Marine n-3 Fatty Acids, Wine Intake, and Heart Rate Variability in Patients Referred for Coronary Angiography

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Background—Dietary n-3 polyunsaturated fatty acids (PUFAs) derived from fish may reduce the incidence of sudden cardiac death (SCD). In addition, wine drinking is suggested to have a protective effect against cardiovascular death.

Methods and Results—We included 291 patients referred for coronary angiography in whom ischemic heart disease was suspected and all of whom completed a food questionnaire regarding fish and wine intake. The n-3 PUFA composition of granulocyte membranes and of adipose tissue was measured. In addition, 24-hour heart rate variability (HRV) was analyzed. Fish intake was positively associated with the level of n-3 PUFAs in adipose tissue. Significant positive correlation coefficients were found between HRV indices and the levels of n-3 PUFAs in granulocytes. Wine intake was also significantly positively related to HRV, but the patients with the highest wine intake also had the highest intake of fish, as documented by a high n-3 PUFA content in adipose tissue. Multiple linear regression analysis revealed that traditional factors such as treatment with β-blockers, smoking, age, and previous myocardial infarction were independently related to HRV, and furthermore that n-3 PUFAs (but not wine intake) were significantly independently associated with HRV.

Conclusions—The close positive association between n-3 PUFAs and HRV in patients suspected of having ischemic heart disease may indicate a protective effect of n-3 PUFAs against SCD. This may partly explain the reduction in SCD observed in humans with a modest intake of n-3 PUFA. Wine intake was also positively correlated with HRV, but this correlation was no longer significant after controlling for the cellular level of n-3 PUFA. (Circulation. 2001;103:651-657.)

Key Words: fatty acids ■ death, sudden ■ arrhythmia ■ nutrition ■ alcohol

Dietary n-3 polyunsaturated fatty acids (PUFAs) may reduce the incidence of sudden cardiac death (SCD), the most common cause of death in Western countries. Such a reduction was striking in the GISSI Prevenzione trial,1 in which patients with a previous myocardial infarction (MI) given n-3 PUFAs experienced a 45% reduction in the risk of SCD. A similar reduction in the risk of SCD by n-3 PUFA has been observed in other studies in patients without a previous MI.2,3

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The reduced incidence of SCD in relation to dietary n-3 PUFAs has been explained by an antiarrhythmic effect of n-3 PUFAs demonstrated in animal and in vitro studies.4–6 In addition, findings that heart rate variability (HRV), an independent predictor of mortality7–9 and arrhythmic events,10 is increased by n-3 PUFAs in humans11–14 may support an antiarrhythmic effect of n-3 PUFAs.

Another dietary approach suggesting a protective effect against cardiovascular death is wine drinking.15 The explanation for this effect is largely unknown, but among other factors, antioxidants present in wine may be beneficial.16 However, recent data also suggest that persons who drink wine may simply eat a healthier diet than abstainers and beer drinkers.17

The aim of the present study was to examine dietary habits concerning fish consumption and wine intake in patients referred for coronary angiography because of suspected ischemic heart disease (IHD) and to relate these findings to biomarkers of fish intake and to 24-hour HRV.

Methods

Study Population

From February 1997 to April 1999, 295 patients referred for elective coronary angiography due to suspected IHD were included in the study at the Department of Cardiology, Aalborg Hospital, Aalborg,
Denmark. Patients with the following conditions were not enrolled: (1) acute MI during the past 6 months; (2) cardiac surgery or angioplasty during the past 6 months; (3) nonischemic cardiomyopathy; (4) implanted pacemaker; or (5) permanent tachyarrhythmias.

At least 2 weeks before coronary angiography, the patients were examined at our outpatient clinic, where they completed a food questionnaire and where blood samples were taken, an adipose tissue biopsy sample was obtained, and an ambulatory 24-hour Holter recording was performed. The procedures followed in the study were approved by a regional ethics committee, and signed informed consent was obtained from all the patients.

### Food Questionnaire

Patients were asked about their fish consumption at lunch and at dinner. A score was given according to the following: never eat fish=1; eat fish once a month=2; eat fish 2 to 3 times a month=3; eat fish once weekly=4; eat fish 2 to 3 times a week=5; and eat fish at least once daily=6. Thus, the accumulated fish score for both lunch and dinner could range from 2 to 12.

Patients were also asked about their drinking habits. The questions were similar to those for fish consumption, ie, never drink wine=1, drink wine once a month=2, etc.

### Blood Samples

Blood samples were drawn after $\geq 10$ hours of fasting. Granulocytes were isolated from whole blood, and lipids were extracted and fatty acids esterified as described previously. The content of n-3 PUFAs in granulocytes and in adipose tissue was measured by gas chromatography with a Chrompack CP-9002 gas chromatograph (Chrompack International) and expressed as percent of total fatty acids.

