British Journal of Nutrition (2003), **89**, 267–272 DOI: 10.1079/BJN2002747

Serum fatty acid levels, dietary style and coronary heart disease in three neighbouring areas in Japan: the Kumihama study

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(Received 16 July 2001 – Revised 9 May 2002 – Accepted 6 September 2002)

CHD mortality is extremely low in Japan, particularly in rural districts, when compared with that in Western countries. This has been partly attributed to the difference in dietary lifestyle. We investigated the factors influencing CHD mortality in a rural coastal district of Japan, comprising mercantile, farming, and fishing areas with distinct dietary habits. We prospectively examined the incidence of CHD from 1994 to 1998, as well as coronary risk factors and serum fatty acid concentrations. The incidence of angina pectoris was significantly ($P=0{\cdot}01$) lower in the fishing area than in the mercantile and farming areas. Blood pressure, physical activity, prevalence of diabetes, serum levels of uric acid and HDL-cholesterol were similar between the three areas. Total- and LDL-cholesterol levels were significantly lower but the smoking rate was markedly higher in the fishing area than in the other two areas. Serum levels of saturated fatty acids and *n*-6 polyunsaturated fatty acids (PUFA) were lowest in the fishing area, but *n*-3 PUFA did not differ significantly. The *n*-6:*n*-3 PUFA ratio was lowest and eicosapentaenoic:arachidonic acid was highest in the fishing area. Although many previous studies have emphasized the beneficial effect of *n*-3 PUFA in preventing CHD, the present study indicated that a lower intake of *n*-6 PUFA and saturated fatty acids has an additional preventive effect on CHD even when the serum level of *n*-3 PUFA is high because of high dietary fish consumption.

Coronary heart disease: Dietary style: Fatty acids: Coronary risk factors

The mortality from CHD in Japan is 22 to 26/100 000 population per year (Fukiyama *et al.* 2000), which is very low in comparison with the United States and northern Europe (Crombie *et al.* 1987). A lower CHD mortality is also observed in Mediterranean Europe and France (Criqui & Ringel, 1994). These regional differences are thought to be partly due to differences in dietary habits. The average dietary intake of fish in Japan is about five times that in the United States (Crombie *et al.* 1987). Wine and Mediterranean fish dishes have also been reported to reduce CHD mortality (Renaud & de Lorgeril, 1992; Criqui & Ringel, 1994; de Lorgeri *et al.* 1999). Recently, the incidence of CHD has been increasing in urban areas of Japan as westernization of the diet has become widespread, while that in rural areas still remains low (Konishi *et al.* 1987).

Kumihama town is a coastal rural community in Kyoto Prefecture, facing the Sea of Japan with an area of $145{\cdot}04\,km^2$ and a population of 12 584 in the 1995 census. Kumihama town contains mercantile, farming, and fishing areas. The inhabitants in the mercantile area are mostly the owners of small shops and their families. The population and mean age in each area are: 2069 and 45 years in the mercantile area, 7571 and 45 years in the farming area, and 2944 and 46 years in the fishing area. The inhabitants of the three areas, each with similar incomes and educational backgrounds, move rarely from one area to another. Since Kumihama Municipal Hospital is the only institution in the district with a coronary care unit for intervention and there are no cardiologists in other hospitals, all the patients with CHD in Kumihama town were referred to and treated at this hospital.

In routine clinical practice, we had noticed that patients with CHD were few in the fishing area. The analysis of

Abbreviations: AA, arachidonic acid; AMI, acute myocardial infarction; AP, angina pectoris; EPA, eicosapentaenoic acid; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids.

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such a regional difference within a small town may confirm the presence of a relationship between CHD and dietary lifestyle, the relationship having been suggested in many reports covering study populations from distant districts or different countries with various environmental and genetic conditions. Therefore, we performed a cross-sectional, prospective survey to analyse the relationship between CHD incidence and lifestyle of the inhabitants of the three neighbouring areas in Kumihama town.

Subjects and methods

The present study, conducted between 1994 and 1998, consisted of two parts. First, CHD incidence was analysed to confirm our notion that the incidence of CHD is low in the fishing area. Second, serum fatty acids levels and lifestyles were examined in apparently healthy subjects who participated in the annual health check in June 1998.

