STELLINGEN

1. Consumptie van vette vis heeft een gunstig effect op het risico voor coronaire hartziekten.

2. De betere samenstelling van voedingsvetten door de verlaging van het transvetzuurgehalte heeft de gemiddelde kans op de ontwikkeling van coronaire hartziekten in Nederland sinds 1990 verminderd.

3. Zutphenaren zijn geen vetkleppen.
   Reactie op Het Parool, 15 maart 2001

4. Voor het verkrijgen van een evenwichtig beeld over de relatie tussen voedingsstoffen en het risico op coronaire hartziekten moeten editors en onderzoekers gestimuleerd worden ook negatieve resultaten te publiceren.

5. *Openbaarheid van wetenschappelijke bevindingen stimuleert de productie van* voedingsmiddelen met een gezondheidsclaim.

6. De kwaliteit van de wetenschapsjournalistiek kan verbeterd worden als journalisten zich niet alleen zouden richten op internationale tijdschriften met een hoge impact factor.

7. Bij het beoordelen van de gezondheidseffecten van nieuwe genetisch gemodificeerde voedingsmiddelen is meer sprake van angst dan van risico.

8. Voor een duikende voedingsonderzoeker heeft de uitspraak 'zo gezond als een vis' meerdere betekenissen.

Stellingen behorende bij het proefschrift 'Prospective studies on diet and coronary heart disease. The role of fatty acids, B-vitamins and arginine'.

Claudia Oomen
Wageningen, 26 juni 2001

Prospective studies on diet and coronary heart disease

The role of fatty acids, B-vitamins and arginine

Claudia M Oomen

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Prospective studies on diet and coronary heart disease

The role of fatty acids, B-vitamins and arginine

Claudia Maria Oomen

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 dinsdag 26 juni 2001
des namiddags te half twee in de Aula.

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Prospective studies on diet and coronary heart disease. The role of fatty acids, B-vitamins and arginine
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in herinnering aan mama
voor papa

ABSTRACT

In this thesis, the results of prospective studies on fatty acids, B-vitamins and arginine and the occurrence of coronary heart disease have been described. The results presented are mainly based on the Zutphen Elderly Study. In this study of 939 men aged 64-84 years, detailed information was available on dietary intake, biological risk factors, incidence of major chronic diseases, vital status and causes of death between 1985 and 1995. In addition, data of 5 cohorts from Finland, Italy and the Netherlands, and of all 16 cohorts of the Seven Countries Study were used to investigate specific hypotheses on diet-heart relations in an international setting.

Using repeatedly collected data of serum cholesterol, dietary and lifestyle factors in the Zutphen Elderly Study, we observed that changes in intake of saturated fat, polyunsaturated fat, dietary cholesterol and alcohol, and changes in weight were significantly related to changes in total and/or HDL cholesterol concentrations. The strengths of the observed associations are comparable to those obtained in experimental studies performed in younger populations. This indicates that public health strategies on dietary interventions and weight reduction in order to improve cholesterol concentrations are relevant also at old age.

The average trans fatty acid intake decreased 2.4% of energy in the Zutphen Elderly Study between 1985 and 1995, mainly due to industrial lowering of the trans fatty acid content in edible fats. Such a decrease in trans fatty acids intake is compatible with a 23% reduction in risk of coronary heart disease. Changes in food composition with respect to the trans fatty acid content are therefore important from a public health perspective.

Using the Finnish, Italian and Dutch data of the Seven Countries study, fatty fish consumption was estimated to reduce the relative risk of coronary heart disease mortality with 34%. For total or lean fish consumption no effect was observed. People at risk for coronary heart disease are therefore advised to eat fish, preferably fatty fish at least once a week, to reduce their risk for coronary heart disease.

