N-3 fatty acids from fish and markers of cardiac arrhythmia

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Dit onderzoek is uitgevoerd binnen de onderzoekschool VLAG.

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Proefschrift

ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van Wageningen Universiteit
Prof. dr. ir. L. Speelman
in het openbaar te verdedigen
op woensdag 15 september 2004
des namiddags om half twee in de Aula

Geelen, Anouk
N-3 fatty acids from fish and markers of cardiac arrhythmia

Thesis Wageningen University – with references – with summary in Dutch

ISBN 90-8504-084-1

Aan mijn ouders

Abstract

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N-3 fatty acids from fish may protect against heart disease mortality by preventing fatal arrhythmias. The objective of this thesis was to investigate whether this possible antiarrhythmic effect of n-3 fatty acids is supported by short-term effects on electrophysiological markers. We performed two human intervention studies comparing the effects of fish oil capsules supplying 1.5 g very-long-chain n-3 fatty acids with placebo capsules on several markers of arrhythmia risk.

In the first intervention study, we observed no effect of n-3 fatty acids on heart rate variability and baroreflex sensitivity in an apparently healthy population. This finding does not support the hypothesis that n-3 fatty acids prevent arrhythmia in healthy subjects via effects on cardiac autonomic control. We also observed no effects of n-3 fatty acids on the standard electrocardiogram of these healthy humans. Additionally, supplementation with n-3 fatty acids did not lower C-reactive protein concentrations. This makes it less likely that beneficial effects on inflammation are involved in a mechanism explaining the protective effect on heart disease risk of n-3 fatty acids, although we cannot exclude an effect on elevated C-reactive protein concentrations during systemic inflammation.

In the second intervention study, we studied a marker closer to arrhythmia endpoints in a more susceptible population. We investigated the effect of n-3 fatty acids on premature ventricular complexes, a common form of arrhythmia that can provide the trigger for life-threatening arrhythmia. This study was conducted in patients with frequent premature ventricular complexes. N-3 fatty acids were not significantly effective in the treatment of premature ventricular complexes. This makes it less likely that n-3 fatty acids reduce the risk of sudden cardiac death through preventing triggers of arrhythmia. However, we did find a decrease in heart rate of 2.1 beat/min on n-3 fatty acids. Such a decrease may predict a risk reduction for sudden cardiac death of about 6% and that can partly explain the cardioprotective effect of n-3 fatty acids. Like in the study with healthy subjects, we did not find effects of n-3 fatty acids on the standard electrocardiogram in this more susceptible population.

The only effect found in our studies that would predict an effect of n-3 fatty acids on arrhythmia risk and subsequent sudden death is a decrease in heart rate. Whether intake of n-3 fatty acids from fish can indeed reduce the incidence of life-threatening cardiac arrhythmia remains to be resolved by clinical trials in high-risk patients.

Contents

Chapter 1

Introduction

RATIONALE

Evidence from earlier studies indicates that fish and its n-3 fatty acids can reduce the risk of heart disease.[1-3] Especially, intake of n-3 fatty acids may protect against fatal heart disease by preventing cardiac arrhythmias.[4-6] Cardiac arrhythmias are a major health problem; they are the main direct cause of sudden cardiac death.[7] Sudden cardiac death is the most common manifestation of coronary heart disease and is responsible for about 50% of the mortality from cardiovascular disease in the United States and other developed countries.[8] Risk is not limited to diagnosed patients; about half of the cases of sudden death do not have a history of previous heart disease. Thus, food substances that could reduce the risk of arrhythmias have a huge preventive potential.

We wanted to investigate whether and how dietary n-3 fatty acids from fish can prevent cardiac arrhythmias in humans and for that purpose we used markers of arrhythmia risk in short-term experiments. Short-term effects on biomarkers would provide clues as to possible underlying mechanisms of action and may also support positive health effects of n-3 fatty acids. Also, existing and new biomarkers that predict health outcome will provide efficient tools to test the bioefficacy of various food substances in future studies. Therefore, the major research question behind the studies described in this thesis was:

Is the possible antiarrhythmic effect of n-3 fatty acids supported by short-term effects on electrophysiological markers?

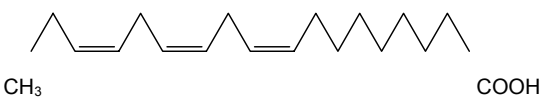
The next paragraphs give background information on n-3 fatty acids and their effect on cardiovascular disease and arrhythmia. This is followed by a description of electrophysiological markers of arrhythmia. Finally, the introduction ends with the outline of this thesis.

STRUCTURE, SOURCES AND INTAKE OF N-3 FATTY ACIDS

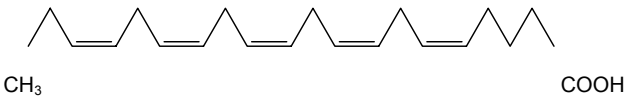
N-3 fatty acids, also called omega-3 fatty acids, are polyunsaturated fatty acids with the first double bond three carbons back from the methyl end (Figure 1.1). N-3 fatty acids are present in the diet in vegetable oils and nuts as alpha-linolenic acid (ALA; C18:3n-3) and in fish as very long-chain polyunsaturated fatty acids, primarily eicosapentaenoic acid (EPA; C20:5n-3) and docosahexaenoic acid (DHA; C22:6n-3). The essential fatty acid ALA can be converted to EPA and DHA by a series of desaturations and elongations. However, in humans the conversion of ALA to EPA

and DHA is limited.[9-12] Table 1.1 presents EPA + DHA content of various fish products.[13]

In the US, Europe and Australia, the mean intake of ALA varies between 1 and 2 g per day and that of very long-chain fatty acids from fish between 0.1 and 0.5 g per day.[14-16] In the Netherlands, the recommendation for the dietary intake of ALA is 1% of energy intake and for n-3 fatty acids from fish 0.2 g per day.[17] In this thesis, we will focus on the very long-chain n-3 fatty acids from fish unless otherwise mentioned.



Alpha-linolenic acid (ALA, C18:3n-3)



Eicosapentaenoic acid (EPA, C20:5n-3)

Docosahexaenoic acid (DHA, C22:6n-3)

Figure 1.1 Structure of the n-3 fatty acids alpha-linolenic acid, eicosapentaneoic acid and docosahexaenoic acid. These polyunsaturated fatty acids have the first double bond three carbons back from the methyl end.

N-3 FATTY ACIDS AND MECHANISMS OF PREVENTION OF CARDIOVASCULAR DISEASE

Human observational studies and clinical trials provide strong indications that n-3 fatty acids from fish can prevent cardiovascular disease.[1-3;18;19] However, multiple mechanisms could be involved. The triglyceride-lowering effects of 3 to 5 g/d of n-3 fatty acids are well established.[20] Also, high doses n-3 fatty acids seem to have a

small, dose-dependent effect on blood pressure, especially in hypertensive populations.[21;22] Furthermore, n-3 fatty acids modestly prolong bleeding time, but their effect on thrombosis remains unclear.[23] Additionally, possible effects of n-3 fatty acids on endothelial function, arterial compliance, nitric oxide production, adhesion molecules, inflammatory mediators, eicosanoids and LDL oxidation are mentioned that may contribute to an antiatherogenic effect of n-3 fatty acid.[24] Altogether, most effects on intermediates are not consistent among studies or only seen with high intakes of n-3 fatty acids. However, low to moderate doses of n-3 fatty acids are associated with protection in observational studies.[1;18;19] Intervention studies show an effect of n-3 fatty acids on mortality and sudden cardiac death, which is already visible after only a few months, and no effect on non-fatal events.[4;6] This suggests an effect of n-3 fatty acids on arrhythmia rather than on atherosclerosis or thrombosis. In animal studies, n-3 fatty acids prevented and reduced the severity of arrhythmias, increased the threshold for ventricular fibrillation and improved the electrical stability of animal hearts.[25-29] The n-3 fatty acids also prevented and terminated tachyarrhythmias in cultured neonatal cardiomyocytes.[30;31] Therefore, in our opinion, the most likely hypothesis is that n-3 fatty acids from fish reduce the risk of fatal coronary heart disease through antiarrhythmic effects.

Table 1.1 Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in fish products

Species	Gram EPA + DHA per 100 gram
Mackerel	2.1 to 6.0
Salmon	1.3 to 2.8
Sardines	2.1
Herring	1.1 to 2.6
Conger (eel)	0.8 to 1.4
Trout	1.1
Mussels	0.6 to 0.9
Shrimp	0.4 to 0.8
Haddock, cod, whiting	0.4 to 0.6
Flat-fish (flounder, sole, dab)	0.3 to 0.7
Tuna	0.4

There are several mechanisms by which n-3 fatty acids could affect arrhythmia risk. In vitro studies suggest that n-3 fatty acids directly affect ion transport through heart cell membranes, which is essential for heart rhythm.[32] Such effects are expected to produce detectable and relevant changes in the electrophysiology of the whole heart in living organisms, but this has hardly been studied. Studies on heart rate variability, a predictor of arrhythmia risk, suggest that n-3 fatty acids may work via effects on the autonomic nervous system that controls heart rhythm.[33;34] Thus, the mechanisms by which n-3 fatty acids could affect arrhythmias are not clear.

ELECTROPHYSIOLOGICAL MARKERS OF ARRHYTHMIA

In this thesis, we use the term "*electrocardiographic characteristics*" for measurements within one heartbeat derived from a standard 12-lead electrocardiogram, i.e. heart-rate corrected QT duration, QRS duration, apex-to-end-T duration, amplitude T loop, width T loop, spatial QRS-T angle, and spatial U-wave amplitude. We use the term "*electrophysiological markers*" for the broader group of potential predictors of arrhythmia not only derived from the standard electrocardiogram, but also from the 24-hour Holter recording and from an electrocardiogram combined with blood pressure measurements. Short-term intervention studies on effects of n-3 fatty acids on electrophysiological markers of arrhythmia may provide indications for antiarrhythmic effects. In this thesis, we focus on heart rate variability, baroreflex sensitivity, electrocardiographic characteristics and premature ventricular complexes.

Heart Rate Variability

In healthy subjects, the sinus node initiates each beat of the heart at a relatively constant frequency. Input of the autonomic nervous system can modify this rate. The sympathetic nerve has an accelerating effect, while the parasympathetic nerve slows heart rate down as needed. Thus, the frequency of the heartbeat varies over time, constantly responding to various biological conditions. Heart rate variability reflects autonomic control of the heart and is quantified by analysis of variations of the intervals between consecutive normal heartbeats. A high variability in heart rate is an indication of good adaptive ability, indicating a healthy person with well functioning autonomic control mechanisms.

Table 1.2 Selection of time and frequency domain measures of heart rate variability

Variable	Description	Physiological explanation
Time domain		
SDNN (ms)	Standard deviation of all normal RR intervals	Estimate of overall heart rate variability, influenced by both short-term (for example, respiratory) and long-term (for example, circadian) factors.
SDANN (ms)	Standard deviation of the mean of all 5-min segments of normal RR intervals of the entire recording	Estimate of long-term variation, influenced by both short-term and long-term factors.
RMSSD (ms)	The square root of the mean of the sum of the squares of differences between adjacent normal RR intervals over the entire recording	Estimate of short-term variation, predominantly reflects parasympathetic tone.
Frequency domain		
HF (ms^2)	High frequency power (0.15-0.40 Hz)	Parasympathetically mediated, represents primarily respiratory variation.
LF (ms^2)	Low frequency power (0.04-0.15 Hz)	Modulated by both sympathetic and parasympathetic nervous systems, strongly affected by the oscillatory rhythm of the baroreceptor system.
VLF (ms^2)	Very low frequency power (0.003-0.04 Hz)	Not well defined.
Total power (ms^2)	Variance of all normal RR intervals	Total variance in the signal.

Heart rate variability analysis can be performed on short electrocardiogram segments (0.5 to 5 minutes) or on 24-hour electrocardiogram registration (Holter monitoring). There are two approaches to measure heart rate variability: analysis in the time or in the frequency domain (Table 1.2). Time domain values result from statistical calculations performed on the set of interbeat intervals. Frequency domain analysis

yields information about the amount of the overall variance in heart rate resulting from periodic oscillations of heart rate at various frequency bands.[35;36] The evaluation of heart rate variability is an established tool for the assessment of cardiac autonomic status. Low heart rate variability predicts mortality, sudden cardiac death, and arrhythmic events in postinfarction patients[37-41] and mortality and cardiac events in the general population.[42-45] Depressed heart rate variability reflects the predominance of sympathetic activity. This may compromise cardiac electrical stability, which may favor the development of ventricular fibrillation in the presence of an arrhythmic substrate.

Baroreflex Sensitivity

The baroreflex is a negative feedback system that buffers blood pressure. Baroreceptors are pressure-sensitive nerve endings mainly found in the wall of the aortic arch and in the carotid sinuses. The baroreflex is stimulated by a blood pressure rise. This results in a reduction of heart rate and cardiac contractility and consequently a fall in blood pressure. A decrease in blood pressure has opposite effects. Baroreflex sensitivity, the response of heart rate to a given change in systolic blood pressure,[46] is an indicator of cardiac autonomic regulation.

Baroreflex sensitivity can be calculated using various indirect techniques by measuring changes in heart rate (in ms) against the changes in blood pressure (in mmHg). Baroreflex sensitivity can be assessed by analyzing the spontaneous fluctuations of arterial pressure during metronome respiration.[47] The slope of the linear relationship between the length of RR intervals and blood pressure represents baroreflex sensitivity. Low baroreflex sensitivity predicts mortality in patients with chronic heart failure[48;49] and cardiac and arrhythmic mortality in postinfarction patients.[41;50;51]

Electrocardiographic characteristics

An electrocardiogram is a recording of the electrical activity of the heart from electrodes placed on the skin in specific locations (Figure 1.2A). Apparent effects on the electrophysiology of the heart may be detectable in a surface electrocardiogram of humans. Several characteristics that indicate specific cardiac activity can be measured from electrocardiographic tracings. For example, the QT interval on the electrocardiogram reflects the duration of activation and recovery of the ventricular muscle and may be a relevant measure for arrhythmia risk. In the general population, several studies, although not all,[52] showed that subjects with a longer duration of QTc

have an increased risk of all-cause and cardiac mortality.[53-55] Furthermore, QRS duration is a measure of ventricular depolarization. Apex-to-end-T duration,[56;57] T-loop morphology,[58] and the spatial QRS-T angle[59;60] have been proposed as markers of the heterogeneity of ventricular repolarization, which provides the condition for the genesis of ventricular arrhythmias.

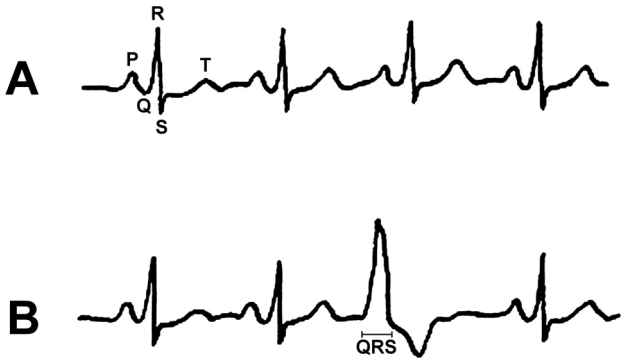


Figure 1.2 A. Normal electrocardiogram. B. Electrocardiogram with a premature ventricular complex. The premature ventricular complex comes too early and can be recognized by its wide QRS-complex. Usually a long compensatory pause follows the premature ventricular complex.

Premature Ventricular Complexes

Premature ventricular complexes, a rather common form of arrhythmia, arise from the ventricular myocardium rather than from the normal pacemaker, the sinus node. As a result, there is a disruption of the normal rhythm (Figure 1.2B). Clinical symptoms depend on the frequency of the premature ventricular complexes. As premature ventricular complexes may provide the trigger for a more serious arrhythmic event, it is mechanistically plausible that changes in premature ventricular complexes are relevant for risk. Premature ventricular complexes can be an indication that a so-called normal heart has a group of cells with enhanced automaticity.[61] Thus a reduction in premature ventricular complexes results in fewer triggers and may in that way decrease the risk of ventricular tachycardia and ventricular fibrillation. It should be noted however, that effects on premature ventricular complexes do not necessarily imply effects on endpoints. In the Cardiac Arrhythmia Suppression Trial (CAST),

encainide and flecainide reduced the number of premature ventricular complexes, but increased rather than decreased mortality.[62]

Furthermore, frequent premature ventricular complexes are independent predictors of sudden cardiac death and mortality in survivors of myocardial infarction.[63;64] Also in middle-aged men without cardiovascular disease, frequent premature ventricular complexes during exercise predicted long-term risk of cardiovascular death.[65]

OUTLINE OF THIS THESIS

We performed two human intervention studies on the effects of n-3 fatty acids on markers of arrhythmia. We describe the results in this thesis. Before that, a literature review of human studies addresses the hypothesis that n-3 fatty acids reduce the risk of fatal coronary heart disease through antiarrhythmic effects (**chapter 2**).

In the first intervention study, we investigated the effect of supplemental intake of n-3 fatty acids on heart rate variability and baroreflex sensitivity in apparently healthy subjects (**chapter 3**). In this way, we tested whether n-3 fatty acids could prevent risk of arrhythmia through effects on cardiac control by the autonomic nervous system. We also studied the effect of n-3 fatty acids on several electrocardiographic characteristics in these healthy subjects (**chapter 4**). Demonstration of such effects could provide clues concerning how n-3 fatty acids may prevent arrhythmia and sudden cardiac death. They might also suggest biomarkers, which could be used to study potentially antiarrhythmic food ingredients in humans.

In the second intervention study, we studied a marker closer to arrhythmia endpoints in a more susceptible population. We investigated the effect of n-3 fatty acids on premature ventricular complexes, a common form of arrhythmia that can provide the trigger for life-threatening arrhythmia, in patients with frequent premature ventricular complexes. Demonstration of such an effect would provide clues as to possible underlying mechanisms and it may also add to the evidence that n-3 fatty acids prevent fatal heart disease by preventing serious ventricular arrhythmia. In addition, we studied whether n-3 fatty acids were able to reduce heart rate, as measured by continuous 24-hour monitoring, and in this way reduce the risk of sudden death (**chapter 5**). Also in this population, we studied the effect of n-3 fatty acids on several electrocardiographic characteristics (**chapter 6**).

