PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Pakistan Journal of Nutrition 7 (4): 603-606, 2008
ISSN 1680-5194

Effect of Seafoods (Periwinkle, Bonkafish and Crayfish) and Vegetable Oils Enriched Meal on Cardiovascular Disease

J.I. Ndem, M.I. Akpanabiatu* and E.U. Essien
Department of Biochemistry, Faculty of Basic Medical Sciences,
University of Calabar, P. M. B. 1115, Calabar, Nigeria

Abstract: Periwinkle (Tympanotonus fustcatus), Crayfish (cambarellus diminutus) and Bonka fish (Ethimalosa fimbriata) are local marine food sources of omega-3 fatty acid. Groundnut oil, corn oil and soybean oil are notably high in omega-6 fatty acids. The present study compared changes in haematological and biochemical indices in rats fed with local marine foods (periwinkle, bonka fish and crayfish) and vegetable oils (groundnut, soybean and corn oil) enriched meals. Rats in all the experimental groups had a significant ($P< 0.05$) increase in the Hb, PCV and RBC values and a non-significant decrease ($P> 0.05$) in the WBC counts, when compared with the control. The results of the lipid profile of the test groups on omega-3 and omega-6 enriched pellets were significantly lower than that of control but the HDL-C concentrations were significantly higher in these groups. Similarly rats on pellets enriched with local marine foods (periwinkle, bonka fish and crayfish) considered to be rich in omega-3 fatty acid had significant decreased ($P < 0.05$) cholesterol, and HDL – C concentrations while TG, VLDL and LDL-C increased significantly when compared with control. These results suggest that consumption of diet enriched with periwinkle, bonka fish, crayfish and oil rich in omega-3 and omega-6 polyunsaturated fatty acids may prevent cardiovascular disease. This may be one mayor reason for low incident of coronary heart disease among the poor rural people that consumed basically periwinkle, bonka fish and cray fish as their main sources of protein

Key words: Periwinkle, bonka fish, crayfish, groundnut oil, corn oil, soybean oil, cardiovascular disease

Introduction

Human diet is a key player in the development of degenerative human disease such as cardiovascular disease and the mechanism is multifaceted. Today, human attention is turn to the consumption of natural food, sea food products and food derived antioxidants such as vitamins, as a means of freeing our world of the devastating effect of degenerative diseases. Consumption of food rich in saturated fats have been associated with degenerative diseases such as coronary heart disease, cancer and cerebra-vascular disease (Renaud and Lorgeril, 1992; Stephen and Sieber, 1994; Lapinskas, 2001).

An important fact is that we need both omega-3 and omega-6 polyunsaturated fatty acids for normal functioning of the body but most people consume far more of one type than the other (Rudin and Clara, 1996). This is because westernized dietary pattern seem to favor the consumption of more omega-6 than omega-3 fatty acids. The main reason for this deluge is the growing reliance on vegetable oil such as corn, safflower, sunflower, groundnut and cotton seed oils (Willet, 1994). This imbalance has therefore resulted in high rate of heart disease, cancer, obesity, autoimmune disease, allergies, diabetes and depression (Werbach, 1993; Shils *et al*., 1999). In Nigeria, especially among the affluent class, congestive heart failure, hypertension and other degenerative disease may be attributed to reduced intake of balanced ratio of omega-3 and omega-6 fatty acids enriched diet. It is well known that Eskimos eating their traditional marine diet have no record of heart disease compared to other nationalities. They also have lower levels of rheumatoid arthritis and myocardial infarction (Linder, 1992). Their cardio protective effects have been reported to be due to their high consumption of PUFA (Christensen *et al*., 1997). It has also been reported that for maximum benefit from the two kind of essential fatty acid a ratio of 1:1 is required (Hass, 1992). This is however contrary to the report that most people consumed 20g to 30g of omega-6 to every 1g of omega-3 (Simopoulos, 1999).

Our goal is to use locally available sea food rich in omega-3 and oils rich in omega-6 essential fatty acids to feed experimental animals and analyze their lipid profile as an index of cardio-protective effect of these meals. The haematological indices are also assay to assess the health status of the experimental animals, couple with their immunological state.

