Heart rate variability and fatty acid content of blood cell membranes: a dose-response study with n−3 fatty acids[1–3]

Jeppe Hagstrup Christensen, Merete Stubkjær Christensen, Jørn Dyerberg, and Erik Berg Schmidt

ABSTRACT

Background: Dietary intake of long-chain n−3 polyunsaturated fatty acids (PUFA) may protect against sudden cardiac death, an event that may be predicted by measurement of heart rate variability (HRV).

Objective: The objectives of this study were to *1*) examine the correlations between the content of fatty acids in blood cell membranes (platelets and granulocytes) and HRV in healthy subjects, and *2*) assess the effect on HRV of dietary intervention with n−3 PUFA in different doses.

Design: Sixty healthy volunteers (25 women and 35 men) were randomly assigned to 3 treatment groups in a double-blind design. Subjects received a daily supplement of either 6.6 g n−3 PUFA, 2.0 g n−3 PUFA, or placebo (olive oil). A 24-h Holter recording was obtained for each subject before supplementation and after 12 wk of supplementation; the 24-h HRV was then related to the content of fatty acids in granulocytes and platelets.

Results: Before supplementation, positive correlations were observed in men between the content of docosahexaenoic acid in cell membranes and HRV indexes ($r = 0.50$, $P < 0.01$), whereas such correlations were not found in women. Dietary intervention revealed a dose-dependent effect of n−3 PUFA on HRV in men, whereas no effect was found in women.

Conclusion: The study showed a beneficial effect of n−3 PUFA on HRV in healthy men, suggesting an antiarrhythmic effect of n−3 PUFA. No such effect was observed in healthy women. *Am J Clin Nutr* 1999;70:331–7.

KEY WORDS n−3 fatty acids, omega-3 fatty acids, fish oil, sudden cardiac death, cardiac arrest, ventricular arrhythmias, heart rate variability, granulocytes, platelets

INTRODUCTION

The most common cause of death in Western countries is sudden cardiac death (SCD). SCD is often caused by serious ventricular arrhythmias in patients with known ischemic heart disease (IHD), but it may also occur in previously healthy persons. During the past decade there has been increasing interest in the role of fatty acids in the pathogenesis of SCD. In particular, the possible protective effect of long-chain n−3 polyunsaturated fatty acids (PUFA), mainly derived from fish (1–3), has been studied. Victims of SCD have been found to have a lower content of n−3 PUFA in their coronary arteries compared with control subjects (4), which is consistent with evidence of an antiarrhythmic effect of n−3 PUFA reported in animal studies (5, 6) and in vitro experiments with cardiac myocytes (7). However, there is only sparse information that directly shows an antiarrhythmic effect of n−3 PUFA in humans, although dietary n−3 PUFA intake has been associated with reduced incidence of SCD (8–10).

We (11) reported in 1996 that dietary n−3 PUFA significantly increased heart rate variability (HRV) in human survivors of myocardial infarction (MI). This points to an antiarrhythmic effect of n−3 PUFA, because low HRV is strongly associated with increased mortality and arrhythmic events in post-MI patients (12–15). Furthermore, we found the content of n−3 PUFA in cell membranes to be positively correlated with HRV in these patients (16). This finding was reproduced in another group of patients with increased risk of SCD, namely patients with chronic renal failure treated with maintenance hemodialysis (17).

The aim of the present study was to examine the possible correlation between fatty acids in blood cell membranes and HRV in healthy subjects. Furthermore, the subjects were randomly assigned to dietary supplementation with either n−3 PUFA in 2 different doses or an olive oil placebo to investigate the possible effect of dietary supplementation with n−3 PUFA on HRV.

SUBJECTS AND METHODS

Sixty healthy subjects were recruited from among the medical staff, bank employees, and students at institutions in Aalborg, Denmark. None of the volunteers took any medications and none had any known diseases. The procedures followed in the study were in accord with the ethical standards of the responsible regional committee on human experimentation and in accord with the Helsinki Declaration of 1975 as revised in 1983. Characteristics of the study population are given in **Table 1**.

[1] From the Department of Nephrology, Aalborg Hospital, Aalborg, Denmark; the Department of Medicine, Hjørring/Brønderslev Hospital, Hjørring, Denmark; and Medi-Lab, Copenhagen, Denmark.

[2] Supported by The Medical Research Foundation of the County of Northern Jutland, and Aalborg Stifts Julelotteri. LUBE A/S of Hadsund, Denmark, provided the Pikasol capsules.

[3] Address reprint requests to J Dyerberg, Medi-Lab, 5–7 Adelgade, PO Box 2, 1001 Copenhagen K, Denmark.

