with diabetic control.

Table 5. Effect of O3FA on lipid peroxidation in plasma, RBC and HDL

Group	Plasma(nM)	RBC (µM)	HDL (nM)
Normal control	2.63±0.27^{a}	0.31±0.13^{a}	2.14±0.65^{a}
Diabetic control	4.38±0.55^{d}	0.52±0.13^{c}	5.64±0.47^{d}
Omega-3 control	1.96±0.51^{b}	0.35±0.14^{a}	2.16±1.11^{a}
Diabetic omega-3	3.08±0.58^{c}	0.35±0.21^{a}	2.56±0.40^{a}
Omega-3 diabetic	3.14±0.64^{c}	0.40±0.03^{a}	3.46±0.74^{b}
Control baseline	2.63±0.09^{a}	0.37±0.12^{a}	1.90±0.70^{a}
Diabetic baseline	4.23±0.37^{d}	0.43±0.11^{b}	4.28±1.48^{c}

Values are mean ±S.D (n=5). Values with different superscript are significantly different from one another (p<0.05)

Figure 1 shows the graph of the effect of O3FA on activity of aryl esterase in the plasma of the animals. Diabetes significantly (p<0.05) reduced the activity of the enzyme, aryl esterase. There was no significant (p>0.05) difference between the aryl esterase activity of the normal control, omega-3 control and control baseline groups. Aryl esterase activity of the normal control group was about 4 fold of the diabetic control group. O3FA increased aryl esterase activity of the diabetic groups by 2.1 and 2.4 folds in the diabetic omega-3 and omega-3 diabetic groups.

Figure 2 depicts the graph of the effect of O3FA on activity of aryl esterase in the VLDL of the animals. The activity of the enzyme was reduced in the diabetic state of the animals. The activity of aryl esterase was significantly (p<0.05) reduced by 133% in the diabetic control compared with the normal control group. The administration of

O3FA increased the activity by 24% and 38% in the diabetic omega-3 fatty acid omega-3 diabetic groups. There was no significant ($p<0.05$) difference between omega-3 control, diabetic omega-3, omega-3 diabetic and control baseline groups.

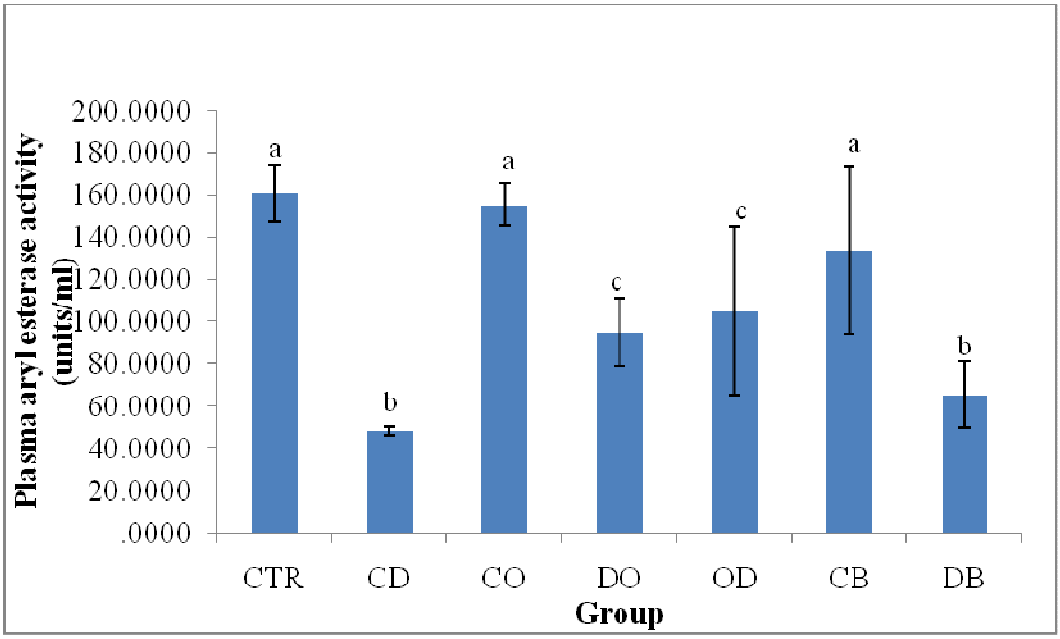

Figure 1. Graph of effects of O3FA on aryl esterase activity (units/ml) in the plasma of the animals
Each bar represents the mean±S.D of 5 rats. Bars with different alphabets are significantly different at p<0.05. CTR: Normal control, CD: Control diabetic, CO: l Omega-3 control, DO: Diabetic omega-3, OD: Omega-3 diabetic, CB: Control baseline, DB: Diabetic baseline

