Impacts on fish consumption on cardiovascular risk profiles.

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Abstract

The influence of fish consumption on cardiovascular risk profiles was studied extensively in different community. The dietary omega-3PUFAs of fish exert beneficial effects by reducing platelet aggregation and improving blood lipoprotein profiles and have been consistently associated with triglyceride-lowering effects. A interventional study was carried out by feeding fish curry prepared by Asian style and fried fish in coconut oil in randomly selected healthy individual. The experiment, due to feeding of omega-3 PUFA content fish, noticeable differences were observed on concentration of lipid cholesterol fractions and lipid indices among the subjects. Concentration of TC, TG, VLDL-C, Non-HDL and LDL-C showed an increase among fish curry eaters than fried fish eaters whereas HDL-C and lipid indices such TC/HDL, LDL/HDL, AC and API were higher in fried fish eaters than fish curry eaters. However, the significant differences were observed in TG, VLDL-C and non-HDL-C in fish curry eaters. Significantly higher TG, VLDL-C and hs-CRP in male than female was observed in this study where males ate fish curry. Both types of fish consumption influenced cardiovascular risk profiles in different ways. Overall, non-HDL showed increase due to feeding of both type of fish consumption. Although, fish flesh consists of high amount of essential fatty acids, these roles on cardiovascular profiles was prevented due to the style of consumption of fish.

Keywords: Fish consumption, Cardiovascular risk profiles, Interventional study, Omega3 fattyacid, Lipid profiles.

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Introduction

Fish is the major source of omega-3 polyunsaturated fatty acid (PUFAs), including eicosapentaenoic acid (EPA; 20; 5n-3), docosahexaenoicacid (DHA; 22:6n-3) and docosapentaenoic acid (DPA; 22:5n-3) which is found in smaller amounts in fish. The most concentrated food source of EPA and DHA is fatty fish such as albacore tuna, salmon, mackerel, sardine, and herring [1]. The beneficial health effects of fish and seafood consumption on cardiovascular risk factors have mainly been attributed to long chain omega-3 polyunsaturated fatty acids (LC omega-3 PUFAs [2]. This has been due to the abundance of EPA and DHA [3]. The dietary omega-3PUFAs of fish exert beneficial effects by reducing platelet aggregation and improving blood lipoprotein profiles and have been consistently associated with triglyceride-lowering effect [4]. The most consistent effects of omega-3 PUFA are the reduction of serum cholesterol [5], trigycerides, Very-Low Density Lipoprotein Cholesterol (VLDL-C) and Low Density Lipoprotein Cholesterol (LDL-C) [6]. Further, intake of omega-3 PUFAs increases HDL-C [7], but however some studies have shown contradictory results regarding the atherogenic effects of omega-3 PUFAs from fish [8]. Low HDL-Cholesterol (HDL-C) as well as high LDL-C is associated with the development of Coronary Heart Disease (CHD [9]. Epidemiological studies have shown that fish consumption is associated with lower body weight [10]. The fish consuming population had a lower atherogenic risk as opposed to the non-fish consuming population [11] and the intake of fish may have substantial implications on public health and the health economy by decreasing the risk of CVD. Japanese people eat about 3 ounces /week on average while typical Americans perhaps eat fish twice a week. Nutritional studies have shown that the intake of omega-3 fatty acids from fish averages 1.3 gram per day in Japan as compared to 0.2 gram per day in the US [12]. TG and LDL-C were only significantly lower in intervention that provided more than 4g/day of n-3 PUFA through increased fish consumption. Two trials of 4 weeks [13] and 8 weeks in duration showed that consumption of 125-150 g/day (3.4-5.4g/day of omega-3 PUFA) of fish reduced of LDL-C by 14%-15% [14]. Participants consuming oily fish diet (2.6-3.0g/day of omega-3 PUFA) for 8 weeks experienced non-significant reductions in Total- C and LDL-C. A trend towards lowered TG levels (by 3.1%) was observed when compared to a red meat diet (1.2 -1.4 g/day of n-3 PUFA) [15]. Potential mechanisms for the cardioprotective effects of omega-3 fatty acids include: Anti-atherogenic effects such as reduction in non-HDL-C levels, TG and VLDL-C levels, chylomicrons, VLDL-C and chylomicron remnants, increase in HDL-C levels, improvement in LDL-C and HDL-C particle size and plaque stabilization and antithrombotic effects, decreased systolic and diastolic blood pressure (Bays, 2010) [16]. Diets with high PUFAs to SFA ratio (PUFA: SFA) lower serum cholesterol, particularly, LDL-C. This is considered to be beneficial in view of the relationship between serum cholesterol and CHD [17]. The lower incidence of heart disease with diminished platelet aggregation and prolonged clotting time in Greenland Eskimos have been attributed to their high intake of fish and fish oils [18] which contain relatively high concentration of EPA and DHA. In addition, the total cholesterol, TG, LDL-C are low in Eskimos whereas high HDL-C is raised.