TABLE 1
Characteristics of the study participants before dietary supplementation[1]

	Women (n = 25)	Men (n = 35)
Age (y)	38 ± 11	38 ± 10
Body mass index (in kg/m^2)	23 ± 2	25 ± 3[2]
Heart rate variability indices		
RR (ms)	782 ± 90	820 ± 90
SDNN (ms)	142 ± 41	168 ± 39[3]
SDNNindex (ms)	57 ± 15	67 ± 19[3]
SDANNindex (ms)	131 ± 41	159 ± 42[4]
pNN50 (%)	11 ± 10	13 ± 11
RMSSD (ms)	32 ± 13	36 ± 15

[1] $\bar{x}$ ± SD. Eight women and 8 men were smokers. RR, mean of all normal R-R intervals; SDNN, SD of all normal R-R intervals; SDNNindex, mean of SDs of all normal R-R intervals for all 5-min segments; SDANNindex, SD of mean of R-R intervals measured in successive 5-min periods; pNN50, percentage of successive R-R interval differences ≥50 ms; RMSSD, square root of mean of sum of the squares of differences between adjacent intervals.
[2–4] Significantly different from women (t test): [2]P = 0.001, [3]P = 0.02, [4]P = 0.01.

Study design

The study design was double-blind and placebo-controlled with respect to the 3 dietary groups (*see* below). Both women and men were included in each group. However, because significant differences between the sexes were found before supplementation, the dietary groups were also analyzed after dividing each group according to sex.

Dietary supplements

In a double-blind design, the subjects were randomly assigned to 3 groups. Group 1 received daily supplements of 6.6 g n−3 PUFA as 3.0 g eicosapentaenoic acid (EPA) and 2.9 g docosahexaenoic acid (DHA) [10 capsules of Pikasol; LUBE A/S, Hadsund, Denmark, a reesterified triacylglycerol (EPAX 5500) Pronova Biocare A/S, Sandefjord, Norway]. Group 2 received supplementation with 2.0 g n−3 PUFA daily (0.9 g EPA and 0.8 g DHA, in 3 capsules of Pikasol) and 7 capsules of placebo (olive oil). Group 3 received 10 capsules of placebo daily. The subjects took 3 capsules in the morning and 7 capsules with the evening meal, taking the capsules from 2 different boxes to maintain the blinding. The supplements were given for 12 wk.

Heart rate variability analyses

A 24-h Holter recording was obtained for each subject on a flash card by using a 3-channel digital monitor (Diagnostic Monitoring, Santa Ana, CA). This was done before and after 12 wk of dietary supplementation. The recordings were analyzed by using a commercially available software program, DIAGNOSTIC MONITORING (Diagnostic Monitoring). The following time-domain HRV variables were analyzed: *1*) RR, the mean of all normal R-R intervals during the 24-h recording; *2*) SDNN, the SD of all normal R-R intervals during the 24-h recording; *3*) SDNNindex, the mean of the SDs of all normal R-R intervals for all 5-min segments of the 24-h recording; *4*) SDANNindex, the SD of the mean of R-R intervals measured in successive 5-min periods; *5*) pNN50, the percentage of successive R-R interval differences ≥50 ms; *6*) RMSSD, the square root of the mean of the sum of the squares of differences between adjacent intervals. QRS complexes with abnormal morphology were excluded from HRV analysis and the recordings were processed without knowledge of other subject variables.

Fatty acid analysis

Blood samples were drawn in the fasting state before the Holter recording, both before and after 12 wk of dietary supplementation. Granulocytes and platelets were isolated and their fatty acid composition was measured by gas chromatography and expressed as a percentage of total fatty acid content, as described previously (18).

Statistical analysis

Paired t tests were used to test for any differences within the groups before and after supplementation. Differences among the groups (placebo, low-dose n−3 PUFA, and high-dose n−3 PUFA) were tested by one-way analysis of variance. If significant differences were found when comparing the 3 groups, Tukey's test was applied. Correlations between the cellular concentrations of fatty acids and HRV variables were tested by simple linear regression analysis using the SPSS software package, version 6.0 (SPSS, Chicago). Bonferroni-corrected correlations were used if multiple correlations were made. Furthermore, we used Lowess' locally weighted regression smoothing, which is an iterative weighted least-squares method (19). A P value < 0.05 (two-tailed) was considered statistically significant.

RESULTS

Values before supplementation

In general, women had lower body mass index and lower HRV values than men before supplementation (Table 1). The 3 intervention groups were comparable before supplementation regarding HRV indexes, cellular content of fatty acids **(Table 2)**, and plasma lipids and lipoproteins.

Fatty acids in granulocytes and platelets in women and men

The contents of the fatty acids 18:1n−9 and 18:2n−6 in granulocytes and 18:2n−6 in platelets were higher in women than in men, whereas the contents of 20:4n−6 and 22:5n−3 in granulocytes were higher in men (Table 2). Although these differences were significant, they were rather small.

There were distinct differences in fatty acid contents between platelets and granulocytes in both women and men, particularly for 18:1n−9 and 20:4n−6. However, significant correlation coefficients in the range of 0.25–0.85 were found between the contents of the fatty acids in granulocytes and in platelets, except for 18:1n−9, for which no correlation was found (r = 0.00). The highest correlation coefficient was found for EPA (r = 0.85, $P < 0.001$).

Fatty acids in granulocytes and HRV in women and men

In women, significant positive correlations were found between the content of 20:4n−6 in granulocytes and the 6 HRV indexes used. There was also a significant positive correlation between the EPA content of granulocytes and RR in women (r = 0.50, $P < 0.01$).

