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Research Article

Evaluation of fatty acid profile with special reference to hypertension intake from marine edible fishes

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Abstract

The present study describes the changes in fatty acid profile in hypertension patients by up taking the marine edible fishes *Elutherenema tetradactylum*, *Sphyraena obtusata* and *Siganus javus* because these marine edible fishes are rich in ω –fatty acids. In this study the total cholesterol, HDL and LDL were significantly decreased from 211.9 – 202.1 mg/dl, 177-159.6 mg/dl. The palmitic acid (C16:0) was found significantly higher in all of peoples compared with other SFAs. This study revealed that the most abundant in individual FAs 16:0,18:0,18:1 n9 and 20:2 n6 were present in blood in both before and after dietary intake. The minimal changes of SFAs levels were decreased averagely from 59.2 to 52.2%. In addition to above PUFAs also increased from 27.7-30.5%. The essential FAs like ALA (C18:3n3), EPA (C20:5n3) and DHA (C22:6n3) were accounting in the range of 2.64-2.92%, 3.67-3.94% and 3.65-4.38%. Omega – 6/3 ratio were recorded from 1.77-2.45%. This study proves the marine edible fishes reduce the hypertension of the patients.

Keywords: Edible fishes, ω –fatty acids, SFAs, HDL and LDL

1. Introduction

Hypertension or high blood pressure is a cardiac chronic medical condition in which the systemic arterial blood pressure is elevated. Hypertension is classified as either primary (essential) hypertension or secondary hypertension; about 90–95% of cases are categorized as "primary hypertension," which means high blood pressure with no obvious medical cause (Carretero and Oparil, 2000). The remaining 5–10% of cases (Secondary hypertension) is caused by other conditions that affect the kidneys, arteries, heart or endocrine system. Treating hypertension has been associated with about a 40% reduction in the risk of myocardial infarction (Collins et al., 1990). Hypertension is estimated to contribute 4.5% of current global disease burden. Indeed, hypertension accounts for more than 5.8% of total deaths, 1.9% of years of life lost and 1.4% disability adjusted life years globally. In industrialized countries, the risk of becoming hypertensive for an individual with a family history of hypertension have been estimated to be up to four times higher than average (Corvol, 1992). Hypertension is already a highly prevalent risk factor for cardiovascular diseases throughout the industrialized world. It is becoming an increasingly common health problem worldwide because of increasing longevity and prevalence of contributing factors such as obesity, physical inactivity and an unhealthy diet (Mohan et al., 2004).

Recently, there has been considerable interest concerning the potential cardioprotective effect of consuming fish oil supplements containing high quantities of ω -3 polyunsaturated fatty acids. Numerous prospective and retrospective trials from many countries, including the U.S., have shown that moderate fish oil consumption decreases the risk of major cardiovascular (CV) events, such as myocardial infarction (MI), sudden cardiac death (SCD), coronary heart disease (CHD), atrial fibrillation (AF) and most recently, death in patients with heart failure (HF) (GISSI-HF Investigators, 2008). Fish oil is obtained in the human diet by eating oily fish, such as herring, mackerel, salmon, albacore tuna, and sardines or by consuming fish oil supplements or cod liver oil. However, fish do not naturally produce these oils, but obtain them through the ocean food chain from the marine microorganisms that are the original source of the ω-3 polyunsaturated fatty acids (-3 PUFA) found in fish oils. Fatty acid intake and metabolism may play a role in the pathogenesis of essential hypertension. Higher saturated fatty acid intake increases blood pressure, while polyunsaturated fatty acids, particularly linoleic acid and the n-3 series, decrease blood pressure. Considerable attention has been directed at the various classes of fatty acids and their impact on the prevention and treatment of cardiovascular diseases. It has been postulated that consumption of fish oil may reduce blood pressure by altering prostaglandin synthesis. ω-3 fatty acids have potentially favorable effects that may protect against cardiovascular diseases and in some reports shows improve renal function in chronic progressive renal disease and kidney graft recipients (Busnach et al., 1998). The nutritional importance of fish consumption is in great extent associated with the content of ω- 3 and ω- 6 fatty acids. Analysis of fatty acids and trace elements in marine fishes promotes understanding of potential relationship between fish and health of human nutrition. Such observations suggest a role for these fatty acids in the management of hypertension. This work describes an investigation of fatty acid profile in human blood with dietary intake of marine edible fishes *Elutherenema tetradactylum, Sphyraena obtusata* and *Siganus javus*.