Among SFAs, palmitic acid compared to mirystic or lauric acids or their combination lower cholesterol levels (total-LDL-C and HDL-C) [19]. Palm oil had no substantial differences in the serum lipid profile, except for an increase in HDL [20]. Also palm oil raised the total cholesterol (both HDL and LDL-C), but TC/HDL ratio was not affected. Palmitic acid slightly increases both LDL-C and HDL-C and the HDL/LDL ratio, which is a valuable predictor of cardiovascular disease risk, is relatively neutral. However, [21] supports that palmitic acid in palm oil raises LDL-C, but underlines the wide variability of dose-response relation with a considerable number of negative results. The reduction of total SFAs, is one of the main targets of dietary recommendations in order to lower morbidity and mortality due to CVD [22]. The FAO and the WHO set recommended intakes at 20-35% for total fat, 10% for SFAs, up to 15%-20% for MUFAs and 6%-11% PUFAs, in relation to the total energy intake [23]. Coconut oil is used either to fry or cook fish. Few studies have investigated the effect of coconut oil on human health and its negative role is related to its high content of SFAs that increases the risk of coronary heart diseases. Coconut oil contains many fatty acids not only palmitic acid but also oleic acid and antioxidants which could be compensatory. A population based case-control study looked at the association between the dietary pattern and risk of first non-fatal acute myocardial infarction in Costa Rican adults [24]. Sugar-based amphiphilic macromolecules complexed with alpha-tocopherol is a new anti-athero-inflammatory nanotherapeutics, which reduces the inflammatory act of human atherosclerotic plaque [25]. Increased baked/broiled fish intake may lower heart failure risk, whereas increased fried fish intake may increase heart failure risk in postmenopausal women [26]. The influence of fish consumption on cardiovascular risk profiles was studied extensively in different community (Table 1). The fish consumption showed direct effect on the cardio vascular risk profiles by decreasing LDL-C and increasing HDL-C and decreasing LDL: HDL ratio and TC: HDL ratio [27]. Research revealed the decrease of TG in serum with fish consumption [28]. [29] Reported that omega-3 intake from non-fried fish is inversely associated with interleukin-6 and C-reactive protein levels and these associations were independent of age, body mass index, physical activity, smoking, alcohol consumption, and dietary variables.

Obesity

Obesity is often described by classical parameters such as Body Mass Index (BMI) and Waist Circumference (WC), and Waist-to-Hip Ratio (WHR) [30]. The relationship of obesity parameters and lipid profile is stated in some studies [31].

Methodology

Selection of subjects and approach

A challenge experiment was set up to do a research on the influence of omega-3 fatty acid content on cardiovascular risk profiles of healthy people. Hundred healthy undergraduates (subjects) who were between the ages of 23 to 30 years and full time resident in the hostels of the Eastern University in Vantharumoolai, Sri Lanka, were randomly selected for this study. A cohort design was chosen, and approval was obtained from the Ethical Clearance Board of the Faculty of Health Care Sciences, Eastern University, Sri Lanka.

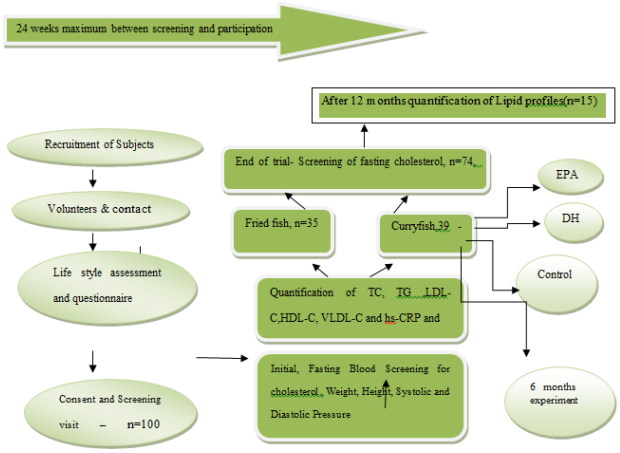

Figure 1: *Overview of participant recruitment and screening in the experiment approach.*

Consumption of omega-3 fatty acids containing fish

Fish was collected from a nearby marine landing center that supplied to a cafeteria where it was cleaned, cut and weighed (66.51 ± 12.29 g) into pieces of muscle. These pieces of fish muscle were made into a fish curry with coconut cream. The total weight of the fish curry including gravy was 80.41 ± 9.43 g which was fed to subjects (n=39). Another set of subjects (n=35) were fed with 62.50 ± 11.04 g fish fried in coconut oil (Vimal, Sri Lanka) daily. A six months trial commenced on December 2015 and ended May 2016.