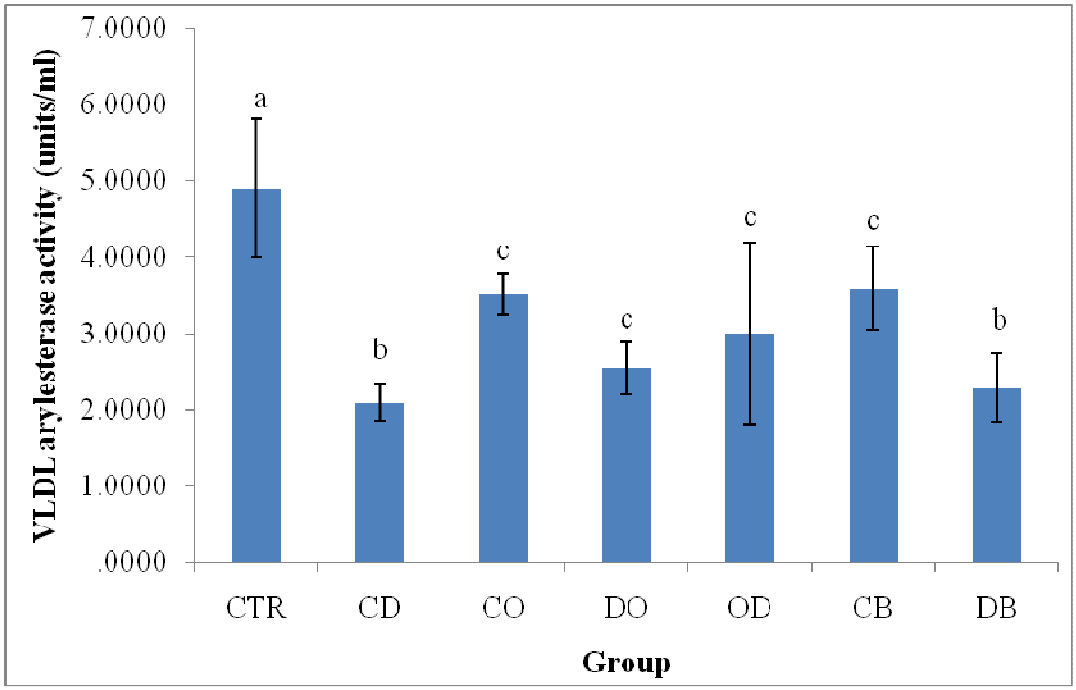

Figure 2. Graph of effects of O3FA on aryl esterase activity in the VLDL of the animals
Each bar represents the mean±S.D of 5 rats. Bars with different alphabets are significantly different at p<0.05. CTR: Normal control, CD: Control diabetic, CO: l Omega-3 control, DO: Diabetic omega-3, OD: Omega-3 diabetic, CB: Control baseline, DB: Diabetic baseline

Figure 3 shows the graph of the effect of O3FA on activity of aryl esterase in the HDL of the animals. The activity of aryl esterase was significantly ($p<0.05$) reduced in the diabetic groups compared with normal control group and the decrease was by 86% comparing diabetic control with normal control. The supplementation of omega O3FA increased the activity of aryl esterase by 36% in the diabetic omega-3 group while a decrease of 21% was observed in omega-3 diabetic group compared with diabetic control. There was no significant ($p<0.05$) difference between the activities of aryl esterase in normal control, omega-3 control and control baseline groups.