### Adipose Tissue Biopsy

A subcutaneous adipose tissue biopsy sample was obtained in each patient by a previously described method, with the content of n-3 PUFAs determined after the fatty acids had been esterified and extracted as described above.

### HRV Analyses

A 24-hour Holter recording was obtained in each patient on a flash card with a 3-channel digital monitor. The recordings were analyzed with commercially available software from Diagnostic Monitoring, which also provided the flash cards and monitors. The following time-domain HRV variables were analyzed:

1. RR: mean of all normal RR intervals during the 24-hour recording.
2. SDNN: standard deviation of all normal RR intervals in the entire 24-hour recording.

### Statistical Analysis

Comparisons of differences between 2 groups were tested by nonpaired $t$ test for continuous variables (expressed as mean values and SD), whereas comparisons between more than 2 groups were analyzed by Kruskal-Wallis test. The $\chi^2$ test or Fisher exact test was used for discrete variables and frequencies. Nonparametric correlation coefficients (univariate) were calculated between HRV indices and parameters related to fish consumption and wine intake. To determine independent correlates of HRV, multivariate linear backward regression analysis was performed. A $P$ value $<0.05$ (2-tailed) was considered statistically significant.

### Results

### Patient Data

Four patients had to be excluded owing to technical HRV errors, leaving 291 patients available for final analysis. There were 106 women with a mean age of 59.6 years ($\pm 7$ years) and 185 men with a mean age of 60.0 years ($\pm 9$ years). Women tended to have lower HRV than men, reflected in an SDNN in women of 119 $\pm 31$ ms and in men of 126 $\pm 34$ ms ($P<0.05$). Also, patients with a previous MI had a lower SDNN (119 $\pm 31$ ms) than patients without a previous MI (126 $\pm 33$ ms, $P<0.05$). In Table 1, the 291 patients are characterized according to angiographic findings.

### Fish Consumption and n-3 PUFA

In Table 2, patients’ fish scores are given, as well as the content of marine n-3 PUFA (total n-3 PUFA, eicosapenta-
enoic acid, and docosahexaenoic acid [DHA]) in cell membranes in granulocytes and in adipose tissue. Increases in fish intake were reflected in increasing levels of n-3 PUFAs in both granulocyte membranes and adipose tissue (correlation coefficients ranging from 0.38 to 0.44, \( P < 0.001 \)).

**HRV and n-3 PUFA**

n-3 PUFAs are incorporated into the bilayer of cell membranes in the body. Significant positive correlations were found between n-3 PUFA–related parameters and HRV indices, and as shown in Table 3, these correlations were most profound between DHA levels in granulocytes and HRV.

In Table 4, the 291 patients have been characterized according to the content of DHA in granulocytes. When the patients were divided into DHA quartiles, the groups were comparable with respect to age, body mass index, left ventricular ejection fraction, cardiovascular medications used, number of smokers, number of patients with a previous MI, and degree of coronary artery disease. There was a trend toward greater HRV among those with the highest DHA content with significantly higher RR and SDNNindex (Table 4).

**HRV, Wine Intake, and Fish Consumption**

The HRV indices SDNN, SDNNindex, and SDANNindex increased with increasing intake of wine (\( r = 0.157, P = 0.01 \); \( r = 0.184, P < 0.01 \); and \( r = 0.132, P < 0.05 \), respectively), but patients with a high wine intake also had higher fish consumption, reflected by a higher concentration of DHA in adipose tissue (Figure). The linear correlation between SDNN, SDNNindex, and SDANNindex and wine intake became insignificant after controlling for the cellular level of DHA (data not shown). However, the positive correlation between DHA in granulocytes and the HRV indices SDNN, SDNNindex, and SDANNindex remained significant after controlling for wine intake (\( r = 0.128, P < 0.05 \); \( r = 0.155, P = 0.01 \); and \( r = 0.120, P < 0.05 \), respectively).

When patients with no wine intake were compared with those with daily wine intake, the 2 groups were comparable with respect to age, body mass index, ventricular ejection fraction, smoking habits, cardiovascular medications used, degree of coronary artery disease, and number of patients with a previous MI (data not shown). With regard to beer intake, no association was found with either HRV or fish intake.

**Independent Correlates of HRV**

In a multiple linear regression analysis, traditional modifiable and nonmodifiable parameters were included as independent factors along with factors related to fish intake. The dependent parameters in the analysis were HRV indices. From Table 5, it is seen that treatment with \( \beta \)-blockers, fish score,
and the content of n-3 PUFAs in adipose tissue and in granulocytes were each independently correlated with HRV indices; in addition, smoking, age, and previous MI independently had an impact on HRV.