2. Materials and Methods

2.1. Study design and patient population

Ten patients averagely aged (35-65 years) were screened for hypertension, using 24 hrs ambulatory blood pressures monitoring, during annual follow-up from 2008 to 2009. Patients with a 24 hrs average systolic blood pressure above 139 mmHg or diastolic above 87 mmHg were regarded as hypertensive, as proposed by the meta-analysis of Staessen et al. (1991). Initially blood samples of all patients were collected for measure the total blood cholesterol, HDL, LDL levels and fatty acid profiles. All patients are advice to dietary intake every day of marine edible fishes *Elutherenema tetradactylum*, *Sphyraena obtusata* and *Siganus javus* for three months. The diet was controlled by dieteticians. All patients were following the same control diet of the 90 days prior to blood sampling. There were no modifications of the treatment followed by the patients. After three month hypertension was ruled out in all these individuals on clinical examination prior to blood sampling.

2.2 Sample preparation and analysis of FAMEs

2.2.1 Saponification

Blood samples aliquots were centrifuged to separate RBC from plasma and oven dried at 67 ℃ for 24 hrs. From this dried samples add 1 ml of 1.2M NaOH in 50% aqueous methanol and maintained in screw cap tubes. Incubated the tubes at 100 ℃ for 30 mins in a water bath.

2.2.2 Methylation

The samples were cooled at room temperature for 25 mins. Then acidified and methylated by adding 2 ml of 54% 6 N Hcl in 46% aqueous methanol. The samples were incubated at 80 ℃ for 10 mins in water bath. Then rapid cool the samples.

2.2.3 Extraction

In extraction process, methylated fatty acids were extracted by adding 1.2 ml of 50% Methyl tert-butyl ether in hexane to the samples then mixed for 10 mins. Remove bottom phase.

2.2.4 Base wash

In extraction process, methylated fatty acids were extracted by adding 1.2 ml of 50% Methyl tert-butyl ether in hexane to the samples then mixed for 10 mins. Remove bottom phase.

2.2.5 Separation of FAMEs by GC

Fatty acids were analyzed by gas phase chromatography according to method of Lepage (1986). The fatty acid samples were analysed by gas phase chromatography (Network Gas Chromatograph model 6890N, Agilent Technologies, USA). Samples were injected by Split injector, split ratio 100:1, used column was Ultra -2 capillary column. Used oven initial temperature 170 ℃, Ramp 1 was 5 ℃ up to 260 ℃ and Ramp 2 was 40 ℃ up to 310 ℃. Used detecter was Flame Ionization Detector (FID). Used carrier gas Hydrogen, Flow rate 30 ml/min, makeup gas, Nitrogen, Flow rate 30 ml/min, air 400 ml/min. Used software Sherlock version 4.5 with EUKARY data base (MIS Ver. No. 3.8, Microbial ID. Inc., Newark, Delaware). The Fatty Acid standard purchased from Sigma Aldrich Company.

3. Results and Discussion

Efforts to control the epidemic of high hypertension and its cardiovascular and renal complications have traditionally focused on pharmacologic treatment of persons with established hypertension. Such efforts reflect an impressive body of evidence that has documented the beneficial effects of antihypertensive drug therapy in preventing hypertension related clinical complications. In the present study marine edible fishes *Elutherenema tetradactylum*, *Sphyraena obtusata* and *Siganus javus* were choose for investigate the changes in fatty acid profile in hypertension patients because these marine edible fishes are rich in ω –fatty acids and commonly available in every session of India. In our investigation we found that total cholesterol, HDL and LDL were significantly decreased from 211.9 – 202.1 mg/dl, 177-158.6 mg/dl (Fig. 1 and 2.). This study specifically examines the effect of fish in take approximately 50-100mg (40Z)/week reduced the hypertension in man. Table 1 and 2 express the variation of fatty acids profile between before intake of fish and after intake of fish.

