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OXIDANT AND ANTIOXIDANT STATUS IN VEGETARIANS AND FISH EATERS

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ABSTRACT

An adequate antioxidant reserve which is usually present in a vegetarian diet is associated with higher life expectancy. On the other hand habitual consumption of fish is associated with lower risk of cardiovascular diseases which is attributed to the polyunsaturated fatty acids (PUFA) present in it. However the PUFA are readily susceptible to oxidation leading to the formation of free radicals which is known to be involved in the causation of several diseases.

The oxidant-antioxidant status of 23 vegetarians and 22 fish eaters was studied by determining the plasma lipid peroxides measured as malondialdehyde (MDA) and the antioxidants, viz. glutathione (GSH), ascorbic acid, ceruloplasmin and uric acid.

Results show that the ascorbic acid values were significantly higher in vegetarians. In addition, MDA correlated negatively with ascorbic acid only in vegetarians. However, correlation of MDA with glutathione showed a significant negative correlation only in fish eaters. There was no statistically significant difference in the MDA, GSH, ceruloplasmin and uric acid levels. The mechanism behind these findings are not clear and needs to be explored.

KEY WORDS

Antioxidants, ceruloplasmin, free radicals, glutathione, lipid peroxide, malondialdehyde

INTRODUCTION

An increased concentration of end products of lipid peroxidation is the evidence most frequently quoted for the involvement of free radicals in human diseases. Several studies support the hypothesis that lipid oxidation products ingested with food or produced endogenously represent a health risk (1). It is well established that free radicals and reactive oxygen species are continuously produced *in vivo.* Numerous detoxifying mechanisms in the body protect the cells from the toxic effects of free radicals and the associated peroxidation of lipids. These include protective enzymes (superoxide dismutase, glutathione peroxidase, glutathione reductase, glutathione transferase, catalase, etc.) and several low molecular weight water and lipid soluble antioxidants (reduced glutathione, uric acid, ubiquinol-10, etc). Apart from these endogenous antioxidants, there are several essential antioxidants which include vitamins E, A , C and carotenoids.

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There is increasing evidence that under certain circumstances these protective systems may be overwhelmed. Free radical mediated oxidation of lipids particularly affects the polyunsaturated fattyacids (PUFA). The primary products of free radical oxidation undergo rapid and spontaneous fragmentation. Some have considerable lethal potential. The peroxidation of lipids has gained significance in recent times because of its implication in the causation of a number of diseases (1,2).

Epidemiological studies all over the world have shown that vegetable-rich diets are associated with higher life expectancy. The reason for this could be due to the higher antioxidants present in such diets (3). Higher fruit and vegetable intake was associated with a lower risk of myocardial infarction and other cardiovascular diseases (4), cancers of the mouth, pharynx, larynx, stomach, colon, etc (5,6). Habitual consumption of fish on the other hand is also associated with reduced mortality from coronary heart diseases (7,8). The beneficial effects, namely, lowered plasma lipids, diminished thrombogenecity and decreased blood pressure have been attributed to the accumulation of n-3 fatty acids over time in the individuals consuming fish regularly. The PUFAs account for 30-50% of the total fatty acids in fishes from Indian waters (9).

In contradiction to these beneficial effects of a fish - enriched diet it is found that high PUFA content in the diet increases the ratio of the PUFAs to the saturated fatty acids in all biological membranes. Hence it can be expected that an increase in the PUFA content of the biological membranes enhances their susceptibility to oxidation. Evaluation of the oxidant and antioxidant status in the vegetarians as compared to the fish eaters was the objective of the study.

MATERIALS AND METHODS

Healthy male subjects in the age group of 40-55 years were chosen for the study. Study included two groups of subjects. The first group consisted of twenty three vegetarians. The second group consisted of twenty two volunteers consuming at least 4-6 fish dishes per week amongst other foods including vegetables and meats. The inclusion and exclusion criteria were as follows.

Inclusion Criteria

Vegetarians : Subjects consuming purely lacto vegetarian diet.

