Traditional fish intake and fatty acid composition in fish consuming and non-fish consuming populations

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To evaluate the validity of habitual marine fish intake, the relation between fatty acid composition of serum phospholipids and dietary patterns were investigated. Dietary intake and serum fatty acid concentrations were measured in healthy subjects of coastal fish consuming and non-fish consuming populations. Amongst fish consumers, the intake of total energy ($p<0.01$) and carbohydrate ($p<0.05$) is significantly lower and protein intake higher than in non-fish consumers. The mean percentages of saturated and monounsaturated fatty acids do not show significant variation. However, in the ω-6 fatty acid series, the percent of linoleic acid, 22:4 ω-6 and 22:5 ω-6 is significantly lower in fish consumers, whereas dihomo-gamma linolenic acid is higher than in the non-fish consumers. The percentage of ω-3 fatty acids in fish consumers, eicosapentaenoic acid, docosapentaenoic acid and docosahexaenoic acid are significantly greater ($p<0.01$) than those in non-fish consumers probably attributable to differences in fish intake. These differences in fatty acid profiles, particularly in the long-chain ω-3 series, are highlighted with the consumption of fish being a possible explanation between fish consuming and non-fish populations. The findings of this study suggest that the therapeutic efficacy of fish consumption is worthy of further study.

Key words: fish intake, India, Andhra Pradesh, Nellore district, serum phospholipid, fatty acid consumption, essential fatty acids (EFA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), docosahexaenoic acid (DHA), arachidonic acid (AA)

Introduction

Marine fish consumption has attracted increased interest over the past two decades because of its potential role in the prevention and treatment of cardiovascular disease (CVD)[1,2]. Dietary fish and fish oils contain long chain polyunsaturated fatty acids (PUFA) of the ω-3 family, eicosapentaenoic (EPA; 20:5 ω-3 or timnodonic acid), docosapentaenoic (DPA; 22:5 ω-3 or clupanodonic acid) and docosahexaenoic acids (DHA; 22:6 ω-3 or cervonic acid)[3]. The results of case-controlled studies reported that the consumption of a small amount, even one or two fish meals per week, compared with no fish at all protects against CVD[4,5].

There is substantial cross-cultural and longitudinal evidence that both quality and quantity of fats ingested are important causes of nutrition-related diseases[6]. Since mammals lack enzymes necessary to synthesise ω-6 or ω-3 PUFA *de novo,* they must be obtained from the diet as either linoleic acid (LA; 18:2 ω-6) or alpha-linolenic acid (ALA; 18:2 ω-3). Further elongation and desaturation of these essential fatty acids (EFA) leads to the synthesis of long-chain PUFA in the circulating lipoproteins[7].

If there is benefit in consuming fish or fish products, a simple indicator of this intake would be useful as a partial measure of protection against risk factors. Accurate assessment of dietary fat in individuals and populations remains difficult and time consuming, therefore often biomarkers are used instead. Deposition of certain PUFA in body tissues is a valid biomarker of long-term dietary intake of these fatty acids. Saturated fat intake can not be assessed in this way and is impossible to differentiate between the importance of the two factors using such methods. The percentage of ω-3 and ω-6 PUFA in serum phospholipids is a well-recognised estimate of dietary intake, and the concentrations of EPA, DPA and DHA in serum or plasma lipids are directly related to the intake of fish oils[8].

In order to more directly evaluate how the consumption of fish affects biological functions, a method of analysing serum concentrations of ω-3 PUFA in fish consuming and non-fish consuming populations was investigated. There are no studies on fatty acid profiles in India, and, to our knowledge, nothing has been published on populations in relation to dietary habits.

Methods

India is one of the nine major fish-producing countries of the world and the catch of seafood has touched 30 billion US dollars in which India's contribution is 0.82%. At present, it produces about 3.4 million tonnes of fish, out of which, 1.6 million tonnes come from culture fisheries inland and 1.8 million tonnes from marine. The vast coastline stretches over 7,516 km in length; the continental shelf has an area of 414,868 km^2. The river systems together with irrigation canals cover a length of 140,000 km. The inland

