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BIOCHEMICAL STUDIES ON MARINE FISH OIL PART-I: EFFECTS OF FISH OIL AND LIPID LOWERING DRUGS ON HDL/LDL CHOLESTEROL LEVELS

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ABSTRACT

ω-3 fatty acids present in fish oil are potent cholesterol lowering agents. This fact has drawn our attention to investigate their effects alone and in combination with competitive inhibitors, (hydrooxymethylglutaryl coenzume-A reductase). The naturally occurring ω-3 fatty acids i.e., polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in fish oil have been proved as anticholesterolemic agents. In order to observe their actions as lipid lowering agents, the present study has been carried out which deals with the synergistic effect of fish oil with that of Lovastatin and Gemfibrozil.

INTRODUCTION

Atherosclerosis is a primary cause of cardiovascular diseases leading many of the deaths throughout the world. Several risk factors have been implicated in the etiology of atheroselerosis and ischemic heart disease. These include the levels of various plasma constituents, e.g., increase in cholesterol, triglycerides, lipoprotein, and the genetic background of individuals (Castelli 1984). Gertler *et al*. (1950) showed that plasma cholesterol concentration was higher in patients with coronary heart disease (CHD). Gofman *et al*. (1950 and 1954) explained that the increase concentration of low density lipoprotein (LDL) was responsible for coronary heart diseases (CHD).

Importance of lipoprotein such as HDL, LDL, VLDL and chylomicron as reported by Fredrickson *et al*. (1967a, b) that HDL- and LDL-apoprotein were found in α- and β-lipoprotein respectively. Both apoproteins, found in VLDL and chylomicron take part in transporting cholesterol in blood. Due to the presence of ω-3 PUFAs, fish oil may be useful as antihypertriglyceridemic because it decreases the synthesis of VLDL and triglyceride. Antihyperlipidemic drugs may be recommended when dietary and risk factor management fail, one of them is lovastatin that impairs the synthesis of cholesterol and VLDL by inhibiting Hydrooxy Methyl Glutaryl (HMG) CoA reductase.

In this context, comparative studies have been done on fish oil, lovastatin and gemfibrozil with the aim to search out such a drug having high-potency and lesser side effects. The experiments were performed on rabbits and found synergistic effects of these compounds.

MATERIALS AND METHODS

- Electrical centrifuge 1000-4000 rpm (China)

- Disposable syringe 10 ml, 21 gauge BD
- Centrifuge tubes
- Glass tubes
- Pasture pipettes
- Juster 0.1ml to 1ml
- Microlab 100 E. Merck

Reagents:

All the reagent kits were purchased from Federal Republic of Germany.

Experimentally prepared food materials:

- Butter (Lurpak, Denmark)
- Lovastatin (purchased from local market)
- Gemfibrozil (purchased from local market)
- Fish oil extracted from marine fish livers (Faheem, 1995)
- Chick pea (gram seed) local supplier
- Alfa alfa (loosan or green grass) local supplier

Animal and their feeding schedule:

A study of 22 weeks was carried out on 45 healthy, white male rabbits of 1.0 to 1.5 kg. Animals were divided into nine groups at random. Each group consisted of five animals (n=5) receiving their respective diets and water ad libitum. During the experimental period animals were fed according to the schedule. The whole procedure from feeding schedule to the biochemical analysis and determination of LDL-cholestrol and HDL-cholesterol were explained comprehensively elsewhere in the thesis (Faheem Ahmed, 1995).

RESULTS

Fish oil obtained from two marine fish *Parastromateus niger* (Black Pomfert) and *Scomberomorus guttatus* (Surmai*)* were examined alone and in combination with lovastatin and gemfibrozil. The results are presented in the Tables 1-14. Normal and pathological values for LDL cholesterol and HDL cholesterol are given in the Tables 1, 2 and 8, 9 respectively. The results are summarized as:

1) Effects of various antilipidemic agents on HDL cholesterol:

Fish oil non-significantly reduced the HDL-cholesterol level i.e., 34.20% (Table 3) while lovastatin and gemfibrozil decreased the HDL-cholesterol level by 56.93% ($P<0.01$) and 40.35% ($P<0.05$) respectively (Tables 4 and 5). Fish oil combining with lovastatin and with gemfibrozil decreased the HDL-cholesterol by 50.67% ($P<0.01$) and 32.89% ($P<0.01$) respectively (Tables 6 and 7).

