

## Research article

**Influence of fish on biochemical markers: A comparative study among male subjects of fish eaters and vegetarians**Asha Prabhu<sup>1,3</sup>, H. P. Kedilaya<sup>1</sup>, Manjula Shantaram<sup>2,3</sup><sup>1</sup>Department of Biochemistry, Srinivas Institute of Medical Sciences & Research Centre, Srinivasnagar, Mukka, Surathkal, Mangalore, Karnataka, India<sup>2</sup>A. J. Research Centre, A.J. Institute of Medical Sciences & Research Centre, Mangalore, Karnataka, India<sup>3</sup>Yenepoya University, Deralakatte, Mangalore, Karnataka, India

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Corresponding author: **Manjula Shantaram**. Email: manjula59@gmail.com**ABSTRACT**

**Introduction and Aim:** Cardiovascular diseases (CVD) are the major causes of morbidity and mortality in India. Intake of a high or moderate amount of fish has shown a decreased risk of CVD. This is due to the presence of long-chain n-3 polyunsaturated fatty acids (PUFA), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in fish oils. According to past studies, regularly consuming these fatty acids lowers the risk of heart failure, myocardial infarction, endothelial dysfunction, inflammation, arrhythmias, and sudden death. The beneficial effects of a fish-rich diet appear to conflict with PUFA's susceptibility to oxidation. As a result, the study aimed to compare the lipid profile, cardiac markers, and lipid peroxidation status in male adults who consume moderate amounts of fish regularly.

**Materials and Methods:** Total cholesterol (T.C.), HDL-cholesterol (HDL-C), triglycerides (T.G.), high sensitive C-reactive protein (hs-CRP), Lipoprotein (a) [Lp(a)], Malondialdehyde (MDA) and antioxidant activity (AOA) were measured in healthy male individual of 85 fish eaters and 77 vegetarians in the age group of 25-40 years. The atherogenic indices TC/ HDL-C, LDL-C/HDL-C, a Student's 't' test, and non-HDL-C/ HDL-C were determined. The two groups' parameters were compared using the Student's t-test.

**Results:** In Fish eating male subjects, the lipid profile and cardiac markers were decreased except for HDL-C (which was increased). Fish eaters exhibited significant decreases in atherogenic indices, and significant variation was not observed in the oxidant status of the study groups ( $p>0.05$ ).

**Conclusion:** According to the present study's findings, regular intake of moderate amounts of fish is linked to a decrease in lipid profiles and cardiac markers without affecting the individual's oxidation status.

**Keywords:** Fish eaters; vegetarians; lipid profile; hs-CRP; Lp(a); cardiovascular disease.

**INTRODUCTION**

Cardiovascular diseases (CVD) are a range of disorders that impact the structure and functioning of heart and blood vessels (1). Compared to the preceding decade, CVD-related deaths have increased by 12.5% (2). High-income countries experience a significant drop in death rates (3).

By 2030, deaths from non-communicable illnesses will rise from 38 million to 52 million, and more than 23 million people may die each year from cardiovascular diseases alone, according to the "WHO Global Status Report published in 2014 on 'Non-Communicable Diseases' (4).

Large prospective studies from India suggest that around 30 - 42% of deaths are associated with CVD, and the mortality rate is higher in men than women (5). Obesity, hyperlipidemia, type 2 diabetes, hypertension, and cardiovascular disease (CVD) are common in South Asians and are increasingly prevalent in the Asian Indian population (6,7). Several findings have explained that altered lipid profiles cause endothelial dysfunction. The risk

factors for CAD were increased levels of blood total cholesterol (T.C.), LDL cholesterol (LDL-C), and decreased levels of HDL cholesterol (HDL-C) (8). Inflammation is a new risk factor for coronary artery disease. Vascular inflammation is a significant contributor to the development of atherosclerosis. Hs-CRP, a marker of low-grade inflammation, is elevated in individuals with metabolic syndrome and is linked to an increased risk of CVD (9,10).

Studies on  $\omega$ -3 fatty acids have demonstrated that their frequent consumption lowers circulating triglycerides, endothelial dysfunction, and arrhythmias, suppresses the pro-inflammatory process, and decreases inflammatory mediators. Additionally, EPA and DHA also showed antihypertensive and anticoagulant effects. Dietary  $\omega$ -3 fatty acids enhance and alter the protein part in the HDL, stabilize the cardiac membrane, and change the electrophysiological properties of the myocardium (11-13). Oxidative stress plays a role in various clinical conditions, such as malignant diseases, diabetes, and atherosclerosis. RBCs are most vulnerable to oxidative stress that results in

lipid peroxidation, leading to hemolysis. However, a diet rich in fish increases the risk for oxidation because of PUFAs in them (14).