Fish eaters : Subjects consuming 4 - 6 fish dishes per week along with vegetables and occasionally other meats.

Exclusion Creteria : For both the groups, subjects who were smokers, alcoholics or diabetics were excluded from the study.

An informed consent was taken from the subjects. Height, weight and blood pressure were recorded and the body mass index (BMI) was calculated by using the formula,wt in Kg /ht in metres^2. Five ml of heparinised venous blood was drawn from the anticubital vein of the subjects in the post absorptive state. Within 2 hours of collection the samples were analyzed for lipid peroxides, reduced glutathione, ascorbic acid, ceruloplasmin and uric acid.

Reduced GSH in the whole blood was estimated by the method of Beutler *et al* (10). This method is based on the development of a relatively stable yellow colour when 5-5' dithio bis - 2 nitro benzoic acid (DTNB) is added to sulphydryl compounds. The yellow colour developed was measured spectrophotometrically at 412 nm within 10 minutes.

Ascorbic acid was estimated by the dinitro phenyl hydrazine method (11). Ascorbic acid is oxidized by Cu^{+2} to form dehydro ascorbic acid, which reacts with acidic 2, 4 dinitro phenyl hydrazine to form a red bis-hydrazone which was measured spectrophotometrically at 520 nm.

Ceruloplasmin in the plasma was estimated by the method of Sunderman *et al* (12). At pH 5.4 ceruloplasmin catalyses the oxidation of p-phenylene diamine dihydrochloride (PPD) to yield a purple coloured product which was measured spectrophotometrically at 530 nm.

Uric acid in the plasma was estimated by the modified Trinder's method (13) using Transasia BioMedicals Manual kit. Uric acid is oxidised by uricase to give allantoin, carbondioxide and hydrogen peroxide. The hydrogen peroxide formed oxidizes the phenol, 2, 4, 6 tribromo 3 - hydroxy benzoic acid (TBHB) and forms a coloured product (quinone- imine) with 4 amino antipyrene (4 A A P). The pink colour obtained was measured spectrophotometrically at 510 nm.

Plasma lipid peroxides was estimated as malondialdehyde by using the TBA method of Yagi (14) and was modified (15). The procedure adapted was as follows.

0.5ml plasma was precipitated with 2.5ml of 10% phosphotungstic acid. After letting it stand for 10 minutes, centrifuged at 3000g for 10min. The sediment was suspended in 4ml distilled water. This was followed by addition of 0.5 ml glacial acetic acid and 0.5ml 0.33% TBA. It was kept in a water-bath at 97°C for 45 minutes. The tubes were cooled and 0.05ml 5M HCl was added to the tubes to lower pH of the solution to <2 (1.6-1.7). The pink colour was extracted with 5ml butanol. The butanol layer was transferred to a spectrophotometer cuvette. Absorbance was read at 535nm. MDA values were expressed as mmol /L (molar absorbance of MDA = 1.56 10^5). 4 ml distilled water treated similarly was used as the blank.

STATISTICAL ANALYSIS

Results obtained were subjected to statistical analysis. Students 't' test or the Mann Whitney 'U' test was used depending on the nature of the data.

Correlation between the variables was estimated by the Pearson's correlation coefficient.

RESULTS

Anthropometric data of the two groups are presented in Table 1. Both the groups were compatible in age and BMI. All the subjects were normotensive. The oxidative stress assessed by estimating the malondialdehyde is presented in Table 2. The mean content of plasma MDA is 4.78 ± 2.65 mmol/L in vegetarians and 4.11 ± 1.93 mmol/L in fish eaters. No statistically significant difference was observed between the two groups as studied by the unpaired 't' test. Results of the antioxidant parameters, viz. GSH, ascorbic acid, ceruloplasmin and uric acid are given in Table 3. The unpaired 't' test was used for testing the difference in means of GSH, ascorbic acid and ceruloplasmin. For uric acid the Mann Whitney 'U' test was used as the variance showed a statistically significant difference.