2) Effect of various antilipidemic agents on LDL cholesterol:

Lovastatin alone exhibited a significant decrease by 87.32% ($P<0.01$) and gemfibrozil by 82.25% ($P<0.01$) the level of LDL-cholesterol respectively (Tables 11 and 12). Fish oil significantly decreased the level of LDL-cholesterol by 63.73% ($P<0.01$, Table 10), whereas fish oil with lovastatin and with gemfibrozil decreased the LDL level by 75.18% ($P<0.01$) and 53.31% ($P<0.01$) respectively (Tables 13 and 14).

DISCUSSION

Our experimental results have shown specific effects of fish oil alone and in combination with other antilipidemic drugs to study a relationship between naturally occurring ω-3 fatty acid and drugs. Most significant effect was observed in case of fish oil with lovastatin which may be attributed to the overall selective inhibition of HMG-CoA reductase enzyme by lovastatin in the same way as that of compactin and mavalonin (Tobert *et al*., 1982; Brown and Goldstein, 1984). This enzyme is responsible for the conversion of HMG-CoA into mevalonic acid being a substrate for bile acid synthesis in liver causes an increase in hepatic LDL receptors and thereby promotes influx of LDL-cholesterol from plasma. The net result is a decrease in plasma LDL-cholesterol and VLDL cholesterol.

Gemfibrozil, on the other hand, inhibits hepatic secretion of VLDL into the plasma, as well as increases the rate of its degradation by lipoprotein lipase. This action might contribute to a reduction in the hepatic synthesis and secretion of VLDL.

Fish oil did not show any significant increase in HDL, whereas increase in HDL was observed in case of gemfibrozil as compared to the control samples (Kinsella, 1987). Generally it was indicated that increase in HDL-cholesterol was not observed in feeding trials, but it rather exhibited a descending trend. Kinetic studies of the metabolism of LDL led to the conclusion that plasma LDL levels were decreased by a reduction in the rate of synthesis of apolipoprotein B component (Illingworth *et al.,* 1984). It was observed during experiment that fish oil reduced the HDL-cholesterol level while LDL and VLDL remained unchanged provided it is combined with diet.

CONCLUSION

The study of fish oil and its combination with antilipidemic drugs was carried out to ascertain or to confirm these synergistic effect of fish oil with that of gemfibrozil or lovastatin in order to reduce the quantity of these drugs to attain a better profile of activity thus avoiding side effects and toxicity of these drugs. Fish oil being a dietry component would not cause any toxic effect.

The observation of synergistic action of lovastatin and fish oil led to the conclusion that the site of action of fish oil may be the same as that of lovastatin (Huff *et al*., 1992; Swahn and Olsson, 1993).

Table 1
Effect of normal diet on the serum HDL-cholesterol mg/100 ml in Group 1 normal control (N.C.) Rabbits

Weeks	1A	1B	1C	1D	1E	Mean	± SE
0	6	6	7	9	9	7.40	0.61
4	9	7	8	10	10	8.80	0.52
8	10	9	10	11	15	11.00	0.94
12	11	10	12	14	19	13.20	1.43
16	14	12	15	16	25	16.40	2.01
18	15	18	16	18	26	18.60	1.73
20	14	25	18	22	27	21.20	2.11
22	16	33	24	24	29	25.20	2.55

Table 2
Effect of Cholesterol + Butter rich diet on the serum HDL-cholesterol mg/100 ml in Group 2 Pathological control (P.C.) Rabbits