Analysis of coronary heart disease incidence

We studied patients from April 1994 to March 1998, who came to our hospital because of initial onset of chest pain or electrocardiographic abnormalities detected at the annual health check. Patients having past history of CHD were excluded from the present study. The diagnosis of angina pectoris (AP) was based on the exercise electrocardiography test using the Bruce protocol, and a positive electrocardiographic finding was defined as ischaemic ST segment depression ≥ 1 mm. However, its diagnostic accuracy is relatively low (60–65 %). In order to exclude false-positive electrocardiographic results, coronary angiography was performed, to which the subjects consented. Acute myocardial infarction (AMI) was diagnosed on the basis of chest pain lasting >20 min with ST elevation and an increase in the plasma creatinine phosphokinase level to more than twice the upper limit of normal. Of 100 patients with a positive exercise test, seventy-two gave consent to coronary angiography. Significant coronary stenosis was defined as ≥ 75 % luminal narrowing (American Heart Association Committee, 1975).

Annual health check

The public health office of Kumihama town sent personal invitations for the annual health check to all inhabitants ≥ 40 years by mail. The invitation also mentioned that individuals who were regularly visiting the hospital did not need take the examination. Thus, 2996 out of 7438 individuals (40·3 %) participated in this examination. The rate of response was: 45·1 % in the mercantile; 38·0 % in the farming; 43·3 % in the fishing area. Because a large number of subjects were surveyed, the health check was held in June and October every year. Thus, we selected 1344 participants (389 in the mercantile, 587 in the farming, and 368 in the fishing area) of the June 1998 health check to exclude the possible influence of the seasonal difference in diet on serum fatty acid levels.

We used a quasi-random sampling frame in which we approached every *n*th apparently healthy subjects from the age–sex registers. A total of 294 individuals consented to the present study. The number of participants in each area was: ninety-five (thirty-eight men, fifty-seven women) in the mercantile, 118 (forty-seven men, seventy-one women) in the farming, and eighty-one (thirty-two men, forty-nine women) in the fishing area, with no regional difference in the sampling of population ($P=0·28$). To obtain uniform information, experienced public health nurses carried out an interview using a questionnaire for the assessment of medical history, smoking habits, diet and physical activity. Diet was evaluated on the basis of weekly frequency of the intake of meat, fish, and vegetables using a recall method for the past week. Physical activity was estimated by using the Framingham Study questionnaire (Kannel & Sorlie, 1979) and physical activity index was calculated from the weighted sum of hours spent at five levels of activity during a normal working day. Height was measured in stocking feet with an anthropometer in the upright position. Body weight was recorded by a weighing machine with the subjects clothed and 0·5 kg was subtracted from the recorded weight. Blood pressure was measured with a fully automated sphygmomanometer on the right arm with the subject in the sitting position after 5 min of rest, and calculated as the mean value of two serial measurements. Diabetes mellitus was defined by a plasma fasting blood glucose level $\geq 6·99$ nmol/l plus a haemoglobin A1c value $\geq 6·5$ %, according to the guidelines of the Japanese Diabetes Society.

Blood samples were taken from an antecubital vein after overnight fasting and were centrifuged at 3000 ***g*** for 15 min at room temperature. Then the serum was stored at -80°C until assay. Total cholesterol and triacylglycerol were measured by enzymic methods. HDL-cholesterol was analysed by the phosphotungstate precipitation method (Wako, Osaka, Japan). The LDL-cholesterol level was calculated from the following formula: LDL cholesterol = total cholesterol − HDL cholesterol − triacylglycerol/5 (Friedewald *et al.* 1972). Serum uric acid levels were determined by an enzymic method (Kyowa Medics, Tokyo, Japan).