In this study Palmitic acid (C16:0) was found significantly higher in all of peoples compared with other SFAs. This study revealed that the most abundant in individual FAs 16:0,18:0,18:1 n9 and 20:2 n6 were present in blood both before and after dietary intake with minimal changes the level of SFAs were decreased averagely from 59.2 to 52.2%. In addition to above PUFAs also increased from 27.7-30.5%. The essential FAs like ALA (C18:3n3), EPA (C20:5 n3) and DHA (C22:6 n3) were accounting in the range of 2.64-2.92%, 3.67-3.94% and 3.65-4.38%. ω – 6/3 ratio were recorded from 1.77-2.45%. These findings are consistent with previous report, which showed that combining a daily fish meal with a weight-loss regimen led to additive effects on ambulatory blood pressure and decreased heart rate (Bao et al., 1998). The effects were greatest on waking blood pressures, which fell by 6.0 and 3.0 mm Hg (systolic and diastolic, respectively) with dietary fish alone, 5.5 and 2.2 mm Hg with weight loss alone, and 13.0 and 9.3 mm Hg with fish and weight loss combined relative to control values (Bao et al., 1998). Dietary fish also significantly decreased 24 hrs ($P = 0.036$) and waking ($P = 0.013$) ambulatory heart rates (Bao et al., 1998). Although several recent trials have demonstrated that consumption of ω-3 polyunsaturated fatty acids results in a modest decrease in blood pressure, the precise effect and amount of fish oil needed to produce an antihypertensive effect have been inconsistent. A study by Knapp and FitzGerald, (1989) reported that very large doses of fish oil (15 go fo ω-3 polyunsaturated fatty acids or 50 mL of fish oil) reduced blood pressure in men with mild essential hypertension, whereas a lower dose of fish oil (3 g of ω-3 polyunsaturated fatty acids or 10 mL of fish oil) produced no significant changes. Kestin et al. (1990) noted a significant reduction in subjects' systolic but not diastolic blood pressure after consumption of 3.4 g of ω-3 polyunsaturated fatty acids over a 6-week period. Radack et al. (1991) found that consuming 2.0 g of ω-3 polyunsaturated fatty acids decreased systolic blood pressure by 4.1 mm Hg and diastolic blood pressure by 2.4 mmHg. Margolin and coworkers, (1991) in another randomized crossover trial, noted a significant reduction in systolic and diastolic blood pressures in both the fish oil group (consuming 4.7 g of ω-3 polyunsaturated fatty acids per day) and control groups. Researchers at the University of Western Australia have released the results of a study that clearly demonstrates that a weight-loss diet combined with daily fish consumption is highly effective in reducing blood pressure, lowering triglyceride levels while increasing "good" (HDL 2) cholesterol levels and in improving glucose tolerance. In their experiment they observed waking blood pressures decreased by 5.5 mm Hg (systolic) and 2.2 mm Hg (diastolic) in the calorie-restricted group and by 13.0 mm Hg and 9.3 mm Hg in the group combining a daily fish meal with a calorie-restricted diet. The combination of fish consumption and weight loss improved glucose and insulin metabolism significantly and also resulted in a 38% reduction in triglyceride levels and a 24%

increase in the level of "good" cholesterol (HDL2). The researchers conclude that a combination of weight loss and daily fish consumption significantly reduces the risk of cardiovascular disease among obese, hypertensive patients (Mori et al., 1999). To conclude from this study the incorporation of a daily meal of fish rich in omega 3 fatty acids into a reduced-fat, energy restricted diet in obese treated hypertensive subjects resulted in additive effects on blood pressure reduction. Thus a healthy diet rich in ω-3 FAs is beneficial for all age groups.).