In men, a significant positive correlation was found between DHA in granulocytes and all the HRV indexes, with correlation coefficients ranging from 0.38 ($P < 0.05$) to 0.50 ($P < 0.01$). The correlation in men before supplementation between DHA in granulocytes and the clinically important parameter, SDNN, is

TABLE 2
Fatty acid content of granulocytes and platelets before dietary supplementation[1]

	Fatty acids in granulocytes		Fatty acids in platelets	
	Women (n = 25)	Men (n = 35)	Women (n = 25)	Men (n = 35)
	% of total fatty acids		*% of total fatty acids*	
14:0	0.5 ± 0.4	0.5 ± 0.3	0.3 ± 0.1	0.4 ± 0.2
16:0	11.8 ± 0.7	11.7 ± 1.0	15.5 ± 1.0	15.6 ± 1.2
18:0	17.0 ± 0.8	17.1 ± 0.8	19.0 ± 0.8	19.3 ± 0.7
18:1n−9	32.3 ± 1.6	30.8 ± 1.4[2]	18.1 ± 0.7	18.2 ± 0.8
18:2n−6	10.9 ± 1.2	10.1 ± 1.2[3]	6.2 ± 0.6	5.8 ± 0.7[4]
18:3n−3	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1
20:4n−6	12.1 ± 1.1	13.3 ± 1.4[5]	26.0 ± 1.4	26.0 ± 1.4
20:5n−3	0.5 ± 0.2	0.6 ± 0.4	0.8 ± 0.3	0.8 ± 0.3
22:5n−3	1.4 ± 0.4	1.8 ± 0.5[5]	1.7 ± 0.4	1.9 ± 0.4
22:6n−3	1.5 ± 0.4	1.6 ± 0.5	2.6 ± 0.5	2.4 ± 0.4

[1] $\bar{x}$ ± SD.
[2–5] Significantly different from women (t test): [2] $P < 0.001$, [3] $P = 0.018$, [4] $P = 0.02$, [5] $P = 0.001$.

shown in **Figure 1**. In this figure, it is apparent that Lowess regression smoothing (dotted line) showed a dose-response relation between 22:6n−3 and SDNN with a steep rise in SDNN when the content of 22:6n−3 in granulocytes was between 1.3% and 2.1%. The correlation coefficient in this interval was 0.68 ($P < 0.001$).

Fatty acids in platelets and HRV in women and men

In women, no correlation was found between HRV and the content of 20:4n−6 in platelets, but a significant correlation was found between EPA content and RR ($r = 0.47$, $P < 0.05$). Furthermore, in women, negative correlations were observed between HRV indexes and 18:2n−6 and 18:3n−3, whereas positive correlations were found between 16:0 and both pNN50 and RMSSD (data not shown).

In men, there were significant positive correlations between the content of DHA in platelets and RR, SDNN, SDNNindex, SDANNindex, and pNN50, with correlation coefficients ranging from 0.32 ($P < 0.05$) to 0.46 ($P < 0.01$).

DHA and HRV in men

The group of 35 men was dichotomized according to median content of DHA in platelets and in granulocytes and the HRV indexes were calculated for each subgroup. In **Table 3** it is apparent that the men with the highest content of DHA in their cell membranes also had the highest HRV indexes.

Dietary supplementation

The dietary supplements were well tolerated and no subjects dropped out of the study. Subjects receiving 2.0 or 6.6 g n−3 PUFA daily for 12 wk had a significant increase in cellular concentrations of EPA and DHA, with the largest increase occurring in the 6.6-g group. A decrease in plasma triacylglycerol was simultaneously observed, whereas other plasma lipids and lipoproteins were unaffected by dietary supplementation with n−3 PUFA. These results, together with the HRV indexes before and after dietary supplementation, are shown in **Table 4**. It is apparent that when results for women and men were analyzed together, dietary n−3 PUFA supplementation had no effect on HRV. The 3 groups had normal serum concentrations of potassium, magnesium, and calcium before and after dietary supplementation (data not shown).

Women and men with low HRV

A possible beneficial effect on HRV after n−3 PUFA supplementation could be expected in men with the lowest HRV (Figure 1); thus, the subjects (both women and men) were divided into 2 groups according to the median SDNN (150 ms) before supplementation. There was a significant increase in RR after supplementation with n−3 PUFA (both doses) and a nonsignificant increase in other HRV indexes in subjects with before-supplementation SDNN < 150 ms (**Table 5**). No such effect was found in the group with the highest SDNN before supplementation (data not shown).

