Epidemiological Evidence of Relationships between Dietary Polyunsaturated Fatty Acids and Mortality in the Multiple Risk Factor Intervention Trial (43413)

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Abstract. **This evaluation of the Multiple Risk Factor Intervention Trial database investigated the effects of dietary PUFA on disease outcomes that may relate to polyunsaturated fatty acid (PUFA) biochemistry. The Multiple Risk Factor Intervention Trial was a randomized clinical trial in coronary heart disease (CHD) primary prevention involving 12,866 middle-aged men determined to be at high risk of CHD. They were assigned to either a special intervention group or a usual care group and returned to clinics on an annual basis for assessment of risk factor status. Only data on the usual care men (n = 6,250) are presented, since the multi-intervention effects on the special intervention group introduce considerable analytic complexities. Mean PUFA intake estimates were calculated from four dietary recall interviews at baseline and follow-up Years 1, 2, and 3 and estimates for PUFA were established using absolute grams, percentage of total kilocalories, and ratios. Proportional hazards regression analysis controlling for age, race and baseline diastolic blood pressure, smoking, high and low density lipoprotein cholesterol levels, and alcohol was used to analyze dietary PUFA intakes on 10.5-year mortality rates. Results were more significant when PUFA were expressed as percentage of total kilocalories. No significant associations with mortality were detected for linoleic acid (18:2n-6), the predominant dietary PUFA. Significant inverse associations were observed for linolenic acid (18:3n-3) on mortality from CHD ($P < 0.04$), all cardiovascular diseases (CVD) ($P < 0.03$), and all cause mortality ($P < 0.02$); the sum of fatty acids primarily derived from fish oils (20:5n-3 + 22:5n-3 + 22:6n-3) on CHD ($P < 0.02$), CVD ($P < 0.006$), and all cause ($P < 0.02$) mortality; and, the ratio of 18:3n-3 to 18:2n-6 on cancer mortality ($P < 0.05$). Analysis using the total n-3 (18:3 + 20:5 + 22:5 + 22:6) to total n-6 (18:2 + 18:4 + 20:4) ratio was also significant on cancer mortality ($P < 0.04$). These findings support reports that n-3 fatty acids are protective against CVD and suggest that composition of dietary PUFA may influence CVD and cancer rates.**

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Most studies of dietary polyunsaturated fatty acids (PUFA) on physiological and disease-related mechanisms have been experimental or clinical in nature. Since epidemiological investigation of dietary PUFA intakes on disease occurrence would provide additional insight into determining optimal intake levels, an evaluation of the Multiple Risk Factor Intervention Trial (MRFIT) database was initiated. This report expands on results of dietary PUFA intake analyses that were presented at the Second International Conference on Omega 3 Fatty Acids in March 1990 (1).

Specific dietary PUFA intakes by the middle-aged American men who participated in this trial from 1973 through early 1982 were described using calculated averages of four 24-hr dietary recall interviews obtained during the study. Reported PUFA intake levels were compared with trends observed in the Japanese population over time. Relationships between dietary PUFA intakes and mortality outcome groups were examined.

Methods

Background. MRFIT was a multicenter, clinical trial supported by the National Heart, Lung, and Blood

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Institute to study the primary prevention of coronary heart disease (CHD). Men aged 35–57 years at entry who were determined to be at high risk of developing CHD based upon smoking status, diastolic blood pressure, and serum cholesterol levels comprised the study population. The trial was conducted at 22 clinical centers, where the men were followed for 6 to 8 years. From 361,662 screenees, 12,866 men were selected to be participants during three screening visits. Approximately half were randomized to each of two study groups. The special intervention group received programs to reduce smoking, blood pressure, and blood cholesterol, while the usual care (UC) group participants were referred to their usual source of medical care and returned annually for examination at their respective clinical centers. Only data on the UC group were analyzed for this evaluation to avoid the analytic complexities introduced by the multi-intervention effects on the special intervention group. A more detailed description of the MRFIT background, design, and organization is provided elsewhere (2).

Principal Dietary Data Collection Method. The principal dietary method chosen for the MRFIT was the 24-hr dietary recall, since it is appropriate for measuring the dietary intake of groups (3). Dietary recall interviews were conducted at the baseline third screen visit and at Years 1, 2, 3, and 6 for participants in the UC group. Data generated from 24-hr recall interviews have been used to describe the MRFIT population at baseline and to monitor the dietary patterns and food selection trends by both the special intervention and UC groups over the follow-up period (4, 5).

