Study of the effects of dietary fish intake on serum lipids and lipoproteins in two populations with different dietary habits

Isabel C. Torres[1]*, Lurdes Mira[2], Cristina P. Ornelas[3] and Amélia Melim[3]

[1]*Departamento de Química, Universidade da Madeira, Campus Universitário da Penteada, 9000 Funchal, Portugal*
[2]*Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, R. Ernesto de Vasconcelos, Edifício C1, 1749–018 Lisboa, Portugal and*
Centro de Metabolismo e Endocrinologia, Faculdade de Medicina de Lisboa, Av. Prof. Egas Moniz, 1749–018 Lisboa, Portugal
[3]*Centro de Investigação e Tecnologia da Madeira (CITMA), 9000 Funchal, Portugal*

(Received 12 April 1999 – Revised 13 September 1999 – Accepted 24 September 1999)

Increased concentrations of *n*-3 polyunsaturated fatty acids (PUFA), namely eicosapentaenoic acid (20 : 5; EPA) and docosahexaenoic acid (22 : 6; DHA), have been shown to be beneficial in coronary artery disease (CAD). In the present study, the relationships between fish intake and concentrations of serum EPA and DHA and the effects of these fatty acids on serum lipids and lipoproteins were investigated. Two groups of men, one living in a fishing village and the other in a farming village, participated in this study. The daily fish consumption was ten times greater in the fishing village group than in the rural village group and the mortality from IHD in the rural village was four times higher. Serum concentrations of EPA and DHA were significantly higher in the fishing village group ($P < 0{\cdot}001$). In this group, the serum concentration of arachidonic acid (20 : 4; AA), was significantly lower ($P < 0{\cdot}001$), and the ratio EPA : AA was twice that of the rural village ($P < 0{\cdot}001$). Moreover, in the fishing village group, the serum triacylglycerol and total cholesterol levels were significantly lower than those observed in the rural village ($P < 0{\cdot}01$ and $P < 0{\cdot}05$ respectively). In the fishing village group the serum LDL-cholesterol concentration was also lower, although the difference was not significant. Our results reinforce the hypothesis that a high intake of *n*-3 PUFA provides protection against CAD.

_n_-3 Fatty acids: Serum lipids: Eicosapentaenoic acid: Docosahexaenoic acid

Coronary artery disease (CAD) is the main cause of death in Western industrialized countries and hypercholesterolaemia is a major risk factor for CAD (Castelli, 1983; Kannel *et al*. 1986). It has been suggested that the development of CAD may be partly altered by diet, especially by the intake of *n*-3 polyunsaturated fatty acids (PUFA) (Leaf & Weber, 1988*a*; Nordøy, 1991; Harris, 1994). The *n*-3 PUFA eicosapentaenoic acid (20 : 5; EPA) and docosahexaenoic acid (22 : 6; DHA) are essential constituents of human diets and are found in considerable amounts in fatty fish species like mackerel (*Scomber colias*), sardines (*Clupea pilchardus*), Atlantic salmon (*Salmo salar*), Albacore tuna (*Orcynus albacora*) and scabbard fish (*Aphanopus carbo*) (Leaf & Weber, 1988*b*; Nordøy, 1991).

Several studies have claimed that the *n*-3 fatty acids EPA and DHA have physiological effects in man, particularly hypolipidaemic as well as anti-thrombotic and anti-atherogenic effects (Bang *et al*. 1971, 1980; Dyerberg *et al*. 1978; Illingworth *et al*. 1984; Hirai *et al*. 1987; Harris, 1989, 1997; Nordøy, 1991). In the 1970s epidemiological studies related the low incidence of IHD in Greenland Eskimos with their traditional diet, which consists almost exclusively of marine food (Bang *et al*. 1971, 1980; Bang & Dyerberg 1972; Dyerberg *et al*. 1975). Low death rates from CHD among Japanese people were also correlated with their high fish consumption (Kagawa *et al*. 1982; Hirai *et al*. 1987). In the Zutphen study (the Netherlands), an inverse dose–response relationship was observed between fish intake and death from CHD during 20 years of follow-up (Kromhout *et al*. 1985). In another prospective population study an inverse association between fish consumption and the 30-year risk of death from CHD was also reported (Daviglus *et al*. 1997). Despite the many studies carried out, there is still some controversy about the benefits of the intake of fish in the prevention of death from cardiovascular diseases (Ascherio *et al*. 1995; Daviglus *et al*. 1997), as well as the mechanisms

Abbreviations: AA, arachidonic acid; CAD, coronary artery disease; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; PUFA, polyunsaturated fatty acids.

* **Corresponding author:** Dr Isabel C. Torres, fax +351 91 705149, email isat@dragoeiro.uma.pt

responsible for the protective effects of *n*-3 fatty acids (Bronsgeest-Schoute *et al*. 1981; Delany *et al*. 1990; Reis *et al*. 1990; Lovegrove *et al*. 1997).

The aim of the present study was to analyse the relationships between fish intake, serum fatty acid composition, and serum lipids and lipoproteins of residents of two villages of Madeira Island, Câmara de Lobos and Curral das Freiras, with different CAD mortalities. Câmara de Lobos is a fishing village, the population of which has a high fish consumption and a relatively low death rate from CAD. Curral das Freiras is an inland farming village and its population has a low fish intake and relatively high death rate from CAD.