## MATERIALS AND METHODS

### Selection of subjects

In this comparative analysis based on a population, 85 primarily fish eating males and 77 vegetarian males aged 25-40 years were enrolled. These subjects were selected from certain parts of the coastal regions of the Dakshina Kannada and Udupi districts of Karnataka.

### Inclusion criteria

Vegetarians: Individuals who follow a strict lacto-vegetarian diet. Fish eaters: Those who eat fish five or more times a week, along with vegetables and intermittently with various meats (an approximation of quantity was determined by dividing the amount of fish cooked by the family members consuming it).

### Exclusion criteria

The following persons were detained from the study: Diabetes mellitus, hypertension, pregnancy, smoking, chronic kidney disease, and those with disorders with chronic inflammation, such as leprosy and tuberculosis. The institutional ethics committee granted an ethical clearance. The study was explained by the investigator to the subjects, and informed consent was obtained from the subjects. Anthropometric measurements were assessed, including weight, height, and waist circumference. A questionnaire was used to determine the subjects' dietary profile.

### Sample collection

After an overnight fast of 8 to 10 hours, About 7 ml of venous blood was collected from the subject. 3.5 ml of whole blood was collected in plain vacutainer tubes, and 3.5 ml was collected in tubes containing EDTA. Serum separated from whole blood collected in the plain tube was used for the estimation of lipid profile, high sensitive C-reactive protein (hs-CRP) and Lipoprotein (a) [Lp(a)], and Antioxidant activity (AOA). Blood in the EDTA tube was used to estimate oxidant status in RBC suspension.

The cholesterol-oxidase-peroxidase (-CHOD-PAP) endpoint method was used to estimate total cholesterol (15). The selective inhibition endpoint oxidase (-CHOD-PAP) technique directly determined HDL-C (16). Triglycerides (T.G.) were estimated by the GPO-PAP method (17). and VLDL-C calculated using the formula  $VLDL-C = TG/5$ . LDL-C was calculated using Friedwald's formula:  $[Total\ cholesterol - HDL-C - (Triglycerides/5)]$  (18). Non-

HDL cholesterol is the cholesterol found in lipoprotein particles considered atherogenic and defined as the difference between total cholesterol and HDL-C (19). High-sensitive C-reactive protein (hs-CRP) was assessed using Nephelometry (20). Lipoprotein (a) [Lp (a)] was measured using an immuno-turbidimetric assay (21). Whole blood collected in an EDTA tube was used to estimate Malondialdehyde.

### Statistical analysis

The evaluated parameters were expressed as mean  $\pm$  S.D. The SPSS program was used to analyze the differences between the parameters using the Student's t test.  $p < 0.05$  was considered significant, and  $p < 0.01$  was considered highly significant.

## RESULTS

This comparative study includes 85 predominantly fish eating and 77 vegetarian male subjects. The current study evaluated anthropometric parameters such as height and body weight. BMI ( $Kg/m^2$ ) was calculated using the above anthropometric measurements. Male subjects who consumed fish had a BMI of  $26.04 \pm 4.83\ kg/m^2$ , and vegetarians had a BMI of  $24.71 \pm 4.49\ kg/m^2$  ( $p > 0.05$ ). The Student's t test was used to compare the waist circumference data of males of both groups ( $p > 0.05$ ). Male fish eaters had larger waist circumferences than the vegetarian male subjects.

Male subjects of the vegetarian group had a higher mean  $\pm$ SD value of all the parameters except HDL-C (which was decreased). The marker T.G. was  $124.42 \pm 56.12\ mg/dL$  in male fish eaters and  $159.36 \pm 54.96\ mg/dL$  in vegetarian male subjects, which showed a statistically highly significant difference ( $p < 0.01$ ). The mean  $\pm$ SD value of Lp (a) was  $11.70 \pm 5.03\ mg/dL$  in fish eaters and  $14.83 \pm 4.68\ mg/dL$  in vegetarians, showing a statistically highly significant difference ( $p < 0.01$ ). The non-HDL-C parameter showed a significant increase ( $p < 0.05$ ) in the male vegetarian subjects. According to Table 2, all atherogenic indices were considerably higher in male vegetarian subjects than in male fish eaters. The indices TC/HDL-C ( $p < 0.01$ ), non-HDL-C/HDL-C ( $p < 0.01$ ), and LDL-C/HDL-C ( $p < 0.05$ ) increased significantly. The value of MDA is expressed in terms of mean  $\pm$ SD. At the end of 1 hour, MDA was  $3.83 \pm 0.52\ (\mu\ mol/L)$  in fish eaters and  $3.76 \pm 0.45\ (\mu\ mol/L)$  in vegetarians ( $p > 0.05$ ). MDA measured at the end of 2 hours using hydrogen peroxide as an oxidizing agent was  $5.93 \pm 0.80\ (\mu\ mol/L)$  in fish eaters and  $5.99 \pm 0.47\ (\mu\ mol/L)$  in vegetarians ( $p > 0.05$ ).