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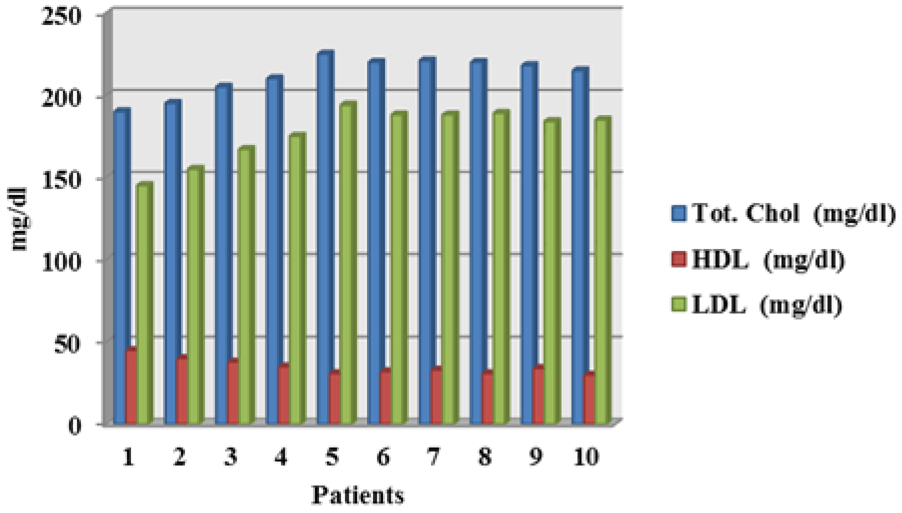