Subjects and methods

Study participants

Madeira is a small Portuguese island situated in the middle of the North Atlantic Ocean, 500 km from the African coast and 1000 km from the Portuguese mainland. Madeira covers an area of 741 km^2 with a population of about 260 000. The two communities studied, Câmara de Lobos, the most important piscatorial village, with a population of 14 991, and Curral das Freiras, a rural village, with a population of 2388, were the target for the present study. Fifty male inhabitants from the coastal village and thirty-seven from the inland village participated in the study. The two groups were selected randomly from electoral rolls. We did not include more than one participant from each household. The age range was 25–65 years. Correspondence with the subjects was by mail, informing them about the aims and possible benefits that could be derived from the study. They and their wives were invited to an interview at the local health centre. The interviews were conducted by trained nurses and focused on: medical history (e.g. cardiovascular disease), health behaviours, medication, smoking habits and lifestyle. In addition, a socio-cultural questionnaire was completed for each subject. Blood pressure, weight and height were measured. Venous blood samples were collected, after 12 h fasting.

Characteristics of participants

The mean age, BMI, blood pressure, disease status and socio-cultural characteristics of the two groups studied are presented in Table 1. The medication taken by the participants in the two groups studied included drugs for blood pressure (hypertension) and circulation, sedatives and antidepressants. Subjects with insulin-dependent diabetes mellitus injected themselves with insulin.

Dietary assessment

Dietary intake was assessed by two nutritionists using an interviewer-administered, sixty-seven-item food-frequency questionnaire, a slightly modified version of a sixty-one-item instrument developed by Willett *et al*. (1983) who validated their food-frequency questionnaire against a 28 d record (Willett *et al*. 1985). Three modifications were made: (1) separation of a few items into detailed subcategories (e.g. the questions addressing fish consumption were separated into four specific fish items: frequency, amount, seasoning and food preparation); (2) description of the types of fat and oils normally used for frying, cooking and food preparation; (3) creation of separate and more detailed questions about consumption of wine, beer, whisky and brandy. Participants were asked, how often, on average, during the last year, they had consumed each food. They were asked, on average, if they had consumed a specified portion of a fish item. Photographs of portions of different types of fish were also available. Each participant's wife was also interviewed about the husband's usual food consumption. There were seven choices for frequency of consumption, ranging from 'never' to 'at least two times per day'. Food and utensil models were used to establish the quantity of food consumed. The food-intake data were converted into energy and nutrients by using the Portuguese food composition tables (Gonçalves Ferreira & Da Silva, 1985) and PIABAD software (unpublished), from the Becel Food Institute, Portugal. Additional information about some typical Madeiran foods was obtained from the Department of Human Nutrition, National Health Institute, INSA, Lisbon, Portugal.

Other measurements

Body weight was measured by a digital scale with an accuracy of ± 100 g, after venepuncture and a light breakfast. The subjects were weighed without shoes and heavy clothing. Standing height was measured without shoes. Blood pressure was taken by a nurse with an Hg sphygmomanometer and was measured twice with an interval of about 3 min between each measurement. The participants were in a sitting position and relaxed before the measurements were taken.

Analytical methods

Blood sampling. Blood samples were collected after 12 h fasting overnight. Serum was obtained by low-speed centrifugation at 1000 ***g*** at 4° for 10 min, within 1 h of venepuncture, transferred to plastic tubes in portions and stored under N_2 at −20° until analysis.

Serum lipids and lipoproteins. Serum lipids and lipoproteins were assayed with an Auto-Multi-Analyzer Hitachi System 704 (Boehringer Mannheim Hitachi, Mannheim, Germany). Total serum cholesterol and triacylglycerol levels were measured enzymically with the triacylglycerol GPO-PAP–cholesterol CHOD-PAP kit (Boehringer Mannheim, Mannheim, Germany). Serum HDL-cholesterol was determined enzymically using the CHOD-PAP kit (Boehringer Mannheim), after precipitation of the chylomicrons, VLDL and LDL with phosphotungstic acid and Mg^{2+}. The within-assay CV for these assays (*n* 10) were 1·4, 1·4 and 2·4 % for total cholesterol, HDL-cholesterol and triacylglycerols respectively, and the between-assay CV (*n* 10) were 0·9, 1·1 and 0·95 % respectively. LDL-cholesterol was calculated using the formula of Friedewald *et al*. (1972) (total cholesterol − HDL-cholesterol − triacylglycerol/2·2), when the triacylglycerol level was $\leqslant 4{\cdot}6$ mmol/l.

Serum lipid fatty acid composition. Before the extraction of serum lipids by the Folch method (Folch *et al*. 1957),

Table 1. Characteristics of participants in the present study

Characteristics	Fishing village (*n* 50) Mean	SD	Rural village (*n* 37) Mean	SD
Age (years)	41·4	11·0	48·2**	11·3
Body weight (kg)	72·0	4·8	61·8***	7·1
Height (m)	1·70	0·036	1·67***	0·022
BMI (kg/m^2)	24·95	1·76	22·13***	2·78
Blood pressure (mmHg)				
Systolic	133·3	13·4	133·2	16·9
Diastolic	79·2	6·9	82·1	10·6
Disease status (%)				
Diabetes (IDDM)	2		5·4*	
Hypertension	6		2·7*	
Psychiatric disease	0		8**	
IHD	2		3	
Income				
(mean annual family income in Euro)	4550		3700**	
Primary occupation (%)				
Building construction	–		60	
Farming	8		32	
General labour	12		8	
Fishing	70		–	
Driving	10		–	
Education (%)				
Minimum school level	84		60**	
No education	16		40**	
Physical activity (%)				
Subjects with no sporting activities	94		96	
Subjects with sporting activity once weekly	6		4	
Subjects walking daily (%)				
Subjects walking rarely (< 1 km)	81		79	
Subjects walking moderately	19		21	
Smoking status				
Non smokers (%)	38		55*	
Average number cigarettes smoked/d for smokers	15		13	

IDDM, insulin-dependent diabetes mellitus.
Mean values were significantly different from those for the fishing village: *$P < 0·05$, **$P < 0·01$, ***$P < 0·001$ (Student's *t* test).