Materials and Methods

Animals/feed materials: Twenty-eight adult male and female albino Wistar rats were obtained from the Animal house of the Department of Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, Calabar

Table 1: Diet formulation and composition

Components	GRP I (control) (g)	GRP II (g)	GRP III (g)	GRP IV (g)
Grower Mash	4200	3360	3360	3360
Periwinkle	-	280	140	-
Bonka Fish	-	280	140	-
Cray Fish	-	280	140	-
Groundnut oil	-	-	420	-
Corn Oil	-	-	-	420
Soy Bean Oil	-	-	-	420
Total (g)	4200	4200	4200	4200

for this study. The animals weighed between 260 – 280 grams each. All the animals were kept in plastic cages with stainless mesh at the bottom to prevent faeces and feed dropping from mixing with the experimental animal feed. The experimental animals were divided into four groups of seven animals each (four males and three females). The males were kept in separate cages from the female to prevent mating during the course of the experiment. The rat pellets was obtained from Bendel feed and flour mill limited Benin City, Nigeria. Crayfish, periwinkle and bonka fish were purchased from Uyo Main Market, Uyo, Akwa Ibom State, Nigeria. The edible portions of the periwinkle were removed from the shell and dried in the oven (Plus II Gallankamp) at 50°C overnight. The dry periwinkle, crayfish and bonka fish were individually ground into powder form with the use of an electric blender (National blender) and store in polyethylene sac. Groundnut oil, corn oil and soybean oil were obtained off counter from Uyo Main Market in Uyo, Akwa Ibom State, Nigeria. The oils were manufactured by Grand Cereals and Oil Mills Limited, Jos, Nigeria.

Experimental design and diet formulation: The experimental animals were randomly assigned into four groups. Group 1 animals served as the control and were fed with rat pellets only (Diet I). Group II animals were fed with rat pellets enriched with seafoods highly rich in omega-3 fatty acids (*cambarellus diminutus*, *Tympanotonus fuscatus* and *Ethmalosa fembriata*) (Diet II). In Group III, an attempt was made to balance omega-3 and omega-6 fatty acids by combining the seafoods (crayfish, periwinkle and bonka fish) with groundnut oil in equal proportions (Diet III). Group IV animals were fed pellets enriched with corn oil and soybean oil as good sources of omega-6 fatty acid (Diet IV). The different diets were prepared in the ratio of 1:4 as represented in Table 1. All the experimental animals were fed *ad libitum* for five weeks and they had free access to drinking water.

Collection of blood: At the end of the experimental period all the animals were anaesthetized using chloroform. The animals were dissected and blood samples were obtained by cardiac puncture into EDTA tubes for haematological indices determination and heparinized sample tube for plasma preparation. Plasma samples were obtained from whole blood by centrifugation at 3000 g for 10 minutes, using a bench top centrifuge (MSE, England) and stored in the refrigerator at 4°C. Haematological indices and Lipid profile determinations were carried out within 24 hours of sample collection.

Biochemical and haematological determinations: Plasma lipid profile was determined using standard reagent kits obtained from Randox Laboratory UK. Serum total cholesterol determination was according to the enzymatic colorimetric endpoint methods (Richmond, 1972; Roeschlau *et al*., 1974; Trinder, 1969). HDL-cholesterol was measured by combination of the methods (Trinder, 1969; Lopes-Virella *et al*., 1977). LDL-cholesterol was obtained by calculation using the formula provided in Randox HDL-cholesterol kit booklet. Plasma TG was assayed by colorimetric methods (Trinder, 1969; Tietz, 1990). VLDL and LDL were obtained by calculation according to method in the HDL-cholesterol kit manual (Randox Kit)

The percent packed cell volume (% PCV), white blood and Red blood cells count were determined by method of Dacie and Lewis, 1975. Haemoglobin was measured using the method of Alexander and Griffith, 1996

Statistical analysis: Data are expressed as mean ± SD. Comparisons of data were by the student's t-test and $P \leq 0.05$ was considered significant.