Whereas the 24-hr recall is generally not intended to characterize usual intake by individuals considered desirable when studying diet-disease relationships, multiple recalls on the same individual improve accuracy in terms of establishing more reliable usual-intake estimates. On this premise, data from recalls obtained at baseline and at follow-up years were used for this evaluation.

Mortality Ascertainment. Mortality was ascertained from the beginning of the trial and continues to be monitored by the MRFIT coordinating center in Minneapolis, Minnesota. Clinics assumed responsibility for follow-up while the trial was in progress until February 28, 1982, at which time the National Death Index became the primary mortality follow-up method. The data presented include deaths ascertained through December 31, 1985 representing 10.5 years of follow-up. Cause-specific mortality assignments were based on the ninth revision of the International Classification of Diseases. All death certificates and supporting records were independently coded by two nosologists without knowledge of treatment group, and where differences existed, adjudication was achieved by a third nosologist.

Statistical Analysis. MRFIT recalls were analyzed in 1985 using the University of Minnesota Nutrition Coordinating Center Food Table version No. 11, which contains very complete information on individual fatty acids, thereby making an evaluation of PUFA intake possible (6). Mean values available from recall data analysis at baseline and at the first 3 follow-up years were calculated for each PUFA estimate under study. PUFA intake estimates in grams and as a percentage of total kilocalories were established for total PUFA, linoleic acid (18:2n-6), linolenic acid (18:3n-3), arachidonic acid (20:4n-6), eicosapentaenoic acid (20:5n-3), docosapentaenoic acid (22:5n-3), and docosahexaenoic acid (22:6n-3). The sum of 20:5n-3, 22:5n-3, and 22:6n-3, as well as the ratios of 18:3n-3 to 18:2n-6 and total n-3 to total n-6, were also calculated for analytic purposes.

Four mortality outcome groups were established based upon numbers of deaths from specific causes. The groups were CHD, all cardiovascular diseases (CVD), all cancers, and all causes (AC). Deaths and documented clinical myocardial infarctions during the first 3 years of follow-up were excluded from the analysis.

Proportional hazards regression analysis with PUFA estimates as the linear term in the log-relative risk model was applied to evaluate dietary effects on mortality outcome groups. Each PUFA estimate was entered into the model as a continuous variable using log transformations as appropriate with the regression coefficient and significance level given. Participants were divided into quintiles based on their average intake of each PUFA estimate. Relative risks of mortality were calculated comparing Quintiles II, III, IV, and V to the first quintile using proportional hazards regression analysis. Adjusted relative risks were also determined by including age, race and baseline values of diastolic blood pressure, cigarettes smoked per day, high and low density lipoprotein-cholesterol, and alcohol in the model.

Results

Dietary Polyunsaturated Fatty Acid Intake Description. Table I shows the dietary PUFA intake pattern observed in the MRFIT UC group. Linoleic acid (18:2n-6) contributed most of the total PUFA. About 10% of total PUFA included 18:3n-3. Very small quantities of the precursor of eicosanoids, 20:4n-6, were reported (see Table I). About 20% of the group reported no intake of the long chain n-3 fatty acids found in fish oils 20:5, 22:5, and 22:6. The mean sum of these three fatty acids was about 175 mg/day and the mean intake ratios of 18:3n-3 to 18:2n-6 and total n-3 to total n-6 were approximately 0.12 and 0.13, respectively.

Mortality Findings. Table II shows the number of deaths available for analysis. CHD deaths numbered 175, and CHD was the only specific disease that could

Table I. Dietary Polyunsaturated Fatty Acid Distribution in the Multiple Risk Factor Intervention Trial Usual Care Group (n = 6250)

		% Total PUFA
Grams		
Total PUFA	16.828 ± 7.663[a]	
18:2n-6	14.603 ± 6.957	86.78
18:3n-3	1.688 ± 0.736	10.03
20:4n-6	0.222 ± 0.107	1.32
20:5n-3	0.069 ± 0.155	0.41
22:5n-3	0.024 ± 0.057	0.14
22:6n-3	0.082 ± 0.193	0.49
Ratio		
$\frac{18:3n-3}{18:2n-6}$	0.122 ± 0.034	
$\frac{n-3s}{n-6s}$	0.133 ± 0.051	

[a] Mean ± SD.

Table II. Number of Deaths by Cause in the Usual Care Group of the Multiple Risk Factor Intervention Trial

Category	Deaths
Coronary heart disease	175
Cardiovascular diseases	232
Cancer	132
All causes	522

be analyzed separately. All other CVD deaths amounted to only 57. The cancer mortality outcome group included over 30 anatomic sites, contributing to a total of 132. A total of 158 deaths from other causes in addition to CVD and cancer comprised the AC mortality outcome group.