0·5 ml docosaenoic acid (*cis*-13-22 : 1; Sigma Chemical Co., St Louis, MO, USA) (100 μg/ml in chloroform) was added to 0·5 ml serum as internal standard and butylated hydroxytoluene (Sigma Chemical Co.) was added as antioxidant. Fatty acids were transmethylated with methanolic-HCl (Christie, 1989) and the methyl esters were extracted into *n*-heptane and analysed with a Hewlett-Packard 5890 series II GC with flame ionization detector (supplied with air and high purity H_2). A 1 μl portion was injected into a 50 m BP5 fused silica capillary column with 0·20 mm i.d., 0·33 μm film thickness and a split ratio of 1 : 20. High purity He (N56) was used as the carrier gas at a linear velocity of 0·3 m/s and N_2 was used as the make-up gas. The initial oven temperature was 50°, followed by temperature programming in three steps: a first rate of 25°/min up to 160°, followed by a second rate of 4°/min up to 256° and by a third rate of 2°/min up to 280° which was maintained for 15 min. The injector and flame ionization detector temperatures were maintained at 280° and 300° respectively (Jennings, 1987). Peaks were identified by comparison with known pure standard mixtures (Sigma Chemical Co.) and quantified by automatic integration of areas. Response factors for each fatty acid were determined using pure standards (Sigma Chemical Co.) and a correction factor was applied to compensate for the lower ionization detector response to unsaturated fatty acids.

The results were expressed in absolute concentrations (mg/l serum), using an internal standard. A mixture of pure fatty acid methyl esters, dissolved in *n*-heptane and stored at –20°, was used as a control. By this technique, the within-assay CV at *n* 10 were 1·21, 1·96 and 0·56 % respectively, for 20 : 4, 20 : 5 and 22 : 6; and the between-assay CV at *n* 10 were 5·06, 8·35 and 4·08 % for those fatty acids respectively.

Statistical analysis

Data are expressed as means and standard deviations. The level of significance chosen was $P < 0·05$. In order to test

whether the differences between the mean values of the items studied in both groups were significant, the Student's *t* test was used. When the conditions of applicability failed, the Mann-Whitney test was used instead. Medians and 25th–75th percentiles were used for dietary data. Correlations between the serum lipids, lipoproteins and dietary intakes of fatty acids in both groups were assessed using Pearson product-moment coefficients (*r*), when data were distributed normally (Glantz, 1992). For the ANOVA of lipid findings with alcohol consumption the participants were classified into four groups (C_0, control, 0 g/d, who never consumed alcohol; C_1, who consumed 1–26 g/d, the maximum recommended for males; C_2, who consumed 27–100 g/d and C_3 who consumed >100 g/d) and we used the Systat software (1992; System for Statistics, Evanston, IL, USA). For pair-wise comparison with the control group (0 g/d) we used the Dunnet test. ANOVA was used to study the variance between the four age groups. The software used was Microsoft Excel 7.0 for Windows 95 (Microsoft Corp., USA).

Results

Dietary intake

The mean, median and 25th–75th percentile nutrient intakes of the participants are shown in Table 2. In the rural village, one of the participants presented very low mean intakes of protein and total fat. However, in this case, about 50 % of dietary energy was supplied by alcohol and the remainder was supplied by the intake of foods rich in carbohydrates, such as potatoes, rice and bread. Among nutritional variables there were significant differences in the mean intakes of total energy, protein, carbohydrate and fat between the two groups studied. Significantly higher intakes of monounsaturated fatty acids and PUFA were observed in the fishing village group. EPA, DHA and AA intakes were significantly higher ($P < 0{\cdot}001$) in the fishing village, with DHA intake almost ten times higher. The cholesterol intake in the men from the fishing village was higher than that for men from the rural village ($P < 0{\cdot}05$). The alcohol intake in the fishing village group exceeded the intake in the rural village group ($P < 0{\cdot}05$). The study participants from Câmara de Lobos had a significantly higher daily fish intake *per capita* than the participants from Curral das Freiras (Table 3). In particular, the intake of scabbard fish (the most widely consumed fish) was extremely low in the rural village, with an intake frequency of two to three times per month. In the rural village group only four (10 %) out of the thirty-seven individuals studied ate scabbard fish, while in the fishing village group thirty-two (64 %) out of the fifty individuals studied ate this type of fish.

Serum lipid and lipoprotein levels

The mean serum lipid and lipoprotein concentrations for both groups under study are presented in Table 4. The most significant difference observed between the two groups studied was the triacylglycerol concentration, which was lower (by 27·9 %) in the fishing village group ($P < 0{\cdot}01$). In this group, the total serum cholesterol level was also lower (by 10 %) ($P \leqslant 0{\cdot}05$). However, there were no significant differences in LDL-cholesterol or HDL-cholesterol levels, or in the ratio HDL-cholesterol : total cholesterol.