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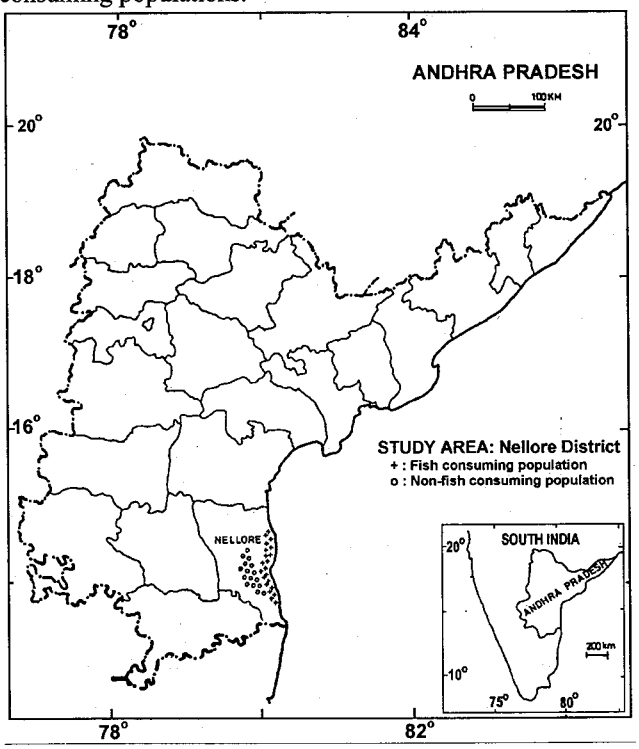
water bodies of natural lakes as well as man made reservoirs cover an area of 29,000 km^2 and have largely been responsible for the prominence of fishing. The estimated population who thrive solely on fishing is 5.38 million of which 3.28 million live along the 3,000 km coastline and the remainder on river banks, lake sides or near backwaters[9].

Andhra Pradesh is the fifth largest state of India with an area of 275,068 km^2, with 8.4% of the total population. The state lies between 12°14' to 19°54' north latitudes and 76°50' east longitudes. It has a coast line of 966 km along the Bay of Bengal on the south eastern part. The fishermen population is 326,304 of which 21,693 inhabit the coastal Nellore district. There are 453 fishing villages, 62 in the Nellore district alone, situated on the east coast between 13°30' to 15°10' north latitudes and 79°50' to 80°15' east longitudes. The fishery resources are tremendous and provide a major source of income for the coastal population[10].

The present study contrasts two populations, fish consumers and non-fish consumers, selected from the coastal Nellore district. The distribution of the study area is shown in Figure 1. Ethics approval was granted by Sri Venkateswara University in Tirupati. All subjects were healthy and not on any medication during the few days before investigation. In order to establish rapport, the study objectives were explained to all the subjects and informed consent was obtained. The fish consuming populations were defined as those who ate fish regularly, whilst the non-fish consumers ate no fish at all. The frequency of fish intake ranged 40-100g per meal and 5-7 times a week with an average of 20-30g/day.

Figure 1. Study area of fish consuming and non-fish consuming populations.



A total of 1000 healthy individuals belonging to fish consuming (266 men and 234 women) and non-fish consuming (263 men and 237 women) populations aged 20-70 years were studied for atherogenic risk factors (data not shown). A sub-sample of the population was selected for this study by the systematic sample technique. Four per cent (40) and ten per cent (100) of subjects were chosen at random for the estimation of fatty acids and dietary assessment, respectively. Data on individual dietary intakes for three consecutive days were collected with 24-hour recall method. Cooking methods were similar in both populations. Boiling was the most common method of cooking, but dry roasting and frying were occasionally used. Subjects generally used vegetable oils such as ground nut, sunflower and palm oil, supplied through civil supplies. Non-fish consumers occasionally used animal fats like butter and ghee. Average individual oil consumption was 10-15g/d, calculated from questions probing for the usual weekly intake of oil/ghee. The intake of mean total calories, carbohydrates, fats and proteins were calculated from the Nutritive Values of Indian Foods[11].