Results

The haematological indices of experimental animals are as shown in Table 2. The results showed that the haemoglobin concentration increases with increase in the content of essential fatty acid in the supplement although when the test groups (II, III and IV) were compared with the control group 1; there was no significant change ($P \leq 0.05$). Animals on diet enriched with omega-3 and omega-6 fatty acids (group III) had the highest value of % PCV, which was significantly different from the control ($P \leq 0.05$) and other groups. Animals in group II and IV, which were fed omega-3 and omega-6 enriched rat pellets respectively, had significantly lower % PCV than the control group ($P \leq 0.05$). The RBC

Table 2: Haematological indices of adult albino Wistar rats fed with different ratios of n-3 and n-6 polyunsaturated fatty acids supplemented diets

Parameters+/Groups	Hb(g/dl)	PCV (%)	RBC ($x10^{12}$/l)	WBC ($x10^{9}$/l)
Group I(Control)	9.26 ± 1.60	47.63 ± 0.48	1.945 ± 0.053	4.43 ± 0.10
Group II	10.32 ± 0.42	45.25 ± 0.96	2.140 ± 0.054*	5.25 ± 0.13
Group III	12.23 ± 0.29	50.75 ± 0.96*	2.385 ± 0.081*	5.58 ± 0.17
Group IV	11.29 ± 0.28	43.00 ± 0.81*	2.287 ± 0.084*	5.35 ± 0.13

+ = Mean ± SD. * Significantly different from the control value ($P<0.05$)

counts for the experimental groups II, III, IV showed a significant change ($P \leq 0.05$) when compared with the control. These results also showed that there were no significant changes ($P \geq 0.05$) in WBC counts when the test groups were compared with control.

Table 3 depicts the plasma lipid profile of Wistar albino rats fed with different ratios of n-3 and n-6 fatty acids enriched pellets. The plasma total cholesterol and triacylglycerol (TG) showed that group III recorded the lowest values with the highest value recorded by the control group I. The lipid profile showed significant change ($P \leq 0.05$) when compared with control and among the groups except in the plasma total cholesterol concentration of group II. Rats fed n-3 and n-6 fatty acids enriched pellets had significantly elevated plasma HDL-cholesterol when compared with the control and when compared with omega-3 and omega-6 enriched pellets, respectively. Plasma VLDL-cholesterol recorded significantly ($P \leq 0.05$) lower concentration for rats fed n-3 and n-6 enriched pellets with rats fed n-6 enriched pellets only recording the highest VLDL value. Group to group comparison also showed significant change ($P \leq 0.05$) in the VLDL values. Experimental animals on diet enriched with the mixture of n-3 and n-6 fatty acids (group III) recorded the lowest value of LDL-cholesterol but were not significant ($P \geq 0.05$) when compared with that of control animals and other groups, excepts when group II was compared with group IV. However, when all the test groups (II, III, and IV) were compared with control group, there were significant changes ($P \leq 0.05$).

Discussion

Essential fatty acids (omega-3 and omega-6) and their derivatives have been known to lower cholesterol levels in the blood, which is an important parameter for reducing the incidence of coronary heart disease (CHD). Marine foods, especially fish have been reported to be rich in n-3 fatty acids while omega-6 fatty acids are found mostly in vegetable oil (Shils *et al.*,1994). Eskimos and Japanese fishermen have been observed to have low incidence of myocardial infarction although they consume a diet high in animal protein, fat and cholesterol (Dyerberg and Jorgensen, 1982). This has been attributed to high levels of omega-3 fatty acid in their diets.

Commonly eaten in Nigeria especially among people in the riverine areas and low income earners are seafoods, which include bonka fish, crayfish and periwinkles. Vegetable oils such as groundnut, corn and soybean oil are commonly eaten by the affluent groups in Nigeria. Essien and others have reported that oils from vegetables are good source of n-3 and n-6 essential fatty acids (Essien *et al.*, 1995). In this study rat pellets supplemented with different ratios of these essential polyunsaturated fatty acids sources were fed to experimental animals in order to determine their effect on the risk of CHD in experimental animals.