Men with low HRV

Because a positive correlation between DHA content and HRV before supplementation was observed only in men, the sub-

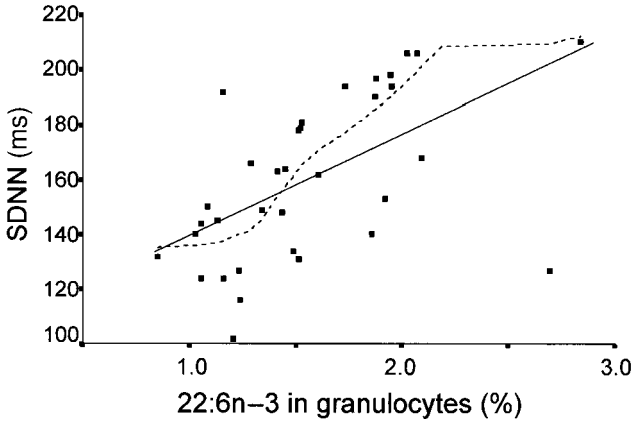

FIGURE 1. The relation between the content of docosahexaenoic acid (22:6n−3) in granulocytes and the heart rate variability parameter SDNN (the SD of all normal R-R intervals during the 24-h recording) before supplementation in 35 healthy men. The solid line represents the linear regression and the dotted line represents Lowess' weighted regression smoothing.

TABLE 3
Heart rate variability indexes before supplementation in 35 healthy men after dichotomizing the group of men into 2 subgroups according to the median content of docosahexaenoic acid (DHA) in platelets and in granulocytes[1]

	DHA in platelets			DHA in granulocytes		
	<2.5%	≥2.5%	P^2	<1.5%	≥1.5%	P^2
RR (ms)	789 ± 81	854 ± 80	0.01	790 ± 93	852 ± 68	0.02
SDNN (ms)	150 ± 29	185 ± 36	<0.01	153 ± 39	182 ± 29	0.01
SDNNindex (ms)	63 ± 17	73 ± 21	0.09	60 ± 17	76 ± 19	<0.01
SDANNindex (ms)	141 ± 30	178 ± 42	<0.01	145 ± 42	174 ± 34	0.02
pNN50 (%)	16 ± 11	10 ± 8	0.09	10 ± 8	17 ± 11	0.03
RMSSD (ms)	32 ± 12	40 ± 16	0.09	31 ± 12	41 ± 16	0.02

[1] $\bar{x}$ ± SD. RR, mean of all normal R-R intervals; SDNN, SD of all normal R-R intervals; SDNNindex, mean of SDs of all normal R-R intervals for all 5-min segments; SDANNindex, SD of mean of R-R intervals measured in successive 5-min periods; pNN50, percentage of successive R-R interval differences ≥50 ms; RMSSD, square root of mean of sum of the squares of differences between adjacent intervals.
[2] *t*-Test.

jects were divided according to sex. An increase, although not significant, was found for almost all HRV indexes after dietary supplementation with 2.0 g n−3 PUFA daily (compared with before supplementation) in men with a low SDNN before supplementation. This trend was more pronounced when 6.6 g n−3 PUFA was given, with significant increases in SDNN, pNN50, and RMSSD. When these 2 dietary groups were pooled, this finding was further substantiated and was significant (**Table 6**).

DISCUSSION

Low HRV is a powerful predictor of mortality, SCD, and arrhythmic events in post-MI patients (12–15). HRV also appears to be a valid marker of the risk for arrhythmic events in healthy subjects (20). Our findings therefore suggest that high concentrations of DHA in cell membranes may protect against serious ventricular arrhythmias in men. In both women and men, the strong positive association between RR and n−3 PUFA

TABLE 4
Heart rate variability indexes, the content of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in cell membranes, and plasma lipids and lipoproteins in the 3 treatment groups (including both women and men) before and after dietary supplementation for 12 wk with placebo, 2.0 g n−3 PUFA daily, or 6.6 g n−3 PUFA daily[1]