Only the ascorbic acid levels were significantly higher in vegetarians with a mean value of 1.03 ± 0.76 mg /dl in vegetarians and 0.60 ± 0.46 mg/dl in fish eaters. Pairwise correlation analysis carried out indicate that the correlation of glutathione with MDA showed a significant negative correlation in fish eaters (r = -0.441) and a weak positive correlation in vegetarians (r = 0.053). Figure 1 depicts the distribution of values in the two groups. On the contrary correlation of MDA with ascorbic acid levels showed no correlation in vegetarians as well as fish eaters. (r = 0.065 and 0.122). Figure 2 depicts the distribution of values in the two groups. The correlation of MDA with uric acid does not show any particular trend. The vegetarian and the fish eating group had 'r' values of 0.02 and 0.001. The plot for the uric acid levels of the two groups are shown in Figure 3. Correlation of MDA with ceruloplasmin showed a negative correlation for both the groups (r = -0.126 and -0.382). Though the correlation is stronger in fish eaters the values are not statistically significant. Figure 4 shows the correlation of MDA with ceruloplasmin.

DISCUSSION

Current understanding of *in vivo* oxidation of PUFAs by free radicals is based on the *in vitro* estimation of break down products of hydroperoxides formed during lipooxidation. In the present study significant difference in the peroxide levels between the two groups was not observed (4.78 and 4.11 mmol/L). The values are close to the range given by Esterbauer *et al* (11) who reported the normal range as 1.8 - 3.9 mmol/L. This finding is in agreement with the study of Higdon *et al* (16) who studied *in vivo* oxidation by measuring plasma MDA and F2 isoprostanes (a more specific indicator of lipid peroxidation). Similar studies by Calvilleo *et al* (17) have shown that susceptibility of rat erythrocytes to oxidative stress is not affected by supplementation of n-3 fatty acids. In contradiction, Meydani *et al* (18) and Piche *et al* (19) have reported increased concentration of plasma MDA in humans given supplements of fish oil. In the present study though significant differences in the peroxide levels are not observed, the vegetarian group showed a marginally higher value. This may probably be due to the higher rate of peroxidation of n-6 PUFA as quoted by Thomas *et al* (20).

The plasma levels of ascorbic acid in vegetarians were about twice that of the fish eaters. However no statistical difference in the GSH levels are observed. Ascorbic acid functions as an antioxidant both *in vitro* and *in vivo* (21). Frei *et al* (22) have shown that ascorbic acid acts as a primary defence in blood against aqueous radical attack. The work of Meister *et al* (23) supports the idea that GSH and ascorbate mutually spare each other. Higher doses of vitamin C were shown to rescue GSH deficient animals. Conversely, GSH enhancing agents were shown to delay the onset of scurvy. A significant negative correlation of GSH with MDA in fish eaters and negative correlation of ascorbic acid with MDA in vegetarians is an important observation in the study, probably indicating that ascorbic acid is spared in fish eaters and glutathione in vegetarians. Uric acid levels do not show statistically significant differences in vegeterians and fish eaters. Studies by Davies *et al* using model systems for lipid peroxidation show that the antioxidant effect of urate can be due to sequestration of catalytic iron (24). Sevanian *et al* (25) have reported that the antioxidant activity of urate is not associated with its depletion because a stable non catalytic urate - iron complex is formed. This may probably be the reason for non significant difference and the lack of correlation observed between MDA and uric acid in both the groups. The copper containing enzyme ceruloplasmin, is relatively susceptible to oxidative stress. Yamoshji *et al* (26) have reported elevated levels of ceruloplasmin in acute infections. Though, no significant difference in ceruloplasmin levels was observed between the vegetarians and fish eaters, vegetarians showed a marginally higher value.