High-sensitivity C-reactive protein, a marker of systemic inflammation, is a powerful predictor of cardiovascular risk. Dietary effects on markers of inflammatory processes

have hardly been studied and are not yet established.[66] Yet the inflammatory component may influence atherosclerotic plaque stability and thereby occurrence of acute myocardial infarction and sudden death. Therefore, we used serum samples of the first intervention study to investigate whether n-3 fatty acids reduce C-reactive protein concentrations in healthy middle-aged subjects (**chapter 7**).

The final chapter addresses the main outcome and implications of our studies and discusses the use of markers of arrhythmia and recommendations for future research (**chapter 8**).

Chapter 2

Antiarrhythmic effects of n-3 fatty acids: evidence from human studies

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Current Opinion in Lipidology 2004;15:25-30

ABSTRACT

Purpose of review

N-3 fatty acids from fish reduce cardiovascular mortality including sudden cardiac death. In this paper, the authors discuss the results of human studies with regard to the hypothesis that n-3 fatty acids reduce the risk of fatal coronary heart disease through antiarrhythmic effects.

Recent findings

Results from two recent clinical trials do not support a protective effect of n-3 fatty acids. In light of the earlier published bulk of evidence that n-3 fatty acids reduce cardiovascular mortality and sudden cardiac death, it is hard to explain these findings. Two recent observational studies confirmed that intake of n-3 fatty acids from fish is associated with less cardiovascular disease in the general population. They indicated that the protective effect of a fish meal may depend on the n-3 fatty acid content or preparation method and suggested a protective effect on arrhythmia rather than on atherosclerosis. Intervention studies on electrophysiological predictors of arrhythmia do not clearly confirm a beneficial effect of n-3 fatty acids. However, most of these studies were small or performed in healthy populations.

Summary

The available evidence still suggests that n-3 fatty acids may prevent fatal cardiac arrhythmia, but more conclusive studies are urgently needed.

INTRODUCTION

Human observational studies and clinical trials suggest that low to moderate intakes of fish or its n-3 fatty acids (0,2 - 1 g/day) reduce cardiovascular mortality and sudden cardiac death.[1-3;18] Several intermediates in the cardioprotective effect of n-3 fatty acids have been proposed. However, most effects on intermediates are not consistent among studies or only seen with high intakes of n-3 fatty acids. For example, high doses (approximately 4 g/day) of n-3 fatty acids reduce serum triglyceride concentrations,[20] but the low doses of fish oil associated with protection in observational studies have only minimal effects on serum triglycerides. The same holds for blood pressure: high intake of n-3 fatty acids may lower blood pressure, especially in older and hypertensive populations, but the antihypertensive effect of fish oil doses in the normal dietary range remains to be established.[67] Effects on bleeding time and hemostatic factors also require high intakes (2 g or more) of n-3 fatty acids.[23] Therefore, low to moderate doses of n-3 fatty acids can prevent cardiac death, but do not clearly affect conventional predictors of atherosclerosis. In the authors' opinion, the most likely hypothesis is that n-3 fatty acids from fish reduce the risk of fatal coronary heart disease through antiarrhythmic effects. In this paper, the authors discuss the results of human studies on n-3 fatty acids with regard to this hypothesis.

CLINICAL TRIALS ON ENDPOINTS

Four trials have been performed on n-3 fatty acids from fish in the secondary prevention of myocardial infarction. Treatment with 1 g/day n-3 fatty acids significantly lowered the combined risk of mortality, non-fatal myocardial infarction and stroke by 10-15% after 3.5 years in the GISSI-Prevenzione Trial.[3] In a very small trial in India, there were fewer cardiac deaths in the fish oil group than with placebo (11.4 versus 22.0%) after 1 year.[68] In the Diet and Reinfarction Trial (DART), reduced mortality (29%) was seen after the 2-year intervention in those advised to eat fish.[4] However, the long-term, post-trial follow-up data showed that the reduction in mortality was followed by an increased risk (hazard ratio 1.31) over the next 3 years.[69] Also, another recent publication presented results that are not in line with a protective effect of n-3 fatty acids. Advice to eat two portions of fatty fish each week or to take 3 g of fish oil daily was associated with higher risk of cardiac death (HR 1.26) and sudden cardiac death (HR 1.54) in 3114 men with angina.[70] This excess risk was largely seen in the subgroup given fish oil capsules. Unfortunately, compliance to advice was only shown for a subsample of patients and because the trial was not blind, the intake of fish oil

may have modified the patient's or the physician's behavior towards intake of medication or diet and lifestyle. In light of all the earlier evidence it is hard to explain these adverse results.

In the clinical trials that do show a beneficial effect of moderate intakes of n-3 fatty acids (approximately 1 g/day) on mortality and sudden cardiac death, this effect is seen within a few months after starting treatment, with no or a much smaller effect on non-fatal events.[4;6;68] This suggests an immediate effect on arrhythmia rather than a slow effect via regression of atherosclerosis. However, the recent negative findings[69;70] indicate that the evidence is not conclusive and that more clinical trials are needed.

OBSERVATIONAL STUDIES

The majority, but not all, of about a dozen prospective cohort studies show that intake of a low to moderate amount of fish is associated with a reduction of cardiovascular mortality in the general population and that n-3 fatty acids appear to be responsible for this effect.[19;71] The Physicians' Health Study reported that eating fish at least once per week was associated with a reduced risk of sudden cardiac death (RR 0.48), but not with myocardial infarction.[18] In particular, men with blood levels of n-3 fatty acids in the fourth quartile had an 81% lower risk of sudden cardiac death than those in the lowest quartile.[72] This supports the idea that n-3 fatty acids are the fish compound responsible for the protective effect. Also, a population-based case-control study[2] found a strong inverse association between red blood cell membrane n-3 fatty acid levels and the risk of sudden cardiac death.

Two recent observational studies provide further evidence for a protective effect of n-3 fatty acids. Mozaffarian *et al.*[73] showed that cardiac benefits of fish consumption might vary depending on the type of fish meal and its n-3 fatty acid content. In the Cardiovascular Health Study, a population-based prospective cohort study of adults aged 65 years or older, consumption of tuna or other broiled or baked fish was associated with lower risk of fatal coronary heart disease and arrhythmic death but not non-fatal myocardial infarction. On the other hand, consumption of fried fish and fish sandwiches was associated with trends toward higher risk.[73] This may be due to the low n-3 fatty acid content of such fish, the unfavorable effects of frying, or the ingestion of mercury. Tuna or other fish once per week provided about 267 mg/day of n-3 fatty acids, while fried fish and fish sandwiches, typically low in n-3 fatty acids and not associated with n-3 plasma phospholipid levels, provided only 64 mg/day. This supports the concept that n-3 fatty acids in fish oil are responsible for the protective

effect on heart disease. Also, a case-control study nested in the Cardiovascular Health Study showed that higher plasma phospholipid levels of n-3 fatty acids were associated with a lower risk of fatal coronary heart disease, but not with non-fatal myocardial infarction.[74] This again suggests an effect of n-3 fatty acids on arrhythmia rather than on atherosclerosis.

Together, observational studies show a strong relationship of n-3 fatty acids with fatal coronary heart disease and sudden cardiac death, but not with non-fatal heart disease. This is consistent with antiarrhythmic effects of n-3 fatty acids.

STUDIES ON ELECTROPHYSIOLOGICAL PREDICTORS OF ARRHYTHMIA

Indications for antiarrhythmic effects of n-3 fatty acids may also result from short-term intervention studies on electrophysiological predictors of arrhythmia. Here the authors focus on effects of fish oil on heart rate variability, baroreflex sensitivity and premature ventricular complexes (Table 2.1).

Heart rate variability and baroreflex sensitivity

Isolated human hearts beat at a frequency of about 90-100 beats per minute. *In vivo* this rate is modified by the input of the sympathetic and parasympathetic nerves, the so-called autonomic control, which speeds up or slows down heart rate as needed. Both heart rate variability and baroreflex sensitivity reflect autonomic control of the heart. Heart rate variability is quantified by analysis of variations of the intervals between consecutive normal heart beats. A high variability in heart rate is an indication of good adaptability, implying a healthy person with well functioning autonomic control mechanisms. Baroreflex sensitivity is the response of heart rate to a change in blood pressure.[46] Low heart rate variability and low baroreflex sensitivity are powerful predictors of mortality, sudden cardiac death, and arrhythmic events in post-infarction patients.[38;40;50;51] Heart rate variability also predicts mortality and cardiac events in the general population.[43;44]

The effect of n-3 fatty acids on heart rate variability has been investigated in several studies. A positive association between blood levels of n-3 fatty acids and heart rate variability was found in several patient populations,[75-77] as well as in healthy subjects.[33;78] Also, intervention studies by Christensen *et al.* suggest that n-3 fatty acids increase heart rate variability, particularly in post-infarction patients (Table

2.1).[33;34;79] Villa *et al.*[80] carried out a small study in 10 myocardial infarction patients who were treated with 3 and 6 g/day n-3 fatty acids for 4 weeks each, in a different sequence. This study suggested some improvement, but because a placebo group was lacking, the value of this finding is unclear. In contrast, in a study of Geelen *et al.* [81] in 74 healthy middle-aged subjects, heart rate variability did not significantly improve after 12 weeks on 1,5 g/day n-3 fatty acids. Together, these studies show no consistent effect of n-3 fatty acids on heart rate variability (Table 2.1).

To the authors' knowledge, the effect of n-3 fatty acids on baroreflex sensitivity has only been investigated in two studies (Table 2.1). Geelen *et al.*[81] did not find an effect of 3.5 g/day fish oil for 12 weeks versus placebo on baroreflex sensitivity in 73 healthy middle-aged men and women. Weisser *et al.*[82] supplemented 10 participants with 6 g of n-3 fatty acids daily for 6 weeks and measured baroreflex sensitivity before and after supplementation. Their results suggested that n-3 fatty acids increased baroreflex sensitivity. However, they did not use a concurrent placebo control group. It is possible that the change in baroreflex sensitivity was due to chance or caused by adaptation of participants to the measurement procedure.

Thus, human intervention studies on heart rate variability and baroreflex sensitivity show no consistent evidence for an effect of n-3 fatty acids on cardiac autonomic control. However, most studies were conducted in healthy participants. The one study performed in post-infarction patients did find an effect.[34] Post-infarction patients have a high risk of arrhythmia and a suppressed autonomic function. Thus, it is possible that n-3 fatty acids affect heart rate variability and baroreflex sensitivity only in a more susceptible population of post-infarction patients. Alternatively, n-3 fatty acids prevent arrhythmia by a mechanism independent of cardiac autonomic control.

Table 2.1 Human studies on the effect of n-3 fatty acids on potential electrophysiological predictors of arrhythmia

Study	Subjects	Design	Dose n-3 fatty acids	Duration (weeks)	Conclusion
Heart rate variability					
Christensen et al.[34]	49 postinfarction patients	parallel	5.2 g/day	12	HRV improved significantly
Christensen et al.[79]	17 patients with chronic renal failure	parallel	5.2 g/day	12	No comparison between groups because of low number of patients
Christensen et al.[33]	60 healthy subjects	parallel	2.0 or 6.6 g/day	12	HRV improved in subgroup of men with low baseline HRV
Geelen et al.[81]	74 healthy subjects	parallel	1.5 g/day	12	No effect of n-3 fatty acids on HRV
Villa et al.[80]	10 myocardial infarction patients	before-after	3 and 6 g/day	4	Improvement of HRV within groups
Baroreflex sensitivity					
Geelen et al.[81]	73 healthy subjects	parallel	1.5 g/day	12	No effect of n-3 fatty acids on BRS
Weisser et al.[82]	10 normotensive subjects	before-after	6 g/day	6	Improvement of BRS within the small group
Premature ventricular complexes					
Hardarson et al.[84]	18 myocardial infarction patients	cross-over	20 mL cod liver oil	6	No effect of n-3 fatty acids on PVCs
Christensen et al.[85]	19 patients with ventricular tachycardia	parallel	5.2 g/day	16	Nonsignificant reduction in PVCs
Christensen et al.[86]	49 myocardial infarction patients	parallel	5.2 g/day	12	No effect of n-3 fatty acids on PVCs
Sellmayer et al.[87]	68 patients with >2000 PVCs per 24 hr	parallel	2.4 g/day	16	N-3 fatty acids decrease number of PVCs

HRV, heart rate variability; BRS, baroreflex sensitivity; PVC, premature ventricular complex.

Premature Ventricular Complexes

Premature ventricular complexes are the result of electrical impulses arising from one of the cardiac ventricles before the next expected heart beat, that is, prematurely. As a result, the subsequent rhythm is irregular. Clinical symptoms depend on the frequency of the premature ventricular complexes. As premature ventricular complexes are often the triggers of more serious arrhythmias, it is mechanistically plausible that changes in premature ventricular complexes are relevant for risk. It should be noted, however, that effects on premature ventricular complexes do not equate to effects on endpoints. In the Cardiac Arrhythmia Suppression Trial (CAST), encainide and flecainide reduced the number of premature ventricular complexes, but increased rather than decreased mortality.[83] Nevertheless, frequent premature ventricular complexes are independent predictors of sudden cardiac death and mortality in survivors of myocardial infarction.[63;64] Also in middle-aged men without cardiovascular disease, frequent premature ventricular complexes during exercise predicted long-term risk of cardiovascular death.[65]

Premature ventricular complexes are forms of arrhythmia, and it may be hypothesized that n-3 fatty acids decrease their incidence. This was indeed found by Sellmayer *et al.*[87] They tested the effects of n-3 fatty acids in 68 patients with a minimum of 2000 spontaneous premature ventricular complexes per 24 h. The proportion of patients with a reduction in premature ventricular complexes of over 70% was 44% after fish oil versus 15% in the placebo group (P <0.01). However, three smaller studies in different patient populations did not reveal an effect of n-3 fatty acids on premature ventricular complexes (Table 2.1).[84-86] The numbers of premature ventricular complexes were rather low in these studies, which makes it hard to detect an effect because of the large spontaneous variability that exists in the occurrence of premature ventricular complexes. Thus, an effect of n-3 fatty acids on premature ventricular complexes was only found in a relatively large study in patients with high numbers of premature ventricular complexes.

Heart rate and electrocardiographic characteristics

Another supposed predictor of sudden death is high heart rate.[88] A recent observational study reported an inverse association between n-3 fatty acids and heart rate.[89] However, the evidence from intervention studies on n-3 fatty acids and heart rate is not conclusive.[90-92] Furthermore, the apparent effects on the electrophysiology of the heart, as suggested by the observed reduction in risk of sudden cardiac death, may be detectable in a surface electrocardiogram of humans. However, Geelen *et*

al.[93] observed no effect of 3.5 g/day fish oil for 12 weeks compared with placebo on several electrocardiographic characteristics within one heart beat, including QT interval duration, in 84 healthy middle-aged men and women.

POSSIBLE MECHANISMS OF ACTION

Although the human data suggest that n-3 fatty acids may be antiarrhythmic, it is as yet unclear how they could affect cardiac electrophysiology at the cellular or molecular level. N-3 fatty acids may have an indirect effect through cardiac control by the autonomic nervous system, as discussed above. However, it is unclear how n-3 fatty acids would specifically affect cardiac autonomic control. N-3 fatty acids may also have direct effects on electrophysiological processes in the cardiac muscle. They could exert a direct effect by incorporation into cardiac membrane phospholipids.[94] This may increase membrane fluidity.[95] Also, n-3 fatty acids may affect sodium[96-98] and calcium[99;100] currents through heart cell membranes, which are essential for heart rhythm. The n-3 fatty acids are thought to prolong the duration of the inactivated state of these channels and inhibit their conductance.[32] N-3 fatty acids may also improve calcium handling by the cell.[101;102] Finally, incorporation of n-3 fatty acids into cardiac membrane phospholipids might influence the production of a variety of eicosanoids that may lower vulnerability to arrhythmias and in this way prevent ventricular fibrillation during myocardial ischemia and reperfusion.[103]

CONCLUSION

Observational and trial evidence suggests that n-3 fatty acids may prevent sudden cardiac death by suppressing life-threatening cardiac arrhythmia. Although this is an attractive and promising hypothesis, it has not yet definitively been proven. At least three ongoing clinical trials, by the authors' group[104] and by colleagues in Boston and Portland, directly study the effect of n-3 fatty acids from fish on the incidence of life-threatening cardiac arrhythmia. In all three trials, patients with an implantable cardioverter defibrillator, who are at high risk of arrhythmia, receive either fish oil or placebo. The main outcome of the trials is spontaneous ventricular tachyarrhythmia as recorded by the defibrillator. An implantable cardioverter defibrillator detects cardiac arrhythmia, treats it with an electric shock, and keeps a record of all events in its memory chip. This makes it possible to monitor occurrence of arrhythmic events throughout the entire study period and thereby determine the effectiveness of fish oil.

If these trials show that n-3 fatty acids protect cardiac patients from arrhythmia then a benefit for healthy people will also become very likely.

Chapter 3

Effect of n-3 fatty acids on heart rate variability and baroreflex sensitivity in middle-aged subjects

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American Heart Journal 2003;146:e4

ABSTRACT

Background

N-3 fatty acids may reduce the risk of sudden death by preventing life-threatening cardiac arrhythmias. Heart rate variability (HRV) and baroreflex sensitivity (BRS) reflect cardiac autonomic regulation; reduced values predict arrhythmic events and mortality. Effects of n-3 fatty acids on these risk indicators of arrhythmia have not been widely studied. We investigated the effect of supplemental intake of n-3 fatty acids on HRV and BRS in apparently healthy subjects aged 50 to 70 years.

Methods

After a run-in period of 4 weeks, 84 subjects were randomized to receive capsules with either 3.5 g of fish oil or placebo oil daily for 12 weeks. Before and after intervention, electrocardiograms and blood pressure were recorded for 10 minutes with standardized respiration of 15 breaths/min. The SD of the duration of all normal RR intervals ($SDNN_{10}$) and the root mean square successive differences ($RMSSD_{10}$) were calculated from the 10-minute recordings. We also computed low frequency power (LF) and high frequency power (HF). BRS was computed by integrating the spectral systolic blood pressure to interbeat-interval transfer function in the 0.05- to 0.15-Hz band.

Results

The different measures of HRV and BRS did not significantly improve with the intake of n-3 fatty acids. $SDNN_{10}$ decreased by 3.05 ms or 7.7% (95% CI, -8.91 to 2.82 ms), and BRS decreased by 0.92 ms/mm Hg or 0.1% (95% CI, -2.66 to 0.81 ms/mm Hg) in the fish oil group compared with the placebo group.

Conclusions

N-3 fatty acids have no effect on HRV from short-term recordings and BRS in apparently healthy subjects.