**Table 1:** Comparison of lipid profile and cardiac markers in male subjects of the study groups

Parameters (mg/dL)	Males	
	Fish eaters (n=85)	Vegetarians (n=77)
TC	202.11 ± 28.86	209.30 ± 30.43
TG	124.42 ± 56.12	159.36 ± 54.96**
HDL-C	48.58 ± 8.64	46.18 ± 7.63
LDL-C	128.60 ± 23.86	132.36 ± 26.43
Non-HDL-C	153.53 ± 27.56	163.13 ± 28.92*
Lp(a)	11.70 ± 5.03	14.83 ± 4.68**
hs-CRP <sup>d</sup>	0.76 ± 0.35	0.84 ± 0.32

n= number of subjects. All the values are Mean ± S.D. Statistical analysis by Student's t test revealed the difference between the parameters, \*\*p<0.01 is statistically highly significant, \*p<0.05 is statistically significant. hs-CRPd-high sensitive C-reactive protein in mg/L; Lp(a)-Lipoprotein (a).

**Table 2:** Comparison of atherogenic indices in male subjects of the study group

	Males	
	Fish eaters (n=85)	Vegetarians (n=77)
TC/HDL-C	4.26 ± 0.83	4.61 ± 0.83**
LDL-C/HDL-C	2.72 ± 0.64	2.92 ± 0.68*
Non-HDL-C/HDL-C	3.26 ± 0.83	3.61 ± 0.83**

n=number of subjects. All the values are Mean ± S.D. Statistical analysis by Student's t test revealed a difference between the parameters, \*\*p<0.01 is statistically highly significant, \*p<0.05 is statistically significant.

**Table 3:** Comparison of pro-oxidant and antioxidant status in male subjects of the study group

Parameters (μ mol/L)	Males	
	Fish eaters (n=85)	Vegetarians (n=77)
MDA <sup>a</sup>	3.83 ± 0.52	3.76 ± 0.45
MDA <sup>b</sup>	5.93 ± 0.80	5.99 ± 0.47
AOA	1.44 ± 0.36	1.38 ± 0.19

n= number of subjects. All the values are Mean ± S.D. Statistical analysis by Student's t test revealed that the difference between the parameters, p>0.05, is statistically not significant. MDAA - Malondialdehyde measured at the end of 0 hour; MDAB-Malondialdehyde measured at the end of 2 hours.; AOA-Antioxidant Activity.

## DISCUSSION

This study examined the impact of a modest fish intake on cardiac markers and lipid profiles in males aged 25 - 40. According to the study findings, male fish eaters subjects showed healthier lipid profiles and cardiac markers than vegetarian subjects. These findings support the literature findings of Qi et al., who reported that fish oil, which is rich in Omega-3 fatty acids, decreases plasma T.G. (22). According to a comparative study carried out by Mohammad et al., fresh fish consumption had a greater impact than omega supplementation on T.C., non-HDL-C, T.G., TC/HDL, and LDL/HDL ratios. In this study, LDL levels increased in the supplementation group while decreasing significantly in the fish diet group (23). A similar beneficial result was found in Zhu et al.'s study on dialysis patients, where fish oil supplements reduced total cholesterol and T.G., increasing HDL-C (24). The hs-CRP level decreased in fish eaters, which was consistent with the findings of a study involving Japanese men and women and demonstrated that a diet rich in fish is known to reduce inflammation (25). The reduced Lp (a) level in fish eaters was consistent with the literature

findings of Marcovina et al., which demonstrated that a group of Bantu fishermen had much lower levels of Lp (a) than surrounding Bantu populations who were vegetarians (26). The result on oxidative stress in RBCs is consistent with Cariappa et al., who stated that the plasma MDA content remained unchanged between the two groups. (27). The study of Mabile et al. also noted sustained erythrocyte tolerance to oxidative stress in individuals with hypertriglyceridemia (28). However, in an in vivo study, conflicting findings regarding the effects of eicosapentaenoic acid and docosahexaenoic acid consumption on oxidation were also observed (29). The outcome of this study did not agree with the argument that a diet rich in fish may cause more oxidation.

## CONCLUSION

This study's findings show that men aged 25-40 years who regularly consume a moderate amount of fish have healthier lipid profiles and cardiac markers, thereby having a preventative effect on CVD. This study also reveals that habitual fish consumption does not increase lipid peroxidation. A larger-scale study

might yield more accurate findings regarding the health advantages of including fish in a regular diet.

## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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