Fatty acid composition of serum lipids

In spite of the fact that fatty acids of chain lengths ranging from C_{16} to C_{24} were identified, only palmitic acid (16 : 0), stearic acid (18 : 0), linoleic acid (18 : 2*n*-6), α-linolenic acid (18 : 3*n*-3), eicosatrienoic acid (20 : 3*n*-6), AA (20 : 4*n*-6), EPA (20 : 5*n*-3) and DHA (22 : 6*n*-3) were quantified in mg/l (Table 5). The total serum concentration of *n*-3 fatty acids in the fishing village group was significantly higher ($P < 0{\cdot}01$) than in the rural village group. Likewise, significantly higher concentrations of EPA ($P < 0{\cdot}001$) and DHA ($P < 0{\cdot}001$) acids were observed in the fishing village group. In contrast, the total serum concentration of *n*-6 fatty acids in the fishing village group was lower ($P < 0{\cdot}05$) than in the rural village group, the concentration of AA being much lower in the fishing village group ($P < 0{\cdot}001$). The values for EPA : AA and DHA : AA for the fishing village were twice as high as those obtained for the rural village ($P < 0{\cdot}001$). Positive correlations were found between AA and both EPA and DHA concentrations in both villages (*r* 0·46 and *r* 0·73, $P < 0{\cdot}01$ respectively, for the fishing village and *r* 0·33, $P < 0{\cdot}05$ and *r* 0·46, $P < 0{\cdot}01$ for the rural village). EPA and DHA concentrations were also strongly correlated (*r* 0·74, $P < 0{\cdot}001$ for the fishing village and *r* 0·56, $P < 0{\cdot}01$ for the rural village). The correlations studied between fatty acids and serum lipids and lipoproteins indicated that palmitic acid correlated positively with total cholesterol, LDL-cholesterol and triacylglycerols in both groups studied (*r* 0·32, $P = 0{\cdot}05$; *r* 0·20, $P = 0{\cdot}1$ and *r* 0·58, $P < 0{\cdot}01$ respectively, for the fishing village and *r* 0·67, *r* 0·55, *r* 0·64, all $P < 0{\cdot}01$ for the rural village). However, it should be pointed out that the correlations between palmitic acid, total cholesterol and LDL-cholesterol were much stronger in the rural village.

The ANOVA of lipid findings with age in both groups led to the conclusion that there were no significant differences ($P = 0{\cdot}05$) in the mean values obtained for each variable in the different age groups studied. With respect to the ANOVA of lipid findings with alcohol intake in Curral there were no significant differences for any of the variables in the three alcohol consumer groups studied. Nevertheless, in the groups from Câmara de Lobos there was a significant difference in the mean values obtained for HDL-cholesterol between group C_3, who consumed >100 g alcohol/d, and the control group. Although the mean value obtained for HDL-cholesterol in group C_3 was higher, the difference observed between the two village groups was not significant.

Relationship between dietary intake and serum lipid fatty acids

Positive correlations between the dietary *n*-3 PUFA particularly EPA and DHA, estimated through the food-frequency questionnaire, and the serum concentrations of those fatty acids, were observed both in the fishing village and in the rural village. These correlations were stronger in the fishing village group, both for EPA (*r* 0·34) and for DHA (*r* 0·33),

Table 2. Intakes of energy and nutrients estimated from food-frequency questionnaire data, for groups of male subjects from a fishing village and a rural village on the Portuguese island of Madeira

(Mean values and standard deviations, with minimum, 25th percentile, median, 75th percentile and maximum values)