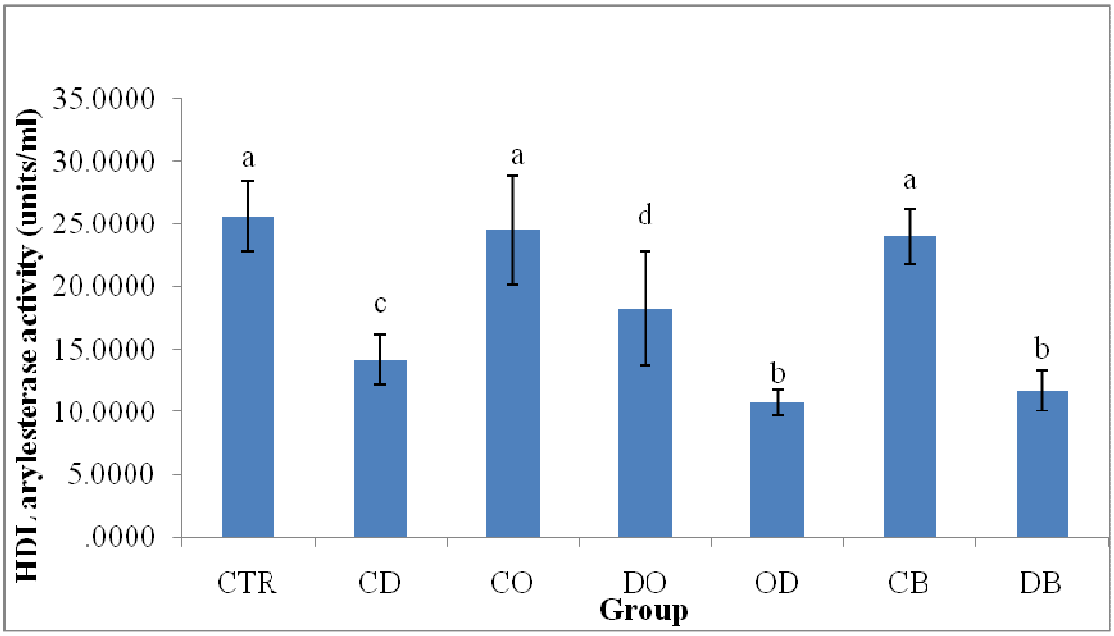

Figure 3: Graph of effects of O3FA on aryl esterase activity in the HDL of the animals

Each bar represents the mean±S.D of 5 rats. Bars with different alphabets are significantly different at $p<0.05$. CTR: Normal control, CD: Control diabetic, CO: l Omega-3 control, DO: Diabetic omega-3, OD: Omega-3 diabetic, CB: Control baseline, DB: Diabetic baseline

Diabetes mellitus is a metabolic disorder that is associated with perturbations in lipid metabolism. In this study, the induction of diabetes in the rats led to a diabetic associated dyslipidemia as illustrated by the significantly increased levels of cholesterol, phospholipid, triacylglycerol and lipid peroxidation in the blood compartments. These observations were consistent with the results of earlier studies [22, 23 and 24] which showed that lipogenesis is elevated in this condition. These expressed the opinion that when this condition is not managed properly, it can lead to the onset of cardiovascular diseases among others [25].

The administration of O3FA alleviated the dyslipidemia observed in the alloxan-induced diabetic rats. This is in accordance with the suggestion that omega-3 fatty acid probably modified the fatty acid components of cells [26]. It may also be an indication of progressive metabolic control of O3FA on mechanisms involved in elimination of the lipids from the body. O3FA supplementation reduced plasma, RBC and lipoproteins lipid profiles (except HDL cholesterol and phospholipid levels) in the O3FA treated diabetic groups compared to diabetic untreated groups. This suggests that O3FA may have beneficial effects on increasing insulin sensitivity in experimentally induced diabetic rats [27].

It could also probably be that it down-regulated by way of modulation of the gene expression for the enzymes of their syntheses [28]. This is shown in the case of cholesterol by the increase in the ratio of hepatic β - hydroxyl - β - methyl glutaryl CoA to mevalonate indicating an inhibition of cholesterogenesis [24]. Another mechanism of cholesterol level control in the system is the HDL. In this study, cholesterol and phospholipid levels in HDL were significantly ($p<0.05$) reduced in the untreated diabetic group. Well documented is the role of HDL in reverse cholesterol transport, it has been recognized to have other important cardioprotective properties which include the ability to protect LDL from oxidative modification [29]. Mackness et al [30] suggested that a direct role for HDL in preventing atherosclerosis is probably by an enzymic process which prevents the accumulation of lipid peroxides on LDL.

