

ASSESSMENT OF LIPID PROFILE AND ATHEROGENIC INDICES FOR CARDIOVASCULAR DISEASE RISK BASED ON DIFFERENT FISH CONSUMPTION HABITS**CHANDRAVATHANI DEVADAWSON^{1*}, CHAMILA JAYASINGHE², SIVAKANESAN RAMIAH³,
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ABSTRACT

Objective: Habitual consumption of moderate amounts of fish is associated with reduced mortality from cardiovascular disease (CVD). However, the beneficial effects of fish-enriched diet seem contradictory due to the oxidation susceptibility of the polyunsaturated fatty acids in them. The atherogenic index is an important tool to identify people with CVD risk. Lipid profile levels are favorably affected by certain fish consumption habits, thereby decreasing the risk of coronary heart diseases. The aim of this study was to evaluate the plasma lipid profile of healthy people with different fish consumption habits and to assess CVD risk using atherogenic indices.

Methods: Fasting blood samples were collected from healthy people for lipid profile estimations with an automated analyzer. Detailed information regarding physical and atherogenic parameters was collected from each participant.

Results: The CVD risk profiles showed varying level with the type of fish consumption. Total cholesterol, triglyceride (TG), and non-high-density lipoprotein (Non-HDL-C) varied significantly among the types of consumers ($p < 0.05$), whereas the high-density lipoprotein cholesterol showed variation ($p < 0.001$). TG/HDL showed the greater level in inland fish consumers. However, atherogenic coefficient and Castelli risk index-2 did not show significant variation among type of fish eating. Atherogenic indices were borderline "high risk" among all fish eaters including beef eating habits.

Conclusion: Fish and beef eaters had high risk among other inland and sea fish eaters. Non-HDL-cholesterol could be a marker for a serum lipid pattern associated with increased risk of heart diseases.

Keywords: Cardiovascular diseases, Lipid profile, Fish consumption, Atherogenic indices.

INTRODUCTION

Dietary fat plays an important role in the onset of heart diseases by affecting atherogenesis. Epidemiological studies on Greenland Eskimos have shown a correlation between the low incidence of coronary heart disease (CHD) and high consumption of fish products [1]. Habitual consumption of moderate amounts of fish may be associated with reduced mortality from CHD and is attributed to the *n-3* fatty acids present in them [2]. Cardiovascular disease (CVD) is the most frequent cause of morbidity and mortality not only in developed nations but also in developing nations such as Sri Lanka [3]. The roles of blood lipids and lipoproteins in predicting morbidity and mortality from CVD have been well reported [4]. Lipoproteins and its associated cholesterol complexes such as high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) have received considerable attention [5]. LDL was identified as the major atherogenic plasma lipoprotein, where elevated levels were a significant coronary risk factor [6]. Variations in LDL concentrations account largely for the variations in CVD incidences for high-risk populations [7]. Prospective epidemiological studies have shown that a low plasma HDL level represents an independent risk factor for CVD [8,9]. Studies have shown that LDL-C/HDL-C and total cholesterol (TC)/HDL-C ratios are the strongest determinants of overall CVD risk [10]. Japanese people are known to have one of the longest life expectancies, and their diet is believed to be the most ideal in the world [11]. Their low mortality rate from CVD has been attributed to their high intake of fish and other seafood. Epidemiological studies have revealed a reduced incidence of CVD in populations with high or moderate fish consumption [12]. This