Proportional Hazards Regression Analyses Results. Table III shows adjusted proportional hazards regression coefficients of analyses for selected PUFA, combined PUFA, and PUFA ratios on the four mortality outcome groups. Unadjusted findings were not substantially different. No significant associations were detected for linoleic acid (18:2n-6) on any mortality group. However, a marginally significant inverse association with CHD appeared when PUFA was expressed as percentage of total kilocalories. When 18:3n-3 was the independent variable in the analysis, a negative pattern was apparent for CHD, CVD, and all cause mortality. The inverse relationship was only significant for AC mortality and marginally significant for CHD, and CVD when 18:3n-3 was expressed in grams, but was significant for all three mortality outcome groups with 18:3n-3 in percentage of total kilocalories as the independent variable. Results for the combined fatty acids predominantly found in fish were similar to those observed with 18:3n-3. Significant inverse associations

Table III. Proportional Hazards Regression Coefficients for Dietary Polyunsaturated Fatty Acids on Mortality in the Multiple Risk Factor Intervention Trial[a]

PUFA	CHD	CVD	Cancer	AC
18:2n-6				
(g)	−0.0143	−0.0102	0.0033	−0.0096
(% kcal)	−0.0724[b]	−0.0552	0.0493	−0.0303
18:3n-3				
(g)	−0.1795	−0.1960[b]	−0.1033	−0.1810[c]
(% kcal)	−0.8493[c]	−0.7801[c]	−0.2133	−0.6569[c]
Fish n-3s				
(g)	−0.9338[c]	−0.9598[d]	−0.0034	−0.3162[b]
(% kcal)	−0.4715[c]	−0.4499[d]	−0.1391	−0.2590[c]
18:3n-3:18:2n-6	0.2764	−0.3599	−5.5047[c]	−1.0729
Total n-3s:n-6s	−0.5447	−0.6878[b]	−1.1812[c]	−0.5212[b]

[a] Deaths and clinical myocardial infarctions from the first 3 years of follow-up excluded. Adjustment: age, race, smoking, baseline diastolic blood pressure, high density lipoprotein, low density lipoprotein, alcohol.
[b] $P < 0.10$.
[c] $P < 0.05$.
[d] $P < 0.01$.

with CHD, CVD, and AC mortality groups, but not for the cancer group, were apparent. Findings from 18:3n-3 to 18:2n-6 ratios showed no association with CHD, CVD, or AC mortality, but a significant inverse relationship was observed between the ratio and cancer mortality. An inverse relationship with cancer mortality was also demonstrated when the total n-3 to total n-6 ratio was the independent variable in the analysis. Marginally significant inverse associations were observed between the total n-3 to total n-6 ratio and mortality from CVD and AC mortality groups, but not for CHD.

Tables IV through VII present proportional hazards regression analysis by quintiles of dietary intake. A protective trend is suggested with increased percentage of total kilocalories from 18:2n-6 for CHD in Table IV. Significant inverse patterns are apparent in Table V for 18:3n-3 on CHD, CVD, and AC mortality. Table VI indicates that the benefit of the combined fish fatty acid intake appeared in the largest intake quintile with a mean ingestion of about 664 mg, or 0.284 %kCAL/day. When compared with zero intake and expressed as percentage of total kilocalories, mortality from CHD, CVD, and all cause mortality was 50%, 45%, and 27% lower, respectively. Thirty-three percent fewer cancer deaths occurred in the highest 18:3n-3 to 18:2n-6 intake quintile when compared with the lowest, shown in Table VII. The pattern of relative risk across quintiles generally showed a smooth trend and gradual decline in cancer mortality as the 18:3n-3 to 18:2n6 ratio increased. Similar results were apparent for total n-3 to total n-6 ratio results.