Weeks	2A	2B	2C	2D	2E	Mean	± SE
0	7	10	13	21	17	13.60	2.22
4	19	27	35	35	36	30.40	2.93
8	48	41	51	62	53	51.00	3.06
12	50	52	69	68	61	60.00	3.52
16	55	55	85	75	65	67.00	5.22
Effect of normal diet*							
18	48	55	80	69	63	63.00	4.96
20	45	52	73	60	55	57.00	4.19
22	40	50	66	54	50	52.00	3.75

*Normal diet: Loosan (alfa alfa), Gram seeds (chick pea)

Table 3
Effect of Cholesterol + Butter rich diet on the serum HDL-cholesterol mg/100 ml in Group 3 Treated 1 (T.1) Rabbits

Weeks	3A	3B	3C	3D	3E	Mean	± SE	
0	23	12	10	17	10	14.40	2.24	
4	55	35	27	30	30	35.40	4.53	
8	69	37	40	35	35	43.20	5.83	
12	79	43	47	36	49	50.80	6.61	
16	85	55	60	42	65	61.40	6.29	
Effect of fish oil (71.43 mg/kg/day)								**P**
18	75	50	44	40	60	53.80	5.62	NS
20	70	50	20	40	58	47.60	7.58	NS
22	55	40	15	35	57	40.40	6.82	NS

Table 4
Effect of Cholesterol + Butter rich diet on the serum HDL-cholesterol mg/100 ml in Group 4 treated 2 (T.2) Rabbits

Weeks	4A	4B	4C	4D	4E	Mean	± SE	
0	3	4	5	3	5	4.00	0.40	
4	18	13	15	14	13	14.60	0.83	
8	25	17	21	19	19	20.20	1.21	
12	28	19	28	25	23	24.60	1.51	
16	31	20	29	29	28	27.40	1.71	
Effect of Lovastatin (0.57 mg/kg/day)								**P**
18	41	19	29	30	27	29.20	3.15	NS
20	15	12	22	23	21	18.60	1.93	<0.05
22	8	4	12	17	18	11.80	2.37	<0.01

Table 5
Effect of Cholesterol + Butter rich diet on the serum HDL-cholesterol mg/100 ml in Group 5 treated 3 (T.3) Rabbits

Weeks	5A	5B	5C	5D	5E	Mean	± SE	
0	4	6	6	3	4	4.60	0.54	
4	13	10	11	10	10	10.80	0.52	
8	15	18	16	15	15	15.80	0.52	
12	18	25	20	18	19	20.00	1.17	
16	21	29	22	19	23	22.80	1.51	
Effect of Gemfibrozil (17.14 mg/kg/day)								P
18	24	19	41	60	22	33.20	6.90	<0.05
20	15	13	25	23	18	18.80	2.05	<0.05
22	12	11	13	19	13	13.60	1.25	<0.05

Table 6
Effect of Cholesterol + Butter rich diet on the serum HDL-cholesterol mg/100 ml in Group 6 treated 4 (T.4) Rabbits

Weeks	6A	6B	6C	6D	6E	Mean	± SE	
0	13	16	14	10	11	12.80	0.95	
4	29	38	40	25	20	30.40	3.40	
8	37	40	42	44	44	41.40	1.19	
12	55	40	49	66	53	52.60	3.78	
16	65	43	55	75	60	59.60	4.75	
Effect of fish oil (71.43 mg/kg/day) +Lovastatin (0.57 mg/kg/day)								P
18	55	37	43	73	55	52.60	5.53	NS
20	50	35	20	64	36	41.00	6.67	NS
22	35	25	10	62	15	29.40	8.24	<0.01

Table 7
Effect of Cholesterol + Butter rich diet on the serum HDL-cholesterol mg/100 ml in Group 7 treated 5 (T.5) Rabbits

Weeks	7A	7B	7C	7D	7E	Mean	± SE	
0	10	10	7	12	11	7.60	0.75	
4	55	55	39	25	35	39.20	5.23	
8	67	63	56	39	46	51.40	4.66	
12	74	69	72	56	67	64.20	2.81	
16	90	83	77	75	77	76.60	2.46	
Effect of fish oil (71.43 mg/kg/day) + gemfibrozil (17.14 mg/kg/day)								P
18	85	75	71	71	75	75.40	2.29	NS
20	81	65	35	70	64	63.00	6.82	NS
22	70	35	40	54	58	51.40	5.64	<0.01