Analysis of serum fatty acids

For the analysis of serum fatty acids, a reference mixture of fatty acid methyl esters (GLC-87) containing the internal standard, methyl heptadecanoate, was purchased from NU-Check-Prep, Inc., Elysian, MN, USA. Standards for methyl esters of eicosapentaenoic acid (EPA), docosapentaenoic acid (Nacalai Tesque, Inc., Kyoto, Japan), eicosadienoic acid, docosatetraenoic acid, lignoceric acid (NU-Check-Prep, Inc.), and 5-8-11 eicosatrienoic acid (Biomol Research Lab., Inc., Plymouth Meeting, PA, USA) were also added to the above reference mixture, and then the mixture was dissolved in *n*-hexane. Serum (100 µl) was first extracted with 1 M-KOH–ethanol in the presence of the internal standard at 60°C for 60 min. After removing non-saponified fractions in *n*-hexane, the fatty acids were extracted in *n*-hexane–diethyl ether, methylated using diazomethane, and subjected to quantification by GC (Hewlett Packard GC system HP 6890; Palo Alto, CA, USA) with flame ionization detector (supplied with air and high-purity H_2). A portion (4 µl) was injected into a 30 m BP5 fused silica capillary column with an internal diameter of 250 µm, 0·2 µm thick film and a split ratio of 1:26. High purity He was used as the carrier gas (1·8 ml/min) and N_2 was used as the make-up gas (25 ml/min). The initial oven temperature was 120°C, followed by temperature programming in three steps: a first rate of 2°C/min up to 170°C, followed by a second rate of 1°C/min up to 200°C and by a third rate of 2°C/min up to 230°C, which was maintained for 10 min. The injector and flame ionization detector temperatures were maintained at 250°C and 260°C, respectively. Peaks were identified by comparison with known pure standard mixtures and quantified by automatic integration of areas. The results were expressed in absolute concentrations (mg/l serum), using an internal standard.

Statistical analysis

Results are expressed as mean values and standard deviations. One-way ANOVA was used for parametric comparisons and significances of individual differences were evaluated by using Scheffe's test if ANOVA was significant. The χ^2 test was applied for discrete variables. In order to investigate whether linoleic acid levels differ between the three areas, we conducted analysis of covariance, with LDL-cholesterol levels as a covariant. Statistical significance was defined as $P<0{\cdot}05$.

Results

Distribution of coronary heart disease patients in the mercantile, farming and fishing areas

There were no significant differences in the percentage of outpatients attending our hospital or in the rate of participation in the annual health checks between the three areas. The proportion of positive stress electrocardiograms was far lower in the fishing area. There were twelve AMI patients (two in the mercantile, nine in the farming and one in the fishing area) and thirty AP patients (nine in the mercantile, twenty in the farming and one in the fishing area). The overall incidence of AMI and AP (/100 000 population per year) in Kumihama town was 24 and 60, which were only one-third and one-sixth of the average in Japan, respectively (Ito, 1987). Incidence of AMI did not differ significantly between the three areas, but that of AP was far lower in the fishing area (Table 1). We also assessed death certificates between 1994 and 1998, but no cases were observed of death by a cardiac cause.

Clinical features, diet and smoking habits in the three areas

There were no significant differences between the three areas with regard to gender, age, height, weight, BMI, physical activity index, mean blood pressure, fasting blood glucose, rate of diabetes and serum uric acid concentration. The serum levels of total cholesterol and LDL-cholesterol were lower in subjects from the fishing area than in those from the mercantile area ($P<0{\cdot}001$ for total cholesterol and $P<0{\cdot}05$ for LDL-cholesterol), although the levels of HDL-cholesterol and triacylglycerol did not differ significantly.

The weekly frequency of meat intake was significantly ($P<0{\cdot}0001$) lower in the fishing area (2·6 (SD 1·0)) than in the mercantile area (3·3 (SD 1·0)) or the farming area (3·2 (SD 1·0)). In contrast, the weekly frequency of fish intake was significantly ($P<0{\cdot}0001$) higher in the fishing area (5·2 (SD 1·3)) than in the mercantile area (3·2 (SD 1·3)) or the farming area (2·7 (SD 1·0)). Vegetables were eaten significantly ($P<0{\cdot}0001$) more frequently in the farming area (4·4 (SD 1·1)) than in the mercantile area (3·5 (SD 0·8)) or the fishing area (3·9 (SD 1·0)). The prevalence of smoking was markedly higher in the fishing area than in the other areas ($P=0{\cdot}01$) (Table 2).