Fish processing using traditional method

The fish was cleaned thoroughly, and the muscle (trunk and abdomen) was cut into pieces (65 ± 3 g) and weighed (1.5 kg). The fish pieces were transferred into a bowl and two teaspoons of salt was sprinkled with one teaspoon of turmeric. The dish set aside for 10 minutes. Next, a medium size aluminum pan was kept on medium heat, and coconut oil (50 ml) was added, followed by addition of mustard seeds, fenugreek, fennel seeds, curry leaves, and cinnamon sticks.

The mixture was left for 30 seconds. Then, garlic, green chillies, and chopped onion were added for 4-5 minutes until the onion became translucent. Sliced tomatoes were then added and left for 2 minutes, followed by addition of chilli powder, coriander powder, cumin powder, and salt. After stirring well, 2 liters of diluted coconut cream and tamarind pulp were poured into the mixture and cooked for 4 minutes. When the gravy boiled for 5 minutes, the pieces of fish gently were slid into the gravy and left for another 5-10 minutes to cook under low heat with the pan covered. Finally, two cups of concentrated coconut cream (50 ml) were added into the boiling fish curry and cooked for another 5 minutes at low heat. Fresh coriander leaves were sprinkled on the curry,

and the fish curry was kept away from the flame.

Consumption of fish

The clinical history of subject was recorded. The protocol of the fish curry and fried fish was studied and the consistency of methodology of preparation of curry was monitored weekly. Intake of cooked fish and fried fish were recorded twice a week. The methodology of curry preparation and the volume of coconut cream and gravy and type of oil using for frying were noted twice a week. The temperature and pH of gravy and oil were measured using thermometer (Gallenkamp, Griffin, England) and pH meter (pH ep, Hanna, Itali) respectively once in two weeks. The fish delivering record of subjects was monitored. The students who get fish curry and fried fish from the cafeteria were monitored by daily recording. Subjects were fed 80.41 ± 9.43 g of fish curry or 62.50 ± 11.04 g of fried fish for five days per week.

Determination of cardiovascular risk profiles in subjects

Initial, after 24 weeks and after one year the lipid profiles of the subjects [Total Cholesterol (TC), Triglyceride (TG), Low Density Lipoprotein (LDL-C), high density lipoprotein (HDL-C), Very Low Density Lipoprotein (VLDL-C)] and high sensitive C-reactive proteins (hs-CRP) were estimated in serum samples collected at the commencement and end of the experiment, using automatic biochemical analyzer and the turbidometric method respectively.

Determination of cardio vascular risk lipid indices

Serum cholesterol was estimated using the cholesterol oxidase-phenol-4-aminophenazone method with a lipid clearing agent by enzymatic colorimetric assay (Spinreact®-Spain). The cholesterol in the sample oxidized by the action of cholesterol oxidase enzyme into 4-Cholestenone and hydrogen peroxide which in turn reacts with phenol and 4-aminophenazone in the presence of peroxidase enzyme to produce red color [33]. HDL-C was determined after precipitation of other lipoproteins by sodium phosphotungstate with magnesium chloride reagent (Spinreact®-Spain) and read using analyzer (Indika Chembell, Thermoscientifc, UK). The serum TG level was estimated using an enzymatic method (Indika Chembell, Thermoscientifc, UK). Triglycerides in serum incubated with Lipoprotein Lipase (LPL), liberate glycerol and free fatty acids. Glycerol was converted to glycerol-3-phosphate by glycerol kinase. Glycerol-3-Phosphate (G3P) was then converted by glycerol phosphate dehydrogenase to dihydroxyacetone phosphate. In the last reaction, hydrogen peroxide (H_2O_2) reacts with 4-Aminophenazone (4-AP) and p-chlorophenol in Presence of Peroxidase (POD) to give a red colored dye (Meiattini et al., 1978) [32]. LDL-cholesterol was calculated using Friedewald's formula (LDL-C=TC minus HDL-C minus triglycerides/5) (Friedewald et al., 1972) [33].

The following cutoff points were used as risk factors based on the criteria of the American National Cholesterol Education Program (NCEP): TC >200 mg/dL, HDL-C level <35 mg/dL, LDL-C level >130 mg/dL, LDL-C/HDL-C >2.0, and TC/HDL=4.5, VLDL-C= triglycerides/5, LDL-C= total cholesterol– (HDL-C+VLDL-C)-TG/2.17 (mmol/L) (Friedewald et al., 1972) [33].

Calculation of atherogenic indices

The atherogenic ratios were calculated as follows:

Atherogenic Plasma Index (API) =log TG/HDL-C

Castelli's Risk Index I (CRI-I) =TC/ HDL-C

Castelli's Risk Index II (CRI-II) =LDL-C/HDL-C

Measurement of high-sensitivity C-reactive protein (hs-CRP)

High-sensitivity C-Reactive Protein (hs-CRP) was determined using latex-enhanced immunoturbidimetry with the Cobas C 501 analyser, according to the manufacturer's instructions. Human CRP agglutinates with latex particles that are coated with monoclonal antibodies to human CRP. The precipitate formed is measured turbidimetrically at the wave length of 552 nm. Estimation of serum high-sensitivity C-Reactive protein (hs-CRP) was carried out by turbidimetry latex-high sensitivity kit (Biosystems S.A. Costa Brava, Barcelona [Spain]) method (Rifai et al., 1999) [31].