From the light of results obtained, it can be concluded that i) Vegetarians have a higher concentration of ascorbic acid (an essential antioxidant) and ii) Glutathione (an endogenous antioxidant) is more susceptible for depletion in fish eaters). It may be that either the vegetarians consumed more ascorbic acid or fish eaters were more susceptible to depletion of this vitamin. However, the mechanism behind this finding needs to be elucidated. In the present study the quantity and the quality of the diet consumed by the subjects were not considered. The fish eating volunteers had vegetables and occasionally other meats which is a limitation of the study. The results would have been more clear cut if the volunteers were on absolute fish diet which is practically difficult. Further studies with the large number of subjects which would take into account the above aspects might throw more light on the above findings.

Thus, fish can be consumed regularly for its beneficial effects without fear of the harmful effects of peroxidation of lipids. However, it would be wise to include sufficient amounts of fruits, vegetables, legumes, and nuts to have an adequate antioxidant reserve.

Table 1

Anthropometric data of vegetarians and fish eaters

	Vegetarians (n=23)	**Fish Eaters (n=22)**
Age (yrs)	45.95 ± 4.33	46.05 ± 4.14
BMI (Kg/m^2)	27.17 ± 3.74	27.97 ± 13.32
BP (mm Hg)		
Systolic	125.48 ± 10.08	127.18 ± 9.15
Diastolic	82.78 ± 3.74	85.23 ± 5.11

n = number of subjects

[Values are Mean ± S.D.]

Table 2

Oxidant status of vegetarians and fish eaters as indicated by plasma MDA values

	Vegetarians (n=23)	Fish Eaters (n=22)
MDA (mmol/L)	4.78 ± 2.65	4.11 ± 1.93

n = number of subjects

[Values are Mean ± S.D.]

Table 3

Plasma levels of antioxidants in vegetarians and fish eaters

	Vegetarians (n=23)	Fish Eaters (n=22)
Glutathione (mg/dl)	31.72 ± 7.4	32.48 ± 9.60
Ascorbic acid (mg/dl)	*1.03 ± 0.76	0.60 ± 0.46
Uric acid (mg/dl)	6.01 ± 1.35	6.31 ± 2.11
Ceruloplasmin (mg/dl)	38.27 ± 18.36	31.30 ± 17.97

n = number of subjects

[Values are Mean ± S.D.]