We did not find evidence for a protective effect of intake of α-linolenic acid on coronary heart disease incidence in the Zutphen Elderly Study. However, the strong association between the intake of α-linolenic acid and trans fatty acids complicated studying this relationship. Of the B-vitamins, only the intake of folate and vitamin B6 were independently inversely associated with homocysteine in the Zutphen Elderly Study. In this cohort, intake of different B-vitamins did not protect against coronary heart disease, although there is a suggestion for a protective effect of vitamin B2. Arginine intake was not associated with the risk of coronary heart disease mortality, neither in the Seven Countries Study, nor in the Zutphen Elderly Study. Results of prospective and intervention studies are needed before definite statements can be made about the potential protective effect on coronary heart disease of B-vitamins, arginine and α-linolenic acid.

CONTENTS

General introduction

about 20,000 persons died from coron
Netherlands, about 80% of all deaths fi
people over 65 years of age.[2] Increasing
increase the number of elderly people in
this is expected to be accompanied by a
coronary heart disease between 1994 and

Coronary heart disease occurs when
myocardium is reduced or stopped. T
coronary heart disease is atherosclerosis.
accumulations of lipids and fibrous eleme
changes in the endothelium and the m
muscle cells in the intima.[3] The most imp
of coronary heart disease is rupture o
complicated by occlusive thrombi.[4]

High serum cholesterol is a major cause o
metabolized in the liver, is transported t
density lipoproteins (LDL) carry choleste
high-density lipoproteins (HDL) transpor

cholesterol.[6,7] Only a small (non-significant) inverse relation between cis monounsaturated fatty acids and serum cholesterol was observed. Recent controlled dietary intervention studies investigated the effect of different fatty acids and dietary cholesterol on LDL and HDL cholesterol.[8-10] These studies show that LDL cholesterol is increased by a high intake of saturated fatty acids and dietary cholesterol and is decreased by a high intake of cis polyunsaturated fatty acids. High intake of saturated fatty acids, mono- and polyunsaturated fatty acids and dietary cholesterol increase HDL cholesterol. This effect diminishes with increasing unsaturation of the fatty acids and is smallest for dietary cholesterol.

In conclusion, strong indirect evidence is available that confirms the hypothesis relating dietary saturated, mono- and polyunsaturated fatty acids to coronary heart disease. However, in primary and secondary prevention trials, only modest reductions in coronary heart disease events were shown by dietary fat modification (mainly on the replacement of saturated fatty acids, often associated with changes in polyunsaturated fatty acids and/or dietary cholesterol).[11] Several inadequacies of these trials have been discussed, including limitations in study design (e.g. sample size and follow-up period) or the inclusion criteria (e.g. subject's susceptibility based on baseline diet and absolute risk).[12,13] The results of secondary prevention trials generally suggest that an overall reduction in fat is less effective compared to replacement of saturated to unsaturated fatty acids.[14] The extent to which coronary heart disease risk can be reduced by dietary fat modification is generally small and depends on the type of modification (including the intensity of the dietary change),[15,16] and the population at risk. In addition, other dietary factors may also be important for the prevention of coronary heart disease.[17]

Trans fatty acids

Trans fatty acids are formed when oils are partly hydrogenated to produce fats with better texture and stability. Trans fatty acids are also naturally produced in the rumen of cattle. In the eighties, levels of trans fatty acids in commonly used sources with hydrogenated fat such as hard margarines amounted to 50% in the Netherlands.[18] Up to that point, there had generally been little concern about the large quantities of commercially produced trans fatty acids.[19] At that time, dietary experiments on the effect of trans fatty acids on serum cholesterol were difficult to interpret because changes in trans fatty acids intake were often associated with changes in intake of saturated or cis unsaturated fatty acids.[20]

Specific experiments on trans fatty acids examined only their effect on total cholesterol concentrations and showed inconsistent results.[21,22]

Interest in trans fatty acids was renewed in the early nineties, when several controlled dietary intervention studies showed that a high intake of trans fatty acids not only increased total and LDL cholesterol, but also decreased HDL cholesterol concentrations.[20] Consequently, the food industry in the Netherlands decided to reduce the trans fatty acids content in margarines and spreads to less then 5% in 1995.[23] This resulted in a decline in trans fatty acid intake in several European countries including the Netherlands.[24] Information on the size of this decline in intake of trans fatty acids and, consequently, its effect on the prevention of coronary heart disease is lacking.