	Fishing village (*n* 50)							Rural village (*n* 37)						
Nutrients	Mean	SD	Minimum	25th percentile	Median	75th percentile	Maximum	Mean	SD	Minimum	25th percentile	Median	75th percentile	Maximum
Total energy (MJ/d)	10·0	2·2	4·2	8·9	10·2	11·4	16·8	7·7***	2·8	4·2	5·8	7·1	9·5	16·2
Protein														
(g/d)	19·9	39·9	42·3	94·3	109·4	150·7	230·7	74·1***	35·8	8·0	45·1	64·5	93·8	149·3
(% total energy)	119·7	5·2	11·3	16·4	19·4	22·8	37·7	16·0*	4·9	3·3	12·4	15·9	17·7	28·9
Total fat														
(g/d)	68·0	22·9	18·2	50·7	68·0	83·8	118·3	48·0*	24·5	3·4	33·5	43·5	63·5	104·3
(% total energy)	25·4	6·4	11·3	17·5	23·6	28·9	37·7	23·4	9·0	2·6	25·3	32·9	48·0	78·9
Saturated fat														
(g/d)	23·4	9·1	4·0	17·3	22·3	31·2	37·6	16·7*	9·4	0·6	11·1	15·5	23·4	38·2
(% total energy)	8·8	2·9	7·1	7·1	9·2	10·8	14·4	7·9	3·5	0·6	5·7	7·7	10·2	18·6
Monounsaturated fat														
(g/d)	26·3	9·4	11·5	16·2	25·8	37·2	47·2	19·8*	10·0	1·2	13·6	17·6	26·7	44·2
(% total energy)	9·8	2·7	1·8	6·5	9·0	11·6	16·3	9·8	4·2	0·9	10·3	13·3	20·2	33·4
Polyunsaturated fat														
(g/d)	9·9	3·8	2·5	6·9	10·1	12·8	17·4	6·8*	3·2	1·4	4·3	5·9	9·7	13·4
(% total energy)	3·7	1·2	0·9	2·4	3·5	4·4	6·0	3·4	1·4	1·1	3·3	4·5	7·3	10·1
Linoleic acid (g/d)	7·4	3·2	1·4	4·8	7·3	9·8	14·9	5·5*	2·9	0·5	3·2	4·9	7·9	11·6
α-Linolenic acid (mg/d)	489·1	295·6	39·0	280·3	415·0	655·3	1140·0	358·5*	305·2	0·0	170·0	230·0	437·0	1153·0
Eicosapentaenoic acid (mg/d)	218·1	357·7	0·0	37·8	71·0	187·5	1358·0	26·7†	75·3	0·0	0·0	0·0	24·0	1558·0
Docosahexaenoic acid (mg/d)	536·0	788·3	0·0	90·5	238·0	678·3	3949·0	55·9†	79·7	0·0	0·0	37·0	84·0	386·0
Arachidonic acid (mg/d)	166·3	97·4	18·0	104·3	147·5	211·8	477·0	126·1†	137·5	0·0	28·0	93·0	160·0	487·0
Cholesterol (mg/d)	378·3	166·2	132·0	250·0	347·5	485·8	915·0	240·8*	166·7	0·0	142·0	196·0	324·0	815·0
Carbohydrate														
(g/d)	248·4	70·1	115·4	198·5	254·5	294·2	385·1	236·0*	102·7	43·4	164·3	229·8	268·6	538·8
(% total energy)	42·2	13·6	11·7	30·4	39·0	45·0	59·0	51·9*	13·6	14·6	52·2	77·2	90·3	181·1
Dietary fibre (g/d)	19·1	7·0	5·4	14·9	20·9	23·4	33·1	16·2*	7·3	2·6	11·5	14·7	20·9	34·1
Alcohol														
(g/d)	45·4	65·0	0·0	4·1	16·3	51·3	260·7	21·9*	29·5	0·0	0·0	7·7	36·2	95·7
(% total energy)	12·3	15·9	0·0	1·1	4·4	13·7	69·8	8·6*	12·2	0·0	0·0	4·5	21·3	56·3

Mean values were significantly different from those for the fishing village: *$P < 0·05$, ***$P < 0·001$ (Student's *t* test); †$P < 0·05$ (Mann-Whitney test).

Table 3. Mean daily *per capita* intakes (g) of different fish species, estimated from food-frequency questionnaire data, in groups of male subjects from a fishing village and a rural village on the Portuguese island of Madeira

	Mean daily *per capita* intake (g)	
Species	Fishing village (*n* 50)	Rural village (*n* 37)
Scabbard (*Aphanopus carbo*)	120·88	4·72
Mackerel (*Scomber colias*)	30·09	1·97
Cod (*Gadus morrhua*)	13·15	12·87
Tuna (*Orcynus albacora*)	11·78	6·46
Chicharro (*Tracuro trachurus*)	5·69	0·0
Others	36·90	0·0
Total fish intake	218·50	26·0

Table 4. Serum lipid and lipoprotein concentrations of groups of male subjects from a fishing village and a rural village on the Portuguese island of Madeira

(Mean values and standard deviations)

	Fishing village (*n* 50)		Rural village (*n* 37)	
Lipids and lipoproteins	Mean	SD	Mean	SD
Total cholesterol (mmol/l)	4·8	1·0	5·4*	1·5
HDL-cholesterol (mmol/l)	1·3	0·1	1·2	0·1
LDL-cholesterol (mmol/l)	2·9	0·6	3·4	0·9
Triacylglycerols (mmol/l)	1·3	0·6	1·8**	1·1
HDL : lDL	0·5	0·3	0·4	0·2
HDL : total cholesterol	0·3	0·1	0·2	0·1

Mean values were significantly different from those for the fishing village: *$P < 0·05$, **$P < 0·01$ (Student's *t* test).

Table 5. Serum fatty acid concentrations of groups of male subjects from a fishing village and a rural village on the Portuguese island of Madeira

(Mean values and standard deviations)

	Fishing village (*n* 50)		Rural village (*n* 37)	
Fatty acid (mg/l)	Mean	SD	Mean	SD
16 : 0	687·8	240·5	716·7	202·4
18 : 0	235·6	61·9	246·7	63·9
18 : 2 *n*-6	627·4	182·4	666·2	149·0
18 : 3 *n*-3	75·9	22·5	59·6**	18·7
20 : 3 *n*-6	43·6	16·3	53·3**	17·6
20 : 4 *n*-6	110·2	31·0	145·0***	48·9
20 : 5 *n*-3	35·3	17·9	20·4***	10·7
22 : 6 *n*-3	102·3	49·0	61·3***	23·2
EPA : AA	0·33	0·16	0·15***	0·08
DHA : AA	0·92	0·27	0·47***	0·24
Total PUFA *n*-3	197·0	69·5	157·6**	40·6
Total PUFA *n*-6	782·7	212·6	864·5*	191·9
n-6 : *n*-3	4·2	1·1	5·8**	1·6

EPA, eicosapentaenoic acid; AA, arachidonic acid; DHA, docosahexaenoic acid; PUFA, polyunsaturated fatty acids.

Mean values were significantly different from those for the fishing village: *$P < 0·05$, **$P < 0·01$, ***$P < 0·001$ (Student's *t* test).