INTRODUCTION

Sudden cardiac death is the most common manifestation of coronary heart disease and is responsible for about 50% of the mortality from cardiovascular disease in the United States and other developed countries.[8] Most sudden deaths are caused by acute ventricular tachyarrhythmias.[7] Intake of n-3 fatty acids has been found to protect against coronary heart disease, especially fatal events and sudden cardiac death.[3;4] This suggests that n-3 fatty acids exert their protective effect through lowering the susceptibility for arrhythmias. Animal experiments and *in vitro* studies support this hypothesis,[5] but direct evidence in humans is lacking. Possible underlying mechanisms might involve direct electrophysiological effects of n-3 fatty acids on heart cell membranes or effects through cardiac autonomic control that may be observed by changes in heart rate variability (HRV) or baroreflex sensitivity (BRS). Depressed HRV and BRS reflect the predominance of sympathetic activity. This may compromise cardiac electrical stability, which may favor the development of ventricular fibrillation in the presence of an arrhythmic substrate.

The evaluation of HRV is an established tool for the assessment of cardiac autonomic status. Reduced HRV is predictive of mortality and arrhythmic events in patients after infarction[37-41] and mortality and cardiac events in the general population.[42-45] We showed a positive association between HRV and blood levels of docosahexaenoic acid in healthy subjects.[78] Christensen et al. also showed positive associations between HRV and n-3 fatty acids in granulocytes and platelets in several patient groups[75-77] and in healthy subjects.[33] In a double-blind intervention trial in patients after infarction, Christensen et al. found an increase in HRV after 12 weeks of supplementation with n-3 fatty acids.[34]

The baroreflex buffers blood pressure. BRS, the response of heart rate to a given change in systolic blood pressure,[46] is another indicator of cardiac autonomic regulation. Depressed BRS is predictive of mortality in patients with chronic heart failure[48;49] and cardiac and arrhythmic mortality in patients after infarction.[41;50;51] One small, uncontrolled study suggested that n-3 fatty acids may increase BRS in healthy subjects.[82]

A favorable effect of n-3 fatty acids on arrhythmia risk in the general population would have an enormous preventive potential. Previous studies on HRV and BRS suggest that n-3 fatty acids may prevent cardiac arrhythmia via effects on the autonomic nervous system. However, this hypothesis needs elaboration and confirmation. Therefore, the aim of this study was to investigate the effect of n-3 fatty acids on risk

indicators of arrhythmia that reflect autonomic cardiac control, HRV and BRS, in apparently healthy subjects.

SUBJECTS AND METHODS

Subjects

The Medical Ethical Committee of Wageningen University and Research Centre approved the study protocol. Subjects gave their written informed consent after we explained the study protocol to them. Subjects were eligible when they were 50 to 70 years old and did not use drugs known to affect heart rhythm or autonomic regulation. In addition, subjects with past or present cardiovascular disease were excluded, as were subjects with diabetes mellitus, asthmatic complaints, mean systolic blood pressure >170 mm Hg, or mean diastolic blood pressure >100 mm Hg. Female subjects had to be post-menopausal.

At the start of the run-in period, we recorded a resting 12-lead electrocardiogram and performed blood pressure measurements for screening purposes in 97 subjects. To assess their habitual fish consumption, subjects were interviewed using a questionnaire on the frequency of fish intake. Eight subjects were excluded from participation because abnormalities in their electrocardiogram gave rise to further medical examination. We referred these subjects to their general practitioners. Two subjects were excluded because they had mean systolic blood pressure >170 mm Hg during repeated measurements, and 1 subject was excluded because of an allergy to gelatin (component of capsules). Two subjects dropped out during the study, 1 because of personal reasons and the other because of admission to hospital. Thus, 84 subjects successfully completed the study.

Design and treatment

The study was placebo-controlled and double-blind. We performed the baseline measurements of HRV and BRS after a run-in period of 4 weeks in which all subjects received placebo capsules and were asked not to consume any fish, seafood, or fish oil capsules. Subjects were randomized to receive either a daily dose of 3.5 g of fish oil or placebo oil (high oleic sunflower oil; Loders Croklaan, Wormerveer, the Netherlands) during the 12-week intervention period. We conducted the random allocation into 2 groups separately for the men (N = 44) and the women (N = 42). Within each of these 2 strata, we stratified for diastolic blood pressure in 2 categories:

higher than and less than the median of that stratum. In each of the resulting 4 strata, we subsequently ranked the subjects according to their habitual fish consumption and randomized them into permuted blocks of 2 patients. The oils were administered in 7 soft gelatin capsules per day that each contained 500 mg of oil and 3000 ppm of tocopherol as antioxidant (Banner Pharmacaps Europe B.V., Tilburg, the Netherlands). Fish oil and placebo capsules were indistinguishable from each other. The daily dose of fish oil provided approximately 700 mg of eicosapentaenoic acid (C20:5n-3, EPA), 560 mg of docosahexaenoic acid (C22:6n-3, DHA), and 260 mg of other n-3 fatty acids. The placebo capsules mainly contained oleic acid (C18:1n-9). At the end of the 12-week intervention period, we repeated the measurements of HRV and BRS.

Subjects were asked to maintain their usual diet and lifestyle, but not to eat any fish or seafood. Compliance of the subjects with the protocol was objectively checked with an analysis of n-3 fatty acids in serum cholesteryl esters. Also, the number of leftover capsules that was returned by the subjects suggested that 98% of the capsules were indeed ingested. Diaries kept by the subjects did not reveal any deviations from the protocol that could have affected the outcome.

Intakes of energy, fatty acids, cholesterol, and alcohol were estimated twice by using a telephone-administered 24-hour dietary recall. Height and weight were measured at the start of the run-in period. Weight was monitored during the study. Physical activity was estimated with the Dutch version of the Physical Activity Scale for the Elderly (PASE) questionnaire.[105;106]

Protocol for HRV and BRS measurement

We instructed the subjects not to consume alcoholic drinks after 6 p.m. on the day preceding the measurements and to eat low fat meals on the day of the measurements. Subjects were not allowed to engage in heavy physical activity, smoke, or drink coffee during the 4 hours preceding the measurements. After at least 15 minutes of rest, we made 4 measurements of blood pressure and heart rate with an automatic sphygmomanometer (Dinamap PRO 100, Critikon LTD). The first measurement was discarded, and the last 3 measurements were averaged. After 30 minutes of rest, we recorded the 12-lead electrocardiogram and finger arterial blood pressure for 10 minutes. The fingercuff of a non-invasive continuous blood pressure measurement device (Finapres, Ohmeda, Englewood, NJ) was attached to the middle finger of the left hand. To assess BRS, the subjects were asked to breathe at a fixed rate of 15 breaths/minute (0.25 Hz), at a freely chosen tidal volume, during the 10

minutes of recording. An indicator for metronome respiration was visualized on a computer monitor. An impedance respiration module was connected to the lateral sides of the lower part of the thorax to monitor respiration. We recorded all signals (finger arterial blood pressure, electrocardiogram, and respiration) simultaneously on a modified Mortara ST-Surveyor computer (Mortara Intrument - Mortara Rangoni Europe, Bologna, Italy).

Signal analysis

The recordings were processed without knowledge of treatment type or other subject variables. During the first minute of the 10-minute recordings, the subjects were still developing a regular respiration rate and tidal volume; therefore, the first minute was not included in the analysis. All signals were manually inspected for signal quality and arrhythmias. Signal quality of all recordings was satisfactory. The parts of the recordings with incidental ectopic beats were excluded from the analysis, because HRV and BRS can only be determined during sinus rhythm. When the part of the recording that was suitable for analysis was shorter than 4 minutes, because of exclusion of ectopic beats, HRV and BRS analysis was not feasible. This was the case in the baseline recordings of 8 subjects and in the end recordings of 7 subjects. Therefore, the number of subjects with complete and valid recordings for HRV and BRS analysis was 74. HRV and BRS were computed automatically on the arrhythmia-free parts of the recordings.

The SD of the duration of all normal RR intervals (SDNN) from the 10-minute recordings ($SDNN_{10}$) was calculated as an estimate of overall HRV. The root mean square successive differences (RMSSD), which is the square root of the mean of the sum of the squares of differences between adjacent intervals, was calculated from the 10-minute recordings ($RMSSD_{10}$) as an estimate of short-term components of HRV. Because we calculated SDNN and RMSSD from 10-minute recordings rather than from conventional 24-hour recordings, we use the abbreviations $SDNN_{10}$ and $RMSSD_{10}$. We also computed low frequency power (LF; 0.05-0.15 Hz; a marker of fluctuations in either sympathetic or sympathetic plus vagal activity) and high frequency power (HF; 0.15-0.50 Hz; a marker of vagal activity) according to the procedure described by Bootsma et al.[107] In brief, intervals were normalized to the mean interval. Then, linear trend removal and 10% left and right tapering was done. After padding the data with zeros to the nearest power of 2, the power density spectrum was computed by means of a fast Fourier algorithm.

BRS was assessed from the HRV and the blood pressure variability. BRS was computed by integrating the spectral systolic blood pressure to inter-beat-interval transfer function in the 0.05- to 0.15-Hz band, using a previously published algorithm.[47] The phase angle between heart rate and blood pressure should always be negative, because baroreflex-induced heart rate changes by definition come later in time than the blood pressure changes inducing the reflex. For 1 subject, we found a positive phase angle between heart rate and blood pressure in the end recording. This result was therefore rejected. Thus, the number of subjects with complete results was 74 for HRV and 73 for BRS.

Fatty acid analysis

We took non-fasting blood samples at the start of the run-in period and at the start, middle, and end of the intervention period. Serum cholesteryl fatty acids were analyzed as previously described[108;109] with minor modifications.[110]

Statistical analysis

A pre-trial power calculation showed that 35 subjects per group would be sufficient to detect a significant difference (P<0.05) in response of $SDNN_{10}$ between the fish oil group and the placebo group with a power of 80%, if the real population effect exceeded 27 ms. Because of the skewed distribution of the high and low frequency spectral HRV components, log-transformation of these parameters was performed. Individual responses were calculated as the value obtained for a subject at the end of the intervention period minus the value obtained for the same subject at the start of the intervention period. Differences in response of HRV and BRS between the fish oil group and the placebo group were analyzed with a 2-tailed Student *t* test.

RESULTS

The results concern the 36 men and 38 women for whom HRV analysis was feasible. Characteristics of the study population are given in Table 3.1. Characteristics of the 2 treatment groups were comparable and were within the normal range.

Table 3.1 Characteristics of the 36 men and 38 women for whom HRV analysis was performed (mean ± SD)

	Fish oil	Placebo
Men/Women, no.	18/21	18/17
Age, y	58 ± 4	59 ± 5
Body mass index, kg/m^2	27 ± 3	27 ± 4
Systolic blood pressure, mm Hg	128 ± 15	135 ± 17
Diastolic blood pressure, mm Hg	74 ± 8	77 ± 9
Heart rate, beats/min	64 ± 9	64 ± 9
Habitual fish intake, g n-3 fatty acids per month	8.4 ± 7.9	8.7 ± 7.3
Smokers, no.	6	7
PASE-score	134 ± 55	133 ± 63

PASE, physical activity scale for the elderly.

Compliance was confirmed by a change in EPA concentration in cholesteryl esters during intervention of on average +407% in the fish oil group and -5% in the placebo group. In each of the subjects in the fish oil group, the EPA concentration more than doubled during intervention. For DHA, the change was on average +94% in the fish oil group and -6% in the placebo group (Table 3.2).

Table 3.2 The content of EPA and DHA as percentage of total fatty acids in serum cholesteryl esters at start and end of the intervention period, and the response (mean ± SD)

	Treatment	Start intervention	End intervention	Response
EPA	Fish oil	0.76 ± 0.29	2.91 ± 0.67	2.15 ± 0.54
	Placebo	0.69 ± 0.27	0.65 ± 0.24	-0.05 ± 0.11
DHA	Fish oil	0.49 ± 0.11	0.94 ± 0.17	0.44 ± 0.13
	Placebo	0.48 ± 0.10	0.45 ± 0.09	-0.03 ± 0.07

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

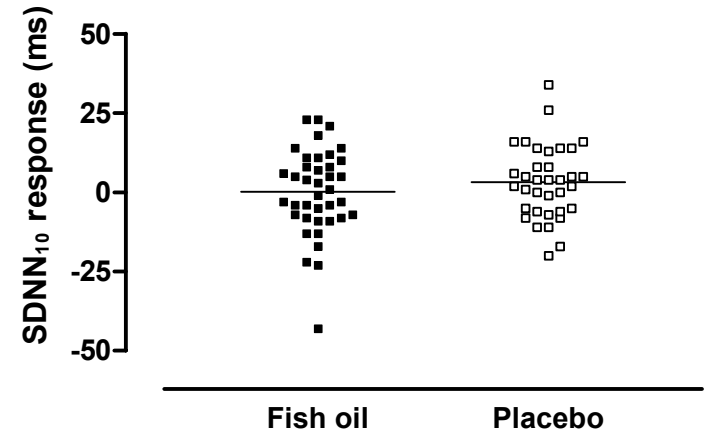

Figure 3.1 $SDNN_{10}$ (SD of all normal RR intervals from the 10-minute recordings) response of 39 subjects who took 3.5 g of fish oil daily for 12 weeks (closed squares) and of 35 subjects who took placebo (open squares).

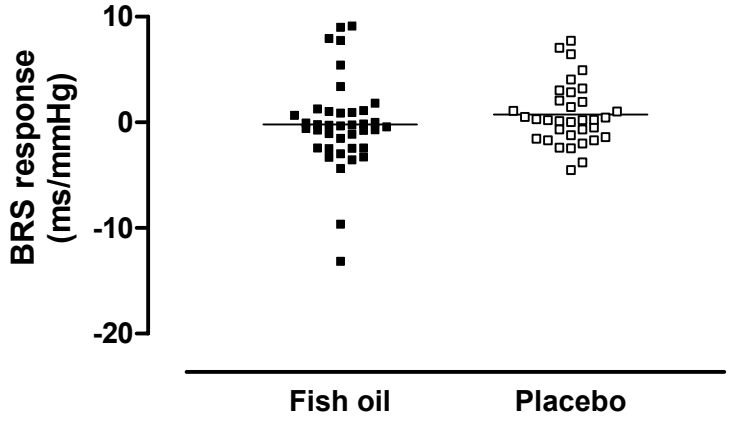

Figure 3.2 BRS response of 39 subjects who took 3.5 g of fish oil daily for 12 weeks (closed squares) and of 34 subjects who took placebo (open squares).

Table 3.3 HRV parameters and BRS at the start and the end of the intervention period, their response (mean ± SD), and the difference in response between the fish oil group and the placebo group (95% CI)

	Treatment	No.	Start intervention	End intervention	Response	Difference in response
$SDNN_{10}$, ms	Fish oil	39	34.5 ± 16.1	34.7 ± 16.2	0.15 ± 13.6	-3.05 (-8.91 to 2.82)
	Placebo	35	33.7 ± 15.2	36.9 ± 16.4	3.20 ± 11.5	
$RMSSD_{10}$, ms	Fish oil	39	27.1 ± 19.5	27.4 ± 18.9	0.26 ± 21.5	-3.72 (-12.7 to 5.23)
	Placebo	35	26.7 ± 20.7	30.6 ± 19.3	3.97 ± 16.5	
Log(LF), 10^{-2} log ms^2	Fish oil	39	224 ± 42.9	223 ± 39.7	-1.11 ± 27.9	-5.45 (-18.0 to 7.12)
	Placebo	35	228 ± 38.6	233 ± 44.3	4.33 ± 26.1	
Log(HF), 10^{-2} log ms^2	Fish oil	39	248 ± 45.4	246 ± 41.2	-1.97 ± 36.2	-13.2 (-30.1 to 3.63)
	Placebo	35	244 ± 44.5	255 ± 43.7	11.2 ± 36.3	
BRS, ms/mm Hg	Fish oil	39	6.08 ± 4.41	5.89 ± 4.17	-0.19 ± 4.28	-0.92 (-2.66 to 0.81)
	Placebo	34	5.84 ± 3.24	6.58 ± 4.58	0.74 ± 2.90	

$SDNN_{10}$, Standard deviation of all normal RR intervals from the 10-minute recordings; $RMSSD_{10}$, the root mean square successive differences from the 10-minute recordings; Log(LF), the log of the power in the low frequency range (0.05-0.15 Hz); Log(HF), the log of the power in the high frequency range (0.15-0.50 Hz).

Different measures of HRV ($SDNN_{10}$, $RMSSD_{10}$, LF, and HF) and BRS were not significantly affected by treatment (Table 3.3). $SDNN_{10}$ decreased on average by 3.05 ms (7.7%) in the fish oil group as compared with the placebo group (95% CI, -8.91 to 2.82 ms; Figure 3.1). BRS decreased by 0.92 ms/mm Hg (0.1%) in the fish oil group as compared with the placebo group (95% CI, -2.66 to 0.81 ms/mm Hg; Figure 3.2).

During the intervention period, systolic blood pressure decreased by 2.3 ± 8.6 mm Hg in the fish oil and by 3.5 ± 10.0 mm Hg in the placebo group. Diastolic blood pressure decreased by 1.3 ± 4.6 mm Hg in the fish oil and by 1.7 ± 5.9 mm Hg in the placebo group. Heart rate decreased by 1.5 ± 6.0 beats/min in the fish oil group and by 2.1 ± 4.7 beats/min in the placebo group. These changes were not significantly different between the 2 groups. Body mass index did not change in either group.

Background dietary intake was similar in the 2 treatment groups. The fish oil group consumed 35% of the energy as fat, 0.5% of the energy as n-3 fatty acids, 6% of the energy as alcohol, and 23 mg/MJ cholesterol. In the placebo group, the corresponding figures were 33%, 0.4%, 5%, and 23 mg/MJ.

DISCUSSION

We observed no effect of a daily intake of 3.5 g of fish oil for 12 weeks versus a placebo oil on HRV and BRS in an apparently healthy middle-aged population.

The effect of n-3 fatty acids on HRV has been investigated in several other studies. A positive association between HRV and blood levels of n-3 fatty acids was found in several patient populations[75-77] and in healthy subjects.[33;78] The major limitation of these studies is the cross-sectional design, which precludes causal inference.