**Discussion**

There is growing evidence that n-3 PUFA may reduce the risk of SCD.1–3,20 Our results show that patients suspected of having IHD who had a high level of DHA in their granulocyte membranes had a higher 24-hour HRV than patients with a low level of DHA in granulocyte membranes. This may suggest a protective effect of n-3 PUFAs against SCD, because low HRV is a strong marker of SCD and arrhythmic events,10 especially in patients with a previous MI but also in patients with stable IHD and preserved left ventricular function.21 The results were further substantiated by multiple regression analysis, in which not only were factors known to affect HRV8 (such as treatment with β-blockers, smoking, age, and previous MI) independently correlated with HRV indices, but more interestingly, n-3 PUFA–related factors were independent correlates.

HRV was also positively related to wine intake. This could in and of itself be an explanation for the better survival among wine drinkers compared with abstainers, but a more likely explanation may be that fish and wine intake are associated.17 Thus, patients with the highest wine intake also had the highest consumption of fish, as documented by the high n-3 PUFA content in adipose tissue, and the positive association between SDNN and wine intake was dependent of the cellular level of DHA. Thus, wine intake was not independently correlated with any of the HRV indices.

SCD is often the first manifestation of IHD and is responsible for ≈50% of the mortality from IHD in Western countries.22 The magnitude of the problem is illustrated by a

<table>
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<tr>
<th>TABLE 4. Patients Characterized According to Content of DHA in Granulocytes (Quartiles)</th>
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<tbody>
<tr>
<td>DHA Quartiles in Granulocytes</td>
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<tr>
<td>1st (n=73)</td>
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<tr>
<td>---------------------------------------------------------------</td>
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<tr>
<td>Men, n</td>
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<tr>
<td>Age, y</td>
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<tr>
<td>Body mass index, kg/m²</td>
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<tr>
<td>Ejection fraction</td>
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<td>Smokers, n</td>
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<td>Previous MI, n</td>
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<tr>
<td>Adipose tissue</td>
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<tr>
<td>EPA, %</td>
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<td>DHA, %</td>
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<td>HRV</td>
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<td>RR, ms</td>
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<td>SDNN, ms</td>
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<tr>
<td>SDNN index, ms</td>
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<tr>
<td>RMSSD, ms</td>
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<td>SDANN index, ms</td>
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<tr>
<td>PNN50, %</td>
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<tr>
<td>Cardiovascular medications, n</td>
</tr>
<tr>
<td>ACE inhibitors</td>
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<tr>
<td>Calcium inhibitors</td>
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<td>β-Blockers</td>
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<td>Nitrates</td>
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<td>Statins</td>
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<tr>
<td>Aspirin</td>
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<tr>
<td>Diuretics</td>
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<tr>
<td>Angiographic findings, n</td>
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<tr>
<td>0-vessel disease</td>
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<tr>
<td>1-vessel disease</td>
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<tr>
<td>2-vessel disease</td>
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<tr>
<td>3-vessel disease</td>
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<tr>
<td>1-, 2-, or 3-vessel disease</td>
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EPA indicates eicosapentaenoic acid. Values are mean (SD) or exact numbers.

*P<0.05, †P<0.01 (1st quartile vs 4th quartile, nonpaired t test).
recent British study reporting that 74% of fatal events among patients with heart disease happened outside the hospital. Because of their low cost and small number of side effects, n-3 PUFAs may thus play a role in the secondary prevention of SCD, not only for high-risk patients but for all patients suspected of having IHD.

In our patients, n-3 PUFAs were derived from fish, as demonstrated by the close positive association between fish intake and the level of n-3 PUFA in adipose tissue. Thus, adipose tissue DHA content is the biomarker of choice for the assessment of long-term habitual dietary intake of n-3 PUFAs from fish. The cell membrane phospholipid fatty acid composition of n-3 PUFAs, on the other hand, probably reflects the effect of dietary n-3 PUFA intake on changes responsible for the reduction in SCD and is merely a physiological marker of dietary n-3 PUFAs. This is in accordance with the significant positive correlations observed particularly between cellular DHA levels and HRV indices in our study. We previously reported a beneficial effect of n-3 PUFA on HRV in patients at high risk for SCD. In an intervention trial comprising 55 post-MI patients with a ventricular ejection fraction ≤0.40, 24-hour HRV significantly increased in the n-3 fatty acid group compared with the placebo group. Also, in these patients, a positive significant correlation was found between baseline levels of n-3 PUFAs in cell membranes and HRV. In addition, we found a positive association between cellular levels of n-3 PUFAs and HRV in patients with chronic renal failure and in healthy subjects in whom HRV increased in a dose-dependent way after dietary supplementation with n-3 PUFA.