Baseline data collection

Demographic data (age, gender, and race/ethnicity), cardiovascular risk factors (smoking, diabetes, and hypertension), clinical parameters (systolic pressure and diastolic pressure blood pressure) were collected using a structured questionnaire before enrolling the subjects for experiment. The BMI was calculated as per the standard guidelines [34]. The type of fish used for preparation of curry and fry were recorded. Amount of fish curry or fried fish given to the subjects were weighted daily.

Anthropometric parameters

Body mass index (BMI): The weight (kg) and height (m) of the subjects were measured before the experiment started and after 24 weeks of eating fish during lunch or dinner using the mechanical bathroom scale (Sumbow, China). The scale was calibrated by adjusting dial to zero before weighing the body weight.

Body weight in kilograms divided by height in meters squared

BMI= X (Kg)/(ym*ym), Where X = bodyweight in Kg; y=height in meter

Basal metabolic rate (BMR): The basal metabolic rate was calculated in female and male subjects pre and post interventional period of fish consumption. This Calorie Calculator is based on the Mifflin-St Jeor equation. With this equation, the Basal Metabolic Rate (BMR) is calculated by using the following formula:

BMR= 10 × Weight+6.25 × Height (CM) -5 × Age+5 (Male)

BMR=10 × Weight+6.25 × Height(CM)-5 × Age-161 (Female)

Blood pressure: Standard mercury sphygmomanometer, (EL-DI-003, Jiangsu, China) (38.1-cm (15-inch) stethoscopes, and appropriately sized cuffs were used to measure the blood pressure. Systolic and diastolic pressure were measured monthly from the subjects for six month. Mean value of BP was taken.

Statistical Analysis

Multiple comparison tests were performed by using one-way Analysis of Variance (ANOVA), using SPSS version 19 and Excel 2010 (Armonk, NY: IBM Corp) in order to dissolve the data lipid profiles with the type of fish consumed. Bivariate correlation and a paired T-test were also performed. IBM SPSS statistics software was used to describe data.

Results and Discussion

This study was conducted to assess the effect of fish consumption (omega-3 fattyacid) on serum lipid profile in healthy young subjects along with their BMI and blood pressure. It was observed that the mean BMI (Kg/cm^2) in male (25.47 ± 2.48) and female (21.75 ± 3.49) before the experiment and after the experiment it was in male (25.78 ± 2.50) and in female (21.93 ± 3.37) respectively and showed significantly different ($p<0.001$). Among the subjects 18 % had within the healthy BMI (18.5-22.9), 50 % of subjects had above health range (23-26.9), 18% were obese (>27) and 14% were below health range (<18.5) as per cut off values of Asian population .There was no significant difference in BMI between before and after experiment among subjects ($p>0.01$). Initially, the mean total systolic pressure (mm Hg) was (111.25 ± 6.79) in male and in female (109.38 ± 6.47) and at the end of the experiment in male (112.92 ± 4.64) and in female (110.00 ± 6.32) respectively. Likewise, at the beginning of the experiment, the mean diastolic pressure was 76.66 ± 4.36 in males and 74.11 ± 6.82 in females and at end it was 77.66 ± 4.36 in males and 75.65 ± 5.32 in female. But, there were no significantly differences seen among systolic and diastolic pressure before and after the experiment between both sexes ($p<0.01$) (Table 2).

The Basal metabolic rate (BMR) showed differences among sex, male (1365.19 ± 413.30) and female (1206.88 ± 116.85) at pre-intervention whereas in post intervention, a increase of BMR male (1369.89 ± 414.62) and female (1213.55 ± 120.14) was observed.

Table 1: *Polyunsaturated fatty acids (Omega-3 PUFA) in raw, fried and curried fish.*

Fish name (Common name)	Raw fish(mg/100 g) omega-3FA	Fried fish(mg/100 g) omega-3FA	Curry fish (mg/100 g) omega-3FA
Leiognathus bindus (Pony fish)	23.65(0.73)	2.44(0.36)	11.27(0.59)
Mugil cephalus (Mullet)	15.53(0.42)	5.38(0.77)	13.20(1.01)
Rasterlligerkanagurata (Mackeral)	23.90(0.08)	5.40(1.01)	24.29(1.49)
Chirocentrus dorab (Wolf)	25.04(0.70)	6.96(0.92)	15.50(0.74
Selar crumenophtthalamus (Scad)	23.68(3.12)	4.09(1.26)	4.35(0.34)
Katsuwonus pelamis (Tuna)	33.91(2.25)	7.61(0.63)	15.65(1.16)
Sphyraenea jello (Baracuda)	22.82(0.16)	5.21(1.13)	22.13(1.00)
Dussumieria acuta (Herring)	26.10(0.26)	3.97(0.65)	8.03(2.60)