	Placebo (n = 20)			2.0 g n−3 PUFA (n = 20)			6.6 g n−3 PUFA (n = 20)		
	Before	After	Difference[2]	Before	After	Difference[2]	Before	After	Difference[2]
Heart rate variability indexes									
RR (ms)	821 ± 105	812 ± 92	10 ± 94	802 ± 91	813 ± 90	−11 ± 54	788 ± 77	801 ± 88	−13 ± 66
SDNN (μs)	170 ± 43	157 ± 36	13 ± 33	164 ± 44	155 ± 38	10 ± 34	136 ± 27	136 ± 33	0 ± 27
SDNNindex (ms)	65 ± 15	65 ± 12	0 ± 11	65 ± 19	63 ± 18	2 ± 8	58 ± 20	59 ± 22	−1 ± 10
SDANNindex (ms)	163 ± 48	145 ± 43	18 ± 42	155 ± 44	144 ± 42	11 ± 39	125 ± 29	124 ± 34	1 ± 32
pNN50 (%)	13 ± 9	12 ± 6	1 ± 8	14 ± 11	12 ± 9	2 ± 5	9 ± 10	11 ± 10	−2 ± 7
RMSSD (ms)	35 ± 12	35 ± 8	0 ± 9	37 ± 15	34 ± 12	3 ± 6	30 ± 16	33 ± 15	−3 ± 9
n−3 PUFA in granulocytes (% of total fatty acids)									
EPA	0.51 ± 0.2	0.55 ± 0.3	−0.04 ± 0.3	0.56 ± 0.5	1.82 ± 0.8[3]	−1.24 ± 0.6[5]	0.58 ± 0.2	4.07 ± 1.0[3]	−3.49 ± 1.0[6]
DHA	1.54 ± 0.4	1.54 ± 0.5	−0.01 ± 0.4	1.48 ± 0.4	1.85 ± 0.6[3]	−0.36 ± 0.4[5]	1.58 ± 0.5	2.14 ± 0.4[3]	−0.56 ± 0.4[5]
n−3 PUFA in platelets (% of total fatty acids)									
EPA	0.73 ± 0.3	0.74 ± 0.4	−0.01 ± 0.2	0.72 ± 0.3	2.06 ± 0.6[3]	−1.33 ± 0.6[5]	0.85 ± 0.3	4.66 ± 1.3[3]	−3.81 ± 1.3[6]
DHA	2.45 ± 0.5	2.40 ± 0.6	0.05 ± 0.4	2.42 ± 0.4	2.81 ± 0.4[3]	−0.39 ± 0.3[5]	2.66 ± 0.5	3.57 ± 0.5[3]	−0.91 ± 0.4[6]
Plasma lipids and lipoproteins (mmol/L)									
Total cholesterol	5.0 ± 1.1	5.0 ± 1.2	0.0 ± 0.4	5.0 ± 0.9	5.0 ± 0.9	−0.1 ± 0.5	5.1 ± 1.2	4.9 ± 1.1	0.2 ± 0.5
LDL cholesterol	3.0 ± 1.0	3.0 ± 1.0	0.0 ± 0.3	3.3 ± 0.9	3.3 ± 0.9	0.0 ± 0.4	3.2 ± 1.1	3.2 ± 1.1	0.0 ± 0.4
HDL cholesterol	1.4 ± 0.4	1.4 ± 0.3	0.0 ± 0.2	1.3 ± 0.2	1.2 ± 0.2	0.1 ± 0.1	1.3 ± 0.3	1.3 ± 0.4	0.0 ± 0.2
Triacylglycerols	1.2 ± 0.7	1.2 ± 0.9	0.0 ± 0.4	1.2 ± 0.6	1.0 ± 0.5[4]	0.2 ± 0.3	1.3 ± 1.2	0.8 ± 0.7[3]	0.4 ± 0.7[5]

[1] $\bar{x}$ ± SD. RR, mean of all normal R-R intervals; SDNN, SD of all normal R-R intervals; SDNNindex, mean of SDs of all normal R-R intervals for all 5-min segments; SDANNindex, SD of mean of R-R intervals measured in successive 5-min periods; pNN50, percentage of successive R-R interval differences ≥50 ms; RMSSD, square root of mean of sum of the squares of differences between adjacent intervals.
[2] Defined as before−after.
[3,4] Significantly different from before supplementation (paired *t* test): [3] $P < 0.01$, [4] $P < 0.05$.
[5] Significantly different from placebo, $P < 0.05$.
[6] Significantly different from placebo and the 2.0-g n−3 PUFA group, $P < 0.05$ (one-way ANOVA).

TABLE 5
Heart rate variability indexes in healthy subjects (14 women and 13 men) with before-supplementation SDNN < 150 ms, before and after dietary supplementation for 12 wk with either 2.0 or 6.6 g n−3 PUFA daily[1]

	Before supplementation	After supplementation
RR (ms)	761 ± 65	785 ± 79[2]
SDNN (ms)	126 ± 18	131 ± 26
SDNNindex (ms)	51 ± 12	53 ± 14
SDANNindex (ms)	116 ± 21	121 ± 29
pNN50 (%)	7 ± 6	9 ± 6
RMSSD (ms)	27 ± 9	29 ± 9

[1] $\bar{x}$ ± SD. RR, mean of all normal R-R intervals; SDNN, SD of all normal R-R intervals; SDNNindex, mean of SDs of all normal R-R intervals for all 5-min segments; SDANNindex, SD of mean of R-R intervals measured in successive 5-min periods; pNN50, percentage of successive R-R interval differences ≥50 ms; RMSSD, square root of mean of sum of the squares of differences between adjacent intervals.

[2] Significantly different from before supplementation, $P = 0.02$ (*t* test).

content in cell membranes suggests a lower heart rate in subjects with a high intake of n−3 PUFA, because RR is negatively correlated with heart rate. This is consistent with a recent study showing reduced heart rate in healthy men after dietary supplementation with DHA (21). The positive associations we found between HRV indexes and the cellular concentration of DHA in men were substantiated by the dichotomized DHA concentrations and the corresponding HRV indexes before supplementation (Table 3).

We found small differences between women and men in the content of certain fatty acids, especially in granulocytes. Such sex differences were described previously in a study that related dietary intake of fatty acids to the same fatty acids in plasma phospholipids (22). It seems likely that women and men consume fatty acids in slightly different proportions.