The mechanism by which increased levels of n-3 PUFA affect HRV indices is not fully understood but may be explained in part by the antiarrhythmic properties of n-3 PUFAs observed in animal and in vitro studies. Considerable evidence exists that a change in the lipid composition of biological membranes is closely associated with alterations in their function, and in almost all studies with dietary supplementation with n-3 PUFAs, an increase in phospholipid eicosapentaenoic acid and DHA in cell membranes has been observed. Often, these alterations in the fatty acid composition of the cell membrane change the membrane fluidity, which may be crucial because the fluidity of the cell membrane may provide a dynamic basis for enzyme and receptor functions that secondarily leads to changes in Ca²⁺ fluxes across the cell membranes and may change the affinity of β-receptor complexes. Other mechanisms could also be involved in the antiarrhythmic actions of n-3 PUFAs (and actions on HRV), and further research in this area is needed.

Our results are in line with large human trials with hard end points that indicated an antiarrhythmic effect of n-3 PUFAs. In the recently published GISSI-Prevenzione trial, 11 324 patients with a previous MI were randomized to 0.85 g daily of fish oil or control for up to 3.5 years. A reduction in cardiovascular mortality by 15% to 20% and a 45% reduction in SCD was observed in the n-3 PUFA group. This trial thus confirmed the results from the landmark Diet And Reinfarc- tion Trial (DART) comprising >2000 men with a previous MI, in which men advised to eat fatty fish at least twice a week had a 29% reduction in total mortality after 2 years, which was thought to be due to an antiarrhythmic effect of n-3 PUFAs.

Two studies have related fish intake to the risk of SCD in patients without prior MI. A population-based case-control study with 334 cases of primary cardiac arrest and 493 controls concluded that dietary intake of n-3 PUFA equal to 1 fatty fish meal per week was associated with a 50% reduction in the risk of primary cardiac arrest compared with no intake of fish. In the US Physicians’ Health Study, 20 551 male physicians were followed for up to 11 years, and consumption of fish at least once a week was associated with a 52% reduction in the risk of SCD compared with men who...
TABLE 5. Linear Multiple Regression Analysis (Backward) With HRV Indices as Dependent Factors

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<tr>
<th>Medatable Factors</th>
<th>Nonmodifiable Factors</th>
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<tr>
<td></td>
<td>Medication</td>
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<tr>
<td>RR</td>
<td>β-Blocker†</td>
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<tr>
<td>SDNN</td>
<td>β-Blocker*</td>
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<tr>
<td>SDANNindex</td>
<td>β-Blocker†</td>
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<tr>
<td>RMSSD</td>
<td>β-Blocker*</td>
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<tr>
<td>PNN50</td>
<td>β-Blocker*</td>
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EPA indicates eicosapentaenoic acid.

Backward linear multiple regression analysis was conducted with the following independent modifiable factors: (1) body mass index; medical therapy with (2) ACE inhibitors, (3) β-blockers, or (4) calcium inhibitors; (5) wine intake; (6) tobacco use; (7) EPA in granulocytes [EPA(g)]; (8) DHA in granulocytes [DHA(g)]; (9) total n-3 PUFAs in granulocytes [PUFA(g)]; (10) EPA in adipose tissue [EPA(a)]; (11) DHA in adipose tissue [DHA(a)]; and (12) total n-3 PUFAs in adipose tissue [PUFA(a)]. Nonmodifiable factors included in the test were (13) age, (14) left ventricular ejection fraction, (15) previous MI, (16) sex, and (17) the extent of coronary artery disease. Significant independent factors are given.

*P<0.05, †P<0.01.

consumed fish less than monthly. In these 2 studies, increasing the fish intake further to more than once a week seemed not to influence the risk of SCD, although in the case-control study, the risk of SCD decreased linearly with increasing levels of n-3 PUFAs in cell membranes. The authors stated that the effect of dietary fatty acids on the risk of SCD was mediated through changes in cell membrane fatty acid composition.

Epidemiological data suggest that drinking wine is associated with decreased cardiovascular mortality. In a prospective population study, mortality decreased with increasing intake of wine, whereas this was not observed for intake of beer or spirits. However, a recent cross-sectional study including 48,763 men and women found that wine drinking including 48,763 men and women found that wine drinking increased fish intake among wine drinkers as a mutually beneficial factor. Further studies are needed, although an intervention trial with alcohol is not realistic.

Acknowledgments

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