Table 8
Effect of normal diet on the serum LDL-cholesterol mg/100 ml in Group 1
normal control (N.C.) Rabbits

Weeks	1A	1B	1C	1D	1E	Mean	± SE
0	11	10	12	12	14	11.80	0.59
4	13	10	13	13	16	13.00	0.85
8	14	11	15	18	21	15.80	1.53
12	15	15	18	22	27	19.40	2.05
16	18	16	20	24	32	22.00	2.53
18	19	25	21	27	33	25.00	2.19
20	20	33	24	28	34	27.80	2.37
22	23	40	33	31	37	32.80	2.60

Table 9
Effect of Cholesterol + Butter rich diet on the serum HDL-cholesterol mg/100 ml in Group 2
Pathological control (P.C.) Rabbits

Weeks	2A	2B	2C	2D	2E	Mean	± SE
0	15	20	18	21	27	20.20	1.78
4	85	81	97	95	103	92.20	3.60
8	190	198	351	186	205	226.00	28.10
12	316	317	486	342	332	358.60	28.82
16	345	368	655	363	392	424.60	51.95
Effect of normal diet*							
18	313	260	429	303	285	318.00	26.10
20	210	173	350	253	204	238.00	27.52
22	95	109	290	161	165	164.00	30.77

*Normal diet: Loosan (alfa alfa), Gram seeds (chick pea)

Table 10
Effect of Cholesterol + Butter rich diet on the serum LDL-cholesterol mg/100 ml in Group 3
Treated 1 (T.1) Rabbits

Weeks	3A	3B	3C	3D	3E	Mean	± SE	
0	24	22	50	26	18	28.00	5.06	
4	125	180	180	131	80	139.20	16.86	
8	139	217	280	176	170	196.40	21.75	
12	195	291	386	205	180	251.40	34.71	
16	230	380	550	250	200	322.00	57.92	
Effect of fish oil (71.43 mg/kg/day)								P
18	210	310	97	197	178	198.40	30.52	<0.05
20	180	200	27	140	165	142.40	27.25	<0.01
22	135	150	20	130	149	116.80	21.92	<0.01

Table 11
Effect of Cholesterol + Butter rich diet on the serum LDL-cholesterol mg/100 ml in Group 4 Treated 2 (T.2) Rabbits

Weeks	4A	4B	4C	4D	4E	Mean	± SE	
0	6	7	10	10	8	8.20	0.72	
4	103	78	102	77	81	88.20	5.26	
8	181	108	127	124	109	129.80	11.95	
12	221	138	163	153	141	163.20	13.52	
16	263	143	194	177	154	186.20	18.92	
Effect of Lovastatin (0.57 mg/kg/day)								**P**
18	165	78	69	154	97	112.60	17.66	<0.01
20	45	27	26	120	56	54.80	15.43	<0.01
22	16	8	20	42	32	23.60	5.38	<0.01

Table 12
Effect of Cholesterol + Butter rich diet on the serum LDL-cholesterol mg/100 ml in Group 5 Treated 3 (T.3) Rabbits

Weeks	5A	5B	5C	5D	5E	Mean	± SE	
0	7	10	13	8	10	9.60	0.92	
4	135	141	76	32	203	117.40	26.23	
8	208	236	104	43	272	172.60	38.29	
12	299	301	113	61	385	231.80	55.16	
16	347	361	121	76	509	282.80	72.21	
Effect of Gemfibrozil (17.14 mg/kg/day)								**P**
18	141	210	111	66	246	154.80	29.23	NS
20	114	119	30	37	132	86.40	19.52	<0.05
22	93	24	27	21	86	50.20	14.41	<0.01

Table 13
Effect of Cholesterol + Butter rich diet on the serum LDL-cholesterol mg/100 ml in Group 6 Treated 4 (T.4) Rabbits