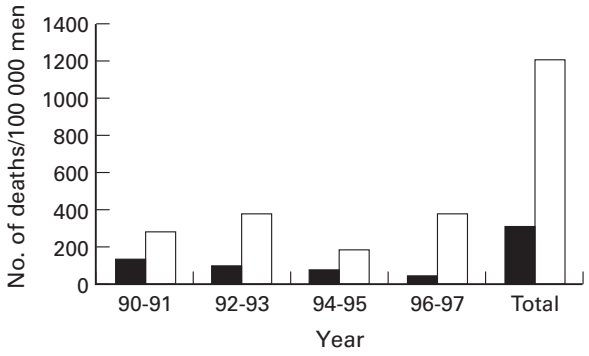

Fig. 1. Number of deaths from IHD per 100 000 men in a fishing village (■) and a rural village (□) on the Portuguese island of Madeira, for the years 1990–7.

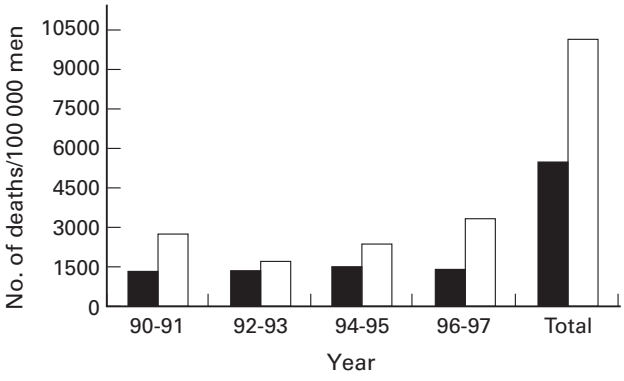

Fig. 2. Number of deaths from all causes per 100 000 men in a fishing village (■) and a rural village (□) on the Portuguese island of Madeira, for the years 1990–7.

than in the rural village (*r* 0·31 and *r* 0·18 respectively). Fish intake and serum EPA and DHA levels correlated positively in both villages. However, the correlation in the fishing village (*r* 0·39, $P < 0·01$ for EPA and *r* 0·36, $P = 0·01$ for DHA) was higher than in the rural village (*r* 0·28 for EPA and *r* 0·21 for DHA, $P = 0·1$).

Mortality rate

From 1990 to 1997, the numbers of deaths/100 000 men in Curral and in Câmara de Lobos resulting from IHD (International Classification of Diseases codes 410 to 413), ascertained from death certificates, were 1205 (the actual number of deaths was thirteen out of 1079 men) and 310 (the actual number of deaths was twenty-two out of 7066 men) respectively (Fig. 1). In the same period, the total numbers of deaths/100 000 men in Curral and in Câmara de Lobos were 10 193 and 5489 respectively (Fig. 2). The mortality from IHD represented 5 and 10 % of total mortality in Câmara de Lobos and Curral respectively. The mortality data were obtained from the Department of Statistics of the Public Health Service of Madeira Island.

Discussion

The aim of the present study was to examine the relationships

between dietary fish intake, serum fatty acid composition and serum lipids and lipoproteins in two populations with different dietary habits and different mortality rates from CAD.

Dietary intake data for the two groups studied (Table 2) revealed that there were differences in intakes of total energy, protein and total fat, which were significantly lower in the rural group. The differences observed in the rural group can be explained by its geographical isolation. Certain places are remote and cannot be easily accessed. These characteristics may have led to voluntary self-sufficiency, resulting in monotony and homogeneity in the diet. It is also important to emphasize that 16·2 % of the participants in Curral das Freiras lived in areas considered extremely poor, at the time of the study. In both villages, only about 25 % of the dietary energy was provided by fats, with 8–9 % of the energy derived from saturated fats. These values differ from those of the typical Western diet, in which almost 40 % of total energy is supplied by fats (Leaf & Weber, 1988*a*). In both groups the percentage of the total energy supplied by protein was above the value of 10–15 % recommended by the World Health Organization (1990). In the fishing village the greatest contributor to protein supply in the diet was the fish. In the rural village, however, protein supply was not due to the fish, since its consumption in this village was very low. The higher fish intake in the fishing village group must have resulted in the higher intakes of the *n*-3 fatty acids EPA and DHA and therefore raised their serum concentrations. Both the consumption of fish and the intakes of EPA and DHA correlated positively with the serum concentrations of these fatty acids. Indeed, the plasma concentrations of EPA and DHA seem to be supplied essentially by fish and its derivatives ingested in the diet (Glomset, 1985). The two groups studied showed a small significant difference in the serum levels of total *n*-6 fatty acids, the level of the fishing village group being lower. The accumulation of *n*-3 fatty acids in the plasma and phospholipids of the membranes was associated with a reciprocal decrease in *n*-6 fatty acids, especially AA due to the inhibiting effect of *n*-3 fatty acids in the synthesis of AA from linoleic acid (Leaf & Weber, 1988*b*; Parkinson *et al*. 1994). This effect of *n*-3 fatty acids may explain the lower concentrations of AA, and its precursor eicosatrienoic acid, that were observed in the fishing village group. The decrease in AA which takes place when there is an increase in the concentration of *n*-3 fatty acids, results in a decrease in thromboxanes and an increase in prostacyclins, the net effect of which is an inhibition of platelet aggregation and of vasoconstriction providing a favourable antithrombotic situation (Leaf & Weber, 1988*a*; Goodnight, 1996). The average EPA : AA value in the fishing village group (0·33 (SD 0·16)) was twice as high as that of the rural village. However, it is lower than those obtained for the Japanese (0·58 (SD 0·26)), Greenland Eskimos (7·0) and Alaskan Eskimos (1·16) but higher than the value determined in Europeans (0·1) (Hirai *et al*. 1980). The high EPA : AA values observed in those groups were ascribed to the high seafood intake, and may explain the low incidence of thrombotic disorders (Hirai *et al*. 1980). The lower EPA : AA value, observed in the more recent study with Alaskan Eskimos when compared with the Greenland Eskimos, has been attributed to a higher intake of typical Western foods in their diet nowadays (Parkinson *et al*. 1994).