has been attributed to the abundance of long-chain *n-3* polyunsaturated fatty acids (*n-3* LCPUFAs) in fish oils including eicosapentaenoic acid (EPA; 20:5*n-3*), docosapentaenoic acid (DPA; 22:5*n-3*), and docosahexaenoic acid (DHA; 22:6*n-3*) [13]. Dietary *n-3* LCPUFAs from fish oils exert beneficial effects by reducing platelet aggregation and improving blood lipoprotein profiles. Fish and fish oil consumption have been consistently associated with triglyceride (TG)-lowering effects. The most consistent effects of *n-3* LCPUFAs are the reduction of serum cholesterol, TG [14], very low-density lipoprotein cholesterol (VLDL-C), and LDL-C levels [15,16]. Furthermore, intake of *n-3* LCPUFAs increases HDL-C levels in healthy subjects [17]. However, some studies have shown contradictory results regarding the anti-atherogenic effects of *n-3* LCPUFAs from fish and fish oils [18]. Many studies have shown that cholesterol is one of the key components of atherosclerotic plaques; therefore, hyperlipidemia is considered as an essential risk factor for atherosclerosis [19,20]. The basic laboratory exponent for cardiovascular risk assessment is the elevated concentration of TC that mostly results from elevated LDL-C [21,22]. Modified LDL particles, especially their oxidized forms (ox-LDL) may be freely taken up by macrophages whose scavenger receptors interact with apolipoprotein B100 (apoB). Subsequently, arising foam cells form fatty deposits on artery walls, which are the starting point for plaques [23,24].

Non-HDL-C is the sum of cholesterol accumulated in all lipoproteins apart from HDL such as chylomicrons, VLDL, and their remnants intermediate-density lipoprotein and LDL [25]. The concentration of non-HDL-C (mg/dl) is calculated using a simple equation: Non-HDL-C = TC - HDL-C. Liu *et al.* compared the diagnostic value of non-HDL-C as

a prognostic factor for acute coronary events and myocardial infarction among healthy subjects and diabetics [26]. Atherogenic index of plasma (AIP), Castelli risk index (CRI), and atherogenic coefficient (AC) are the three ratios studied in predicting the risk of coronary artery disease (CAD). These are the calculated fractions that can be used in a clinical setting for assessing the CVD risk beyond the routine lipid profile tests. AIP is based on two important parameters, TG and HDL-C, both of which are independent risk factors for CAD [27]. CRI 1 and 2, calculated as $CRI-I=TC/HDL-C$ and $CRI-II=LDL-C/HDL-C$, are fractions which involve the independent risk factors for CAD as well [28,29]. AC calculated as $(TC-HDL-C)/HDL-C$ is yet another ratio relying on the significance of HDL-C in predicting CAD risk [30].

No previous studies have described the relationship between different fish consumption habits and CVD lipid risk profiles. Therefore, the objective of this present study was to investigate the atherogenic risk factors using lipoprotein lipids of healthy people from a habitual-fish-consuming population of the Eastern Sri Lankan community who eat three different types of fish. The objective of this study involved in assessing the significance of lipid ratios such as AIP, CRI, and AC to identify CAD risk among healthy people with different fish consumption habits, beyond the routinely done lipid profile especially in insufficient resource situations.

METHODS

Study location

This study was a cross-sectional evaluation of the coastal villages of Batticaloa District, situated in the Eastern part of Sri Lanka. The district extends from latitude 7.75 N and longitude 81.7 E, from Verugal to Kallar along the east coast. The study area was selected by stratified random sampling of 14 rural and urban villages, 10 to 120 km from the town of Batticaloa. All the villages had similar geographical locations, environmental and cultural settings, and average living standards.

Selection of subjects

We studied 371 healthy subjects aged between 25 and 75 years, matched for sex and age, and divided into different groups based on types of fish consumed. All the subjects provided informed consent before participation. The subjects were selected based on their habitual and regular fish consumption during the previous years. They were asked to categorize whether their habitual intake included either sea or inland fish, or all types of fish. Their frequency of fish consumption, quantity of fish consumption per week, and consumption of non-fish meat was determined. We recorded socio-demographic data such as age, sex, and type of physical and lifestyle activities. The investigation was conducted by distributing pre-tested, structured questionnaires to the subjects. We collected data on fish consumption and food habits, along with blood samples for lipid profile assessment simultaneously. The ethical clearance certificate was obtained by submitting the proposal to the Ethical Clearance Committee, Faculty of Medicine, Eastern University, Sri Lanka.

Collection of serum from blood samples

This study aimed to study the lipid profiles of healthy subjects at the Asiri Laboratory, Batticaloa. After 12 h of overnight fasting, blood

samples were obtained from the antecubital vein of the 371 subjects using a syringe (23 gauge), following all aseptic precautions. Blood was collected in a plain bulb (FL Medical, Italy) for estimation of serum lipid profile. Blood samples were incubated at 37°C for 30 minutes and then centrifuged (Scientific, UK) at 1000 rpm for 10 minutes. The separated serum was used for lipid profile estimations by a routine biochemical kit method (Thermo Scientific, UK) with an automated analyzer.