OTHER DIETARY EFFECTS ON CORONARY HEART DISEASE

Besides the effect of diet on serum cholesterol and lipoprotein fractions, dietary factors may also affect the risk of coronary heart disease by other mechanisms. During the 1980s, new trials were initiated based on the idea that dietary habits could be protective in relation to coronary heart disease. In the DART trial, the hypothesis was tested whether fatty fish consumption protects against coronary heart disease.[25] In two other secondary prevention trials, the IEIS and Lyon diet heart trial, comprehensive dietary changes with regard to the Indian diet[26] and a Mediterranean-type of diet[27] were studied. In these two trials, the experimental group consumed a diet low in intake of total fat and saturated fatty acids and high in intake of n-3 fatty acids (from marine and/or plant origin), fruits, vegetables, legumes and cereals compared to the control group. With regard to the clinical result, these dietary trials were remarkably successful in reducing the risk of coronary heart disease and all-cause mortality. These trials suggest that a wider range of dietary factors than fatty acids, acting by different mechanisms, play a role in the development of coronary heart disease.

Based on the available evidence, at least three groups of dietary factors may be associated with the risk of coronary heart disease: n-3 fatty acids, B-vitamins and arginine. Main sources of n-3 fatty acids are (fatty) fish, major sources of B-vitamins are vegetables, meat, milk and potatoes, and sources of arginine are meat, milk and cereals.

N-3 fatty acids

Low rates of coronary heart disease in Greenland, together with a high consumption of fish, have led to the speculation that seafood consumption prevents coronary heart disease.[28] Further epidemiological studies on the association between fish consumption and coronary heart disease, however, showed inconsistent results.[29] Some inconsistencies in the relation between fish consumption and coronary heart disease across populations may be explained by difference in the intake of n-3 fatty acids from fish. N-3 fatty acids eicosapentaenoic (C20:5 n-3) and docosahexaenoic (C22:6 n-3) acid are probably the protective components of fish, and their concentration depends on the type of fish. High-dose marine n-3 fatty acids have been shown to affect beneficially human lipid profiles[30] and blood pressure.[31] N-3 fatty acids from fish have antithrombotic effects in vitro, due to the reduced production of thromboxane A_2 by arachidonic acid, since eicosapentanoic acid competes with archidonic acid for its enzymes[32] (figure 1). Furthermore, in animal models, n-3 fatty acids prevent insulin resistance[33] and the development of ventricular fibrillation.[34] However, human experiments on the effect of a limited intake of n-3 fatty acids obtained from a habitual diet with regular fish consumption were limited and inconsistent. Some prospective studies on the association of both fish consumption and marine n-3 fatty acids with coronary heart disease mortality observed a stronger beneficial effect of fish consumption than of the intake of n-3 fatty acids.[35,36] Therefore, evidence on the protective effect of fish (including the responsible protective components) is inconclusive.

Besides n-3 fatty acids derived from fish, another n-3 fatty acid is present in the green tissue of plants: α-linolenic acid (C18:3 n-3). α-Linolenic acid is the basic n-3 fatty acid and can be elongated and desaturated into eicosapentaenoic acid in animals and humans[37] (figure 1). Eicosapentaenoic and docosahexaenoic acid are found in fish, due to the conversion of α-linolenic acid from phytoplankton.[38] Although the capacity of humans to elongate and desaturate α-linolenic acid is small, α-linolenic acid intake was associated with substantial changes in platelet behavior.[39] α-Linolenic acid itself, or its conversion into eicosapentaenoic acid, may play a role in the protection against coronary heart disease. However, until now, evidence on the relation between α-linolenic acid intake and coronary heart disease is limited.