In the fishing village group the serum triacylglycerol and total cholesterol levels were respectively 28 % and 10 % lower than those observed in the rural village group. In the Câmara de Lobos group the LDL-cholesterol level was also lower, although the difference was not significant ($P = 0·05$). These differences would probably be larger if the fishing village group had not had a significantly higher ingestion of dietary cholesterol and saturated fats. According to Harris (1989), total cholesterol is reduced when the intake of *n*-3 fatty acids is combined with a diet poor in saturated fatty acids. In the present study, this fact seems to be supported by the correlation between the serum concentrations of palmitic acid (16 : 0) and total cholesterol, LDL-cholesterol and triacylglycerols in the two villages. These correlations were not so strong in the fishing village, probably due to the higher intake of *n*-3 fatty acids in this village leading to a hypolipidaemic effect in spite of the higher intake of saturated fats.

Our present study was focused on two populations that had significant differences in age and anthropometric measurements but did not differ significantly in some cardiovascular risk factors, i.e. smoking habits, physical activity and blood pressure. They had, however, several differences in their dietary habits, the fish intake being the most pronounced. In the fishing village group, who consumed ten times more fish than the rural village group, we found higher serum levels of EPA and DHA and lower serum levels of triacylglycerols and total cholesterol, when compared with the rural village group. In addition, in the fishing village group *n*-3 fatty acid concentrations were higher whereas the AA concentration was lower, and the EPA : AA value was twice that of the rural village. Increased EPA : AA values have been related to an observed reduction in mortality due to CAD in many different populations. The two groups had significant differences in the levels of other nutrients in the diet and they also presented significant differences in some of their characteristics. Furthermore, the number of participants was relatively small and the number of inhabitants of both villages is small, making the mortality data from IHD less reliable. For these reasons the present study cannot conclusively determine whether or not high serum concentrations of *n*-3 fatty acids play a protective role in CAD. Nevertheless, it should be noted that the *n*-3 fatty acid level is one of the most striking differences between the two populations. It is also not known at present, whether *n*-3 fatty acids act either by altering favourably the concentrations of lipids and lipoproteins or by decreasing the AA concentration or by means of both effects.

In summary, the fishing village group, who had higher serum levels of the *n*-3 fatty acids EPA and DHA, presented a favourable lipid profile regarding the risk of CAD, i.e. low serum levels of total cholesterol and triacylglycerols. The low level of AA and high level of EPA may also have provided a low thrombus formation tendency. Therefore, the favourable factors observed in the fishing village group may explain, at least in part, the low mortality rate due to IHD in Câmara de Lobos.

Acknowledgements

This work was supported by the Universidade da Madeira, Departamento de Química e Centro de Investigação e Tecnologia da Madeira (CITMA), Portugal. Thanks must go to Professor Amorim Cruz for his assistance in the preparation and supervision of the food-frequency questionnaire, and Dr Ilda Martins for furnishing data related to the nutritional composition of Madeiran recipes (Instituto Nacional de Saúde, Lisboa, Portugal). We are also grateful to Dr Maximiano Martins, President of the Centro de Investigação e Tecnologia Madeira (CITMA), Portugal, for his logistical support in the attribution of grants.

References

Ascherio A, Rimm EB, Stampfer MJ, Giovannucci EL & Willett WC (1995) Dietary intake of marine *n*-3 fatty acids, fish intake, and the risk of coronary disease among men. *New England Journal of Medicine* **332**, 977–982.

Bang HO & Dyerberg J (1972) Plasma lipids and lipoproteins in Greenlandic West-coast Eskimos. *Acta Medica Scandinavica* **192**, 85–94.

Bang HO, Dyerberg J & Nielsen AB (1971) Plasma lipid and lipoprotein pattern in Greenlandic west-coast Eskimos. *Lancet* **i**, 1143–1144.

Bang HO, Dyerberg J & Sinclair HM (1980) The composition of the Eskimo food in north western Greenland. *American Journal of Clinical Nutrition* **33**, 2657–2661.

Bronsgeest-Schoute HC, Van Gent CM, Luten JB & Ruiter A (1981) The effect of various intakes of ω-3 fatty acids on blood lipid composition in healthy humans. *American Journal of Clinical Nutrition* **34**, 1752–1757.

Castelli WP (1983) Cardiovascular disease and multifactorial risk: challenge of the 1980s. *American Heart Journal* **106**, 1191–1200.

Christie WW (1989) The analysis of fatty acids. In *Gas Chromatography and Lipids*, pp. 67–68 Glasgow, Ayr, Scotland: The Oily Press.

Daviglus ML, Stamler J, Orencia AJ, Dyer AR, Liu K, Greenland P, Walsh MK, Morris D & Shekelle RB (1997) Fish consumption and the 30-year risk of fatal myocardial infarction. *New England Journal of Medicine* **336**, 1046–1053.