Figure 1. Before intake of fish total Cholesterol, HDL and LDL values

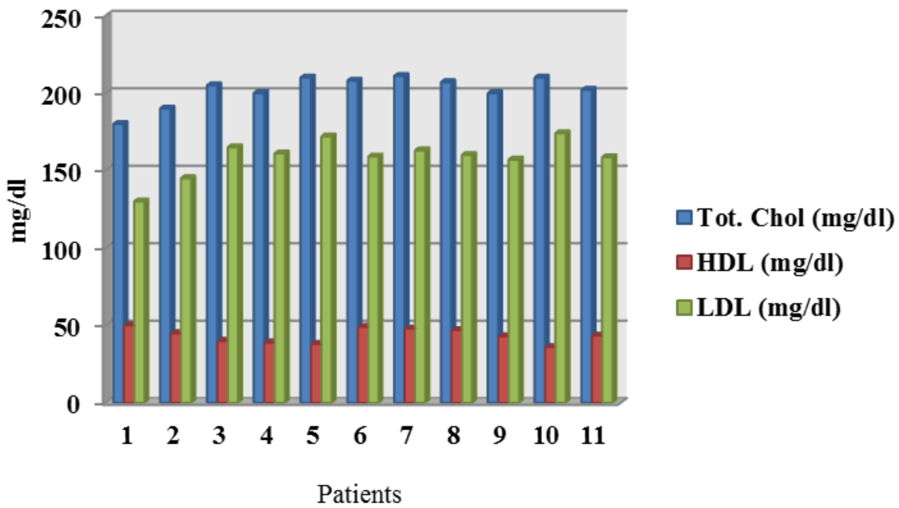

Figure 2. After dietary intake of fish total Cholesterol, HDL and LDL values

Table .1. Fatty acid profile in human RBC (%) before dietary intake of fish

Carbon chain	1	2	3	4	5	6	7	8	9	10	
C13:0	6.14	3.13	4.69	5.14	3.19	4.17	5.19	6.13	5.13	5.19	
C14:0	5.19	4.17	4.63	4.88	7	8.47	8	7.16	6.14	5.83	
C16:0	16.13	27.19	28.61	26.91	20.65	17.81	19.97	20.23	24.31	22.74	
C17:0	4.07	4.09	5.06	4.57	6.14	5.49	6.19	6.14	3.19	4.17	
C18:0	13.12	8.17	8.87	7.18	9.69	6.13	6.43	6.16	3.72	3.19	
C20:0	2.63	3.92	2.34	2.17	3.17	4.16	4	3.64	3.9	4.64	
C21:0	7.14	4.67	1.78	1.19	7.16	4	3.96	2.17	2.8	2.51	
C22:0	2	2.13	2.14	2.63	3.49	3.56	4.53	3.07	2	6.91	
C24:0	1.78	1.63	1.91	1.73	2.61	3.31	4.13	8.97	2.01	5.32	
ΣSFAs	**58.2**	**59.1**	**60.03**	**56.4**	**63.1**	**57.1**	**62.4**	**64.1**	**53.2**	**60.5**	**59.41 (Avg.)**
C14:1ω-7	1.11	2.35	1.16	2.11	1.1	1.82	1.83	1.71	2.01	1.16	
C16:1ω-7	1.38	0.17	0.69	0.93	0.19	0.21	0.6	0.67	0.93	0.64	
C18:1ω-9	9.12	6.13	7.19	6.59	5.12	4.13	4.17	2.91	4.57	6.17	
C19:1ω-8	1.17	1.11	1.13	1.17	1.11	1.17	1.19	0.69	1.11	1.15	
C20:1ω-9	0.63	0.59	0.17	0.63	0.57	0.63	0.83	0.81	0.53	1.59	
C22:1ω-9	2.12	2.11	2	1.11	2.1	0.91	1.11	1.17	0.81	1.14	
C24:1ω-9	0.67	0.14	2.16	0.66	1.91	1.13	1.84	1.09	2.14	1.55	
ΣMUFAs	**16.20**	**12.60**	**14.50**	**13.20**	**12.10**	**10.00**	**11.57**	**9.05**	**12.10**	**13.40**	**12.47 (Avg.)**
C18:2ω-6	0.52	0.63	0.79	0.19	0.41	0.52	0.16	0.61	1.83	1.61	
C18:3ω-3	1.11	1.41	1.81	2.21	1.94	1.81	1.21	1.53	1.96	1.98	
C18:3ω-6	1.02	1.52	0.41	1.64	0.52	2.64	1.54	1.31	1.54	0.69	
C20:2ω-6	9.43	8.61	8.34	9.54	7.84	10.13	9.11	8.11	11.34	6.14	
C20:3ω-6	1.41	2.61	2.04	2.65	1.91	1.52	1.62	2.51	1.91	1.54	
C20:4ω-6	0.84	0.64	0.58	0.64	0.59	0.68	0.11	1.14	0.52	0.19	
C22:2ω-6	0.59	1.11	0.72	1.18	0.5	2.59	0.65	0.24	0.64	0.74	
C20:5ω-3	1.42	1.46	2.18	3.27	0.64	2.54	2.43	2.64	3.67	3.22	
C22:3ω-3	2.13	2.17	2.91	3.11	2.64	3.16	3.69	2.91	2.92	2.95	
C22:4ω-6	3.14	4.16	2.14	2.92	2.53	2.51	2.74	3.14	3.63	2.64	
C22:6ω-3	3.17	3.19	2.64	2.53	2.11	3.65	1.91	2.09	3.96	3.91	
ΣPUFAs	**24.78**	**27.51**	**24.56**	**29.88**	**21.63**	**32.48**	**25.17**	**26.23**	**34.19**	**25.61**	**27.02 (Avg.)**
Unknown and others	0.82	0.79	0.91	0.52	3.17	0.42	0.83	0.62	0.51	0.49	
Total ω-6	16.95	19.28	15.02	20.97	14.3	20.59	15.93	17.06	21.41	13.55	
Total ω-3	7.83	8.23	9.54	11.12	7.33	11.89	9.24	9.17	12.78	12.06	
Ratio (ω-6/ ω-3)	**2.16**	**2.34**	**1.57**	**1.89**	**1.95**	**1.73**	**1.72**	**1.86**	**1.67**	**1.12**	