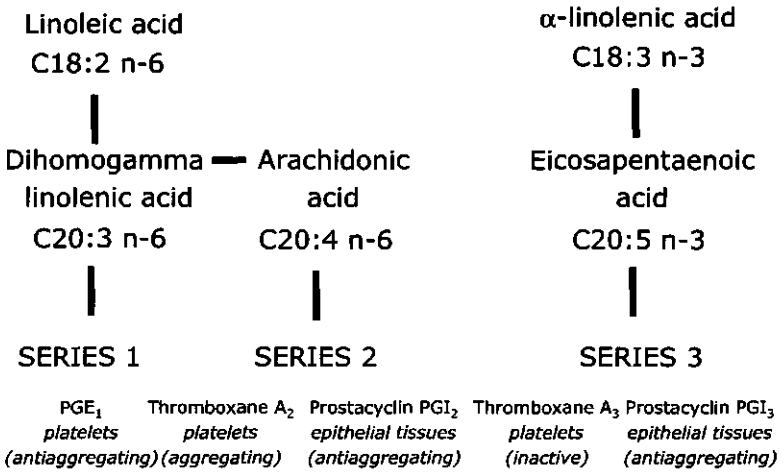

Figure 1: Metabolism of, and prostanoids formed from, n-6 en n-3 unsaturated fatty acids.[17]

B-vitamins

Homocysteine is a sulfur-containing amino acid that may be involved in the development of atherosclerosis. Many case-control and cohort studies showed that elevated blood concentrations of homocysteine are associated with increased risk for cardiovascular diseases.[40,41] Several mechanisms have been suggested to underlie this association, including platelet aggregation, increased blood coagulation or reduced thrombolysis and endothelial dysfunction and injury.[42] However, there is some doubt about the causal association between homocysteine and vascular diseases, since homocysteine concentrations are related with several other cardiovascular risk factors and with an early decline in renal function, which is common in atherosclerosis.[43]

Homocysteine is formed at demethylation of the dietary essential amino acid methionine. Four B-vitamins serve as either coenzyme or substrate for one of the homocysteine metabolic pathways; folate, vitamins B2, B6 and B12.[44] Folate (as a substrate in the form of 5-methyl-tetrahydrofolate) and vitamin B12 (as a coenzyme) are involved in the remethylation reaction converting homocysteine to methionine. Vitamin B2 is the coenzyme of methylenetetrahydrofolate reductase, which catalyzes the conversion of tetrahydrofolate to 5-methyl-

tetrahydrofolate. Vitamin B6 is a coenzyme for two enzymes involved in the transsulphuration of homocysteine to cysteine.

Until now, most studies on the association between B-vitamins and coronary heart disease were case-control studies. The majority studied circulating levels of B-vitamins, instead of dietary intake of the vitamins. The results of these studies have been inconsistent.[41] However, underlying diseases or short-term variations in nutrient intake may have influenced plasma vitamin levels and, consequently, the association with coronary heart disease risk. Prospective studies relating B-vitamin intake to coronary heart disease risk may provide more insight in the causality of the relation between B-vitamin intake, homocysteine and coronary heart disease.

Arginine

Another potential protective dietary factor in the relation with coronary heart disease is arginine. Arginine is a semi-essential amino acid, and is the precursor of nitric oxide. Nitric oxide is an important mediator of vasodilatation. It is synthesized by vascular endothelium to regulate the vascular tone in normal conditions and alterations of such control in disease status. Apart from vasodilatation, nitric oxide has also anti-clotting and anti-adhesive properties that may inhibit atherosclerosis.[45] Impaired availability of nitric oxide has been identified in patients with risk factors for coronary heart disease such as hypercholesterolemia, atherosclerosis and hypertension. From experimental studies, the hypothesis is derived that arginine intake could restore the impaired endothelial function and increased platelet activation in these patients. However, contradictory results were shown in dietary experiments, dependent on the risk profile of the experimental group, dose and duration of arginine therapy.[46] The association between dietary arginine and the risk of coronary heart disease in human populations has not been investigated yet.