The omega-3 fatty acid in raw fish was significantly higher in almost all fishes than curry and fried fishes. By cooking fish with coconut cream and frying in coconut oil, the content of omega-3 fatty acid showed decrease and which can be explained that the impact of omega-3 fatty acid in lipid profiles and atherogenisis did not reveal in the present result (Table 1). The cardio vascular risk parameters and lipid indices showed differences in pre and post intervention of experiment as shown in Table 4 where in curry fish consumers had significant variation between pre and post intervention in TG and VLDL content ($p<0.05$). There was not significant difference in TC, LDL-C and HDL-C. However, the after another one year of post intervention showed that there was no significant difference in the lipid profiles between the intervention while the lipid indices, TC: HDL and AC had significant different ($p<0.05$) between intervention as shown in Table 5. There was no significant different in cardio risk profiles between pre and post intervention in overall both curried and fried fish consumers (n=74) (Table 6).

Table 2: *Body Mass Index and blood pressure during intervention.*

Parameters	Male	Female	95% CI	Significant (2 tailed)
BMI(kg/m2)-pre-intervention	25.30 (2.44)	21.62(3.61)	1.94 to 5.43	0.00*
BMI(kg/m2)-post-intervention	25.60(2.50)	21.91(3.40)	1.98 to 5.39	0.00*
Systolic Pressure(mm Hg)-pre-intervention	111.15(7.11)	109.33(6.06)	-1.95 to5.59	0.34
Systolic Pressure(mm/Hg)-post-intervention	112.69(5.33)	110.00(5.89)	-0.501 to 5.88	0.97
Diastolic pressure(mm Hg)-pre-intervention	76.53(4.85)	74.04(6.96)	-0.0891 to5.88	0.15

Diastolic pressure(mm Hg)-post-intervention	77.46(4.47)	75.71(5.350	-1.044 to4.65	0.21
Data presented as mean (SD).* Significant p<0.00.				

As per the Table 7, the TC had not significantly changed due to curry or fried fish consumption (c^2=1.495, p=0.474). TG lower risk level increased in curry fish consumers from 11.4% to 22.9% whereas it had decreased in fried fish eaters from 5.1%to 2.6%. Over all, high risk level of TG showed decrease not significantly in both curry fish and fried fish consumers (20% to 14.3% and 5.1% to 0.0%) respectively.

Curry fish consumers had an increase of lower risk of LDL-C from 8.6%to 20% whereas in fried fish consumers decreased from 17.9%to 12.8 %, but higher risk level of LDL-C had no change in curry fish consumers whereas in fried fish eaters the high risk level of LDL-C had decreased from 5.1% to 2.6%. HDL-C lower risk level had increased from 48.6% to 80% in curry fish consumers and decrease from 12.8% to 5.1% in fried fish consumers and risk level was considered as per the lipid profile risk levels stated by American Heart Association (www.heart.org). However, TC: HDL ratio lower risk level had increase from 80% to 88.6% in curry fish consumers and increase from 66.7% to 88.6% in fried fish eaters. TC/HDL-C and LDL/HDL-C ratio are risk indicators with greater predictive value than isolated parameters used independently [35].

Fish was prepared into both curry and fried forms with coconut cream and coconut oil respectively. Coconut cream added fish increases the TC level in the present study as [35] studied the effect of coconut flakes on the serum cholesterol levels of healthy individuals and observed highly raised cholesterol of 259 to 283mg/dl. Coconut cream used in processed fish curry would mainly contain the soluble fibre which is thought primarily to bring about reduction or no change in TC. A study of Ekanayake et al., (2013) [36] revealed that coconut cream supplementation was responsible for the reduction in the LDL-C and increase of HDL-C.

As observed by Schaefer et al.,(1981) [37], who observed rise of HDL-C with eating SFA rich coconut cream, this study agreed the rise in HDL-C while ingesting a saturated fat rich coconut cream cooked fish.

TG in the serum showed significantly increase in both fish curry and fried fish consumers post intervention period of the experiment. The Non-HDL-C showed significantly rise during post intervention period of the experiment in both curry and fried fish consumers and this could be explained by the overall rise of VLDL-C, LDL-C and TC in the serum during post intervention (Table 6).