Table .2. Fatty acid profile in human RBC (%) after dietary intake of fish

Carbon chain	1	2	3	4	5	6	7	8	9	10	
C13:0	2.4	2.51	3.61	3.59	4.12	3.19	3.57	2.54	2.91	2.16	
C14:0	2.56	2.57	2.1	2.08	2.18	3.15	2.16	2.57	2.41	2.19	
C16:0	20.43	21.51	23.46	21.59	24.43	28.05	21.54	24.41	22	20.64	
C17:0	3.14	2.15	1.16	2.11	3.61	3.17	3.65	2.15	4.14	5.19	
C18:0	8.51	7.43	8.96	8.51	9.64	7.51	8.59	9.11	6.41	8.59	
C20:0	2.19	2.64	0.25	0.96	0.98	2.62	2.14	3.5	3.15	3.72	
C21:0	5.32	7.62	6.54	5.11	5.32	5.19	4.37	4.12	3.18	5.96	
C22:0	2.11	1.11	3.83	2.86	2.59	3.16	3.19	3.68	3.16	1.65	
C24:0	3.76	4.06	3.11	4.39	0.73	2.16	2.99	3.04	2.28	1.02	
ΣSFAs	**50.42**	**51.60**	**53.02**	**51.20**	**53.60**	**48.20**	**52.20**	**55.12**	**49.64**	**51.12**	**52.61 (Avg.)**
C14:1ω-7	1.61	0.54	1.11	1.06	1.55	1.46	1.39	1.54	2.61	2.19	
C16:1ω-7	1.26	1.57	1.42	1.54	1.85	1.51	1.54	1.78	1.08	1.54	
C18:1ω-9	9.12	6.05	7.91	7.54	8.61	8.18	7.14	8.12	4.61	5.64	
C19:1ω-8	3.16	2.15	1.16	1.12	2.16	2.01	1.38	1.69	2.11	0.14	
C20:1ω-9	2.54	2.11	2.01	1.41	2.16	2.19	2.17	2	1.54	3.12	
C22:1ω-9	2.13	0.54	0.54	0.49	0.91	1.65	0.54	1.98	0.57	1.91	
C24:1ω-9	0.3	1.95	1.98	0.96	0.9	0.61	2.18	2.01	1.09	0.58	
ΣMUFAs	**20.12**	**14.91**	**16.13**	**14.12**	**18.14**	**17.61**	**16.34**	**19.12**	**13.61**	**15.12**	**16.49 (Avg.)**
C18:2ω-6	0.67	0.51	0.64	0.81	1.16	0.54	0.49	0.51	2.13	1.61	
C18:3ω-3	1.11	1.19	1.62	1.54	2.64	2.92	1.65	1.47	2.57	2.52	
C18:3ω-6	0.64	1.34	0.19	2.68	2.11	4.43	2.01	0.32	2.16	1.92	
C20:2ω-6	10.11	11.64	11.59	11.92	12.1	11	10.51	7.54	12.54	7.35	
C20:3ω-6	1.52	1.92	1.77	2.29	2.07	2.16	2.07	1.92	1.61	1.54	
C20:4ω-6	2.37	2.54	1.62	1.46	0.52	3.11	1.14	1.69	1.82	2.69	
C22:2ω-6	3.01	1.14	1.42	1.42	1.19	0.88	2.16	1.52	0.67	1.52	
C20:5ω-3	2.14	3.16	3.12	3.94	2.14	2.14	2.56	2.94	2.98	2.92	
C22:3ω-3	1.16	2.88	2.16	2.04	1.17	0.69	2.59	2.16	3.16	3.65	
C22:4ω-6	2.92	3.01	3.19	3.65	0.59	2.14	3.17	2.19	3.14	3.17	
C22:6ω-3	3.19	4.38	2.72	2.66	2.15	3.17	2.92	2.81	3.45	3.96	
ΣPUFAs	**28.84**	**33.71**	**30.04**	**34.14**	**27.84**	**33.18**	**31.27**	**25.07**	**36.23**	**32.85**	**31 (Avg.)**
Unknown and others	0.64	0.81	0.81	0.54	0.42	1.01	0.19	0.69	0.52	0.91	
Total ω-6	21.24	22.1	20.42	24.23	19.74	24.26	21.55	15.69	24.07	22.06	
Total ω-3	7.6	10.88	9.62	9.91	8.1	8.92	9.32	9.38	12.16	8.79	
Ratio (ω-6/ ω-3)	2.79	2.03	2.12	2.44	2.43	3.71	2.21	1.67	1.97	2.04	