Also, Christensen et al. performed three double-blind intervention trials on n-3 fatty acids and HRV. After infarction, patients (n = 55) received 5.2 g of n-3 fatty acids or olive oil daily for 12 weeks. The SDNN increased significantly in the fish oil group compared with baseline and the placebo group.[34] Patients with chronic renal failure (n = 29) received 5.2 g of n-3 fatty acids or olive oil daily for 12 weeks. The mean of all normal RR intervals during the 24-hour recording increased significantly after supplementation with n-3 fatty acids. However, no effect was found on SDNN.[79] In another study, healthy men and women (n = 60) received 2.0 g or 6.6 g of n-3 fatty acids or olive oil daily for 12 weeks. An increase in HRV after supplementation with n-3 fatty acids was only found in 13 men with a low HRV before supplementation (SDNN < 150 ms).[33] The studies by Christensen together suggest that n-3 fatty acids

increase HRV, particularly in patients. However, we could not confirm this in our study in healthy subjects.

We used SDNN from 10-minute recordings ($SDNN_{10}$) made under highly standardized conditions; the subjects were asked to breathe at a fixed rate of 15 breaths/min, as our primary HRV outcome measure. Other investigators have also measured HRV in the time domain from short-term recordings and found that low HRV is associated with risk of coronary heart disease and death.[45;111;112] It can be argued that short-term recordings made under physiologically stable conditions should be processed in the frequency domain.[35] Therefore, we also processed our recordings by frequency domain methods, but we also found no effect of n-3 fatty acids on frequency domain measures of HRV. From our study, we cannot rule out an effect of n-3 fatty acids on HRV obtained from 24-hour recordings.

In our study, we did not find an effect of dietary n-3 fatty acids on BRS. To our knowledge, the effect of n-3 fatty acids on BRS has only been studied in 1 other study.[82] Weisser et al. gave subjects who were normotensive (n = 10) 6 g of supplemental n-3 fatty acids daily for 6 weeks and measured changes in heart rate associated with the blood pressure increase during norepinephrine infusion before and after supplementation. The decrease in heart rate was more pronounced after fish oil supplementation, suggesting an effect of dietary n-3 fatty acids on BRS. There are several explanations for the discrepancy between our study and that of Weisser et al. We investigated BRS by analyzing the spontaneous fluctuations of arterial pressure during 0.25-Hz metronome respiration, which is a suitable non-invasive BRS assessment.[47] Weisser et al. investigated BRS by manipulation with noradrenaline, which causes large and less physiological changes in arterial pressure. Although the spontaneous baroreflex and drug-induced responses are associated to a certain extent,[113] it is possible that these 2 methods yield different results. Other differences between the study of Weisser et al. and our study are that Weisser et al. included a much smaller number of subjects (n = 10) and did not use a concurrent placebo control group. It is possible that the change in BRS observed by Weisser et al. was caused by chance or adaptation of subjects to the measurement procedure. The placebo-controlled design of our study excluded effects from adaptation or drift with time. Thus, we conclude that n-3 fatty acids do not affect BRS in apparently healthy subjects.

Our study population did not have a history of any heart disease. This might explain why we did not find an effect of n-3 fatty acids on HRV and BRS. Human intervention studies in patients after an infarction suggest that n-3 fatty acids prevent sudden death by preventing arrhythmia.[3;4;68;114] Such patients have a much higher risk of arrhythmia

and a suppressed autonomic function compared with healthy subjects. Also, of the 3 intervention trials on n-3 fatty acids and HRV, only the 1 with patients after infarction showed an increase in SDNN after intake of n-3 fatty acids.[34] Thus, it is possible that n-3 fatty acids affect HRV and BRS only in a more susceptible population of patients after infarction or that the effects in healthy subjects are more subtle and therefore less easy to measure. However, even for healthy persons, it might be important to have a sufficient intake of n-3 fatty acids, because n-3 fatty acids might prevent arrhythmia and sudden death once an arrhythmic substrate develops.

The dose of 3.5 g of fish oil in this study provided approximately 1.5 g of n-3 fatty acids per day, which is comparable to 2 servings of fish per day. It is unlikely that the dose of n-3 fatty acids was too low to find an effect on HRV and BRS. Several epidemiological studies suggest that the cardioprotective effect of n-3 fatty acids is already present at low doses of about 200 mg of n-3 fatty acids per day and that increasing the intake of fish further than 1 or 2 servings per week does not confer any additional benefit.[1;18;115] Also, in the GISSI-Prevenzione trial, 1 g of n-3 fatty acids per day was enough to lower the rate of death, non-fatal myocardial infarction, and stroke.[3] Thus, the dose of n-3 fatty acids in our study was high enough to detect relevant effects on HRV and BRS, if any exist.

Our findings suggest that n-3 fatty acids do not affect HRV from short-term recordings made under highly standardized conditions and BRS. In general, our results do not support the hypothesis that n-3 fatty acids can prevent cardiac arrhythmia through effects on cardiac autonomic control, although an effect in more susceptible populations cannot be excluded. The postulated effect of n-3 fatty acids on arrhythmia might work through some other pathway, such as a direct effect on ion channels in cardiomyocytes.

Chapter 4

N-3 fatty acids do not affect electrocardiographic characteristics of healthy men and women

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Journal of Nutrition 2003;132:3051-3054

ABSTRACT

N-3 Fatty acids may reduce the risk of sudden death by preventing life-threatening cardiac arrhythmia. A standard electrocardiogram (ECG) may be used to detect clues as to the mechanism by which n-3 fatty acids affect the electrophysiology of the heart. An earlier study showed that n-3 fatty acids decreased the duration of the heart-rate corrected QT interval (QTc) in dogs. However, effects of n-3 fatty acids on the standard ECG of humans have not been reported. Therefore, we investigated the effect of n-3 fatty acids on QTc, QRS duration, apex-to-end-T duration, T-loop morphology, and spatial QRS-T angle in apparently healthy men and women aged 50 to 70 y. Subjects (*n* = 42/group) received either capsules providing 1.5 g n-3 fatty acids daily or placebo for 12 wk. ECG were recorded before and after intervention. None of the ECG characteristics were affected by n-3 fatty acids. The QTc decreased by 0.8 ms or 0.2% (95% confidence interval, -6.1 to 4.4 ms) in subjects that consumed n-3 fatty acids compared with the placebo group. These results do not support the hypothesis that n-3 fatty acids prevent arrhythmia through electrophysiologic effects on heart cell membranes. However, an effect on the ECG in more susceptible populations can not be excluded.

INTRODUCTION

There are strong indications that n-3 fatty acids from fish reduce the risk of sudden cardiac death, possibly by preventing cardiac arrhythmia.[2;3;32;72] Intervention studies show an effect of n-3 fatty acids on mortality and sudden cardiac death, which is already visible after only a few months, and no effect on non-fatal events.[4;6] This suggests an effect of n-3 fatty acids on arrhythmia rather than on atherosclerosis or thrombosis. In animal studies, n-3 fatty acids prevented and reduced the severity of arrhythmias, increased the threshold for ventricular fibrillation and improved the electrical stability of animal hearts.[25-29] The n-3 fatty acids also prevented and terminated tachyarrhythmias in cultured neonatal cardiomyocytes.[30;31] Thus, we consider an effect of n-3 fatty acids on arrhythmia the most plausible hypothesis for explaining the protective effect of n-3 fatty acids on heart disease.

In vitro, n-3 fatty acids stabilize the electrical activity of isolated cardiac myocytes by elevating the action potential threshold and prolonging the relative refractory time. These electrophysiologic effects may result from an action of free n-3 fatty acids on sodium[96-98] and calcium[99;100] currents through heart cell membranes, which are essential for heart rhythm. The n-3 fatty acids prolong the duration of the inactivated state of the sodium and calcium channels in addition to inhibiting the conductance of these channels.[32]

The striking effects on the electrophysiology of the whole heart in living organisms are expected to be detectable in a surface electrocardiogram (ECG), regardless of the precise processes that underlie the effects of n-3 fatty acids at the cellular level. In dogs surgically prepared to be susceptible to ventricular fibrillation, infusion with n-3 free fatty acids not only prevented cardiac arrhythmia, but also decreased the duration of the heart-rate corrected QT interval (QTc).[116] The QT interval on the ECG reflects the duration of activation and recovery of the ventricular muscle and may be a relevant measure for arrhythmia risk. In the general population, several studies, although not all,[52] showed that subjects with a longer duration of QTc have an increased risk of all-cause and cardiac mortality.[53-55] Thus, a decrease in the duration of QTc by n-3 fatty acids in humans would support a protective effect of n-3 fatty acids on heart disease.

The n-3 fatty acids may also affect ECG characteristics other than QTc. For instance, QRS duration is a measure of ventricular depolarization. Apex-to-end-T duration (aeT),[56;57] T-loop morphology,[58] and the spatial QRS-T angle[59;60] have been proposed as markers of the heterogeneity of ventricular repolarization, which provides the condition for the genesis of ventricular arrhythmias.

Effects of n-3 fatty acids on the standard ECG of humans have not been reported. Demonstration of such effects at the organ level could provide clues concerning how n-3 fatty acids may prevent arrhythmia and sudden cardiac death. They would also suggest a new class of biomarkers, which could be used to study potentially anti-arrhythmic food ingredients in humans. Therefore, we investigated the effect of n-3 fatty acids on QTc, QRS duration, aeT, T-loop morphology, and spatial QRS-T angle in healthy subjects.

SUBJECTS AND METHODS

Subjects

The Medical Ethical Committee of Wageningen University approved the study protocol. Subjects gave their written informed consent after we had explained the study protocol to them. Subjects were eligible if they were 50-70 y old and did not use drugs known to affect heart rhythm or autonomic regulation. In addition, subjects with past or present cardiovascular disease were excluded, as were those with diabetes, asthmatic complaints, mean systolic blood pressure >170 mmHg, or mean diastolic blood pressure >100 mmHg. Women had to be post-menopausal. At the start of the run-in period, we recorded a standard 12-lead ECG and performed blood pressure measurements for screening purposes in 97 subjects. Eight subjects were excluded and referred to their general practitioner, because their ECG warranted further medical examination. Two subjects were excluded because they had mean systolic blood pressure >170 mmHg during repeated measurements. One subject was excluded because of allergy to gelatin (component of capsules) and two subjects dropped out during the study. Thus, 84 subjects successfully completed the study.

Design and treatment

A power calculation showed that 35 subjects per group would be sufficient to detect a significant difference ($P<0.05$) in response of QTc duration between the fish oil group and the placebo group with a power of 80%, if the real population effect exceeded 10 ms. The study was placebo controlled and double blind. We performed the baseline ECG measurements after a run-in period of 4 wk in which all subjects received placebo and we repeated the ECG measurements at the end of the 12-wk intervention period. Subjects were stratified by habitual fish consumption, diastolic blood pressure and sex and then randomized to receive either a daily dose of 3.5 g fish oil or placebo

oil (high oleic sunflower oil) (Loders Croklaan, Wormerveer, the Netherlands) during the 12-wk intervention period. The oils were administered in seven soft gelatin capsules daily each containing 500 mg oil and ~0.15 mg α-tocopherol, 0.75 mg γ-tocopherol and 0.60 mg δ-tocopherol as antioxidants. The peroxide value of the fish oil was 0.9 mEq/kg and of the placebo oil, 0.3 mEq/kg (Banner Pharmacaps Europe B.V., Tilburg, the Netherlands). Fish oil and placebo capsules were indistinguishable. The daily dose of fish oil provided ~700 mg eicosapentaenoic acid (EPA, C20:5n-3), 560 mg docosahexaenoic acid (DHA, C22:6n-3), and 260 mg of other n-3 fatty acids. The placebo capsules contained mainly oleic acid (C18:1n-9).

Subjects were asked to maintain their usual diet and lifestyle, but not to eat any fish or seafood during the study. Compliance of the subjects with the protocol was objectively checked by analysis of n-3 fatty acids in serum cholesteryl esters. We also counted the number of leftover capsules returned by the subjects. Intakes of energy, fatty acids, cholesterol and alcohol were estimated twice by a telephone-administered 24-h dietary recall. Body height and weight were measured at the start of the run-in period and body weight was monitored during the study.

ECG measurement and signal analysis

After the subjects had lain down for a 30-min rest, we recorded the 12-lead ECG for 10 min on a modified Mortara ST-Surveyor electrocardiograph (Mortara Instrument, Mortara Rangoni Europe, Bologna, Italy). The subjects were asked to breathe at a fixed rate of 15 breaths/min because these recordings were also used in a related study that investigated the effect of n-3 fatty acids on risk indicators of arrhythmia that reflect autonomic cardiac control: heart rate variability and baroreflex sensitivity.[81] The ECG recordings were processed without knowledge of treatment type or other subject variables. We used min 3 of the 10-min recordings for the analysis of the ECG measures. For data processing, the Modular ECG Analysis System (MEANS) was used. MEANS determines the overall QT interval for all 12 leads together on a representative beat, which results from selective averaging of dominant beats.[117] Bazett's formula ($QTc=QT/\sqrt{RR}$) was used to correct for heart rate.[118] The aeT was calculated for leads V2-V5 as the difference between the end of the T wave and the peak of the T wave. T-loop morphology was characterized by two parameters, i.e., the spatial amplitude of the T loop and its width. These parameters were determined as described previously.[58] The QRS-T angle was taken as the spatial angle between the mean QRS axis and T axis.

Fatty acid analysis

We took blood samples from subjects that were not fasting at the start of the run-in period and at the beginning, middle and end of the intervention period. Serum cholesteryl fatty acids were analyzed as previously described.[110]

Statistical analysis

The primary outcome of the study was the response of the QTc. Secondary, more explorative outcomes were the responses of QRS duration, aeT, T-loop morphology, and QRS-T angle. Individual responses were calculated as the value obtained for a subject at the end of the intervention period minus the value obtained for that same subject at the start of the intervention period. Differences in response between the fish oil group and the placebo group were analyzed by a two-sided Student's *t* test.

RESULTS

Characteristics of the subjects were within the normal range and similar for the two treatment groups (Table 4.1). Compliance was confirmed by a change in the proportion of EPA in serum cholesteryl esters from 0.76 ± 0.29 to 2.91 ± 0.67 g/100 g fatty acids in the fish oil group and by no change (0.69 ± 0.27 to 0.65 ± 0.24 g/100 g fatty acids) in the placebo group. The capsule count indicated that 98% of the capsules were ingested. Diaries kept by the subjects did not reveal any deviations from the protocol that could have affected the outcome. Body mass index remained constant in both groups. Background dietary intake was similar in the two treatment groups. The fish oil group consumed 35% of energy as fat, 0.5% of energy as n-3 fatty acids, 6% of energy as alcohol, and 23 mg/MJ cholesterol. In the placebo group the corresponding figures were 33, 0.4 and 5%, and 23 mg/MJ.

The QTc duration was not significantly affected by intake of n-3 fatty acids (Figure 4.1); it decreased by 0.8 ms (95% confidence interval, -6.1 to 4.4 ms) or 0.2% in the fish oil group compared with the placebo group (Table 4.2). Intake of n-3 fatty acids did not affect QRS duration, aeT, T-loop morphology and QRS-T angle (Table 4.2).

Table 4.1 Subject characteristics at baseline (mean ± SD, n = 42/group).

	Fish oil	Placebo
Men/Women, n	21/21	22/20
Age, y	59 ± 5	60 ± 5
Body mass index, kg/m^2	27 ± 3	27 ± 4
Diastolic blood pressure, mmHg	74 ± 8	77 ± 9
N-3 Fatty acids from fish (habitual intake), mg/d	309 ± 312	290 ± 225

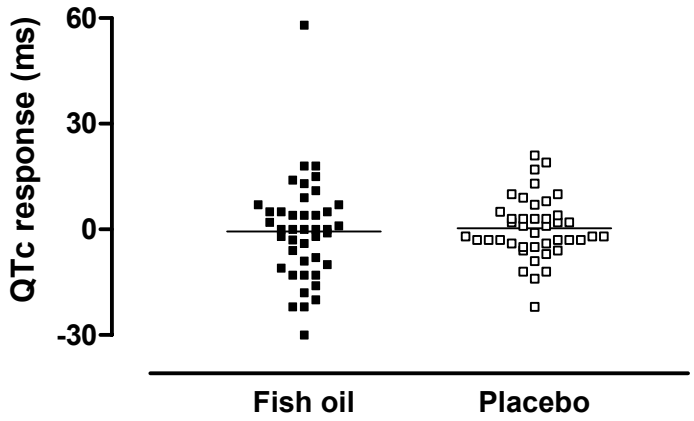

Figure 4.1 Response of the heart-rate corrected QT interval (QTc) of men and women who consumed 3.5 g of fish oil (1.5 g n-3 fatty acids; n = 42) or a placebo (n = 42) daily for 12 wk.

Table 4.2 ECG characteristics at the start and end of the intervention period and the difference in response to treatment between men and women who consumed 3.5 g of fish oil (1.5 g n-3 fatty acids) or a placebo daily for 12 wk

	Fish oil[1]		Placebo[1]		Difference in response[2]
	Baseline	End	Baseline	End	
QTc,[3] ms	422 ± 18	421 ± 21	425 ± 19	425 ± 18	-0.8 (-6.1 to 4.4)
QRS, ms	96.5 ± 9.2	99.0 ± 8.1	97.0 ± 10.7	98.2 ± 10.4	1.2 (-1.2 to 3.7)
aeT V2, ms	101.1 ± 14.9	102.0 ± 15.0	99.8 ± 8.7	101.9 ± 9.0	-1.2 (-4.0 to 1.6)
aeT V3, ms	92.2 ± 13.3	93.2 ± 14.6	93.3 ± 12.7	95.1 ± 12.6	-0.9 (-3.8 to 2.0)
aeT V4, ms	85.2 ± 13.2	84.5 ± 13.6	84.6 ± 13.1	87.7 ± 12.2	-3.7 (-7.5 to 0.1)
aeT V5, ms	77.4 ± 13.7	77.6 ± 13.7	78.4 ± 12.8	79.4 ± 11.7	-0.8 (-4.2 to 2.7)
amplitude T loop, mV	392 ± 159	376 ± 167	419 ± 182	419 ± 177	-16.4 (-46.7 to 13.9)
width T loop, degrees	27.5 ± 14.8	29.8 ± 14.4	26.1 ± 13.0	27.4 ± 14.5	1.0 (-2.4 to 4.3)
spatial QRS-T angle, degrees	64.1 ± 24.8	63.5 ± 20.3	61.8 ± 25.8	62.5 ± 24.2	-1.2 (-6.0 to 3.6)

[1] Values are means ± SD, n = 42/group.

[2] Values are response in the fish oil group minus response in the placebo group (95% confidence interval).