Serum fatty acid levels in healthy inhabitants from the three areas

Data on serum fatty acid levels obtained from the participants of the annual health check are presented in Table 3. Levels of saturated fatty acids (SFA, i.e. myristic acid, palmitic acid and stearic acid) were all significantly lower in the fishing area. Among monounsaturated fatty acids, palmitoleic acid did not differ between the three areas, but the level of oleic acid was significantly lower in the fishing area. With regard to *n*-3 polyunsaturated fatty acids (PUFA), there were no differences of EPA, docosapentaenoic acid, or docosahexaenoic acid between the three areas, although EPA levels tended to be higher in the fishing area. In contrast, *n*-6 PUFA levels (i.e. linoleic acid, γ-linolenic acid, dihomo-γ-linolenic acid and arachidonic acid (AA) were significantly lower in the fishing area. By analysis of covariance with LDL-cholesterol as a covariant, linoleic acid levels showed significant regional difference ($P<0{\cdot}0001$). The *n*-6:*n*-3 PUFA ratio was lowest and EPA:AA highest in the fishing area ($P<0{\cdot}0001$ and $P=0{\cdot}0001$, respectively).

Table 1. Population, positive stress electrocardiograms (ECG), and incidence of acute myocardial infarction (AMI) and angina pectoris (AP) in each area

	Mercantile area	Farming area	Fishing area	*P* value
Population	2069	7571	2944	
Positive stress ECG (/100 000 population per year)	290	238	34	<0·0001
Incidence of AMI (/100 000 population per year)	24	30	8	0·45
Incidence of AP (/100 000 population per year)	109	66	8	0·01

Table 2. Clinical characteristics of the participants in annual medical screening from each area†
(Mean values and standard deviations)

	Mercantile area (*n* 95)		Farming area (*n* 118)		Fishing area (*n* 81)		
	Mean	SD	Mean	SD	Mean	SD	*P* value
Gender							
Male	38		47		32		>0·99
Female	57		71		49		
Age (years)	60	12	60	11	59	11	0·72
Height (cm)	156	9	155	8	158	9	0·19
Weight (kg)	54	9	53	9	57	10	0·06
BMI (kg/m^2)	22	3	22	3	23	3	0·09
Physical activity index	31	7	34	9	33	8	0·10
Mean blood pressure (mmHg)	98	13	100	14	96	13	0·11
Fasting blood glucose (mmol/l)	5·05	1·44	4·83	0·78	4·77	0·72	0·46
Diabetic patients (*n*)	1		1		0		0·67
Uric acid (mmol/l)	0·29	0·08	0·29	0·08	0·32	0·08	0·06
Total cholesterol (mmol/l)	5·62	0·93	5·36	0·88	5·13**	0·70	0·0006
LDL-cholesterol (mmol/l)	3·47	0·85	3·21	0·78	3·13*	0·60	0·01
HDL-cholesterol (mmol/l)	1·63	0·36	1·66	0·36	1·55	0·34	0·08
Triacylglycerol (mmol/l)	1·19	0·57	1·13	1·02	0·93	0·46	0·07
Smoking rate (%)	11·6		16·9		28·4		0·01

Mean values were significantly different from those of the mercantile area by Scheffe's test: *$P<0·05$, **$P<0·001$.
† For details of participants and procedures, see p. 268.

Discussion

The present study demonstrated that the incidence of CHD in a coastal rural district with three distinct populations was extremely low, as compared with the average of Japan (Ito, 1987). In particular, the incidence of CHD was lowest in the fishing area, where the frequency of fish consumption was much higher than that of meat consumption. Similar

Table 3. Serum fatty acid levels (mg/l) of the normal inhabitants of Kumihama town‡
(Mean values and standard deviations)