HDL is the most complicated and diverse of the lipoproteins, as they contain many different protein constituents, whose main purpose is to enable secretion of cholesterol from cells, esterification of cholesterol in plasma, transfer of cholesterol to other lipoproteins, and the return of cholesterol to the liver for excretion – a process that has been termed 'reverse cholesterol transport'. In other words, HDL removes excess cholesterol from peripheral tissues and delivers it to the liver for excretion in bile in the form of bile acids [31]. They have an important function in triacylglycerol transport by facilitating the activation of lipoprotein lipase, in the transfer of triacylglycerols between lipoprotein classes, and in the removal of chylomicron remnants and VLDL enriched in triacylglycerols [32].

Other key components of HDL include anti-oxidative enzymes and phospholipid transfer proteins. It prevents the oxidation of LDL and limits the concentrations of oxidized components, which might otherwise render them atherogenic. Thus, human serum paraoxonase, a calcium-dependent enzyme associated with HDL, is reported to catalyze the hydrolysis of oxidized fatty acids from phospholipids and prevents the accumulation of oxidized lipids in lipoproteins, especially LDL [9 and 33]. Observed in this study was a decrease in the activity of this enzyme which was reversed by O3FA supplementation. This agreed with a study where O3FA was administered to a group of 14 patients with familial combined hyperlipidemia for eight weeks, resulted in a modest (10%) but significant increase in plasma PON1 concentration [15]. PON1 has ability to protect against atherosclerosis by hydrolyzing specific derivatives of oxidized cholesterol and/or phospholipids in oxidized low-density lipoprotein and in atherosclerotic lesions. Therefore being an important modulator of cardiovascular disease risk [10, 11 and 12]. There are a number of epidemiological studies that have demonstrated that low concentrations of HDL cholesterol are associated with a higher risk of atherosclerosis. It has been reported that HDL may protect against atherosclerosis via the promotion of reverse cholesterol transport [32 and 34].

LDL are the main carriers of cholesterol to the adrenals and adipose tissue, where there are receptors requiring Apo B100 that are able to take in the LDL by a similar process to that occurring in liver [31]. Higher concentrations of LDL cholesterol as observed in the untreated diabetic group of this study have been associated with increasing severity of cardiovascular disease, although the experimental correlations are not as good as for HDL. The levels of Apo B and of Apo C3 in plasma may be good predictors of the risk of coronary heart disease. For example, Apo B may mediate the interaction between LDL and the arterial wall, and this may initiate the development of atherosclerosis [34]. Indeed, virtually all of the Apo B-containing lipoproteins can pass through the endothelial layer of arteries and initiate atherogenesis, but the smaller LDL are especially atherogenic because they enter the plaques with relative ease and have a high content of cholesterol [34]. Thus, they provide substrates that trigger plaque initiation and growth.

Thiobarbituric acid reactive species (TBARS) are naturally present in biological species and include hydroperoxides and aldehydes which increase in concentration in response to oxidative stress. The assay values are usually reported in malondialdehyde (MDA) which is one of the final products of polyunsaturated fatty acids peroxidation in the cells, increase in free radicals causes an overproduction of MDA. Malondialdehyde level is commonly known as a marker of oxidative stress and antioxidant status [35]. Lipid peroxidation was significantly increased in the diabetic group in all the compartments. This was in agreement with the studies of Mete et al [36]. In the present study, these interactions have been confirmed by significant decrease in MDA in the omega-3 control group. The specific biological mechanism for the beneficial effect of O3FA on lipid peroxidation has not been fully established. Several data indicated that O3FA may play a key role in decreasing the cardiovascular complications of diabetes [37].

Furthermore, MDA has been shown to affect the intrinsic mechanical properties of the plasma membrane resulting in decreased deformability [38]. Dietary supplementation with (O3FA) has been reported to possibly enhance resistance to free radical injury and therefore reduce lipid peroxidation in the organism through an increased plasma antioxidant capability [39]. This study was in agreement with the findings of Harding et al [40] in which it was discovered that O3FA supplementation led to a significant decrease in MDA level of control O3FA and treated groups. They suggested that O3FA up-regulate gene expression of antioxidant enzymes and down regulate genes associated with production of reactive oxygen species.

CONCLUSION

The results demonstrated that O3FA supplementation significantly improved blood lipid profiles. The effect was however more significant in the pre-treatment than in the post-treatment. It is therefore worthy to suggest that

omega-3 fatty acid should be included in diets to prevent the development of disorders relating to diabetes mellitus, cardiovascular diseases among so many others.

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