[3] QTc, heart-rate corrected QT duration; aeT, apex-to-end-T duration.

DISCUSSION

To date, the effects of n-3 fatty acids on ECG-patterns of humans have not been investigated, despite effects on arrhythmia in *in vitro* and animal studies and indications from epidemiology and clinical trials. We observed no effect of a daily intake of 3.5 g fish oil for 12 wk vs. placebo on ECG characteristics including QTc, QRS duration, aeT, T-loop morphology, or spatial QRS-T angle in a healthy middle-aged population.

On the basis of the results of *in vitro* studies and one study in dogs, we hypothesized that n-3 fatty acids would decrease the QTc duration in humans. Kang et al.[31] showed that n-3 fatty acids shorten the duration of the action potential, which may result in a shorter duration of the QT interval on the ECG. Our findings suggest that the mechanism postulated from *in vitro* and animal studies can not be directly translated to healthy humans because n-3 fatty acids did not affect the ECG. The results of our study are in agreement with the results of a cross-sectional study that did not find a relationship between n-3 fatty acids in cholesteryl esters and QTc duration from 24-h Holter recordings in healthy subjects.[78]

However, our results do not exclude the possibility that n-3 fatty acids could produce detectable effects on the ECG in more susceptible people. Changes in ECG characteristics in healthy subjects may be too subtle to be demonstrated in a study like this. It is possible that n-3 fatty acids affect the abnormal ECG in more susceptible populations of, for example, postinfarction patients, who have a much higher risk of arrhythmia than healthy subjects. Another explanation for our lack of findings might be that effects of diet on the electrophysiology of the human heart are too small to cause appreciable ECG changes. Nevertheless, n-3 fatty acids may be the most effective dietary means to improve cardiovascular health with risk reductions of ~30-50%.[3;4;18;119]

The dose of 1.5 g of n-3 fatty acids in the present study is equivalent to ~2 servings of fish daily. Several epidemiologic studies suggest that the cardioprotective effect of n-3 fatty acids is already present at low doses of ~200 mg n-3 fatty acids daily and that increasing the intake of fatty fish beyond 1 or 2 meals per week does not confer additional benefit.[1;18;115] Also in the GISSI-Prevenzione trial, 1 g of n-3 fatty acids daily was enough to lower the rate of death, non-fatal myocardial infarction and stroke.[3] Thus, the dose of n-3 fatty acids in our study was high enough to detect relevant effects on the ECG.

In general, our results do not support the hypothesis that n-3 fatty acids prevent cardiac arrhythmia through electrophysiologic effects on heart cell membranes, although an effect on the ECG in more susceptible populations should be tested. Our findings also do not suggest that intake of n-3 fatty acids in healthy persons could not prevent future arrhythmias. Long-term intake of n-3 fatty acids may reduce the development of an arrhythmogenic substrate during future ischemic events; such effects, of course, could not be demonstrated in the current study. To further study the mechanism of the antiarrhythmic effect of n-3 fatty acids, research must include more susceptible populations such as postinfarction patients.

Chapter 5

Effects of n-3 fatty acids from fish on premature ventricular complexes and heart rate in humans

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Submitted for publication

ABSTRACT

Objectives

In this study, we investigated the effect of n-3 fatty acids on heart rate and premature ventricular complexes (PVCs), a common form of arrhythmia that may trigger more life-threatening arrhythmias.

Background

A large body of evidence suggests that n-3 fatty acids from fish prevent fatal heart disease. They may be an effective and safe alternative to drug treatment for reducing the risk of arrhythmia and sudden cardiac death.

Methods

Patients (n = 84) with at least 1440 PVCs per 24 hours in a previous Holter recording were randomized to receive either 1.5 g/day n-3 fatty acids or placebo. Two 24-hour Holter recordings were made at baseline and two after the intervention period of ~14 weeks.

Results

Treatment did not significantly affect the number of PVCs, which decreased by on average 867 per 24 hours or 6% in the fish oil group compared with placebo (95% confidence interval, -3187 to 1453). However, 24-hour mean heart rate decreased significantly by on average 2.1 beats/min in the fish oil group compared with placebo (95% CI, -3.9 to -0.3).

Conclusions

Supplementation with 1.5 g/day of n-3 fatty acids from fish does not substantially suppress the number of PVCs in a patient population with frequent PVCs. This makes it less likely that the presumed effect of n-3 fatty acids on risk of sudden cardiac death is mediated by a reduction in the frequency of triggers of arrhythmia. However, n-3 fatty acids significantly decreased heart rate by 2.1 beat/min, which may predict a 6% lower risk of sudden death.

INTRODUCTION

Clinical trials show a beneficial effect of moderate intakes of n-3 fatty acids (≈1 g/d) on mortality and sudden cardiac death. This effect is seen within a few months after starting treatment, with no or a much smaller effect on non-fatal events.[4;6] Also, observational studies show strong inverse relationships of fish consumption or blood levels of n-3 fatty acids with incidence of fatal coronary heart disease and sudden cardiac death, but not with non-fatal heart disease.[2;18;19;72] In addition, experimental studies have indicated that n-3 fatty acids increase the arrhythmia threshold and effectively prevent ventricular fibrillation in *in vitro* and animal models.[120] Thus, results from different types of studies suggest that n-3 fatty acids have an immediate effect on arrhythmia rather than a slow effect via regression of atherosclerosis.

Frequent premature ventricular complexes (PVCs) are independent predictors of sudden cardiac death and mortality in survivors of myocardial infarction.[63;64;121] Also, in middle-aged men without prior symptoms of cardiovascular disease, frequent PVCs during exercise predicted long-term risk of cardiovascular death.[65] PVCs can indicate that there exists a group of cells with enhanced automaticity in an otherwise healthy heart.[61] N-3 fatty acids might intervene with the occurrence of PVCs by slowing down spontaneous beating rate or by prolonging the refractory period.[120] PVCs are a common form of arrhythmia that is in itself innocent but that may trigger more serious arrhythmic events, such as ventricular tachycardia or ventricular fibrillation.[122] Thus a reduction in PVCs results in fewer triggers and may in that way decrease the risk of more serious arrhythmic events. It should be noted however, that effects on PVCs do not necessarily parallel effects on endpoints. In the Cardiac Arrhythmia Suppression Trial (CAST), encainide and flecainide did reduce the number of PVCs, but increased rather than decreased mortality.[62] On the other hand, it is conceivable that n-3 fatty acids prevent PVCs and at the same time reduce fatal arrhythmias and mortality. Therefore, if effective, n-3 fatty acids may be a safe alternative to drug treatment of arrhythmias.

One previous study already suggested that n-3 fatty acids can reduce PVC incidence. Sellmayer et al. tested the potential antiarrhythmic effects of fish oil in patients with spontaneous PVCs.[87] The number of PVCs decreased from 6937 ± 5192 (mean ± SD) at the start to 3591 ± 3884 after 16 weeks in the fish oil group, compared with a decrease from 6306 ± 3363 to 4728 ± 3320 in the placebo group. Although the effects were impressive, the findings of only one study cannot be taken as definitive evidence and this potentially important finding urgently needed confirmation. In addition, n-3 fatty acids may reduce heart rate and in this way reduce the risk of sudden

death.[90;91;123] However, no study has as yet directly addressed effects of dietary n-3 fatty acids on heart rate as measured by continuous 24-hour monitoring. Therefore, the aim of the present study was to investigate the effect of n-3 fatty acids on the incidence of PVCs and heart rate in patients with frequent PVCs. Demonstration of an effect on PVCs would provide clues as to possible underlying mechanisms and it may also add to the evidence that n-3 fatty acids prevent fatal heart disease by preventing serious ventricular arrhythmia.

SUBJECTS AND METHODS

Subjects

The study protocol was approved by the Medical Ethical Committee of Wageningen University. Patients gave their written informed consent after the study protocol had been explained to them. We defined frequent PVCs as on average at least one PVC per minute (1440/24 hours). Thus, cardiologists recruited and enrolled patients aged 18 years or older with at least 1440 PVCs per 24 hours in a previous Holter recording made less than six months before. Patients who used Class I, III or IV antiarrhythmic therapy or digitalis were excluded, as were those with known left ventricular dysfunction, sustained tachycardia, symptomatic ischemia, hemodynamically relevant valvular defects, or other cardiac diseases related with arrhythmia. Also, patients who had used any supplemental n-3 fatty acids during the previous three months were not eligible.

Ninety-two patients who met the inclusion criteria were randomized of whom 84 successfully completed the study. One patient was withdrawn because of prescription of antiarrhythmic medication and one patient died during the study. Four patients dropped out for personal reasons, one for perceived side-effects, and one for hospital admission.

Design and treatment

This placebo-controlled, double-blind study with parallel design was conducted in three hospitals in the Netherlands. Patients were randomized in blocks of two units, stratified for history of myocardial infarction. Patients received a daily dose of 3.5 g of either fish oil or placebo oil (high oleic sunflower oil) (Loders Croklaan, Wormerveer, the Netherlands) during the intervention period of 14 ± 1 (mean ± SD) weeks. The oils were administered in seven soft gelatin capsules daily each containing 500 mg oil and

1.5 mg tocopherol as an antioxidant (Banner Pharmacaps Europe B.V., Tilburg, the Netherlands). The daily dose of fish oil provided ~700 mg eicosapentaenoic acid (EPA, C20:5n-3), 560 mg docosahexaenoic acid (DHA, C22:6n-3), and 260 mg of other n-3 fatty acids. The placebo capsules contained mainly oleic acid (C18:1n-9).

Data collection

Twenty-four hour Holter recordings were made with SEER MC® digital recorders (GE Medical Systems Information Technologies, Milwaukee, WI, USA). We averaged the results of two Holter recordings made at baseline, as well as two recordings made at the end of the study, both at a one week interval, to reduce the large variation in the occurrence of PVCs.

Data on demographics, medical history and cardiac medication were collected at baseline and recorded in the Case Report Form. Intakes of energy, fatty acids, cholesterol and alcohol were estimated once during the intervention by a telephone-administered 24-hour dietary recall. Also, patients were interviewed at baseline and at the end of the intervention using a questionnaire on the frequency of fish consumption to assess and monitor their fish intake. Body mass index and blood pressure were monitored during the study. Patients reported the intake of capsules in a diary. We took non-fasting blood samples a few days prior to the start of treatment and during the last week of the intervention. Serum cholesteryl fatty acids were analyzed as previously described.[110]

Holter analysis

The 24-hour Holter recordings were analyzed with a Marquette Series 8000 Holter analyzer (GE Medical Systems Information Technologies, Milwaukee, WI, USA) by an experienced Holter technician who was unaware of the treatments. Initially, all beats were automatically categorized into different classes based on their morphology. The technician carefully examined whether classes were correctly identified. Additionally, for each class containing more than ten PVCs, all beats within that class were overlaid on top of each other to check for possible misclassification of non-PVCs and all outliers were removed. The frequency of PVCs was calculated by dividing the number of PVCs by the total time that the signal was of sufficiently high quality for analysis. The mean heart rate was calculated as total number of normal beats in sinus rhythm (3 consecutive normal beats) divided by recording duration corrected for noise and episodes of non-sinus rhythm.

Statistical analysis

A pre-trial power calculation showed that 40 subjects per group would be sufficient to detect a significant difference ($P<0.05$) in response of the number of PVCs between the fish oil group and the placebo group with a power of 80%, if the real population effect exceeded 25%. The primary outcome of the study was the change in number of PVCs during the treatment. Differences in change of PVCs and heart rate between fish oil and placebo group were analyzed by a Student's t test. A subgroup analysis for patients with and without prior myocardial infarction was planned a priori and included in the protocol.

RESULTS

Differences in baseline characteristics between the fish oil and the placebo group were not significant (Table 5.1). Compliance was reflected by a change of EPA in serum cholesteryl esters (g/100 g total fatty acids) during intervention of on average 181% in the fish oil group and -2% in the placebo group. For DHA the change was 49% in the fish oil group and -5% in the placebo group (Table 5.2). Two patients in the fish oil group and two in the placebo group stopped the intake of the capsules before the study was finished, because they experienced side-effects that they believed were due to treatment: skin rashes, gastrointestinal complaints and nausea (twice). Analyses were performed including the data from these four patients, but results did not change when they were excluded.

The number of PVCs per 24 hours was not significantly affected by fish oil vs. placebo treatment (Figure 5.1). It decreased by on average 867 PVCs per 24 hours or 6% in the fish oil group compared with the placebo group (95% confidence interval, -3187 to 1453) (Table 5.3). In the small subgroup of 22 patients with prior myocardial infarction, the average response was +2717 (+23%) in the fish oil group compared with the placebo group (95% CI, -2254 to 7689) (Table 5.3). In the subgroup of 62 patients without prior myocardial infarction, the average response was –2129 (-22%) in the fish oil group compared with the placebo group (95% CI, -4764 to 507) (Table 5.3).

Twenty-four hour mean heart rate decreased significantly by on average 2.1 beats/min in the fish oil group compared with the placebo group (95% CI, -3.9 to -0.3) (Table 5.3, Figure 5.2). Results on heart rate for the subgroups with and without prior myocardial infarction were very similar to the results for the complete group of patients (Table 5.3). Blood pressure and body mass index were not affected by treatment (data not shown).

Table 5.1 Baseline characteristics of patients who completed the study

	Placebo (n=43)	Fish oil (n=41)
Age, y	61 ± 13	67 ± 13
Male sex, n (%)	22 (51.2)	28 (68.3)
Body Mass Index, kg/m^2	26 ± 4	26 ± 4
Number of PVCs per 24 hours at screening	7623 ± 7779	9553 ± 8896
Heart rate at screening, beats/min	76 ± 11	71 ± 11
Dietary intake of n-3 fatty acids (ALA + EPA + DHA) from fish g/month	6.8 ± 7.7	9.3 ± 10.1
History of myocardial infarction, n (%)	11 (25.6)	11 (26.8)
Beta-blocker use, n (%)	15 (34.9)	23 (56.1)

PVC, premature ventricular complexes; values are mean ± SD or n (%).

Background dietary intake was similar in the two treatment groups. The fish oil group consumed 33% of the energy as fat, 0.6% of the energy (1.4 g/day) as total n-3 fatty acids (mostly ALA), 4% of the energy as alcohol, and 24 mg/MJ cholesterol. In the placebo group the corresponding figures were 33%, 0.5% (1.1 g/day), 5%, and 28 mg/MJ. Fish intake was similar in the treatment groups and did not change during the intervention.

Table 5.2 The content of EPA and DHA as percentage of total fatty acids in serum cholesteryl esters at start and end of the intervention period, and the response (mean ± SD)

	Treatment	Start intervention	End intervention	Response
EPA	Fish oil	1.28 ± 0.76	3.60 ± 1.65	2.32 ± 1.30
	Placebo	1.07 ± 0.69	1.06 ± 0.75	-0.02 ± 0.74
DHA	Fish oil	0.70 ± 0.20	1.04 ± 0.24	0.34 ± 0.19
	Placebo	0.64 ± 0.21	0.62 ± 0.19	-0.03 ± 0.13

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

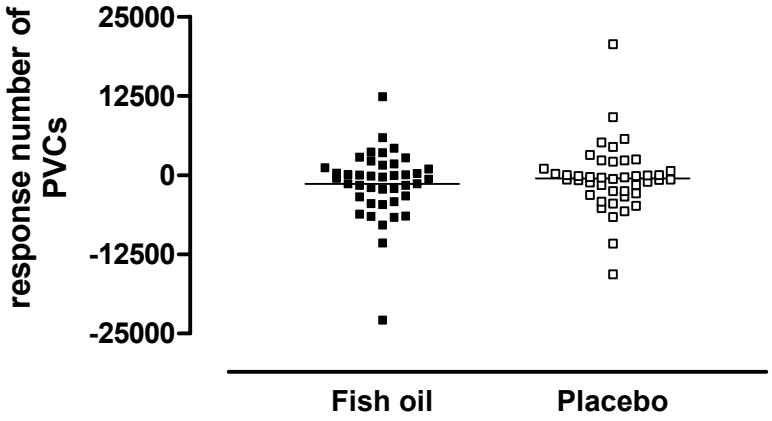

Figure 5.1 Response of number of premature ventricular complexes per 24 hours of 41 patients who took 3.5 g of fish oil daily for 12 weeks (closed squares) and of 43 patients who took placebo (open squares).

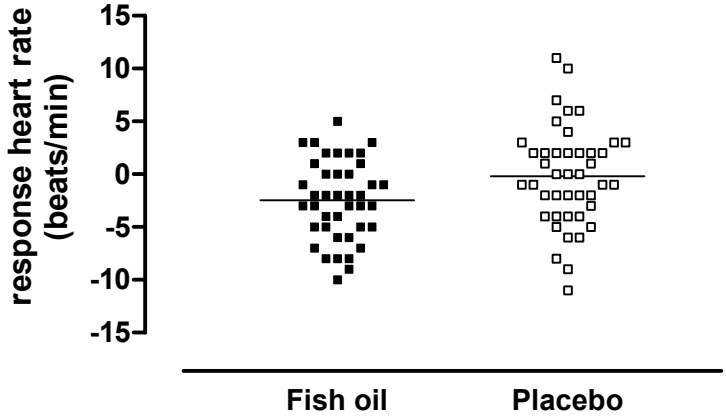

Figure 5.2 Heart rate response of 41 patients who took 3.5 g of fish oil daily for 12 weeks (closed squares) and of 43 patients who took placebo (open squares).