	Mercantile area (*n* 95)		Farming area (*n* 118)		Fishing area (*n* 81)		
	Mean	SD	Mean	SD	Mean	SD	*P* value
Lauric (12 : 0)	3·6	3·1	3·0	2·3	1·9******††	1·1	<0·0001
Myristic (14 : 0)	23·0	12·0	22·7	13·9	16·4****†††	6·5	0·0001
Myristoleic (14 : 1*n*-5)	1·7	1·1	1·6	0·9	1·4*	0·5	0·01
Palmitic (16 : 0)	646·7	146·0	658·1	227·9	548·0***††††	115·9	<0·0001
Palmitoleic (16 : 1*n*-7)	83·7	34·6	83·8	44·1	73·6	29·6	0·12
Stearic (18 : 0)	217·3	32·0	246·3	66·4	198·3*†††††	37·6	<0·0001
Oleic (18 : 1*n*-9)	575·5	137·0	570·1	242·8	485·7**††	118·0	0·0016
Linoleic (18 : 2*n*-6)	857·9	139·2	823·0	201·6	690·4******†††††	149·5	<0·0001
α-Linolenic (18 : 3*n*-3)	21·5	6·7	20·5	9·3	17·3***†	6·1	0·0012
γ-Linolenic (18 : 3*n*-6)	9·7	5·7	11·3	7·4	8·4††	5·5	0·006
Arachidic (20 : 0)	2·2	0·7	3·4******	1·1	3·2******	1·1	<0·0001
Eicosenoic (20 : 1*n*-9)	6·7	1·5	6·5	2·6	5·8*†	1·7	0·006
Eicosadienoic (20 : 2*n*-6)	6·5	2·4	5·8*	1·9	5·0******††	1·3	<0·0001
Dihomo-γ-linolenic (20 : 3*n*-6)	35·1	11·0	36·7	11·4	30·9†††	12·6	0·0026
5-8-11 Eicosatrienoic (20 : 3*n*-9)	2·0	1·0	2·4	2·2	1·9	1·3	0·06
Arachidonic (20 : 4*n*-6)	154·9	26·0	142·3**	33·0	130·6******†	27·9	<0·0001
Eicosapentaenoic (20 : 5*n*-3)	89·5	39·6	83·6	35·5	96·9	46·2	0·07
Behenic (22 : 0)	7·1	4·0	8·0	4·8	5·6	2·5	0·0003
Erucic (22 : 1*n*-9)	2·2	2·0	2·4	1·6	2·2	1·3	0·64
Docosatetraenoic (22 : 4*n*-6)	2·8	0·7	2·4***	0·9	2·1******	1·1	<0·0001
Docosapentaenoic (22 : 5*n*-3)	33·0	9·7	33·1	11·5	32·6	20·3	0·96
Docosahexaenoic (22 : 6*n*-3)	188·1	50·0	174·8	53·3	174·5	60·7	0·14
Lignoceric (24 : 0)	1·7	0·7	2·0	1·2	1·6†	0·9	0·02
Nervonic (24 : 1*n*-9)	2·6	2·4	4·0**	2·1	4·5****	4·7	0·0002
n-6:*n*-3 Polyunsaturated fatty acid ratio	3·44	0·97	3·57	1·30	3·07*****††††	1·29	<0·0001
Eicosapentaenoic acid:arachidonic acid	0·58	0·23	0·60	0·27	0·74*****†††	0·31	0·0001

Mean values were significantly different from those of the mercantile area by Scheffe's test: *$P<0·05$, **$P<0·01$, ***$P<0·005$, ****$P<0·001$, *****$P<0·0005$, ******$P<0·0001$.
Mean values were significantly different from those of the farming area by Scheffe's test: †$P<0·05$, ††$P<0·01$, †††$P<0·005$, ††††$P<0·0005$, †††††$P<0·0001$.
‡For details of participants and procedure see p. 268.

Table 4. Serum eicosapentaenoic acid:arachidonic acid reported in Japan

	Area			
Investigators	Rural mercantile	Farming	Fishing	Urban
Hirai *et al.* (1986)			0·58	
Iso *et al.* (1989)		0·77		0·45
Shiba *et al.* (1980)		0·34	0·68	
Kamada *et al.* (1991)		0·57	0·59	
Nakamura *et al.* (2002) (Present study)	0·58	0·60	0·74	

results have already been obtained in previous studies on native Greenlanders and Japanese (Dyerberg *et al.* 1975; Dyerberg & Bang, 1979; Hirai *et al.* 1980; Hirai, 1985). There were no differences between the three areas in the incidence of hypertension, diabetes, hyperuricaemia and low HDL-cholesterol, which are widely accepted coronary risk factors (Gordon *et al.* 1977). Physical activity did not differ either, despite its beneficial effect on CHD (Andersen *et al.* 1999; Dunn *et al.* 1999; Hambrecht *et al.* 2000). In the fishing area, however, total cholesterol level was lowest but smoking rate was highest. In native Greenlanders also, CHD mortality is extremely low despite their highly prevalent smoking habits (Bjerregaard *et al.* 1997).