Estimation of serum cholesterol, HDL-C, LDL-C, and TG

Serum cholesterol level was estimated using the dynamic extended stability (DES) cholesterol oxidase-phenol-4-aminophenazone endpoint enzymatic method with a lipid clearing agent. Serum HDL-C level was estimated by the phosphotungstic acid endpoint method. Serum TG level was estimated using the DES Trinder endpoint method with a lipid clearing agent. VLDL-C and LDL-C were calculated with Friedewald's formula [31]. TG/5 was calculated from the formula: $VLDL-C=TG/5$, where $TG<400$ mg/dl. LDL-C was calculated from the formula: $TC-(HDL-C+VLDL-C)$. Using these two formulae, LDL-C levels were calculated as follows:

$LDL-C=TC-HDL-C-TG/5$ [32]. The following were cut-off points for different lipoproteins, where elevated levels were determined as risk factors according to criteria of the American National Cholesterol Education Program: HDL-C <35 mg/ml, LDL-C >130 mg/dl, LDL-C/HDL-C >2.0, and TC/HDL-C >4.5 [32].

Calculation of atherogenic indices

The atherogenic ratios were calculated as follows:

$$AIP=\text{Log}(TG/HDL-C)$$

$$CRI-I=TC/HDL-C$$

$$CRI-II=LDL-C/HDL-C$$

Statistical analysis

Multiple comparisons for the means among different variables involved Tukey's *post-hoc* test. $p<0.05$ were considered as significant. Statistical analysis was performed using SPSS for Windows, Version 11. Results were presented as mean±standard error of mean. Tests for significance included Student's t-test and analysis of variance where applicable.

RESULTS AND DISCUSSION

The subjects were categorized as either all fish, inland fish, or sea fish eaters. CVD lipid risk profile values for all fish, inland fish, and sea fish consumers are shown in Table 1. Fish consumption habits influenced the CVD lipid risk profile. TC, TG, and non-HDL-C varied significantly among the three different types of fish eaters ($p<0.05$), while LDL-C showed the most significant variation ($p<0.000$). However, HDL-C showed no significant variation among different fish eaters ($p>0.05$). The atherogenic indices among three different types of fish eaters were showed in Table 2. CRI-II showed the most significant variation among different fish eaters ($p<0.000$), while AIP and TG/HDL-C varied significantly as well ($p<0.05$). TG/HDL-C value was greater for inland fish eaters. However, AC and CRI-I showed

Table 1: The comparison mean±SEM values for lipid profiles of all fish including beef, inland, and sea fish eaters by one-way ANOVA

Parameter (mg/dl)	All fish (n=91)	Inland fish (n=118)	Sea fish (n=162)	F value	p value
TC	208.18±4.70	191.06±3.26	200.58±3.46	4.430	0.013 ^s
TG	125.42±4.93	161.89±9.80	151.79±5.52	5.652	0.004 ^s
LDL-C	135.61±4.24	113.69±3.13	125.12±3.01	8.843	0.000 ^s
HDL-C	47.46±1.26	44.42±0.66	44.97±0.87	2.480	0.085 ^{ns}
VLDL-C	25.08±0.98	32.38±1.96	30.47±1.10	5.681	0.004 ^s
Non-HDL-C	160.73±4.53	146.64±2.98	155.60±3.21	3.585	0.029 ^s

s: Significant; $p<0.05$, ns: Non-significant; $p>0.05$, SEM: Standard error of mean, TC: Total cholesterol, TG: Triglycerides, LDL-C: Low-density lipoprotein cholesterol, VLDL-C: Very low-density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol, Non-HDL-C: Non-high-density lipoprotein cholesterol, ANOVA: Analysis of variance

Table 2: The comparison mean±SEM values for atherogenic indices of all fish including beef, inland, and sea fish eaters by one-way ANOVA