The lipid content and fatty acid composition of Indian marine fish species has been previously reported[12]. The range of lipid content in edible parts is approximately 0.5 to 18%. This depends on seasonal variation in feeding habits and regional differences in basic foods and nutrients. The fatty acid composition provides a better understanding from a nutritional point of view. The grouping into saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and PUFA basically correspond to current health interest. These species show percent variations in major total saturated fatty acids [myristic acid (14:0) 1.6 to 11.3; palmitic acid 16.3 to 35.5, stearic acid 7.0 to 16.0] 36.7 to 63.1, MUFA 15.0 to 39.3, ω-6 PUFA of the ω-6 type 0.3 to 10.4 and ω-3 type 7.1 to 43.0, long-chain ω-3 PUFA (20:5, 22:5, 22:6) being the major constituents and the ratio of ω-3 to ω-6 ranged from 1.2 in kalava to 57.0 in mullet. Long-chain ω-3 PUFA are relatively higher in Indian fish and liver oils.

Venous blood samples were drawn and serum total lipids were extracted by mixing 5mL of chloroform: methanol (2:1 v/v) using the method of Bligh and Dyer (1959). The solvent was evaporated under a stream of nitrogen and the phospholipids were separated from neutral lipids by thin-layer chromatography[13]. The total phospholipid band was scraped into vials containing chloroform in methanol and incubated. The fatty acid methyl esters were extracted with boron-trifluoride-ethanol (BF_3-MeOH) and analysed by Gas-liquid chromatography (GLC)[14]. Samples were converted to methyl esters by heating at 100°C in a nitrogen flushed screw capped tubes for 30 min with 10% BF_3-MeOH and n-hexane. The solvent tubes were screw-capped under a stream of nitrogen. The tubes were heated at 90°C with 2 mL of 0.9% NaCl and 5mL of n-hexane. The hexane layer re-evaporated with nitrogen by using an HP5840A gas chromatograph fitted with two gas columns (0.2 x 180 cm) packed with 5% DEGS on chromosorb equipped with two flame ionisation detectors. The sample was reconstituted with 20 μL of hexane and injected 1 μL into the GLC. The peaks were observed for different fatty acid fractions. The oven temperature of 160°C and ionisation detector of 220°C was maintained with carrier gas pressure of 20 psi. The fatty acid methyl esters were identified by retention times with

known fatty acid standards and expressed as percent by weight of total fatty acids.

The results were expressed as means with standard deviations and statistical analyses were done by Student's t-tests.

Results

Table 1 shows the mean daily nutrient intakes of total energy, proteins, carbohydrates and fats among fish consuming and non-fish consuming populations. The intake of total energy and protein were significantly higher in fish consumers than in non-fish consumers ($p<0.01$). Mean intake of carbohydrates was significantly lower ($p<0.05$), and intake of fat was insignificantly lower in fish consumers than in non-fish consumers.

Table 1. Mean ±SD daily intake of energy and nutrients among fish consuming and non-fish consuming populations.

Nutrient	Fish consuming population (n=50)	Non-fish consuming population (n=50)
Energy (Kcal)	2261.8 ± 356.3 (1786.4-3194.7)	2341.4 ± 407.8** (1645.0-3461.4)
Carbohydrates (g)	331.6 ± 82.2 (196.7-567.4)	376.2 ± 81.2* (206.3-582.3)
Proteins (g)	63.4 ± 13.7 (46.0-100.0)	56.2 ± 9.7** (41.7-85.3)
Fats (g)	75.8 ± 9.8 (38.7-76.4)	79.4 ± 12.8 (30.0-95.0)

Fish consuming population defined as those who consumed fish regularly average at least 20-30 g/day and non-fish consuming population those who ate no fish at all. Figures in parentheses indicate ranges. Comparison between populations. Significant at * $p<0.05$; ** $p<0.01$.

Table 2. Mean ± SD fatty acid composition of serum phospholipids among fish consuming and non-fish consuming populations.