Furthermore, supplementing rats pellets with different ratios of omega-3 and omega-6 PUFA which were: omega-3 fatty acid enriched diet, omega-3 and omega-6 fatty acids enriched diet and omega-6 fatty acid enriched diet showed appreciable improvement in the haematological indices as evidenced by increased Hb, PCV and RBC counts and decreases in WBC counts. The significant increases ($P \leq 0.05$) in Hb, PCV and RBC counts and the insignificant decreases in WBC counts suggested that the enriched diets did not induce anaemia in the experimental animals and this was highly exhibited by the omega-3 and omega-6 fatty acids enriched diet. The lipid profile of animals indicated significantly decreased concentrations of total cholesterol, VLDL-C, TG and increased concentration of HDL-C for the omega-3 and omega-6 fatty acids enriched pellets (supplemented diet). These findings suggest the ameliorating effects of this diet on CHD. Animals on Omega-3 enriched pellets (supplemented diet) showed decreased concentrations of total cholesterol and HDL-C with increased concentration of TG, VLDL-C and LDL-C compared with the control. These results however, suggest an atherogenic potential for the omega-3 rich pellet supplements. Also animals on omega-6 enriched diet showed similar pattern as the animals fed pellets enriched with omega-3 and omega-6 fatty although a higher effect was shown by the later. From the results n-3 and n-6 rich diet is expected to prevent atherogenicity. This is in line with some reports by other authors that a concentrate of free fatty acids of fish oils, prevent ventricular fibrillation and sudden cardiac death in reliable dog model with very high probability (Billman and Hallaq, 1994; Billman *et al.*, 1997).

The results of this investigation suggest that consumption of a diet enriched with both omega-3 and omega-6 would be of more health benefit and may help prevent coronary heart disease. The same tendency but in a lesser extent was exhibited by omega-6 enriched

Table 3: Lipid profile of adult albino Wistar rats fed with different ratios of n-3 and n-6 polyunsaturated fatty acids supplemented diets

Parameters+/Groups	Plasma Total Cholesterol	Plasma TG	Plasma HDL-Cholesterol	Plasma VLDL-Cholesterol	Plasma LDL-Cholesterol
Group I (Control)	125.00 ± 3.78*,**	91.25 ± 0.72	27.57 ± 0.30	18.27 ± 0.13	69.69 ± 3.43
Group II	119.94 ± 2.80*	98.09 ± 3.07*,**	29.26 ± 0.59*,**	19.72 ± 0.60*,**	70.76 ± 3.33*,**
Group III	109.50 ± 2.64*,**	85.23 ± 0.94*,**	37.04 ± 0.81*,**	17.06 ± 0.18*,**	64.97 ± 2.60*
Group IV	114.42 ± 1.92*,**	88.92 ± 0.61*,**	30.56 ± 0.68*,**	17.77 ± 0.13*,**	66.18 ± 2.00*,**

+ = Mean ± SD. * Significantly different from the control value (P<0.05). * Significantly different from the corresponding test group (P<0.05)

diet. However, we observed also that consumption of only omega-3 fatty acid enriched diet though nutritionally very beneficial may not be generally protective against coronary heart disease since long term consumption is likely to increase the risk for coronary heart disease.

In conclusion, we are confident to suggest that the rural Africans, especially those of the coastal regions that consumed mostly the types of fishes/crayfish used in this experiment have good reason for having low incidence of coronary heart diseases. This beneficial health effects are attributed to the supplementation of animal feed with rich sources of n-3 and n-6 fatty acids in the right ratios. Also the nutritional quality of these sea foods may be a remedy to coronary heart diseases, hence the need for African health workers to promote the consumption of marine foods as a strategy of managing CDH, a disease that is alien to the African culture.

References

Alexander, R.R. and J.M. Griffith, 1996. Haemoglobin determination by cyanomethaemoglobin method. In: *Basic biochemical methods*, 2nd ed., John Wiley and Sons, Inc. New York.