Parameter (mg/dl)	All fish (n=91)	Inland fish (n=181)	Sea fish (n=162)	F value	p value
CRI-I	4.61±0.15	4.37±0.09	4.50±0.07	1.303	0.273ns
CRI-II	3.02±0.12	2.55±0.07	2.79±0.6	7.992	0.000s
AC	1.91±0.18	3.04±0.35	2.53±0.17	1.303	0.273ns
API	1.25±0.01	1.30±0.17	1.3±0.01	3.828	0.023s
TG/HDL-C	2.91±0.18	4.04±0.35	3.53±0.17	4.376	0.013s

s: Significant; hp<0.05, ns: Non-significant; p>0.05, SEM: Standard error of mean, CRI: Castelli risk index, TG: Triglycerides, HDL-C: High-density lipoprotein cholesterol, AIP: Atherogenic index of plasma, AC: Atherogenic coefficient, ANOVA: Analysis of variance

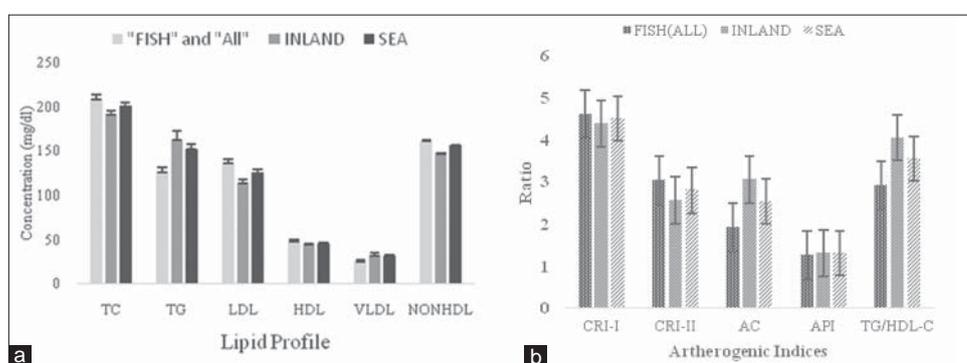


Fig. 1: Comparison of (a) lipid profiles and (b) atherogenic indices between all fish (91), inland (181), and sea fish eaters (162). Data are presented as mean±SEM. TC: Total cholesterol, TG: Triglycerides, LDL-C: Low-density lipoprotein cholesterol, VLDL-C: Very low-density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol, Non-HDL-C: Non-high-density lipoprotein cholesterol, CRI: Castelli risk index, AIP: Atherogenic index of plasma, AC: Atherogenic coefficient, SEM: Standard error of mean

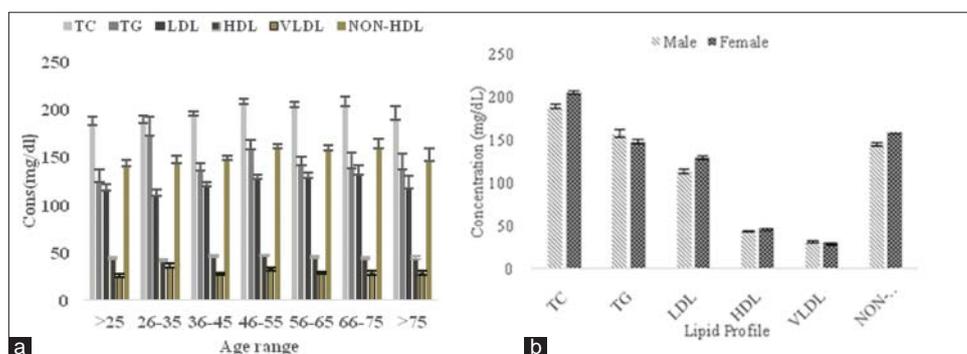


Fig. 2: Comparison of lipid profiles based on (a) age and (b) sex between all fish (91), inland (181), and sea fish eaters (162). Data are presented as mean±SEM. TC: Total cholesterol, TG: Triglycerides, LDL-C: Low-density lipoprotein cholesterol, VLDL-C: Very low-density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol, Non-HDL-C: Non-high-density lipoprotein cholesterol, CRI: Castelli risk index, AIP: Atherogenic index of plasma, AC: Atherogenic coefficient, SEM: Standard error of mean