* : $P < 0.05$

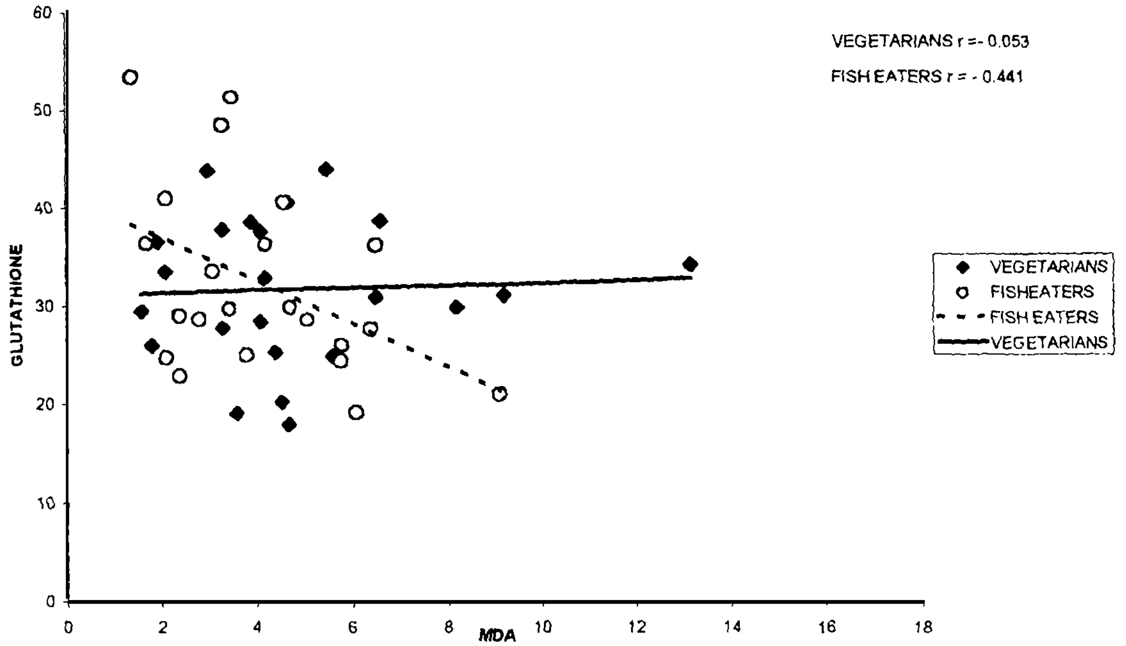

Fig.1:Correlation of MDA with glutathione among vegetarians and fish eaters

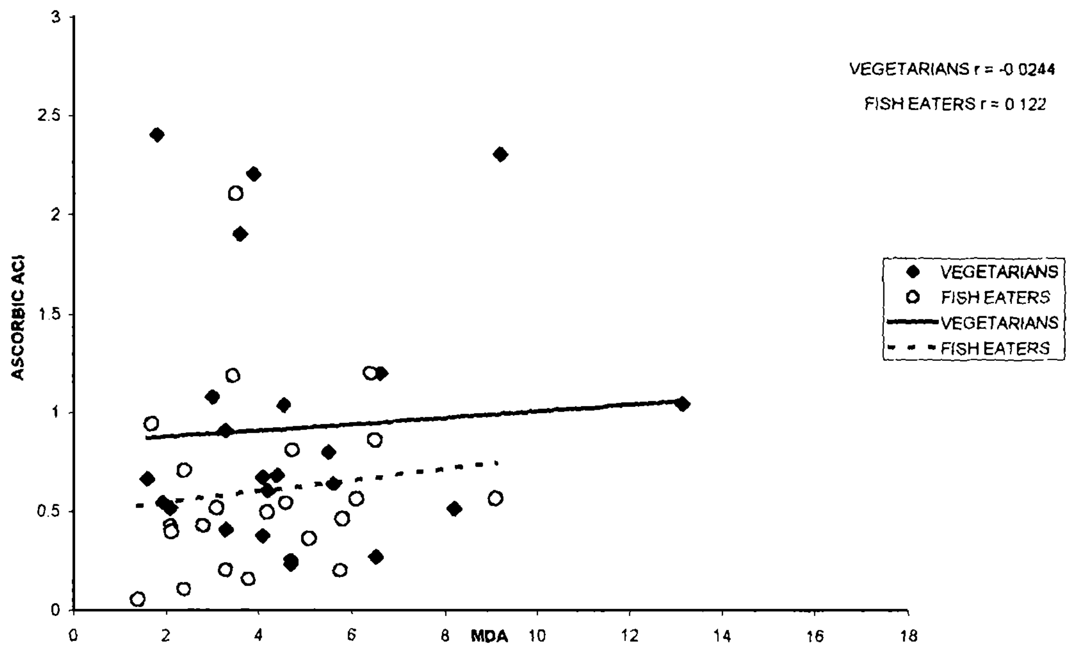

Fig.2:Correlation of MDA with Ascorbic acid among vegetarians and fish eaters