Table 5.3 Number of premature ventricular complexes (PVCs) per 24 hours and heart rate at the start and the end of the intervention period, their response (mean ± SD) and the difference in response between fish oil and placebo group (95% CI), for all patients together and for the patients with and without prior myocardial infarction (MI) separately

	Treatment	No.	Start intervention	End intervention	Response	Difference in response
All patients						
PVC	Fish oil	41	10803 ± 9561	9425 ± 8183	-1378 ± 5368	-867 (-3187 to 1453)
	Placebo	43	7095 ± 8201	6585 ± 8267	-511 ± 5319	
Heart rate, beats/min	Fish oil	41	69.8 ± 8.0	67.5 ± 7.8	-2.3 ± 3.6	-2.1 (-3.9 to -0.3)
	Placebo	43	73.9 ± 10.6	73.7 ± 9.9	-0.2 ± 4.5	
Patients with MI						
PVC	Fish oil	11	10388 ± 10146	10281 ± 9268	-107 ± 5744	2717 (-2254 to 7689)
	Placebo	11	11787 ± 10697	8962 ± 10528	-2824 ± 5430	
Heart rate, beats/min	Fish oil	11	64.3 ± 7.2	62.7 ± 7.0	-1.6 ± 2.9	-2.1 (-5.2 to 1.1)
	Placebo	11	71.6 ± 12.6	72.0 ± 12.5	0.5 ± 4.1	
Patients without MI						
PVC	Fish oil	30	10956 ± 9513	9112 ± 7898	-1844 ± 5247	-2129 (-4764 to 507)
	Placebo	32	5483 ± 6612	5767 ± 7359	284 ± 5126	
Heart rate, beats/min	Fish oil	30	71.8 ± 7.4	69.2 ± 7.4	-2.6 ± 3.9	-2.1 (-4.3 to 0.5)
	Placebo	32	74.7 ± 10.0	74.2 ± 9.0	-0.5 ± 4.7	

DISCUSSION

Daily intake of 1.5 g of n-3 fatty acids for ~14 weeks did not significantly affect the number of PVCs in patients with frequent PVCs. In analogy with Sellmayer et al., we defined an individual positive response to treatment as a reduction in number of PVCs of more than 70% in a particular patient.[87] Only 3 patients in the fish oil group and 7 patients in the placebo group showed a reduction of more than 70%. Sellmayer et al. tested the effects of 2.4 g n-3 fatty acids per day in 68 patients with a minimum of 2000 spontaneous PVCs per 24 hours, using single 24-hour Holter recordings. They found a more than 70% reduction in PVCs in 15 patients (44%) after fish oil and in 5 patients (15%) after placebo. After 16 weeks, the number of PVCs decreased by 48% in the fish oil group and by 25% in the placebo group (P = 0.052). The confidence interval of our study (-3187 to 1453) is wide and includes the effect of -1768 found by Sellmayer et al.[87] Our data are therefore not decisive, but combined with the absence of a significant effect of n-3 fatty acids on the number of PVCs in three other small and probably underpowered studies,[84-86] our study indicates that the large effect as found by Sellmayer et al. may have been due to chance or at least overestimated the true population effect.

Protective effects of n-3 fatty acids on hard endpoints in clinical trials have exclusively been found for post-myocardial infarction patients.[3;4;68] In contrast, no or even adverse effects were found in a trial in 3114 angina patients who were advised to eat two portions of fatty fish each week or to take 3g of fish oil daily.[70] It is hard to explain these adverse results, but it may be suggested that the cardioprotective effect of n-3 fatty acids is restricted to patients with earlier myocardial infarction. N-3 fatty acids may interact with structural abnormalities in hearts with previous infarctions and in that way prevent fatal electrical events. About 25% of the patients in our study had a history of myocardial infarction. When we restricted our analysis to this subgroup of 11 patients per treatment, we saw a non-significant increase of PVCs in patients given n-3 fatty acids. Thus, we have no indications that the mechanism behind the possible antiarrhythmic effect of n-3 fatty acids in post-myocardial infarction patients involves PVCs as potential arrhythmic triggers.

N-3 fatty acids significantly decreased heart rate by 2.1 beats/min (P = 0.022). A recent observational study reported an inverse association between n-3 fatty acids and heart rate.[89] Also, some intervention studies report that n-3 fatty acids or DHA alone decrease heart rate in different patient populations by –2.2 to –3.5 beats/min.[90;91;123] However, so far no study has directly addressed effects of n-3 fatty acids on heart rate as determined by continuous 24-hour monitoring. Increased heart

rate is an independent risk factor for sudden death, but not for fatal myocardial infarction in middle-aged men free of known cardiovascular disease.[88;124-126] The decrease in heart rate of 2.1 beats/min would predict a risk reduction for sudden cardiac death of 6%.[88] It can be speculated that n-3 fatty acids affect heart rate through stabilizing electrical activity of isolated cardiac myocytes by elevating action potential threshold and prolonging relative refractory time.[120] This might affect electrical stimulation of the sinus node resulting in a lower heart rate. However, n-3 fatty acids may also affect sympathetic and parasympathetic control of heart rate by virtue of their interaction with the adrenergic system.[34]

Baseline characteristics of the fish oil and the placebo group were not completely comparable. Most importantly, although not statistically significant, the number of PVCs at screening was somewhat higher in the fish oil group than in the placebo group. However, this would be expected to result in a larger rather than smaller decrease in the number of PVCs, either due to regression to the mean or because higher levels give more room for a decrease. Also, we did find an effect of n-3 fatty acids on heart rate, despite differences in baseline characteristics between groups. Altogether, it seems unlikely that the lack of a distinct effect on the number of PVCs can be explained by baseline differences between the fish oil and the placebo group.

The body of evidence from different types of studies suggesting that n-3 fatty acids may reduce serious ventricular arrhythmia risk justifies further research on this potential protective mode of action.[120] Definitive answers will hopefully come from currently running long-term trials on n-3 fatty acids and arrhythmia incidence in high-risk patients with an implantable cardioverter defibrillator (ICD).[104] We are aware of three such trials, of which one recently reported preliminary results. This study in 200 ICD patients showed a trend towards increased rather than decreased recurrence of ventricular arrhythmias in patients who received n-3 fatty acids over a follow-up of 2 years.[127] The results of the other two trials are required, before definitive conclusions can be drawn.

In the present randomized double-blind study, dietary n-3 fatty acids significantly decreased heart rate by 2.1 beat/min, which predicts a 6% lower risk of sudden death.[88] However, n-3 fatty acids are apparently not very effective in the treatment of PVCs. This makes it less likely that the presumed effect of n-3 fatty acids on the risk of sudden cardiac death is mediated by a reduction in the frequency of triggers of arrhythmia. Nevertheless, further exploration of this hypothesis is warranted and trials on life-threatening arrhythmia and mortality are needed to support dietary recommendations.

Chapter 6

Effect of n-3 fatty acids on electrocardiographic characteristics in patients with frequent premature ventricular complexes

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Submitted for publication

ABSTRACT

N-3 fatty acids may protect against heart disease mortality by preventing fatal arrhythmias. Underlying effects on cardiac electrophysiology may be demonstrable in the standard electrocardiogram (ECG) and provide insight into the mechanism. Therefore, we investigated the effect of dietary n-3 fatty acids on QTc, T-loop width, spatial QRS-T angle, and spatial U-wave amplitude in 74 patients with frequent premature ventricular complexes who received 1.5 g n-3 fatty acids daily or placebo for ~14 wk. None of the ECG characteristics were significantly affected by treatment. Our results do not provide additional support for the hypothesis that n-3 fatty acids prevent cardiac arrhythmia through electrophysiologic effects on heart cell membranes.

INTRODUCTION

Evidence from human observational studies and clinical trials indicates that n-3 fatty acids can protect against fatal heart disease by preventing cardiac arrhythmias.[4;6;72] In addition, experimental studies showed that n-3 fatty acids prevent and terminate arrhythmias in *in vitro* and animal models. N-3 fatty acids stabilized the electrical activity of cardiomyocytes by elevating the action potential threshold and prolonging the relative refractory time. These effects may result from an action of n-3 fatty acids on ion transport through heart cell membranes, which is essential for heart rhythm.[32] Effects on the electrophysiology of the whole heart may be demonstrable in a surface electrocardiogram (ECG) of humans. We earlier reported no effect of n-3 fatty acids on several ECG characteristics in healthy humans.[93] However, effects of n-3 fatty acids may only be detected in abnormal ECGs of more susceptible subjects. We therefore performed a study in patients with frequent premature ventricular complexes, a common form of arrhythmia that may trigger more life-threatening arrhythmias.

The heart-rate corrected QT interval (QTc) on the ECG is a relevant measure for arrhythmia risk. In the general population, subjects with a longer QTc have an increased mortality risk.[53-55] Thus, a decrease in QTc by n-3 fatty acids would support a protective effect of n-3 fatty acids on heart disease. N-3 fatty acids may also affect other ECG characteristics. For instance, T-loop width[58] and the spatial QRS-T angle[128] have been proposed as markers for heterogeneity of ventricular repolarization, which provides the condition for the genesis of ventricular arrhythmias. The spatial QRS-T angle has been recognized as risk predictor of cardiac mortality in the elderly.[128] Furthermore, U-wave changes may predict the occurrence of arrhythmias,[129] although little is known about the origin and physiological meaning of the U-wave. Effects of n-3 fatty acids on specific ECG characteristics could provide insight into the mechanism of a possible antiarrhythmic effect. It could also suggest new biomarkers for the study of the anti-arrhythmic potential of drugs and food ingredients in humans. Therefore, we investigated the effect of n-3 fatty acid intake on QTc, T-loop width, spatial QRS-T angle, and spatial U-wave amplitude in subjects with frequent premature ventricular complexes.

SUBJECTS AND METHODS

The study protocol was approved by the Medical Ethical Committee of Wageningen University. Patients gave their written informed consent after the study protocol had

been explained to them. Cardiologists recruited and enrolled patients aged 18 years or older with at least 1440 premature ventricular complexes per 24 hours in a previous Holter recording made less than six months before the study. Ninety-two patients who met the inclusion criteria were randomized of whom 84 successfully completed the study.

The primary purpose of this double blind, placebo-controlled study with parallel design was to investigate effects of n-3 fatty acids on the occurrence of premature ventricular complexes (results reported elsewhere). Patients were randomized to receive either a daily dose of 3.5 g fish oil or placebo oil (high oleic sunflower oil) (Loders Croklaan, Wormerveer, the Netherlands) during the intervention period of 14 ± 1 (mean ± SD) week. The oils were administered in seven soft gelatin capsules daily (Banner Pharmacaps Europe B.V., Tilburg, the Netherlands). Fish oil capsules provided ~700 mg eicosapentaenoic acid (EPA, C20:5n-3), 560 mg docosahexaenoic acid (DHA, C22:6n-3), and 260 mg of other n-3 fatty acids per day. The placebo capsules contained mainly oleic acid (C18:1(n-9)).

Compliance of the patients was checked by analysis of n-3 fatty acids in serum cholesteryl esters from non-fasting blood samples taken at the beginning and end of the intervention period.[110] Intakes of energy, fatty acids, cholesterol and alcohol were estimated by a telephone-administered 24-h dietary recall. In addition, fish intake was assessed twice by interviewing patients using a questionnaire on frequency of fish consumption.

Complete ECG measurements at both baseline and end of the intervention period were available for 74 patients. Standard 12-lead ECGs were recorded for one minute with a Cardio Perfect Portable recorder and digitally stored on a Cardio Control workstation (Cardio Control NV, Delft, the Netherlands). We processed ECG recordings without knowledge of treatment type or other subject variables, using the Modular ECG Analysis System (MEANS).[117] Bazett's formula ($QTc=QT/\sqrt{RR}$) was used to correct QT duration for heart rate. T-loop width and QRS-T angle were determined as described previously.[58;128] The amplitude of the spatial U-wave gradient vector was calculated by taking the integral of the x, y and z component of the U-wave using the interactive computer program 'Intraval'.[130] Differences in response between fish oil and placebo group were analyzed by a Student's *t* test. A subgroup analysis for patients with and without prior myocardial infarction was planned beforehand and included in the protocol.

RESULTS

There were no substantial differences in baseline characteristics between the fish oil and the placebo group (Table 6.1). EPA in serum cholesteryl esters (g/100 g total fatty acids) confirmed compliance; it changed during intervention from 1.30 ± 0.78 to 3.66 ± 1.68 in the fish oil group and remained constant in the placebo group (1.03 ± 0.70 to 0.98 ± 0.62). QTc was not significantly affected by intake of n-3 fatty acids; it decreased by 0.3 ms (95% confidence interval, -10.7 to 10.1 ms) or 0.1% in the fish oil group compared with placebo (Figure 6.1, Table 6.2). Intake of n-3 fatty acids also did not significantly affect T-loop width, spatial QRS-T angle, and spatial U-wave amplitude (Table 6.2). Subgroup analyses in patients with and without prior myocardial infarction also revealed no significant effects (Table 6.2).

Table 6.1 Characteristics of the 44 men and 30 women for whom complete ECG measurements were available

	Fish oil (n=38)	Placebo (n=36)
Age, y	68 ± 11	62 ± 14
Male sex, n (%)	27 (71.1)	17 (47.2)
Body Mass Index, kg/m^2	27 ± 4	26 ± 3
Background n-3 fatty acid intake from fish (ALA + EPA + DHA), g/month	9.6 ± 10.3	6.1 ± 7.7
History of myocardial infarction, n (%)	11 (28.9)	8 (22.2)

Values are mean ± SD or n (%).

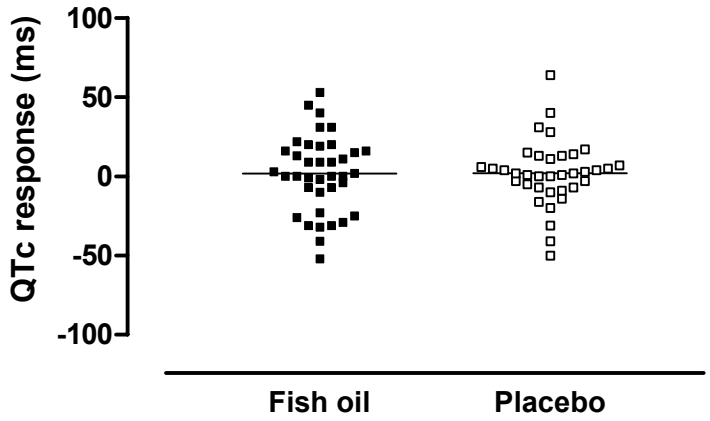

Figure 6.1 Response of the heart-rate corrected QT interval (QTc) of men and women who consumed 3.5 g/d of fish oil (1.5 g/d n-3 fatty acids) (N = 38) or placebo (N = 36) daily for ~14 wk.

Background dietary intake was similar in the two treatment groups. The fish oil group consumed 33% of the energy as fat, 0.6% of the energy (1.5 g/day) as total n-3 fatty acids (mostly alpha-linolenic acid), 4% of the energy as alcohol, and 23 mg/MJ cholesterol. In the placebo group the corresponding figures were 33%, 0.5% (1.1 g), 4%, and 28 mg/MJ. Fish intake was similar in the treatment groups and did not change during the intervention.

Table 6.2 ECG characteristics at the start and the end of the intervention period (mean ± SD) and the difference in response between fish oil and placebo group (95% CI), for all patients together and for the patients with and without prior myocardial infarction (MI) separately

	Fish oil		Placebo		Difference in response
	Baseline	End	Baseline	End	
All patients	(n = 38)		(n = 36)		
QTc, ms	437 ± 22	438 ± 28	435 ± 22	437 ± 26	-0.3 (-10.7 to 10.1)
spatial QRS-T angle, degrees	78.9 ± 44.2	76.2 ± 49.4	70.9 ± 37.6	67.9 ± 39.3	0.4 (-8.0 to 8.7)
width T loop, degrees	34.6 ± 29.0	38.0 ± 32.7	26.4 ± 18.4	26.7 ± 21.6	3.0 (-4.4 to 10.5)
spatial U-wave amplitude, μV	25.3 ± 34.8	19.1 ± 11.4	24.3 ± 30.6	19.5 ± 29.8	-1.4 (-15.8 to 13.0)
Patients with MI	(n = 11)		(n = 8)		
QTc, ms	430 ± 18	436 ± 30	433 ± 27	436 ± 36	2.5 (-26.3 to 31.3)
spatial QRS-T angle, degrees	91.3 ± 42.5	88.9 ± 56.3	114.3 ± 27.0	116.0 ± 27.5	-4.1 (-27.5 to 19.2)
width T loop, degrees	51.2 ± 39.6	50.6 ± 38.7	40.6 ± 20.9	41.3 ± 23.6	-1.2 (-15.2 to 12.9)
spatial U-wave amplitude, μV	32.8 ± 60.4	22.8 ± 15.7	38.5 ± 50.5	24.2 ± 30.7	4.4 (-40.3 to 49.1)
Patients without MI	(n = 27)		(n = 28)		
QTc, ms	439 ± 24	440 ± 27	436 ± 21	437 ± 24	-1.5 (-12.4 to 9.4)
spatial QRS-T angle, degrees	73.9 ± 44.7	71.1 ± 46.4	58.5 ± 30.5	54.1 ± 30.4	1.6 (-7.0 to 10.2)
width T loop, degrees	27.9 ± 20.8	32.8 ± 29.2	22.4 ± 15.8	22.6 ± 19.4	4.7 (-4.4 to 13.7)
spatial U-wave amplitude, μV	22.2 ± 16.8	17.5 ± 9.0	20.2 ± 21.7	18.1 ± 29.9	-2.6 (-16.1 to 10.9)

QTc: heart-rate corrected QT duration.

DISCUSSION

We observed no effect of a daily intake of 3.5 g fish oil for ~14 wk vs. placebo on QTc, QRS-T angle, T-loop width, or spatial U-wave amplitude in these patients with frequent premature ventricular complexes. It is unlikely that this is caused by a lack of power, because the 95% confidence interval of the difference in response of QTc was narrow. A true effect of n-3 fatty acids on QTc of more than 2.5% is not expected. In a previous study, we also observed no effect of n-3 fatty acids on several ECG characteristics in 84 healthy middle-aged men and women.[93] The effects of n-3 fatty acids on the electrophysiology of the human heart may be too small to cause appreciable ECG changes or there may be multiple effects at the cellular level that cancel each other out in the ECG. A standard ECG of short duration (1 minute) may not be useful to demonstrate possible effects of n-3 fatty acids on the heart.

Protective effects of n-3 fatty acids on hard endpoints in clinical trials have exclusively been found for post-myocardial infarction patients[3;4] and not in patients with angina.[70] It could be hypothesized that the cardioprotective effect of n-3 fatty acids is restricted to patients with earlier myocardial infarction. N-3 fatty acids may interact with structural abnormalities in cardiac tissue due to previous infarctions and in that way prevent fatal arrhythmias. If so, n-3 fatty acids would only affect the ECG in postinfarction patients. We did not find effects of n-3 fatty acids on the ECG in our subgroup of such patients, however this group was small (n=19).

A definitive answer as to whether n-3 fatty acids can reduce risk of ventricular arrhythmia will have to come from long-term trials on n-3 fatty acids and arrhythmia incidence in high-risk patients with an Implantable Cardioverter Defibrillator (ICD).[104] We are aware of three such trials; the first in 200 ICD patients recently reported a trend towards increased rather than decreased recurrence of ventricular arrhythmias in patients who received n-3 fatty acids over a follow-up of 2 years.[127] Two other trials are running and will report within two years.