The *n*-6:*n*-3 PUFA ratio was lowest and EPA:AA highest in the fishing area. The high EPA:AA in the fishing area may be attributable to a low AA level, since EPA levels did not differ statistically between the three areas. The *n*-6:*n*-3 PUFA ratio reflects the consumption balance between fats of terrestrial and marine origin, and the ratio was negatively correlated with CHD mortality (Dolecek & Grandits, 1991). In addition to a low dietary EPA:AA, a high dietary *n*-6:*n*-3 PUFA ratio, rather than a high serum cholesterol level, has also been reported to be a major risk factor for CHD (Kondo *et al.* 1986; Okuyama, 2001). These results suggest that the component of dietary fats is more important than the current coronary risk factors.

In the present study, we measured all twenty-four fractions of serum fatty acids but did not quantify dietary fat intake. It has been reported, however, that serum fatty acid levels change in parallel with the amount of dietary fats taken for the past 7 d (Kuriki *et al.* 2000) although the washout and half-lives vary with fatty acids to some extent (Zuijdgeest-van Leeuwen *et al.* 1999). Thus we may safely consider that serum fatty acid levels reliably reflect the dietary style.

All SFA and *n*-6 PUFA showed lower levels in the fishing area, but *n*-3 PUFA levels, except α-linolenic acid, did not differ between the three areas. EPA:AA in each area of Kumihama town was higher than the reported ratio in other rural districts (Table 4, Hirai *et al.* 1980; Shiba *et al.* 1980; Iso *et al.* 1989; Kamada *et al.* 1991) despite similar *n*-3 PUFA levels, which suggests that fish consumption was relatively high even in the mercantile and farming areas, probably because of their location adjacent to the fishing area. The level of α-linolenic acid, the precursor of EPA, was low in the fishing area, as has been found in other studies (Iso *et al.* 1989; Umemura *et al.* 1993). This may reflect the fact that α-linolenic acid is abundant in vegetables and soyabeans and that its intake does not solely depend on fish consumption.

It has been reported that SFA intake is positively correlated with the serum total cholesterol level and CHD mortality (Keys, 1970; Keys *et al.* 1986). Several studies have found no relationship between *n*-6 PUFA intake and CHD mortality (Keys, 1970; Morris *et al.* 1977; Dolecek & Grandits, 1991), but excessive intake of linolenic acid was reported to increase CHD mortality (Taylor *et al.* 1979). In the present study, linolenic acid level was lowest in the fishing area, which was independent of serum LDL-cholesterol levels. Since the serum level of *n*-3 PUFA did not differ between the three areas, the low incidence of CHD in the fishing area may have been related to the lower levels of SFA and *n*-6 PUFA, which may have partly contributed to the suppression of total cholesterol level.

We did not evaluate serum levels of homocysteine, folic acid and vitamins B_6 and B_{12}. Elevated homocysteine levels were recently reported to be related to the increase of cardiovascular risk (Duell & Malinow, 1997), and levels can be lowered by supplementation with folic acid, vitamins B_6 and B_{12} (Malinow *et al.* 1998; Rimm *et al.* 1998). However, homocysteine levels are not affected by dietary fat contents (Grundt *et al.* 1999). Thus, these levels are not considered to have influenced our results greatly.

Many previous studies have emphasized the beneficial effect of *n*-3 PUFA in preventing CHD, but there have been few studies suggesting that a low *n*-6 PUFA intake is useful in this respect. However, the present study indicated that a lower intake of *n*-6 PUFA and SFA has an additional preventive effect on CHD even when the serum level of *n*-3 PUFA is high because of high dietary fish consumption.

Acknowledgements

The authors would like to thank Tomohito Hamazaki (Toyama Medical and Pharmaceutical University, Toyama, Japan) for invaluable suggestions and Chieko Okuda (Louis Pasteur Center for Medical Research, Kyoto, Japan) for helpful suggestions in statistical analyses. Also, we are grateful to Tohru Adachi, Takashi Wada, Shinji Matsuda, and Tsutomu Takemura for their support in the analysis of fatty acids.

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