Table 3: *Cardiovascular risk profiles and lipid indices of the subjects of curry and fried fish consumers.*

	Curry fish eaters				Fried fish eaters			
Lipid profiles	**End profiles**	**Initial profiles**	**R-value**	**P-value**	**End profiles**	**Initial profiles**	**R-value**	**P-value**
TC (mg/dl)	193.86 ± 37.63	188.06 ± 27.26	0.17	0.32	185.78 ± 32.39	183.11 ± 28.63	0.32	0.52
TG(mg/dl)	132.97 ± 54.14	143.91 ± 67.56	0.39	0.03*	113.99 ± 46.13	124.49 ± 60.14	0.38	0.14
LDL-C(mg/dl)	122.14 ± 31.44	113.37 ± 23.55	-0.06	0.73	116.74 ± 26.93	111.76 ± 26.07	0.16	0.22
HDL-C(mg/dl)	45.17 ± 4.85	46.00 ± 9.23	0.12	0.48	45.97 ± 11.81	45.16 ± 8.69	-0.03	0.64
VLDL-C(mg/dl)	26.59 ± 10.83	28.79 ± 13.51	0.38	0.03*	22.79 ± 9.23	24.89 ± 12.03	0.38	0.14
TC:HDL	4.28 ± 0.60	4.19 ± 0.71	-0.17	0.61	4.13 ± 0.46	4.16 ± 0.79	-0.05	0.81
LDL:HDL	2.67 ± 0.53	2.52 ± 0.56	-0.16	0.27	2.61±0.40	2.57 ± 0.61	-0.08	0.66
NON-HDL-C	148.69 ± 34.32	142.06 ± 24.57	0.081	0.64	139.81 ± 29.50	137.95 ± 27.26	0.25	0.65
AC	3.28 ± 0.58	3.18 ± 0.71	-0.182	0.58	3.09 ± 0.55	3.15 ± 0.79	-0.09	0.65
API	0.05 ± 0.00	0.05 ± 0.00	-0.063	0.62	0.05 ± 0.01	0.05 ± 0.01	0.01	0.24
TGHDL	2.22 ± 0.78	3.66 ± 2.07	0.149	0.37	2.53 ± 1.06	2.89 ± 1.68	0.23	0.08
Hscrp	1.89 ± 3.47	1.83 ± 3.58	-0.019	0.95	1.73 ± 3.05	1.34 ± 2.69	-0.02	0.41

*Significant, P < 0.05. AC: Atherogenic coefficient, API: atherogenic index of plasma, HDL-C: high-density lipoprotein cholesterol, hs-CRP: high-sensitivity C-reactive protein, LDL: low-density lipoprotein, LDL-C: low-density lipoprotein cholesterol, TC: total cholesterol, TG: triglycerides, VLDL-C: very low-density.

Table 4: *Status of lipid parameters and lipid indices during pre and post intervention (6 months) and 1 year post intervention.*

Lipid parameters	Pre intervention	Post intervention	Post intervention (1 year)	Significant (2 tailed)
TC(mg/dl)	180.80(17.53)	175.20(27.42)	193.00(38.09)	0.01
TG(mg/dl)	96.40(28.47)	98.00(27.81)	86.93(21.68)	0.25
LDL-C(mg/dl)	110.53(29.89)	111.93(22.00)	131.44(33.19)	0.07
HDL-C(mg/dl)	44.27(6.79)	43.67(5.27)	44.13(3.93)	0.82
VLDL-C(mg/dl)	20.95(9.35)	19.60(5.56)	17.39(4.34)	0.38
NON-HDL-C (mg/dl)	136.53(15.42)	131.53(22.42)	148.87(34.85)	0.07
Lipid Indices				
TC:HDL	4.15(0.53)	4.00(0.21)	4.30(0.53)	0.01*
LDL:HDL	2.70(0.45)	2.54(0.27)	2.95(0.52)	0.08
AC	3.14(0.55)	3.00(0.22)	3.35(0.53)	0.04*
API	0.05(0.01)	0.05(0.01)	0.04(0.01)	0.55

* Significant P <0.05 AC: atherogenic coefficient, API: atherogenic index of plasma, HDL-C: high-density lipoprotein cholesterol, hs-CRP: high-sensitivity C-reactive protein, LDL: low-density lipoprotein, LDL-C: low-density lipoprotein cholesterol, TC: total cholesterol, TG: triglycerides, VLDL-C: very low-density.

[5] Stated that TC, TG and VLDL-C reduction due to the consumption of food with omega-3 PUFA and reached normal. But the present study did not agree with the above finding and omega-3 PUFA effects on cardio risk profiles is modulated by high intake of SFA with fish. [6] reported LDL-C reduction with omega-3PUFA in animal food while our study showed increase of LDL-C with consumption of fish. However, increase of HDL-C with omega-3 PUFA containing fish intake observed by [8] which is similar to the present study too. As stated by [7], results of some studies have shown contradictory results regarding atherogenic effects of omega-3 PUFAs from fish.