The dose-response relation found between the cellular content of DHA and SDNN before supplementation may suggest a threshold effect, although these findings should be viewed with caution because of the small number of men studied. However, a threshold effect was also suggested in a recently published, large prospective cohort study of US male physicians (10). Consumption of ≥1 fish meal/wk was associated with a 52% lower risk of SCD compared with consumption of fish less than monthly, but increasing the intake of fish further did not confer any additional benefit in the prevention of SCD (10). A smaller, population-based case-control study (80% men) with 334 case subjects with primary cardiac arrest and 493 control subjects concluded that dietary intake of n−3 PUFA equal to 1 fatty fish meal/wk was associated with a 50% reduction in the risk of primary cardiac arrest compared with no intake of n−3 PUFA (9). Data from epidemiologic studies (23) also show an apparent cardioprotective effect of n−3 PUFA in populations with a rather low intake of fish.

Two secondary prevention trials that studied dietary n−3 PUFA have been reported. In the Diet and Reinfarction Trial, 2033 men with a previous MI were randomly assigned to receive or not receive advice to eat fatty fish ≥2 times/wk (8). During a 2-y follow-up, the total mortality was reduced significantly, by 29%, in the intervention group, although no decrease in the number of reinfarctions was observed. The authors hypothesized that fish consumption might have reduced the risk of serious ventricular arrhythmias and SCD. In another randomized secondary prevention trial that included 605 post-MI patients (>90% men), the effect of a Mediterranean diet was compared with that of a control diet (24). The experimental diet involved increased intake of bread, fruit, vegetables, fish, and a margarine rich in 18:3n−3. After a mean follow-up of 27 mo there was a striking reduction in the number of cardiac deaths in the intervention group compared with the control group (3 and 16 cardiac deaths, respectively) and no cases of SCD were recorded in the intervention group. However, it is not known whether the high intake of 18:3n−3, the fish-derived n−3 PUFA, or other foods in the Mediterranean diet were responsible for the reduction in SCD (24).

Although there is increasing evidence of a protective effect of n−3 PUFA against SCD in men, such an effect in women has not yet been reported. In the present study, we did not find any correlation between n−3 PUFA content in cell membranes and HRV indexes other than RR in women, and in agreement with this, dietary intervention with n−3 PUFA did not affect HRV in women. The finding of a positive correlation between 20:4n−6 in granulocytes and HRV in women could not be reproduced in platelets. In general, it is believed that 20:4n−6 is arrhythmogenic, but in vitro studies have shown an antiarrhythmic potential of 20:4n−6 similar to that of EPA and DHA, if the activity of cyclooxygenase and lipoxygenase is inhibited (25). Thus, it

TABLE 6
Heart rate variability indexes in men with before supplementation SDNN < 150 ms, before and after dietary supplementation for 12 wk with either placebo or 2.0 or 6.6 g n−3 PUFA daily[1]

	Placebo (*n* = 5)			2.0 g n−3 PUFA (*n* = 7)			6.6 g n−3 PUFA (*n* = 6)			2.0 and 6.6 g n−3 PUFA (*n* = 13)		
	Before	After	Difference	Before	After	Difference	Before	After	Difference	Before	After	Difference
RR (ms)	792 ± 133	774 ± 103	17 ± 95	758 ± 92	783 ± 106	−25 ± 43	760 ± 37	804 ± 69	−44 ± 64	759 ± 64	794 ± 84[2]	−35 ± 53
SDNN (ms)	140 ± 27	134 ± 31	6 ± 22	134 ± 10	137 ± 23	−3 ± 16	133 ± 12	145 ± 14[2]	−12 ± 12	133 ± 11	141 ± 18	−8 ± 14
SDNNindex (ms)	57 ± 17	57 ± 14	0 ± 10	53 ± 11	52 ± 11	1 ± 7	56 ± 15	62 ± 17	−6 ± 7	54 ± 13	58 ± 15	−3 ± 8
SDANNindex (ms)	130 ± 22	120 ± 28	10 ± 20	126 ± 16	132 ± 34	−6 ± 22	122 ± 22	134 ± 15	−12 ± 19	124 ± 19	133 ± 24	−9 ± 20
pNN50 (%)	7 ± 6	10 ± 4	3 ± 8	8 ± 4	9 ± 3	−1 ± 2	7 ± 7	12 ± 7[2]	−5 ± 4	7 ± 5	10 ± 5[2]	−3 ± 4
RMSSD (ms)	27 ± 9	30 ± 3	3 ± 11	28 ± 5	30 ± 5	−2 ± 4	27 ± 9	34 ± 10[2]	−7 ± 5	27 ± 7	32 ± 8[2]	−5 ± 5

[1] $\bar{x}$ ± SD. RR, mean of all normal R-R intervals; SDNN, SD of all normal R-R intervals; SDNNindex, mean of SDs of all normal R-R intervals for all 5-min segments; SDANNindex, SD of mean of R-R intervals measured in successive 5-min periods; pNN50, percentage of successive R-R interval differences ≥50 ms; RMSSD, square root of mean of sum of the squares of differences between adjacent intervals. In the last column, the 2 n−3 PUFA groups are pooled.