Despite the body of evidence that n-3 fatty acids reduce risk of fatal heart disease by preventing ventricular arrhythmia, we could not demonstrate effects on cardiac electrophysiology as measured in body surface ECG. Our results do not provide additional support for the hypothesis that n-3 fatty acids prevent cardiac arrhythmia through electrophysiologic effects on heart cell membranes. Mechanistic studies at the organ level and clinical trials on endpoints will have to provide conclusive answers.

Chapter 7

Intake of n-3 fatty acids from fish does not lower serum concentrations of C-reactive protein in healthy subjects

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European Journal of Clinical Nutrition 2004; in press

ABSTRACT

Objective

High-sensitivity C-reactive protein (CRP), a marker of systemic inflammation, is a powerful predictor of cardiovascular risk. We hypothesized that n-3 fatty acids reduce underlying inflammatory processes and consequently CRP concentrations in healthy middle-aged subjects.

Design

Placebo-controlled, double-blind study.

Subjects

A total of 43 men and 41 postmenopausal women aged 50-70 y. Before and after intervention, we measured serum CRP concentrations with an enzyme immunoassay.

Interventions

Capsules with either 3.5 g/day fish oil (1.5 g/day n-3 fatty acids) or placebo for 12 weeks.

Results

The median CRP change in the fish oil group did not significantly differ from that in the placebo group (0.01 *vs* -0.17 mg/l, $P = 0.057$).

Conclusion

The currently available data –including ours– do not support that beneficial effects on CRP are involved in a mechanism explaining the protective effect on heart disease risk of n-3 fatty acids as present in fish.

INTRODUCTION

Blood markers of low-grade inflammation are emerging as risk indicators for coronary heart disease.[131] High-sensitivity C-reactive protein (CRP) is a marker of systemic inflammation produced by the liver and a powerful predictor of cardiovascular risk in healthy populations.[132;133] Dietary effects on markers of inflammatory processes have hardly been studied and are not yet established.[66;134] N-3 fatty acids are suggested to have anti-inflammatory effects; they appear to reduce the production of inflammatory cytokines associated with several chronic diseases, such as rheumatoid arthritis[135] and inflammatory bowel disease.[136] We hypothesized that n-3 fatty acids from fish reduce underlying inflammatory processes and in this way CRP concentrations.

SUBJECTS AND METHODS

The primary purpose of this placebo-controlled, double-blind study was to study effects of n-3 fatty acids on markers of arrhythmia; details have been published elsewhere.[93] A total of 43 men and 41 postmenopausal women aged 50-70 y were randomized within strata of habitual fish consumption, diastolic blood pressure, and sex[81] to receive either a daily dose of 3.5 g fish oil or placebo (Loders Croklaan, Wormerveer, the Netherlands) for 12 weeks as indistinguishable capsules. The fish oil provided approximately 700 mg eicosapentaenoic acid (C20:5n-3, EPA), 560 mg docosahexaenoic acid (C22:6n-3, DHA), and 260 mg of other n-3 fatty acids per day. The placebo was high-oleic-acid sunflower oil (C18:1n-9). Subjects were instructed to maintain their usual diet and lifestyle. In diaries, they reported illness, irregular medication use, and other deviations from their usual lifestyle. Intakes of energy and nutrients were estimated by 24-h dietary recalls. Subjects abstained from fish, seafood, or (additional) fish oil supplements from 4 weeks before and during the study. We measured CRP concentrations and n-3 fatty acids in cholesteryl esters in nonfasting blood samples at baseline and end.[110] High-sensitivity CRP concentrations were measured with an enzyme immunoassay.[137] CRP standard serum was used for calibration. The intra-assay variation was 5.8% for low (~0.5 mg/l), 2.7% for intermediate (~2 mg/l), and 4.8% for high (~10 mg/l) CRP values. Because of the skewed distribution of CRP values, we present values as medians and interquartile ranges. Differences in response between the fish oil and the placebo group were analyzed using the Mann-Whitney *U*-test.

RESULTS

Three subjects had CRP concentrations greater than 10 mg/l at one of the two blood collections and reported flu-like symptoms in their diary preceding that blood collection. We excluded them from the data reported here.[133] Inclusion of the outliers did not materially affect the results. Compliance was supported by a change in the proportion of EPA (C20:5n-3) in serum cholesteryl esters of 282% (from 0.76 ± 0.29 to 2.91 ± 0.67 g/100 g fatty acids) in the fish oil group and of -6% in the placebo group. Background dietary intake was similar in the two treatment groups. During the study, the fish oil group consumed 0.5% of the energy as n-3 fatty acids, 6% as linoleic acid (C18:2n-6), and 6% as alcohol. In the placebo group, the corresponding figures were 0.4%, 5% and 5%, respectively. The habitual fish intake was 8.4 ± 7.9 g n-3 fatty acids per month in the fish oil group and 8.7 ± 7.3 g n-3 fatty acids per month in the placebo group.

The median CRP change in the fish oil group (0.01 mg/l) was not significantly different (*P* = 0.057) from that in the placebo group (-0.17 mg/l) (Figure 7.1, Table 7.1).

Table 7.1 Serum CRP concentration at the start and after 12 weeks of treatment

	CRP concentration (mg/l); Median and interquartile range	
	Baseline	End
Fish oil	1.14 (0.62 - 1.71)	1.31 (0.63 - 2.17)
Placebo	1.47 (0.72 - 3.02)	1.14 (0.70 - 2.48)

The fish oil group received 3.5 g fish oil; the placebo group received 3.5 g high oleic sunflower oil.

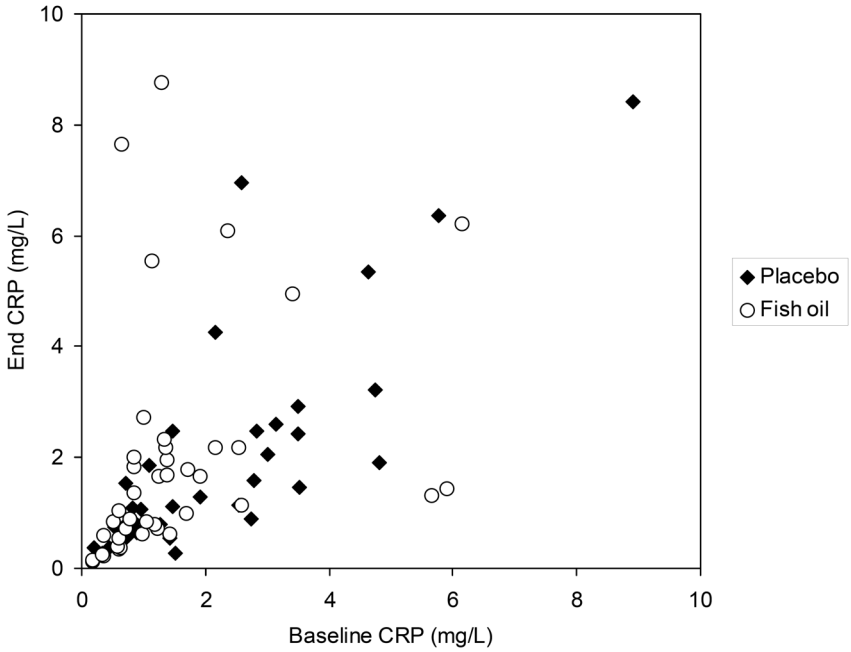

Figure 7.1 Serum CRP concentrations at baseline and after 12 weeks of dietary supplementation with placebo or fish oil.

DISCUSSION

Supplementation with 1.5 g of n-3 fatty acids per day did not lower CRP concentrations in healthy middle-aged subjects. The results would point towards an increase rather than a decrease of CRP with n-3 fatty acids relative to placebo. Other studies on fish oil and inflammation provide conflicting results. In healthy volunteers, increasing fish oil intake suppressed the *in vitro* synthesis of interleukin-2, interleukin-1β, interleukin-1α, and tumor necrosis factor-α.[138;139] However, placebo-controlled studies found that fish oil does not affect *ex vivo* cytokine production[140] or the functional activity of neutrophils, monocytes, or lymphocytes in healthy humans.[141] Few studies investigated effects of n-3 fatty acids on CRP. Pischon et al.[142] performed a cross-sectional analysis in 859 men and women. They reported a modest inverse relation between intake of EPA + DHA and CRP levels. In obese individuals, 4g/day fish oil for 6 weeks did not decrease CRP.[143] A recent study in healthy young subjects (mean age 38y) with lower baseline CRP concentrations (median 0.78 mg/l) than in our study reported no effect of n-3 fatty acids on CRP.[144] Our study shows that n-3

fatty acids also do not affect CRP in a middle-aged population that might be more prone to low-grade systemic inflammations (median CRP concentration 1.24 mg/l). We cannot exclude that n-3 fatty acids affect other inflammatory markers or CRP at higher concentrations during systemic inflammation. However, the currently available data –including ours– do not support that beneficial effects on CRP are involved in a mechanism explaining the protective effect on heart disease risk of n-3 fatty acids as present in fish.

Chapter 8

Discussion

MAIN CONCLUSIONS FROM THE STUDIES IN THIS THESIS

The objective of this thesis was to investigate *whether the possible antiarrhythmic effect of n-3 fatty acids is supported by short-term effects on electrophysiological markers*. Based on the results of our straightforward studies it is doubtful whether n-3 fatty acids affect the investigated markers of arrhythmia. First, we cannot confirm the suggested effect of n-3 fatty acids on heart rate variability.[33;34;79] Second, in our placebo-controlled study, n-3 fatty acids did not affect baroreflex sensitivity in apparently healthy subjects. Third, we do not consider n-3 fatty acids effective in the treatment of premature ventricular complexes as suggested by Sellmayer et al.[87] Additionally, we conclude that the standard electrocardiogram is not useful to study effects of n-3 fatty acids on the healthy human heart. The possible effect of dietary n-3 fatty acids on arrhythmia risk and subsequent sudden cardiac death is only supported by an effect on heart rate. Based on our studies, an effect of n-3 fatty acids on arrhythmia risk has become less likely.

USE OF ELECTROPHYSIOLOGICAL MARKERS OF ARRHYTHMIA

Biomarkers of risk can be used as surrogates for effects on disease outcome. A valid biomarker correlates well with clinical outcome and responds to different treatments in the same way as the clinical outcome. Furthermore, a biomarker should be easily measurable and potentially be modifiable by diet within the time frame of a study.[145] A practical advantage of biomarker studies is that they generally require less subjects and shorter duration than clinical trials on endpoints. In the case of n-3 fatty acids, effects on markers of arrhythmia would add to the body of evidence that already exists from clinical trials, observational studies and laboratory studies in animals and cells.

Short-term favorable effects on electrophysiological markers of arrhythmia may provide support to antiarrhythmic effects of n-3 fatty acids. More importantly, they may yield insight in underlying mechanisms of action. Also, existing and new biomarkers that predict health outcome will provide efficient tools to test the bioefficacy of various food substances in future studies. Effects on biomarkers should never be viewed in isolation from other types of evidence. Randomized clinical trials on disease outcome remain the gold standard for studying health effects of diet.

Heart rate variability and baroreflex sensitivity

Intervention studies by Christensen et al. suggest that n-3 fatty acids increase heart rate variability, particularly in post-myocardial infarction patients.[33;34;79] We were not able to reproduce these results in healthy persons. Christensen et al. used 24-hour recordings in free-living subjects, while we used 10-minute recordings made under highly standardized conditions. Thus, effects of n-3 fatty acids may only be detectable in 24-hour recordings of heart rate variability or be confined to post-myocardial infarction patients. Effect of n-3 fatty acids on baroreflex sensitivity has only been found in one other small study that did not use a concurrent placebo control group.[82] In our placebo-controlled study, n-3 fatty acids did not affect baroreflex sensitivity in apparently healthy subjects. Altogether, our results do not support the hypothesis that n-3 fatty acids can prevent cardiac arrhythmia through effects on cardiac autonomic control.

Premature ventricular complexes

Occurrence of premature ventricular complexes is a form of arrhythmia and it can be hypothesized that n-3 fatty acids decrease their incidence. This was indeed found by Sellmayer et al.[87] In our study, n-3 fatty acids did not substantially suppress the number of premature ventricular complexes. Three other small and probably underpowered studies did not find a significant effect of n-3 fatty acids on the number of premature ventricular complexes.[84-86] Thus the large effect as found by Sellmayer et al. may have been due to chance or may at least have overestimated the true population effect. Altogether, there is no convincing evidence that the presumed effect of n-3 fatty acids on the risk of sudden cardiac death is mediated by a reduction in the frequency of triggers of arrhythmia.

Heart rate and electrocardiographic characteristics

Some intervention studies reported that n-3 fatty acids or docosahexaenoic acid (DHA) alone decreased heart rate in various patient populations.[90;91;123] We were able to confirm these results using heart rate as determined by continuous 24-hour monitoring. We conclude that heart rate may be a marker that is sensitive enough to study possible antiarrhythmic effects of dietary components in different patient populations. It can be speculated that n-3 fatty acids decrease heart rate by an effect on the sinus node or by an effect on autonomic control.

Furthermore, effects of n-3 fatty acids on the standard electrocardiogram of humans have not been reported previously. We observed no effect of n-3 fatty acids on various electrocardiographic characteristics both in healthy middle-aged subjects and in patients with frequent premature ventricular complexes. The effects of n-3 fatty acids on the electrophysiology of the human heart may be too small to cause appreciable changes or there may be multiple effects at the cellular level that cancel each other out in the electrocardiogram.

C-REACTIVE PROTEIN

High-sensitivity C-reactive protein, a marker of systemic inflammation, is a powerful predictor of cardiovascular risk. Supplementation with n-3 fatty acids did not lower C-reactive protein concentrations in healthy subjects. This makes it less likely that beneficial effects on inflammation are involved in a mechanism explaining the protective effect on heart disease risk of n-3 fatty acids, although we cannot exclude an effect on C-reactive protein at higher concentrations during systemic inflammation.

DIETARY RECOMMENDATIONS

In the Netherlands, the recommendation for the dietary intake of n-3 fatty acids from fish is 0.2 g per day.[17] This is equivalent to one portion of fatty fish per week. Although our studies on markers of arrhythmia were mainly negative, they can not exclude an effect of n-3 fatty acids on life-threatening arrhythmia and sudden cardiac death, and thus do not provide a compelling reason to change this advice. Our findings do not exclude that intake of n-3 fatty acids may protect healthy individuals against future arrhythmia once they experience a first coronary event. Long-term intake of n-3 fatty acids may reduce the development of an arrhythmogenic substrate during future ischemic events, although such effects could not be investigated in the studies described in this thesis.

Since 2000 the American Heart Association recommends that all adults eat fish (particularly fatty fish) at least two times a week. For patients with documented coronary heart disease, they recommend ≈1 g of eicosapentaenoic acid (EPA) + DHA per day and also that an EPA + DHA supplement may be useful in patients with hypertriglyceridemia.[146] However, in a recent clinical trial, advice to eat two portions of fatty fish each week or to take 3 g of fish oil daily was associated with higher risk of

cardiac death and sudden cardiac death in 3114 men with angina.[70] This excess risk was largely seen in the subgroup given fish oil capsules. Unfortunately, compliance to advice was only shown for a subsample of patients and because the trial was not blind, the intake of fish oil may have modified the patient's or the physician's behavior towards intake of medication or diet and lifestyle. In light of all the earlier evidence it is hard to explain these adverse results. Although this study by Burr et al. had some methodological limitations, the results should not be ignored, and it stresses the need for double-blind well-controlled trials on life-threatening arrhythmia and mortality in different patient populations to support dietary recommendations.

DIETARY FISH VERSUS FISH OIL SUPPLEMENTS

Both the intake of n-3 fatty acids from fish oil supplements[3] and the intake of fish[4] have been successful in the risk reduction for mortality and sudden cardiac death. We chose to use capsules for practical reasons. However, possible adverse effects of fish oil capsules on cardiac death were reported in men with angina.[70] Based on this unexplainable finding it may be prudent to recommend dietary fish intake and not fish oil capsules. Next to that, replacement of meat in the diet with fish will result in lower intake of saturated fatty acids, which may provide an additional health benefit.

However, concern exists about environmental pollutants in fish, in particular polychlorinated biphenyls (PCBs) and mercury. Fish intake is a major dietary source of mercury and mercury in fish may counteract the beneficial effects of its n-3 fatty acids.[147] Highest mercury contents are found in swordfish, king mackerel, tilefish and shark. Therefore, consumption of a variety of fish, including species with no or low content of mercury, is recommended to minimize any potentially adverse effects due to environmental pollutants and, at the same time, achieve desired cardiovascular disease health outcomes.[24]

Yet another concern is the depletion of the already low stocks of edible fish in the ocean. Farming fish may appear to be a solution, but carnivorous fish species require large inputs of wild fish for feed.[148] Furthermore, farmed salmon have significantly higher concentrations of environmental contaminants such as PCBs than their wild counterparts, according to an analysis of about 700 salmon from around the world.[149] Individual contaminant concentrations in farmed and wild salmon did not exceed U.S. Food and Drug Administration action or tolerance levels for PCBs. The source of the contamination is most likely the farmed salmon’s diet. Therefore the most obvious solution for farmed fish is not feeding them contaminated fish oil and fish meal.

Suitable and sustainable alternatives, as vegetable oils, have to be developed and introduced.[150]

ALPHA-LINOLENIC ACID

The parent compound of the very long-chain n-3 fatty acids from fish is the plant-derived alpha-linolenic acid (ALA). Animal experiments indicate that ALA may prevent heart disease by inhibiting arrhythmias.[5;151] Three clinical trials investigated the effect of increasing ALA intake on the incidence of fatal coronary heart disease.[68;114;152] In all three trials a beneficial effect of ALA was found, but these three trials did not have the ideal double-blind structure and had other limitations in design. In a recent meta-analysis, it was estimated that increasing intake of ALA by 1.2 g/day decreases the risk of fatal coronary heart disease by at least 20%.[153] However, high ALA intake was in several epidemiologic studies associated with increased risk of prostate cancer.[154-156] This does not prove a causal effect, but even if true, the effect on heart disease would outweigh this possible adverse effect. Nevertheless, until further research is undertaken, fish should remain the first recommended source of n-3 fatty acids.