Table 5: *Comparision of pre and post intervention of fish consumption of all subjects.*

	Pre-intervention	Post - intervention		
Lipid parameters	**Mean ± SD**	**Mean ± SD**	**R- value**	**P-value**
TC(mg/dl)	183.10 ± 28.63	185.78 ± 32.39	0.324	0.52
TG(mg/dl)	124.49 ± 60.14	113.99 ± 46.13	0.379	0.139
LDL-C(mg/dl)	111.76 ± 26.07	116.74 ± 26.93	0.164	0.215
HDL-C(mg/dl)	45.16 ± 8.68	45.97 ±11.81	-0.034	0.641
VLDL-C(mg/dl)	24.89 ± 12.03	22.79 ± 9.23	0.379	0.139
Non-HDL	137.95 ± 27.26	139.81 ± 29.50	-0.46	0.647
Lipid indices				
TC: HDL	4.16 ± 0.79	4.13 ± 0.46	-0.05	0.814
LDL: HDL	2.57 ± 0.61	2.61 ± 0.40	-0.084	0.661
AC	3.15 ± 0.79	3.09 ± 0.55	-0.091	0.649
API	0.05 ± 0.01	0.04 ± 0.01	0.006	0.244
Defence protein				
HS-CRP(mg/L)	1.34 ± 2.69	1.73 ± 3.05	-0.018	0.412

(n=74),

* Significant P <0.05 AC: atherogenic coefficient, API: atherogenic index of plasma, HDL-C: high-density lipoprotein cholesterol, hs-CRP: high-sensitivity C-reactive protein, LDL: low-density lipoprotein, LDL-C: low-density lipoprotein cholesterol, TC: total cholesterol, TG: triglycerides, VLDL-C: very low-density.

It was observed that the hs-CRP has no significant difference before and after fish consumption and it reveals it could be a general inflammatory protein and it is subjected to vary with the inflammatory effects on body not only in blood vessels but also in other tissues (Table 6). The % of subjects in hs-CRP high risk group (>3) showed increase from 8.6% to 17.15% in curry eaters whereas it increased from 5.1% to 15.4% fried fish eaters. Since hs-CRP is subjected to change with general inflammation, it could not be used to predict the coronary artery inflammation. It was observed that hs-CRP had the significant difference between the males and females, and the males (0.98 mg/L) had a significantly higher amount of hs-CRP than females (0.88 mg/L).

Table 6: *Effects of fish consumption on lipid parameters.*

Parameter	Curry fish				Fried fish			
	Initial	**End**	χ^2	**P**	**Initial**	**End**	χ^2	**P**
TC								
Baseline <200 mg/dL	65.7% (23)	65.7% (23)	0	1	79.5% (31)	79.5% (31)	0	1
Risk >200 mg/dL	34.3% (12)	34.3% (12)			20.5% (8)	20.5% (8)		
TG								
Baseline <150 mg/dL	68.6% (24)	62.95 %(22)	1.75	0.42	89.75 %(35)	97.4% (38)	2.46	0.29
Lower risk <200 mg/dL	11.4%(4)	22.9% (8)			5.1%(2)	2.6%(1)		
Higher risk >200 mg/dL	20%(7)	14.3% (5)			5.1%(2)	0%(0)		
LDL								
Baseline <130	82.9% (29)	71.4% (25)	1.89	0.39	76.9% (30)	84.6% (33)	0.81	0.67
Lower risk <150	8.6%(3)	20%(7)			17.9% (7)	12.8% (5)		
High risk >160	8.6%(3)	8.6%(3)			5.1%(2)	2.6%(1)		
HDL								
Baseline <40 mg/dL	40%(14)	20%(7)	9	0.01	35.9% (14)	35.9% (14)	1.49	0.47
Lower risk	48.6% (17)	80%(28)			12.8% (5)	5.1%(2)		

50-55 mg/dL								
High risk >60 mg/dL	11.45 %(4)	0%(0)			51.3% (20)	59%(23)		
NON HDL								
Base line <130 mg/dL	0%(0)	5.7%(2)	2.01	0.36	2.6%(1)	0%(0)	1.02	0.59
Lower risk 130-219 mg/dL	65.7% (23)	62.9% (22)			51.3% (20)	53.8% (21)		
High risk >220 mg/dL	34.3% (12)	31.4% (11)			46.2% (18)	46.2% (18)		
TC:HDL								
Base line 3.5-1 mg/dL	14.3% (5)	0%(0)	5.82	0.05	23.1% (9)	0%(0)	15.6	0
lower risk 3.5-5 mg/dL	80%(28)	88.6% (31)			66.7% (260	100%(39)		
High risk >5 mg/dL	5.7%(2)	11.4%(4)			10.3% (40	0%(0)		

Table 7: *hs-CRP content in serum before and after fish consumption.*

Parameter	**Curry fish eaters**				**Fried fish eaters**			
	Initial	**End**	χ^2	**P**	**Initial**	**End**	χ^2	**P**
Base line <1	65.7% (n=23)	65.7% (n=23)	1.6	0.45	74.4% (n=29)	59% (n=23)	2.92	0.23
Inflammation	25.7% (n=9)	17.1% (n=6)			20.5% (n=8)	25.6% (n=10)		
Acute inflammation	8.6% (n=3)	17.1% (n=6)			5.1% (n=2)	15.4% (n=6)		
hsCRP (mg/L)	0.92 ± 0.82	0.98 ± 0.95		0.44	0.72 ± 0.72	0.88 ± 0.74		0.4

Non-HDL-C showed difference before and after fish consumption and it reveals that the type of fish consumption contributes to rise and fall of Non-HDL-C. Fried fish consumption had to increase of Non-HDL-C while curry fish consumption showed to decrease of it (Table 4) [38]. stated that non-HDL-C could be as a prognostic factor of acute coronary events and myocardial infarction among healthy subjects and diabetics. Non-HDL-C concentration, as recommended by the NCEP Adult Treatment Pane III, should be higher by about 30 mg/dl than LDL-C. Non-HDL-C is considered as the second, after the LDL-C goal of CVD therapy in patients with hypertriglyceridemia and should be calculated routinely [39]. However, the LDL-C showed no significant increase due to fish consumption.