DeLany JP, Vivian VM, Snook JT & Anderson PA (1990) Effects of fish oil on serum lipids in men during a controlled feeding trial. *American Journal of Clinical Nutrition* **52**, 477–485.

Dyerberg J, Bang HO & Hjørne N (1975) Fatty acid composition of the plasma lipids in Greenland Eskimos. *American Journal of Clinical Nutrition* **28**, 958–966.

Dyerberg J, Bang HO, Stofferson E, Moncada S & Vane JR (1978) Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis? *Lancet* **ii**, 117–119.

Folch J, Lees M & Stanley GHS (1957) A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry* **226**, 497–507.

Friedewald WT, Levy RI & Fredrickson DS (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry* **18**, 499–502.

Glantz AS (1992) *Primer of Biostatics*. New York, NY: McGraw-Hill.

Glomset JA (1985) Fish, fatty acids, and human health. *New England Journal of Medicine* **312**, 1253–1254.

Gonçalves Ferreira FA & Da Silva ME (1985) *Composição dos Alimentos Portugueses* (*Composition of Portuguese Foods*). Lisbon: Instituto Nacional de Saúde.

Goodnight SH (1996) The fish oil puzzle. *Science and Medicine* **3**, 42–51.

Harris WS (1989) Fish oils and plasma lipid and lipoprotein metabolism in humans: a critical review. *Journal of Lipid Research* **30**, 785–807.

Harris WS (1994) The benefits and pathologies from excesses and deficiencies of dietary fatty acids. *Current Opinion in Endocrinology and Diabetes* **1**, 260–266.

Harris WS (1997) *n*-3 fatty acids and serum lipoproteins: human studies. *American Journal of Clinical Nutrition* **65**, 1645S–1654S.

Hirai A, Hamazaki T, Terano T, Nishikawa T, Tamura Y & Sajiki J (1980) Eicosapentaenoic acid and platelet function in Japanese (letter). *Lancet* **ii**, 1132–1133.

Hirai A, Terano T, Saito H, Tamura Y & Yoshida S (1987) Clinical and epidemiological studies of eicosapentaenoic acid in Japan. In *Polyunsaturated Fatty Acids and Eicosanoids*, pp. 9–24 [WEM Lands, editor]. Champaign IL: American Oil Chemists' Society.

Illingworth DR, Harris WS & Connor WE (1984) Inhibition of low density lipoprotein synthesis by dietary omega-3 fatty acids in humans. *Arteriosclerosis* **4**, 270–275.

Jennings W (1987) *Analytical Gas Chromatography*. New York, NY: Academic Press.

Kagawa Y, Nishizawa M, Suzuki M, Miyatake T, Hamamoto T, Goto K, Motonaga E, Izumikawa H, Hirata H & Ebihara A (1982) Eicosapolyenoic acids of serum lipids of Japanese islanders with low incidence of cardiovascular diseases. *Journal of Nutritional Science and Vitaminology* **28**, 441–453.

Kannel WB, Thomas HE & Kjelsberg MO (1986) Overall and coronary heart disease mortality rates in relation to major risk factors in 325,348 men screened for the MRFIT. *American Heart Journal* **112**, 825–836.

Kromhout D, Bosschieter EB & Coulander CL (1985) The inverse relation between fish consumption and 20-year mortality from coronary heart disease. *New England Journal of Medicine* **312**, 1205–1209.

Leaf A & Weber PC (1988*a*) Omega-3 fatty acids and cardiovascular disease. In *Heart Disease and Update*, pp. 49–60 [E Braunwald, editor]. Philadelphia, PA and London: WB Saunders Company.

Leaf A & Weber PC (1988*b*) Cardiovascular effects of *n*-3 fatty acids. *New England Journal of Medicine* **318**, 549–557.

Lovegrove JA, Brooks CN, Murphy MC, Gould BJ & Williams CM (1997) Use of manufactured foods enriched with fish oils as a means of increasing long-chain *n*-3 polyunsaturated fatty acid intake. *British Journal of Nutrition* **78**, 223–236.

Nordøy A (1991) Is there a rational use for *n*-3 fatty acids (fish oils) in clinical medicine? *Drugs* **42**, 331–342.

Parkinson AJ, Cruz AI, Heyward WL, Bulkow LR, Hall D, Barstaed L & Connor WE (1994) Elevated concentrations of plasma ω-3 polyunsaturated fatty acids among Alaskan Eskimos. *American Journal of Clinical Nutrition* **59**, 384–388.

Reis GJ, David IS, Boucher TM, Sipperly ME, Horowitz GL, Sacks FM & Pasternak RC (1990) Effects of two types of fish oil supplements on serum lipids and plasma phospholipid fatty acids in coronary artery disease. *American Journal of Cardiology* **66**, 1171–1175.

Willett WC, Sampson L, Stampfer MJ, Rosner B, Bain C, Witschi J, Hennekens CH & Speizer FE (1985) Reproducibility and validity of a semiquantitative food frequency questionnaire. *American Journal of Epidemiology* **122**, 51–65.

Willett WC, Stampfer MJ, Underwood BA, Speizer FE, Rosner B & Hennekens CH (1983) Validation of a dietary questionnaire with plasma carotenoid and alpha-tocopherol levels. *American Journal of Clinical Nutrition* **38**, 631–639.

World Health Organization (1990) *Nutrition and Prevention of Chronic Diseases*. *Technical Report Series* no. 797. Geneva: WHO.