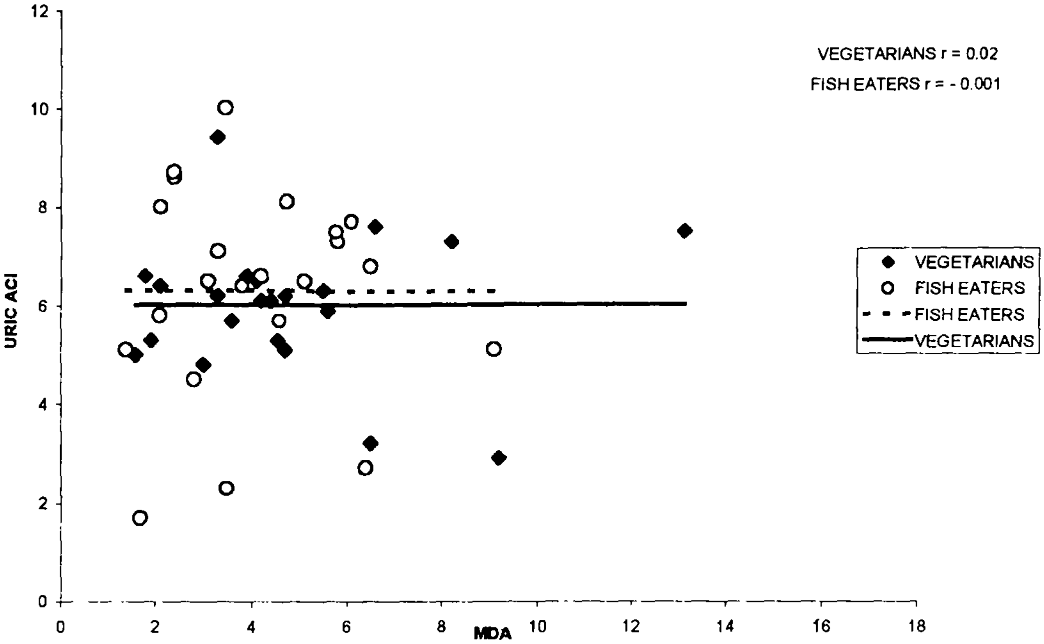

Fig.3:Correlation of MDA with uric acid among vegetarians and fish eaters

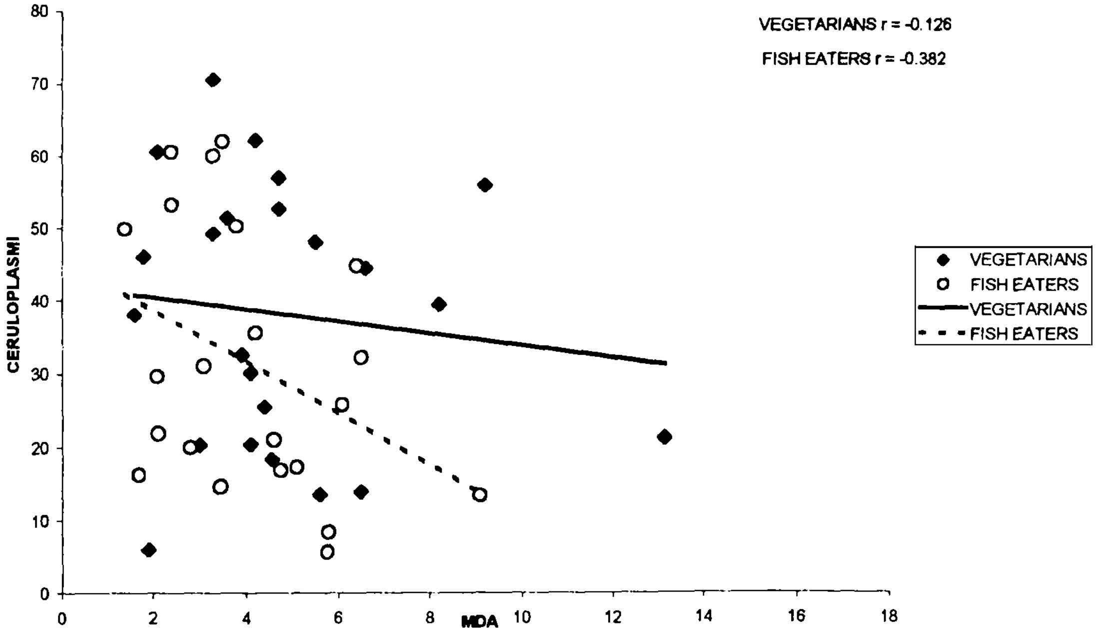

Fig.4:Correlation of MDA with ceruloplasmin among vegetarians and fish eaters

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