With excessive dietary saturated fats and cholesterol, the liver produces a greater load of VLDL and LDL, and because the removal capacity of by the receptors is limited the serum LDL-C levels rise [40].

Among both fish curry and fried fish consumers, TC, LDL-C, TG and VLDL-C showed significantly higher upper quintiles than lower quintiles.

Because of rise of stated cardio risk profiles showed higher than optimum reference level. It is varied with gender and males showed higher upper quintiles in cardiovascular risk profiles. Figure 2 show the correlation of BMI, VLDL-C and LDL-C pre and post intervention (R^2=0.94, 0.14, 0.02).

Fish consumption population had a lower atherogenic risk than the non-fish population as stated by [11]. But the consumption style of fish could be factor to determine the effects on the atherogenic action. In the present study, there was no significant different between curried and fried fish eating population except in TG and VLDL-C.

Two trials of 4 weeks and 8 weeks [14] duration showed that fish consumption of 125 g-150 g/day (3.4-5.4 g/day of n-3 PUFA) of fish reduced the levels of 14-15% respectively.

In this trial subjects consumed on an average 66.5 g/day of fish either as fish curry or fried fish and weekly 250-300 g for 24 weeks and had non- significant role of fish consumption on atherogenic influencing factor [15] reported that the oily fish diet (2.6-3.0 g/day of omega-3 PUFA) for 8 weeks showed non- significant reductions in TC and LDL-C and these results supported the present study.

The increase of TG and VLDL-C in curry fish consumers can be explained by the addition of SFA with curry fish made with coconut cream which contains glyceride.

As [16] stated that anti-atherogenic effects such as reduction in Non- HDL-C, TG and VLDL-C and antithrombotic effects which decreased systolic and diastolic pressure caused by omega-3 PUFA. There is no anti-atherogenic effect by fish consumption had an impact on cardiovascular risk profiles in this study.

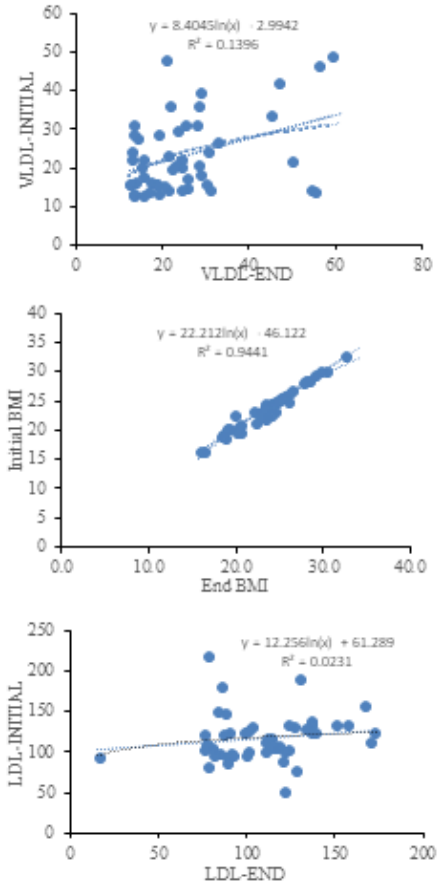

Figure 2: *Correlation between pre and post intervention of BMI and LDL-C and VLDL-C.*

Conclusion

At the end of the trial experiment, due to feeding of omega-3 PUFA content fish, noticeable differences were observed on concentration of lipid cholesterol fractions and lipid indices among the subjects. Concentration of TC, TG, VLDL-C, Non-HDL and LDL-C showed an increase among fish curry eaters than fried fish eaters whereas HDL-C and lipid indices such TC/HDL, LDL/HDL, AC and API were higher in fried fish eaters than fish curry eaters. However, the significant differences were observed in TG, VLDL-C and non-HDL-C in fish curry eaters. Significantly higher TG, VLDL-C and hs-CRP in male than female was observed in this study where males ate fish curry. Both types of fish consumption influenced cardiovascular risk profiles in different ways. Overall, non-HDL showed increase due to feeding of both type of fish consumption. Although, fish flesh consists of high amount of essential fatty acids, these role on cardiovascular profiles was prevented due to the style of consumption of fish.

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Conflict of Interest

The author declared no conflict of interest.

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