RECOMMENDATIONS FOR FUTURE RESEARCH

The adverse results of the trial on fish and fish oil in angina patients are hard to explain[70] and protective effects of n-3 fatty acids on hard endpoints have exclusively been found for post-myocardial infarction patients.[3;4] Therefore, trials on clinical endpoints in angina patients and other high-risk populations are needed to determine whether the effectiveness of n-3 fatty acids is restricted to post-myocardial infarction patients or also applies to other populations. We are aware of several ongoing trials on n-3 fatty acids and clinical outcomes of heart disease in different populations that will report in the coming years. Unfortunately, a primary prevention trial on n-3 fatty acids and clinical endpoints is prohibitive because of time and money constraints; it would take many years of intervention in tens of thousands of subjects.

Most studies until now investigated effects of a mixture of n-3 fatty acids as present in fish oil. Studies on the separate effects of the fish fatty acids EPA and DHA, and also the plant-derived ALA can provide information on differential effects of various n-3 fatty acids. We recommend to start with studies of these specific n-3 fatty acids on 24-hour heart rate, because this can fairly easy be measured. These studies can be performed in healthy subjects, but also in, for example, angina patients. If genuine

differential effects have been found, then specific n-3 fatty acids can be further studied in clinical trials on hard endpoints.

The results of our study do not support the suggested effect of n-3 fatty acids on heart rate variability. However, we cannot entirely reject an effect of n-3 fatty acids on heart rate variability, because we did not measure heart rate variability by 24-hour recordings in post-myocardial infarction patients. Therefore, the effect of n-3 fatty acids on 24-hour heart rate variability should be studied; first in post-myocardial infarction patients and if an effect is found in these patients, the study should be repeated in healthy subjects.

ALA may be an effective alternative to very-long-chain n-3 fatty acids for preventing arrhythmia and concurrent heart disease risk. ALA could work directly or might first need conversion to EPA or DHA in the body. The extent to which humans can convert ALA is unclear, therefore more research on the conversion of ALA to very-long-chain n-3 fatty acids in the human body is necessary.

Also, the possible adverse effect of ALA on prostate cancer risk should be further studied. A first approach would be to study effects of dietary ALA on prostate-specific antigen as a marker for prostate cancer risk.

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Samenvatting

INLEIDING

Uit onderzoek blijkt dat ieder jaar meer dan 50.000 mensen in Nederland overlijden aan hart- en vaatziekten, waaronder iets meer vrouwen dan mannen. Studies laten zien dat het eten van vis samengaat met minder hart- en vaatziekten. Bij patiënten met een eerder hartinfarct vermindert het innemen van extra visolie vooral de kans op plotse hartdood, een onverwacht verlies van hartfunctie. Deze beschermende effecten worden toegeschreven aan de meervoudig onverzadigde **n-3 vetzuren** die in vette vis en visolie voorkomen. Er wordt daarom geadviseerd om één tot twee keer per week vette vis te eten, zoals makreel, zalm en haring. Een mogelijke verklaring voor de beschermende werking van vette vis en visolie is dat mensen die extra n-3 vetzuren innemen minder **hartritmestoornissen** krijgen. Dit wordt ondersteund door resultaten van studies met dieren en experimenten met cellen.

DIT PROEFSCHRIFT

In dit proefschrift staan de resultaten van twee studies bij mensen beschreven. We hebben onderzocht of n-3 vetzuren veranderingen in de werking van het hart veroorzaken die wijzen op een verlaagde kans op hartritmestoornissen. We hebben dus niet direct gekeken naar het optreden van ernstige hartritmestoornissen. In plaats daarvan hebben we **indicatoren** gemeten die verband houden met de kans op het optreden van hartritmestoornissen.

In deze studies slikten de proefpersonen gedurende 12 – 14 weken capsules. De ene helft van de proefpersonen kreeg visolie en de andere helft zonnebloemolie. De hoeveelheid n-3 vetzuren die de **visoliegroep** binnenkreeg is te vergelijken met de hoeveelheid die mensen binnenkrijgen die elke dag (vette) vis eten. De **placebogroep** kreeg zonnebloemolie. Door het vergelijken van de visoliegroep met de placebogroep kunnen we met grotere zekerheid zeggen dat een eventueel effect door de visolie komt en niet door andere factoren.

STUDIE 1: CARDION

De eerste studie hebben we uitgevoerd bij **gezonde proefpersonen** die tussen de 50 en 70 jaar oud waren. Wij wilden in deze studie nagaan of het innemen van n-3 vetzuren invloed heeft op de werking van het hart bij gezonde personen. We bestudeerden de elektrische hartwerking en de aansturing van het hart vanuit het zenuwstelsel. Hiertoe registreerden we het elektrocardiogram (ECG, hartfilmpje) en de bloeddruk.

Het hart klopt niet regelmatig; de hartslag kan door allerlei invloeden sneller of langzamer worden. Zo heeft bijvoorbeeld de ademhaling invloed op de normale hartslag. Deze fluctuaties in hartslag noemt men **hartritme variabiliteit**. Een grotere hartritme variabiliteit is juist een teken voor een gezond hart en een goede controle door het zenuwstelsel. Een hart dat flexibeler om kan gaan met veranderingen is gezonder. Hartritme variabiliteit is een van de indicatoren die we in deze studie gemeten hebben. Ook werden de kleine schommelingen die iedereen in bloeddruk en hartritme heeft met elkaar in verband gebracht. Dit noemen we de **baroreflex gevoeligheid.** De baroreflex is een belangrijk regelmechanisme voor de bloeddruk. Tevens werd met behulp van computerprogramma's het hartfilmpje geanalyseerd, wat verscheidene **ECG-indicatoren** opleverde die iets over de gevoeligheid voor hartritmestoornissen kunnen zeggen.

In deze studie vonden we geen effecten van n-3 vetzuren op hartritme variabiliteit, baroreflex gevoeligheid of ECG-indicatoren. Het lijkt er dus op dat n-3 vetzuren weinig tot geen invloed hebben op de werking van het hart bij gezonde mensen.

STUDIE 2: PREVENC

Een volgende studie hebben we uitgevoerd bij patiënten met relatief onschuldige hartritmestoornissen, namelijk **ventriculaire extrasystolen**. Sommige mensen merken dat ze ventriculaire extrasystolen hebben, doordat ze het hart plotseling sterk voelen kloppen (‘overslaan’), anderen voelen er niets van. Het vaak optreden van ventriculaire extrasystolen duidt op een toegenomen gevoeligheid van het hart voor ritmestoornissen en gaat gepaard met een verhoogde kans op het krijgen van hart- en vaatziekten. In deze studie wilden we nagaan of n-3 vetzuren het aantal extrasystolen kan verminderen. Om dit goed te kunnen vaststellen deden we dit onderzoek bij patiënten bij wie heel veel extrasystolen optreden.

Aan het begin en aan het eind van dit onderzoek hebben we twee maal gedurende een hele dag en nacht een hartfilmpje gemaakt (**Holter**). In deze opnames konden we bekijken of het aantal extrasystolen verminderde bij de patiënten die visolie kregen. We zagen dat dit bij een aantal patiënten het geval was, maar dat er ook patiënten waren waarbij het aantal extrasystolen toenam. Bij elkaar genomen zagen wij geen duidelijk effect van n-3 vetzuren op ventriculaire extrasystolen.

Net als in de studie bij gezonde mensen vonden we bij de patiënten met veel extrasystolen geen effecten op ECG-indicatoren. Wel zagen we dat visolie bij deze patiënten de **hartslag** een beetje vertraagde. Dit wijst op een lager risico voor het optreden van plotse dood, dus een beschermend effect van n-3 vetzuren.

CONCLUSIE

In onze studies vonden we weinig aanwijzingen dat n-3 vetzuren inderdaad de kans op sterfte door hartziekte verlagen via het verminderen van hartritmestoornissen. Een effect van visolie op de snelheid van de hartslag bij patiënten met onschuldige ritmestoornissen is het enige resultaat van onze studies dat wijst op een **beschermend** effect van n-3 vetzuren. Toch sluiten we niet uit dat n-3 vetzuren niet alleen voor patiënten met een eerder hartinfarct maar ook voor nog gezonde personen belangrijk zijn. De beschermende effecten zouden pas merkbaar en meetbaar kunnen zijn op het moment dat later een ernstig hartinfarct optreedt.

Dankwoord

Op het moment dat ik dit schrijf is mijn proefschrift bijna af. Dit is een goed moment om iedereen die op zijn of haar eigen manier een bijdrage heeft geleverd aan mijn onderzoek de revue te laten passeren. Ik hoop maar dat ik niemand vergeet…

Mijn promotoren Martijn Katan en Evert Schouten wil ik als eerste bedanken. Martijn, van een afstandje was je zeer betrokken en vooral je commentaar op mijn manuscripten was erg leerzaam. Door jouw inbreng is de kwaliteit van studies en publicaties gewaarborgd. Evert, je belangstelling voor mijn project was groot; je hebt steeds meegedacht over de richting en inhoud van het onderzoek. Jouw contacten met cardiologen en andere experts kwamen zeer goed van pas.

Vervolgens wil ik noemen: Peter Zock mijn co-promotor en Ingeborg Brouwer mijn ‘dagelijks begeleider’. Peter, ik wil je bedanken voor al jouw input; je onuitputtelijke kennis, inspirerende ideeën en kritische opmerkingen. Ik vond het prettig dat ik bij je binnen kon lopen als ik feedback nodig had. Ingeborg, je hebt al het wel en wee van mijn project van zeer dichtbij meegemaakt. Bedankt voor je eindeloze hulp, goede adviezen en nuchtere kijk op de zaken!

Ik wil de leden van mijn begeleidingscommissie bedanken voor hun kritische commentaar en hun frisse ideeën. Luc Jordaens, Tobias Op’t Hof, Mike Rudrum en Kees Swenne, ondanks jullie drukke agenda wisten jullie tijd vrij te maken om vanuit zeer verschillende perspectieven mijn onderzoek te bekijken.

Alle proefpersonen die hebben deelgenomen aan de CardioN-proef en de PreVenC-trial wil ik hartelijk bedanken voor hun inzet en enthousiasme.

Vera Fierkens en Trudy Jansen waren onmisbaar tijdens de CardioN-proef. Jullie hebben de proefpersonen begeleid en alle metingen uitgevoerd. Dankzij jullie professionele werkwijze en jullie plezierige gezelschap hebben we de studie gezamenlijk tot een zeer goed einde gebracht.

Het was niet mogelijk geweest de PreVenC-trial uit te voeren zonder de medewerking van de cardiologen van de deelnemende ziekenhuizen, met name wil ik noemen Loet van Kempen, Piet van Kalmthout en Luc Cozijnsen. Van begin af aan was duidelijk dat jullie mee wilden werken om deze studie te doen slagen en dat is dan ook gelukt. Zeker ook door de vakkundige werkwijze van de researchverpleegkundigen Anja, Astrid, Desiree, Jeanne, Jolanda, Margreeth en Rina. Bedankt allemaal!

Vervolgens wil ik een aantal mensen bedanken die ervoor hebben gezorgd dat ik de resultaten van gespecialiseerde analyses in mijn studies heb kunnen gebruiken. Kees Swenne heeft veel werk verzet voor de meting en analyse van hartritme variabiliteit en baroreflex gevoeligheid. Annelies Schoneveld heeft de baroreflex analyses verzorgd. Arie Maan was verantwoordelijk voor de analyse van de Holters en heeft de programmatuur aangepast aan mijn wensen. De medewerkers van SEAL (Stichting ECG Analyse Leiden) hebben de analyses van de Holters uitgevoerd. Jan Kors verzorgde de analyse van de ECG's. Henk Ritsema van Eck maakte het mogelijk om ook de U-golf te meten. De analyses hiervoor zijn uitgevoerd door Micky van der Reep. Kees Kluft wil ik bedanken voor de samenwerking in het kader van de CRP-analyses.

Drie studenten hebben als onderdeel van hun studie meegewerkt aan mijn onderzoek: Jacoba Greving, Martinet Streppel en Sabine Quak. Ik vond het leerzaam en gezellig om met jullie samen te werken, bedankt voor al het werk wat jullie hebben gedaan.

Ik heb de afgelopen 4 jaar veel hulp gehad van de afdeling diëtetiek. Natuurlijk voor de planning en uitvoering van het voedselconsumptieonderzoek, maar ook voor de randomisatie en blindering van de capsules en voor advies omtrent velerlei zaken. Karin, Petra, Els, Saskia, Jeanne, Irna en Dieneke, dank jullie wel!

De analyses van de n-3 vetzuren in de cholesterolesters zijn zeer bekwaam uitgevoerd door de medewerkers van het laboratorium van de afdeling Humane Voeding. Truus, Pieter, Jan en Peter, bedankt.

Ik werk sinds een aantal maanden samen met Ellen Kampman en Gerda Pot aan het FISHGASTRO-project. Nu mijn proefschrift af is, heeft dit project mijn onverdeelde aandacht. Bedankt voor de prettige samenwerking tot nu toe.

Alle medewerkers van de afdeling Humane Voeding wil ik hartelijk bedanken voor de prettige werksfeer en alle hulp die ik de afgelopen 4 jaar heb gehad. De collega's van programma 1 wil ik bedanken voor de gezellige en leerzame werkbesprekingen en de medewerkers van het stafbureau van WCFS voor hun behulpzaamheid.

Ik heb het heel erg getroffen met mijn kamergenoten. Margreet, we zijn een goed team op 213: 's ochtends koffie en niet te veel praten en 's middags hele discussies bij de thee. Floor, het is alweer even geleden, maar ik ben niet vergeten hoe gezellig wij het samen hadden.

Het organiseren van de buitenlandse excursie voor de AIO's van onze afdeling was een leuke en leerzame afwisseling van het doen van onderzoek. Onze reis naar Zwitserland, Italië en Duitsland zal ik niet snel vergeten (waar was jij op 11 september 2001?). Excursiecommissie, bedankt voor de gezelligheid en de prettige samenwerking.

De Koffieclub wil ik bedanken voor alle gezelligheid en de vele (soms zinloze) discussies die we gevoerd hebben. Even niet met werk bezig zijn, maar ontspannen op z'n tijd, was voor mij onmisbaar.

Ook buiten werktijd werd er vaak gesproken over onderzoek, begeleiders en andere (afdelings)zaken, dit natuurlijk onder het genot van een hapje, drankje en spelletje. Astrid, Linette, Marleen, Monica en Rosalie, bedankt voor de gezellige avonden en weekendjes, we gaan deze traditie zeker voortzetten. Linette en Astrid, ik ben blij dat jullie op 15 september naast me zullen staan in de aula. Jullie zullen het goed doen als paranimfen en kunnen alvast inspiratie opdoen voor jullie eigen promotie.

Liesbeth en Mariska, onze etentjes zijn altijd erg geslaagd, zeker ook de laatste paar in Frankrijk. We blijven gewoon een rare combi, zelfs over de grens!

Jan & Helma, Gea & John en alle familie uit Maastricht en omstreken, bedankt voor jullie interesse in mijn werk en voor alle gezellige afleiding. Marjan, onze planning ging uiteindelijk weer mooi gelijk op. Ik vond het fijn om met jou over onze AIO-perikelen te kletsen, bedankt en veel succes op 1 oktober.

Lieve papa, mama en Judith, het is alweer 11 jaar geleden dat ik wegging uit Maastricht, maar jullie steun en vertrouwen in mij blijven natuurlijk onmisbaar.

Lieve Robin, deze ereplaats in mijn dankwoord is uiteraard voor jou. Bedankt voor alles wat je de afgelopen jaren voor mij gedaan en betekend hebt!

Curriculum Vitae

Anouk (Maria Martina Elisabeth Eduard) Geelen was born on March 25, 1975, in Maastricht, the Netherlands. In 1993, she passed secondary school, VWO B, at the 'Stedelijke Scholengemeenschap' in Maastricht. In the same year she started the study 'Nutrition and Health' at the former Wageningen Agricultural University. She focused on nutrition and health in western societies. As part of that study she joined the research group of Professor David Jenkins in Toronto for five months to study the effect of wheat gluten on serum lipids. In 1999 she received the MSc degree and started working for the Wageningen Centre for Food Sciences (WCFS) and the Division of Human Nutrition and Epidemiology of Wageningen University. She performed a human intervention trial on the interaction between genetic make up and response of serum cholesterol to plant sterols. In May 2000 she started as a PhD-fellow on the project as described in this thesis. She joined the education program of the Graduate School VLAG (advanced courses in Food Technology, Agrobiotechnology, Nutrition and Health Sciences). She was a member of the daily board of the committee of temporary scientific staff within the Division of Human Nutrition from 2001 through 2003. She was a member of the PhD Study Tour committee that organized a study tour to Switzerland, Italy and Germany in 2001. She is currently working as a post-doc on the project ‘Fish consumption and gastrointestinal health’ at the Division of Human Nutrition of Wageningen University.

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Training and Supervision Plan

Basiscursus electrocardiografie	Boerhaave Commissie	2000
Journal club	Human Nutrition, WUR	2000-2002
N-3 literatuur club	Human Nutrition, WUR	2000-2004
NWO-voeding Papendal	Werkgemeenschap Voeding	2000 2001 2003
PhD-week	VLAG	2000
English Scientific Writing	CENTA	2001-2002
Nutrition & Lifestyle Epidemiology	VLAG	2001
PhD-excursion to Switzerland, Italy, and Germany	Human Nutrition, WUR	2001
Systematisch literatuuronderzoek	Mint/Nutrim	2001
Cursus Persoonlijke Effectiviteit	Sekam	2002
Electrofysiologie club	Human Nutrition, WUR	2002-2004
ISSFAL Congress (International Society for the Study of Fatty Acids and Lipids)	ISSFAL	2002 2004
Thematic Meeting Omega-3 Fatty Acids and Cardiovascular Disease	VLAG/WCFS	2002
Europace Conference	European Society of Cardiology	2003
International Conference on Health Benefits of Mediterranean Diet	EGEA	2003
Thematic Meeting Seafood, Fatty Acids and their role for Human and Fish Health and Development	WIAS/VLAG	2003
Symposium Visvetzuren voor Hoofd en Hart	Nederlands Visbureau	2003
Tutorentraining	OWU, WUR	2003
Effectief Functioneren in Organisaties	VLAG	2004

The studies described in this thesis were funded by the Wageningen Centre for Food Sciences, an alliance of major Dutch food industries, Maastricht University, TNO Nutrition and Food Research, and Wageningen University and Research Centre, with financial support by the Dutch government.

Financial support by the Netherlands Heart Foundation, Wageningen University and Wageningen Centre for Food Sciences for the publication of this thesis is gratefully acknowledged.

Cover design: Drukkerij Cortjens, Maastricht

Printing: Grafisch bedrijf Ponsen & Looijen B.V., Wageningen