Percent of fatty acid	Fish consuming population (n=20)	Non-fish consuming population (n=20)
14:0 (Myristic acid)	1.62 ± 0.62	1.62 ± 0.72
16:0 (Palmitic acid)	28.98 ± 3.26	27.92 ± 2.97
18:0 (Stearic acid)	13.05 ± 4.67	13.84 ± 2.20
18:1 (Oleic acid)	18.95 ± 3.07	19.40 ± 2.97
ω-6 PUFA		
18:2ω-6 (Linoleic acid)	13.56 ± 2.34	17.08 ± 2.43**
18:3ω-6 (Gamma linoleic acid)	0.06 ± 0.03	0.13 ± 0.16
20:3ω-6 (Dihomo-gamma linolenic acid)	1.09 ± 0.43	0.66 ± 0.58**
20:4ω-6 (Arachidonic acid)	11.03 ± 2.79	10.80 ± 2.09
22:4ω-6	0.36 ± 0.35	0.67 ± 0.45*
22:5ω-6 (Osmond acid)	0.35 ± 0.32	1.49 ± 1.22**
Sum of ω-6 PUFA	26.42	30.83
ω-3 PUFA		
18:3ω-3 (Alpha linolenic acid)	0.21 ± 0.24	0.22 ± 0.16
20:5ω-3 (Eicosapentaenoic acid)	1.53 ± 0.95	0.57 ± 0.62**
22:5ω-3 (Docosapentaenoic acid)	1.39 ± 0.85	0.44 ± 0.54**
22:6ω-3 (Docosahexaenoic acid)	5.03 ± 1.42	0.92 ± 0.83**
Sum of ω-3 PUFA	8.16	2.15
Sum of ω-3: ω-6 RATIO	0.31	0.07

Left hand column shows numerical observation of fatty acids. First number of notion indicates number of carbon atoms, second number indicates number of double bonds present in the molecule. ω indicates the position of double bond from the terminal methyl group of the fatty acid. Comparison between populations. Significant at * $p<0.05$; ** $p<0.01$.

The fatty acid compositions of serum phospholipids among fish consuming and non-fish consuming populations are shown in Table 2. Insignificant differences were observed in average percent quantities of SFAs (14:0, 16:0 and 18:0) and MUFAs (18:1) between the fish consuming and non-fish consuming populations. Fish consumers had significantly higher ω-6 PUFA, DGLA and a lower 22:4 ω-6 and 22:5 ω-6 fatty acids compared to non-fish consumers. Further, the sum of ω-6 PUFA was relatively low in fish consumers. However, LA was the major ω-6 PUFA in both population groups, showing statistical significance with lower percent in fish consumers, whereas the difference in AA was insignificant. There was no significant variation for ALA. In fish consumers, the long-chain ω-3 PUFA (EPA, DPA and DHA) were significantly greater than in non-fish consumers. The sum of ω-3:ω-6 PUFA ratio of fish consumers was over four-fold greater than that of the non-fish consuming population.

The study populations were matched by age and gender. Gender variation was found to be significant only for 22:4 ω-6, and EPA in fish consumers. In non-fish consumers, gender difference was observed for DGLA, 22:4 ω-6 and DPA. The percent of ω-3 fatty acids were found to be higher in fish consuming men (8.0) and women (8.6) than in non-fish consuming men (2.3) and women (2.2). The ratio of ω-3 to ω-6 PUFA was relatively greater among fish consuming men (0.31 vs 0.07) and women (0.30 vs 0.07).

Discussion

In this study, the mean values of nutrient intake and serum fatty acid profiles of healthy subjects are reported. Our data show that fish consuming populations have significantly lower total energy and carbohydrate, and higher protein intakes. Analysis of commonly consumed Indian fish indicate that fish with high fat (>5g/100g), medium fat (1-5g/100g), and low fat (<1g/100g) furnish an average of about 1.2, 0.4, and 0.1g long-chain ω-3 PUFA per 100g muscle respectively[15].

The percent of PUFA is a well recognised estimate of dietary intake. In fish consumers, a significantly lower LA and higher DGLA were observed. However, AA were not statistically different. Mammalian cell membranes are rich in AA derived from the diet as LA. Excessive release of AA can lead to many pathophysiological events which serve to dramatise the importance of dietary fatty acids in the regulation of eicosanoid, short-lived regulatory molecules production *in vivo*. Eicosanoids are derived from EFA containing 20-carbon atoms, notably AA and DGLA of the ω-6 and EPA from the ω-3 PUFA. The amount of DGLA will have an influence on the formation of prostaglandin of the 1-series (PGE_1), which lowers blood pressure, inhibits platelet aggregation and produces vasodilation[16].