Weeks	6A	6B	6C	6D	6E	Mean	± SE	
0	27	21	20	16	19	20.60	1.61	
4	87	180	75	210	102	130.80	24.13	
8	255	246	100	280	180	212.20	29.12	
12	315	290	191	317	231	268.80	22.26	
16	420	310	286	364	296	335.20	22.46	
Effect of fish oil (71.43 mg/kg/day) + Lovastatin (0.57 mg/kg/day)								**P**
18	230	160	150	285	156	196.20	23.74	<0.01
20	180	85	35	250	150	140.00	33.38	<0.01
22	82	67	15	210	42	83.20	30.13	<0.01

Table 14
Effect of Cholesterol + Butter rich diet on the serum LDL-cholesterol mg/100 ml in Group 7 Treated 5 (T.5) Rabbits

Weeks	7A	7B	7C	7D	7E	Mean	± SE	
0	25	18	8	20	17	17.60	2.48	
4	180	130	108	90	125	126.60	13.49	
8	209	206	166	130	189	180.00	13.11	
12	247	285	197	212	263	240.80	14.47	
16	315	346	215	356	310	308.40	22.32	
Effect of fish oil (71.43 mg/kg/day) + Gemfibrozil (17.14 mg/kg/day)								P
18	260	280	190	290	256	255.20	15.62	<0.05
20	210	210	89	220	206	187.00	22.01	<0.01
22	180	120	75	170	175	144.00	18.19	<0.01

REFERENCES

Castelli, W.P. (1984). Coronary heart disease risk: importance of joint relationships among cholesterol levels in individual lipoprotein classes. *Am. J. Med.* **76**(2A): 4-12.

Faheem Ahmed (1995). Studies on the fatty acid and other lipid constituents of fish origin. *Ph.D. Thesis*.

Fredrickson, D.S, Levy, R.I. and Lees, R.S. (1967a). Typing of hyperlipoproteinemia. *N. Engl. J. Med.* **276**: 94-98.

Fredrickson, D.S., Levy, R.I. and Lees, R.S. (1967b). A functional view of the plasma lipoproteins. *N. Engl. J. Med.* **276**: 148-159.

Gertler, M.M.; Garn, S.M. and Lerman, J. (1950). Inter-relations of serum cholesterol, cholesterol esters and phospholipids in health and in coronary artery disease. *Circulation*, **2**, 205-14.

Gofman, J.W., Rubin, L., McGinley, J.P. and Jones, H.B. (1954). Hyperlipoproteinemia. *Am. J. Med.* **16**: 514-520.

Gofman, J.W., Jones, H.B., Lindgren, F.T., Lyon, T.P., Elliot, H.A. and Strisower, B. (1950). Blood lipids and human atherosclerosis. *Circulation*. **2**: 161-178.

Harris, W.S., Connor, W.E., Inkeles, S.B. and Illingworth, D.R. (1984). Dietary omega-3 fatty acids prevent carbohydrate induced hypertriglyceridemia. *Metabolism*. **33**: 1016-1019.

Huff, M.W.; Telford, D.E. and Barret, P.H. (1992). Dietary fish oil plus lovastatin decreases both VLDL and LDL apo B production in miniature pigs *Arterioscler. Thromb*. **2**(8): 902-910.

Illingworth, D.R.; Harris, W.S. and Connor, W.E. (1984). Inhibition of low density lipoprotein synthesis by dietary omega3 fatty acids in humans. *Arteriosclerosis* **4**, 270-275.

Kinsella, J.E. (1987). Sea Foods and Fish Oils in Human Health and Disease. Marcel-Dekker, Inc., New York and Basel, pp: 76.

Swahn, E.; and Olsson, A.G. (1993). Omega-3 fatty acids. Metab. Biol. Eff. pp: 217-222.

Tobert, J.A. (1988). New developments in lipid-lowering therapy: The role of inhibitors of hydroxymethylglutaryl coenzyme A reductase. *Am. J. Cardiol*. **62**: 28J-34J.