Billman, G.E., J.X. Kang and A. Leaf, 1997. Prevention of ischemia-induced cardiac sudden death by n-3 polyunsaturated fatty acids. Lipids, 32: 1161-1168.

Billman, G.E. and H. Hallaq, 1994. Prevention of ischemia-induced ventricular fibrillation by n-3 fatty acids. Proc. Natl. Acad. Sci. Uβ A.; 91: 4427-4430.

Christensen, J., E.K. Hagstrup and A. Jens, 1997. 1"Fish consumption, n-3 fatty acids in cell membranes, and heart rate variability in survivors of myocardial infarction with left ventricular dysfunction". Am. J. Cardio., 79: 1670-167.

Hass, E.M., 1992. Staying Healthy with Nutrition, Berkley, Calif: Celestial Arts Publishing, pp: 65-79.

Dacie, J.V. and S.M. Lewis, 1975. Practical Haemology, 5th edn., Churchill Livingstone, London.

Dyerberg, J. and K.A. Jorgensen, 1982. Marine oils and thrombogenesis. Lipid Res., 21: 255-269.

Essien, E.U., G.J. Esenowo and M.I. Akpanabiatu, 1995. Lipid composition of lesser known tropical seeds. Plt. Fd. Hum. Nutr., 48: 135- 140.

Lapinskas, P., 2001. Omega-6 fatty acids – what, why, where and how? Leatherhead FoodResearch Association, Leatherhead, England, pp: 2-7.

Linder, M.C., 1992. Nutrition and metabolism of fats. In: Linder, M.C. (ed.) Nutritional Biochemistry and Metabolism with Clinical Applications, 2nd edn. Elsevier, New York, pp: 51-85.

Lopes-Virella, M.F., P.G. Stone and J.A. Colwell, 1977. Effect of KCD-232, a new hypolipidemic agent on serum lipoprotein changes in hepatoma-bearing rats. *Diabetologia*, 1977, 13: 285-291.

Renaud, S. and M. Lorgeril, 1992. Wine, alcohol, platelets and French paradox for coronary Heart disease. Lancert, 339: 123-1526.

Richmond, W., 1972. Preparation and properties of a cholesterol oxidase from *Nocardia* sp. And its application to the enzymatic assay of total cholesterol in serum. Clin. Chem., 19: 1350-1356.

Roeschlau, P., E. Bernt and W.J. Guber, 1974. Enzymatic determination of total cholesterol in serum. Clin. Chem. Clin. Biochem., 12: 226.

Rudin Donald and Clara Felix, 1996. Omega-3 Oils: To improve mental health, fight degenerative diseases, and extend your life. Garden City Park, NY: Avery Publishing Group.

Shils, M.E., J.A. Olson and M. Shike, 1994. Modern nutrition in health and disease, 8th edn. vol. 1. Williams and Baltimore.

Shils, M.E., J.A. Olson, M. Shike and A.C. Ross, 1999. Modern Nutrition in Health and Disease 9th edn. Baltimore, Md. Williams & Wilkins, pp: 90-92, 1337-1378.

Simopoulos, A.P., 1999. Essential fatty acids in health and chronic disease. Am. J. Clin. Nutri., 70 suppl: 5605-5695.

Stephen, A.M. and G.M. Sieber, 1994. Trends in individual fat consumption of dietary fat in the United States, 1920-1984. Am. J. Clin. Nutri., 52: 457- 469.

Tietz, N.W., 1990. Clinical Guide to Laboratory Tests, 2nd edn. WB Saunders Company, Philadelphia, USA.

Trinder, P., 1969. Quantitative determination of triglyceride using GPO-PAP method. Ann. Biochem., 6: 24 -27.

Werbach, M.R., 1993. Nutritional influence on illness. 2nd edn. Tarzana Calif: Third Line Pres, pp: 13-22, 655-671.

Willet, W.C., 1994. What should we eat? Science, 264: 532-537.