There is reason to believe that the protective effects of fish are attributable to dietary habits over a long period, changing the tissue percent of ω-3 PUFA. It is likely that the way fish is prepared and consumed matters. Furthermore, these fatty acids may be absorbed more efficiently from fish than from fish oil[17]. The habitual intake of fish contributes a major component of ω-3 PUFA (8.16%) in fish consumers compared to non-fish consumers (2.15%) representing a four-fold higher ω-3: ω-6 ratio. The constant consumption of fish and fish oils leads to an increase in the ω-3 fatty acid level in plasma lipids[3]. Incorporation of ω-3 PUFA into

phospholipids is directly linked to the intake of fish and fish oils. EPA may fall more quickly than DHA because of exchange with plasma phospholipids and conversion to eicosanoids[8]. The percent of DPA in phospholipid in fish consumers was greater compared to non-fish consumers. This is potentially of great biomedical interest and the content of DPA in some Indian fish species are greater than EPA (job fish) or even DHA (Gazzard shad, silver jew)[12].

Table 3. Comparative international ethnic differences on fatty acid composition of plasma or serum phospholipids.

Country	AA	EPA	DHA
Present study			
Fish consumer	11.0	1.5	5.0
Non-fish consumer	10.8	0.6	0.9
Denmark			
Greenland Eskimos	0.8	7.1	3.9
Danes	8.0	0.2	3.0
Danish Eskimos	1.3	0.7	1.0
Japan			
Fishing village	6.8	3.8	7.1
Farming village	5.8	2.3	4.5
Norway			
Coastal population	19.7	1.1	2.6
Inland population	20.5	1.1	2.5
Netherlands			
High Fish Group	10.1	1.7	4.2
Low fish group	10.7	0.9	3.2
Europe/ US	2.6	0.5	NA
Australia			
Aboriginal population	11.5	1.1	NA

NA denotes data not available; Compiled from the reference 7
AA = Arachidonic acid; EPA = Eicosapentaenoic acid;
DHA = Docosahexaenoic acid

It is also known that large amounts of ω-3 PUFA replace the ω-6 type and convert it to biologically less active eicosanoids of the 3-series and leukotrienes (LT) of the 5-series from EPA. The implications of these differences in terms of CVD are several including thrombogenicity. Several studies indicate that the thrombogenicity of thromboxane (TXA_3) is much smaller than that of TXA_2, but it should be realised that this is due to the concomitant PGD_3 production, which has stronger platelet deaggregating properties than PGD_2. These fatty acids have increased the production of prostacyclin (PGI_2) and PGI_3, and suppressed that of TXA_2 which in turn can lead to inhibition of platelet aggregation and blood vessel dilation. ω-3 PUFA have been shown to inhibit production of LTB_4 and increase a less potent LTB_5 [18].

The distinctive and principle ω-3 PUFA in fish consumers is DHA, in turn related to the composition of fish fats. The fish eaten included leaner species which provide a general bias in favour of more dietary DHA than EPA. In recent studies, DHA is identified as a particularly protective fatty acid against CVD[19]. Table 3 presents a comparative international perspective on fatty acid composition of plasma phospholipids[7]. The composition of ω-3 PUFA is positively correlated with people consuming more fish. Ethnic differences observed in tissue fatty acid profiles may be due to dietary habits, cooking practices and availability of either lean or fatty fish.

In summary, the traditional intake of marine fish influences the serum ω-3 PUFA composition of phospholipids. Whether an inverse relation exists between high ω-3 PUFA of fish consumers and CVD in Andhra Pradesh needs to be investigated, and such studies are in progress.

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吃魚和不吃魚人群的脂肪酸組成

摘要

作者研究了經常進食海魚的人群，血清磷脂的脂肪酸組成與膳食模式的關系．他們研究了沿海吃魚與不吃魚人群膳食攝取和血清脂肪酸濃度，吃魚人群總能量（P< 0.01）和碳水化合物（P<0.05）明顯低于不吃魚人群，但蛋白質攝取量則明顯增加．飽和與不飽和脂肪酸的攝取，兩組人群無明顯差异．進食海魚人群ω6脂肪酸，亞油酸，22: 4ω－6和22: 5ω－6脂肪酸明顯低于不吃海魚的人群，但是Dihomo-gamma亞油酸則較高，吃海魚的人群ω3脂肪酸，包括EPA，DPA和DHA則明顯高于不吃海魚的人群（P<0.01）．這些脂肪酸的不同，特別是長鏈ω3脂肪的差异是由于進食魚類的緣故．該研究的發現指出魚類膳食的治療效果值得進一步探討．

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