[2] Significantly different from before supplementation within the group, $P < 0.05$.

may be that the cyclooxygenase and lipoxygenase metabolites of 20:4n−6 are arrhythmogenic whereas the free fatty acid of 20:4n−6 may possess antiarrhythmic properties.

In men we found a beneficial effect on HRV of dietary supplementation with n−3 PUFA, but only in those men with low HRV before supplementation. This is perhaps partly explained by the dose-response relation found between DHA and HRV before supplementation. According to Figure 1 it would be expected that only the men with low HRV would show an influence on HRV by increasing their DHA concentration in cell membranes, and this was in fact what was found in a dose-dependent manner. It should be emphasized that the granulocyte membrane concentrations and platelet concentrations of n−3 PUFA are also biomarkers of the composition of other cell membranes, including those of myocardial cells (26).

Our data may suggest that DHA is the principal active n−3 fatty acid that confers protection against arrhythmias, which is consistent with our previous observations in post-MI patients (16). Most animal studies that have shown an antiarrhythmic effect of n−3 PUFA have used mixtures of EPA and DHA, but a recent animal study using both EPA and DHA in separate preparations found DHA to be the active protector against arrhythmias (27). Another recent study suggests that the fluidity of the sarcolemmal membrane is a critical factor concerning asynchronous beating of the myocyte and only DHA, which has the longest chain of the n−3 PUFA, changes the membrane fluidity in a favorable way; this may underlie the antiarrhythmic effect of DHA (28). However, this specific effect may be obscured by the in vivo interconversion between EPA and DHA (26).

We used 24-h HRV as the endpoint because the available data suggest great stability of HRV measures derived from 24-h ambulatory monitoring in normal subjects (29, 30), post-MI patients (31), and patients with previous serious ventricular arrhythmias (32). The importance of measuring HRV in post-MI patients has recently been emphasized (33). Finally, a consensus report on HRV stated that because 24-h HRV indexes appear to be stable and free of placebo effects, HRV may be an ideal method of assessing intervention therapies (34).

Dietary supplementation with n−3 PUFA revealed a dose-dependent reduction in plasma triacylglycerol concentration. No effects on other plasma lipids or lipoproteins were observed, which is in agreement with results from previous studies (35).

With regard to the limitations of this study, the study groups were rather small, thereby increasing the risk of type 2 statistical errors. Also, the differences found between women and men made it necessary to examine the sexes separately, thereby decreasing the numbers of subjects under investigation. In addition, a direct method of evaluating the effects of an intervention therapy on ventricular arrhythmias in humans would be preferable. However, arrhythmias are seldom present, especially in healthy subjects. Therefore, it is necessary to rely on surrogate endpoints, such as HRV, for the development of serious arrhythmias.

This study found a beneficial effect of n−3 PUFA on HRV in healthy men, suggesting an antiarrhythmic effect of n−3 PUFA. Together with our previous observations of an increase in HRV in post-MI patients after dietary supplementation with n−3 PUFA, the results may help explain the reduction in SCD seen in men who regularly eat fish. However, larger studies are needed to clarify the dose-response relation observed in the present study. In addition, more attention should be paid to the effect of n−3 PUFA on arrhythmic tendency in women.