no significant variation among different fish eaters ($p>0.05$). Large F values indicated more than chance variation among different fish eaters in both lipid profiles and atherogenic indices. Overall, lipid profile (TC, TG, LDL-C, and HDL-C) values were greater in females than males (Figs. 1 and 2). TG value was greater for inland fish eaters than sea or all fish eaters (Table 1). All fish eaters were mostly beef eaters as well, whose atherogenic indices were at borderline-high level (Table 2). The atherogenic link between high TG and HDL-C is due to the high plasma concentrations of TG and VLDL-C that generate small dense LDL-C during lipid exchange and lipolysis. These LDL-C particles accumulate in the circulation and form small, dense LDL-C particles, which undergo accumulated catabolism, thus closing the atherogenic circle [33,34]. The TG/HDL-C ratio of AIP was found to be a powerful independent indicator of extensive coronary disease [35]. This ratio was initially proposed by Gaziano *et al.* [36] and is an atherogenic index that has proven to be a highly significant independent predictor of myocardial infarction, even stronger than

that of CRI-I or CRI-II [36], as observed for inland fish eaters. Bambi *et al.* reported that with AIP, it is possible to determine approximately the presence and extent of CAD by non-invasive methods [37]. AIP shows the inverse relationship that exists between TG and HDL-C, and its ratio is a strong predictor of infarction as used by some practitioners in predicting arteriosclerosis [36].

AC is a measure of cholesterol in LDL-C and VLDL-C lipoprotein fractions with respect to good cholesterol or HDL-C. As AC value increases, the risk for developing CVD increases and vice versa [38]. Inland fish eaters had a higher value of AC (3.04 ± 0.35) among the different fish eaters. Recently, non-HDL-C has become a commonly used marker for a blood lipid pattern associated with increased risk of heart diseases [39]. This study showed that non-HDL-C for all fish including beef eaters was borderline-high (160-189 mg/dl), whereas inland and sea fish eaters had an ideal range (130-159 mg/dl). Inland fishes are fatty fishes that have higher saturated fatty acids than sea fishes [40]. People who ate inland fishes had a higher

Table 3: The comparison between lipid profiles and atherogenic indices in males and females by one-way ANOVA

Parameter (mg/dl)	F value	p value
TC	11.850	0.010 ^s
TG	0.878	0.349 ^{ns}
LDL-C	15.085	0.000 ^{s***}
HDL-C	1.878	0.171 ^{ns}
VLDL-C	0.812	0.368 ^{ns}
Non-HDL-C	10.763	0.001 ^{s*}
CRI-I	4.682	0.031 ^s
CRI-II	11.331	0.001 ^{s*}
AC	4.682	0.031 ^s
API	1.942	0.164 ^{ns}
TG/HDL-C	0.848	0.358 ^{ns}

s: Significant; p<0.05, ns: Non-significant; p>0.05, s*: Significant; p<0.001, s***: Significant, TC: Total cholesterol, TG: Triglycerides, LDL-C: Low-density lipoprotein cholesterol, VLDL-C: Very low-density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol, Non-HDL-C: Non-high-density lipoprotein cholesterol, ANOVA: Analysis of variance, CRI: Castelli risk index, AIP: Atherogenic index of plasma, AC: Atherogenic coefficient

TG level in their serum than other types of fish eaters. However, non-HDL-C was high in the serum of people who eat all types of fish and beef (Table 1).

CONCLUSION

The type of fish consumed (all fish including beef, inland fish, or sea fish) had distinct effects on serum cholesterol and lipoprotein fractions (TC, LDL-C, HDL-C, non-HDL-C) and TG level. People who ate all kinds of fish had higher TC levels than those who ate inland or seawater fish. Those who ate inland fish had higher serum TG levels than those who ate other types of fish. All fish including beef eaters had a non-HDL-C level that was borderline high risk for CVD. TC and lipid-protein fractions had age- and sex-related differences. Women had higher TC and LDL-C levels than men. The values of non-HDL-C and TG/HDL-C can be used as improved diagnostic factors for CVD risk.

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