Table IV. Cox Regression Analysis of Dietary Linoleic Acid (18:2n-6) and 10.5-Year Mortality[a]

Quintile	Mean	*n*	CHD RR	CVD RR	CA RR	AC RR
Grams						
I	7.037	1251	1.00	1.00	1.00	1.00
II	10.647	1252	0.73	0.64	1.98	0.80
III	13.387	1252	0.64	0.70	1.44	0.79
IV	16.839	1252	1.07	1.05	1.70	1.09
V	25.067	1251	0.63	0.62	1.65	0.77
P			NS	NS	NS	NS
Percentage of total kilocalories						
I	3.305	1251	1.00	1.00	1.00	1.00
II	4.541	1252	0.69	0.84	1.03	0.80
III	5.431	1252	0.86	0.95	1.34	1.01
IV	6.485	1252	0.80	0.88	1.13	0.93
V	8.836	1251	0.58	0.72	1.35	0.77
P			<0.10	NS	NS	NS

[a] Deaths and clinical myocardial infarctions from the first 3 years of follow-up excluded. Adjustment: age, race, smoking, baseline diastolic blood pressure, high density lipoprotein, low density lipoprotein, alcohol. RR, relative risk.

Table V. Cox Regression Analysis of Dietary Linolenic Acid (18:3n-3) and 10.5-Year Mortality[a]

Quintile	Mean	*n*	CHD RR	CVD RR	CA RR	AC RR
Grams						
I	0.873	1251	1.00	1.00	1.00	1.00
II	1.273	1253	0.96	0.93	1.34	0.96
III	1.577	1251	0.56	0.66	0.85	0.69
IV	1.926	1251	0.96	0.88	1.14	0.89
V	2.802	1252	0.66	0.61	0.87	0.69
P			NS	<0.10	NS	<0.05
Percentage of total kilocalories						
I	0.424	1251	1.00	1.00	1.00	1.00
II	0.544	1252	0.72	0.86	1.12	0.86
III	0.630	1252	0.80	0.97	0.72	0.85
IV	0.734	1252	0.61	0.66	0.90	0.75
V	0.980	1251	0.58	0.66	0.78	0.68
P			<0.05	<0.05	NS	<0.05

[a] Deaths and clinical myocardial infarctions from the first 3 years of follow-up excluded. Adjustment: age, race, smoking, baseline diastolic pressure, high density lipoprotein, low density lipoprotein, alcohol. RR, relative risk.

Table VI. Cox Regression Analysis of Dietary n-3 Fish Fatty Acids in Grams and 10.5-Year Mortality[a]

Quintile	Mean	*n*	CHD RR	CVD RR	CA RR	AC RR
Grams						
I	0.000	1307	1.00	1.00	1.00	1.00
II	0.009	1197	1.08	1.06	1.25	1.09
III	0.046	1251	0.92	0.93	1.16	1.02
IV	0.153	1252	0.89	0.93	0.73	0.85
V	0.664	1251	0.61	0.60	0.97	0.75
P			<0.05	<0.01	NS	<0.10
Percentage of total kilocalories						
I	0.000	1307	1.00	1.00	1.00	1.00
II	0.004	1196	1.07	1.08	1.26	1.09
III	0.019	1252	0.82	0.81	1.13	0.97
IV	0.063	1252	1.12	1.08	0.82	0.92
V	0.284	1251	0.50	0.55	0.90	0.73
P			<0.05	<0.01	NS	<0.01

[a] Deaths and clinical myocardial infarctions from the first 3 years of follow-up excluded. Adjustment: age, race, smoking, baseline diastolic blood pressure, high density lipoprotein, low density lipoprotein, alcohol. RR, relative risk.

Table VII. Cox Regression Analysis of Dietary 18:3n-3/18:2n-6 and Total n-3/Total n-6 and 10.5-Year Mortality[a]

Quintile	Mean	*n*	CHD RR	CVD RR	CA RR	AC RR
18:3 n-3/18:2 n-6						
I	0.080	1251	1.00	1.00	1.00	1.00
II	0.105	1252	0.70	0.70	0.81	0.76
III	0.120	1252	0.82	0.77	0.81	0.83
IV	0.135	1252	0.82	0.79	0.71	0.78
V	0.170	1251	0.96	0.86	0.67	0.82
P			NS	NS	<0.05	NS
Total n-3/total n-6						
I	0.086	1251	1.00	1.00	1.00	1.00
II	0.111	1252	0.76	0.82	1.02	0.93
III	0.127	1252	0.88	0.82	0.80	0.90
IV	0.145	1252	0.77	0.73	0.61	0.72
V	0.199	1251	0.90	0.85	0.62	0.85
P			NS	<0.10	<0.05	<0.10

[a] Deaths and clinical myocardial infarctions from the first 3 years of follow-up excluded. Adjustment: age, race, smoking, baseline diastolic blood pressure, high density lipoprotein, low density lipoprotein, alcohol. RR, relative risk.