REFERENCES

1. Connor SL, Connor WE. Are fish oils beneficial in the prevention and treatment of coronary artery disease? Am J Clin Nutr 1997; 66(suppl):1020S–31S.
2. Leaf A, Kang JX. Dietary n−3 fatty acids in the prevention of lethal cardiac arrhythmias. Curr Opin Lipidol 1997;8:4–6.
3. Stone NJ. Fish consumption, fish oil, lipids, and coronary heart disease. Am J Clin Nutr 1997;65:1083–6.
4. Luostarinen R, Boberg M, Saldeen T. Fatty acid composition in total phospholipids of human coronary arteries in sudden cardiac death. Atherosclerosis 1993;99:187–9.
5. Charnock JS. Lipids and cardiac arrhythmias. Prog Lipid Res 1994;4: 355–85.
6. Billman GE, Hallaq H, Leaf A. Prevention of ischemia-induced ventricular fibrillation by n-3 fatty acids. Proc Natl Acad Sci U S A 1994;91: 4427–30.
7. Kang JX, Leaf A. Antiarrhythmic effects of polyunsaturated fatty acids. Recent studies. Circulation 1996;94:1774–80.
8. Burr ML, Fehily AM, Gilbert JF, et al. Effects of changes in fat, fish, and fibre intakes on death and myocardial infarction: diet and reinfarction trial (DART). Lancet 1989;2:757–61.
9. Siscovick DS, Raghunathan TE, King I, et al. Dietary intake and cell membrane levels of long-chain n-3 polyunsaturated fatty acids and the risk of primary cardiac arrest. JAMA 1995;274:1363–7.
10. Albert CM, Hennekens CH, O'Donnell CJ, et al. Fish consumption and sudden cardiac death. JAMA 1998;279:23–8.
11. Christensen JH, Gustenhoff P, Korup E, et al. Effect of fish oil on heart rate variability in survivors of myocardial infarction: a double blind randomised controlled trial. BMJ 1996;312:677–8.
12. Kleiger RE, Miller JP, Bigger JT, Moss AJ, and the Multicenter Post-Infarction Research Group. Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. Am J Cardiol 1987;59:256–62.
13. Stein PK, Bosner MS, Kleiger RE, Conger BM. Heart rate variability: a measure of cardiac autonomic tone. Am Heart J 1994;127:1376–81.
14. Farrell TG, Bashir Y, Cripps T, et al. Risk stratification for arrhythmic events in postinfarction patients based on heart rate variability, ambulatory electrocardiographic variables and the signal-averaged electrocardiogram. J Am Coll Cardiol 1991;18:687–97.
15. Hartikainen JK, Malik M, Staunton A, Poloniecki J, Camm J. Distinction between arrhythmic and nonarrhythmic death after acute myocardial infarction based on heart rate variability, signal-averaged electrocardiogram, ventricular arrhythmias and left ventricular ejection fraction. J Am Coll Cardiol 1996;28:296–304.
16. Christensen JH, Gustenhoff P, Korup E, et al. Fish consumption, n−3 fatty acids in cell membranes, and heart rate variability in survivors of myocardial infarction with left ventricular dysfunction. Am J Cardiol 1997;79:1671–3.
17. Christensen JH, Aarøe J, Knudsen N, et al. Heart rate variability and n−3 fatty acids in patients with chronic renal failure—a pilot study. Clin Nephrol 1998;49:102–6.
18. Schmidt EB, Varming K, Ernst E, Madsen P, Dyerberg J. Dose-response studies on the effect of n−3 polyunsaturated fatty acids on lipids and haemostasis. Thromb Haemost 1990;63:1–5.
19. Cleveland WS. Bivariate data. In: Cleveland WS, ed. Visualizing data. Summit, NJ: Hobart Press, 1993:86–180.
20. Mølgaard H, Sørensen KE, Bjerregaard P. Attenuated 24-h heart rate variability in apparently healthy subjects subsequently suffering sudden cardiac death. Clin Auton Res 1991;1:233–7.
21. Grimsgaard S, Bønaa KH, Hansen JB, Myhre ES. Effects of highly purified eicosapentaenoic acid and docosahexaenoic acid on hemodynamics in humans. Am J Clin Nutr 1998;68:52–9.
22. Andersen LF, Solvoll K, Drevon CA. Very-long-chain n−3 fatty acids as biomarkers for intake of fish and n-3 fatty acid concentrates. Am J Clin Nutr 1996;64:305–11.
23. Kromhout D, Katan MB, Havekes L, et al. The effect of 26 years of

habitual fish consumption on serum lipid and lipoprotein levels (The Zutphen Study). Nutr Metab Cardiovasc Dis 1996;6:65–71.
24. de Lorgeril M, Renaud S, Mamelle N, et al. Mediterranean α-linolenic acid-rich diet in secondary prevention of coronary heart disease. Lancet 1994;343:1454–9.
25. Kang JX, Leaf A. Effects of long-chain polyunsaturated fatty acids on the contraction of neonatal rat cardiac myocytes. Proc Natl Acad Sci U S A 1994;91:9886–90.
26. Schmidt EB. n−3 Fatty acids and the risk of coronary heart disease. Dan Med Bull 1997;14:1–22.
27. McLennan P, Howe P, Abeywardena M, et al. The cardiovascular protective role of docosahexaenoic acid. Eur J Pharmacol 1996;300:83–9.
28. McMurchie EJ, Leifert WR, McLennan PL, Head RJ. Antiarrhythmic properties of polyunsaturated fatty acids in adult rat cardiomyocytes: a role for membrane fluidity? Prostaglandins Leukot Essent Fatty Acids 1997;57:193 (abstr).
29. Kleiger RE, Bigger JT, Bosner MS, et al. Stability over time of variables measuring heart rate variability in normal subjects. Am J Cardiol 1991;68:626–30.
30. Van Hoogenhuyze DK, Weinstein N, Martin GJ, et al. Reproducibility and relation to mean heart rate of heart rate variability in normal subjects and in patients with congestive heart failure secondary to coronary artery disease. Am J Cardiol 1991;68:1668–76.
31. Kautzner J. Reproducibility of heart rate variability measurement. In: Malik M, Camm AJ, eds. Heart rate variability. Armonk, NY: Futura, 1995:165–71.
32. Bigger JT, Fleiss JL, Rolnitzsky LM, Steinman RC. Stability over time of heart period variability in patients with previous myocardial infarction and ventricular arrhythmias. Am J Cardiol 1992;69:718–23.
33. La Rovere MT, Bigger JT Jr, Marcus FI, Mortara A, Schwartz PJ, for the ATRAMI (Autonomic Tone and Reflexes After Myocardial Infarction) Investigators. Baroreflex sensitivity and heart-rate variability in prediction of total cardiac mortality after myocardial infarction. Lancet 1998;351:478–84.
34. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. Circulation 1996;93:1043–65.
35. Harris WS. n−3 Fatty acids and serum lipoproteins: human studies. Am J Clin Nutr 1997;65(suppl):1645S–54S.