Discussion

The quantity and quality of dietary polyunsaturated fatty acid (PUFA) intake have been shown in experimental studies to influence biochemical and physiological processes. Whether the action is positive or adverse appears to be dependent upon dietary PUFA composition on eicosanoid regulatory mechanisms (7). In addition, considerable attention has also been directed toward actions by the long chain n-3 fatty acids predominantly found in marine animals and seafoods. Numerous studies have suggested that they produce hypolipidemic effects, antiaggregation of platelets, decreased blood viscosity, blood pressure lowering, anti-inflammatory responses, immune competence, and antioncogenic properties (8). Given the unique characteristics of PUFA, mortality rates from various causes might be expected to be influenced by their ingestion, which has been suggested from the results of the MRFIT database.

The protective effects of the long chain n-3 fatty acids on cardiovascular disease are not surprising, since populations consuming large amounts of marine ani-

Table VIII. Trends in Fatty Acid Intake in Japan (g/day/capita) Compared with Multiple Risk Factor Intervention Trial Dietary Fatty Acid Intake Data[a]

	1946	1955	1960	1965	1975	1985	MRFIT late 70s
Saturates	2.89	5.39	6.93	9.96	15.07	16.93	36.49
Monounsaturates	4.34	7.85	9.83	13.97	20.83	20.87	39.27
18:2n-6 PUFA	4.24	6.35	7.18	9.75	13.63	13.86	14.61
20:4 18:4 n-6 HUFA	0.05	0.10	0.15	0.19	0.32	0.35	0.30
18:3n-3 PUFA	0.67	0.96	1.12	1.46	1.99	2.08	1.69
20:5 22:5 22:6 n-3 HUFA	0.79	1.34	1.34	1.32	1.63	1.56	0.18
Total FA	12.98	21.99	26.55	36.65	53.46	55.65	92.54
n-3/n-6	0.340	0.357	0.336	0.280	0.259	0.256	0.133

[a] Data from Japanese Ministry of Health provided by Professor H. Okuyama.

mals and seafoods such as the Greenland Eskimos, fishing villagers of Japan, and Alaskan natives have remarkably low rates of acute myocardial infarction (9–11). Moreover, several epidemiological studies of Western industrialized populations have also reported inverse associations between fish consumption and death, especially from coronary heart disease (12, 13).

However, epidemiological evidence of protective relationships between 18:3n-3 on cardiovascular disease and n-3 to n-6 ratios on cancer mortality is intriguing and has not been reported previously. Although some evidence exists that n-3 fatty acids have antioncogenic properties, the finding may really be serving as a marker for another dietary component responsible for cancer protection. Perhaps further analyses involving other nutrients, food groups, and database evaluations would help clarify the meaning of these findings. Such evaluations combined with clinical and experimental evidence might clarify the absolute PUFA intake levels and n-3 to n-6 ratios necessary for health promotion and disease prevention.

Since few reports express PUFA in terms of fatty acids, it is not possible to compare trends in specific PUFA intake among many studies. Table VIII shows a comparison of Japanese dietary fatty acid data over time. When compared with data from 1975, about the time the MRFIT data were collected, the Japanese consumed less total fat but similar quantities of total PUFA as that reported by the MRFIT men. The composition of PUFA differed considerably. The Japanese consumed more fish oil fatty acids (20:5n-3, 22:5n-3, and 22:6n-3). While the mean Japanese intake was approximately 1.5 g/day, MRFIT UC participants consumed an average of 0.18 g/day. Linoleic acid (18:2n-6) intake was about the same for the Japanese comparison with the MRFIT reported intake, while linolenic acid (18:3n-3) intake was slightly greater for Japanese than MRFIT participants. Overall, a greater n-3 to n-6 fatty acid intake ratio was apparent in the Japanese diet (0.26 compared with 0.13 in the MRFIT group).

It is interesting to note that if the Japanese dietary fatty acid intake data are viewed over time, there is a trend for dietary intake to change in a direction more reflective of the MRFIT fatty acid pattern reported in the 1970s. Given that the disease patterns between Japan and the United States differ considerably, it would seem that dietary PUFA composition may play a role in establishing and changing those patterns over time (14). Indeed, it appears that ischemic heart disease rates are on the rise in Japan (15).

In summary, the results of this evaluation support the hypothesis that fatty acids found primarily in fish oils protect against cardiovascular disease. They also suggest that the composition and balance of PUFA in the diet may influence mortality from cardiovascular disease and possibly various forms of cancer. Further research is needed to define the optimal level and balance of polyunsaturated fatty acids in the diets of humans to promote health and prevent diseases.

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