RATIONALE OF THIS THESIS

The multiple aspects of diet to affect the risk of coronary heart disease outlined above creates challenges for conducting studies to investigate these relationships. Until now most research has been done in dietary intervention studies, investigating the relation between (a high dose of) nutrients and risk factors for coronary heart disease. It is essential to interpret the results of

promising dietary factors in the light of coronary heart disease incidence using prospective population-based studies. Such information will give insight in the impact of nutrient intake at a practical dose, the interrelationships between nutrients and, if consistent with other evidence, can be directly related to public health relevance. The studies described in this thesis focus on three different (classes of) nutrients: fatty acids, B-vitamins and arginine. These nutrients have great potential, however, the evidence from prospective studies on the relation between these dietary constituents and coronary heart disease is limited and/or inconclusive.

STUDY POPULATIONS

In this thesis, data are used of cohorts from the Seven Countries Study.[47] In 1960, Zutphen, a small industrial town located in the eastern part of the Netherlands, was selected for the Dutch contribution to the Seven Countries Study. Another 15 cohorts of men aged 40-59 years from United States, Japan, Greece, former Yugoslavia, Finland and Italy were also enrolled and examined. Although in most cohorts dietary data were collected at baseline in small random samples only, in Zutphen individual dietary information has been collected in 1960, 1965 and 1970, and in the two cohorts from Finland in 1969 and in two cohorts from Italy, in 1965 and 1970. In 1985, the Zutphen cohort was renamed the Zutphen Elderly Study, and the surviving sample was extended with a random sample of other men from the town of Zutphen in the same age category. Hypotheses in this thesis are investigated using data of the Zutphen Elderly Study, and when data are available, using the cohorts of Finland, Italy and the Netherlands and intercohort comparisons of the Seven Countries Study.

OUTLINE OF THIS THESIS

In this thesis, prospective studies on the relations between different dietary constituents and the occurrence of coronary heart disease are addressed. Four studies describe the relation between dietary fatty acids and serum cholesterol concentrations and/or the risk of coronary heart disease. In **CHAPTER 2,** we report on the association between changes in lifestyle and dietary factors, such as different fatty acids on changes in serum total and HDL cholesterol in an elderly population. Concerning the recent changes in trans fatty acid content of

hydrogenated fats, we estimate the decline in trans fatty acid intake between 1985 and 1995 in a Dutch population of elderly men in **CHAPTER 3.** Furthermore, in this chapter, we relate trans fatty acid intake to the 10-year risk of coronary heart disease.

To get more insight in the beneficial effect of n-3 fatty acids, we conducted two studies. In **CHAPTER 4,** we report on the association between lean and fatty fish consumption, which are different in n-3 fatty acid content, and coronary heart disease risk in middle-aged men from Finland, Italy and the Netherlands. In **CHAPTER 5** the association between α-linolenic acid intake and the risk of coronary heart disease in elderly men is described.

Furthermore, three studies deal with (determinants of) amino acids as potential protective nutrients in relation to the risk of coronary heart disease. In **CHAPTER 6**, the association between B-vitamin intake and the risk of coronary heart disease is examined as well as the extent to which the associations are explained by serum homocysteine concentrations. Chapter 7 and chapter 8 deal with the association between arginine and coronary heart disease risk. In **CHAPTER 7**, prospective data from the 16 population cohorts of the Seven Countries Study are used to estimate the association between arginine intake and 25-year coronary heart disease mortality in middle-aged men at cohort level. In **CHAPTER 8**, detailed dietary data of the Zutphen Elderly Study are used to estimate the association between arginine and 10-year coronary heart disease risk at individual level.

Finally, the main results, methodological considerations, public health implications and recommendations for future research are discussed in **CHAPTER 9.**

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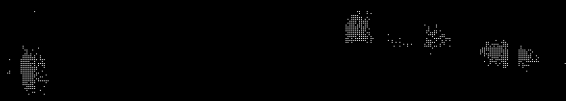
Changes in diet and body weight predict changes in total and HDL



concentrations: A longitudinal study of elderly men

Claudia M Oomen, Marga C Ocké, Edith JM Feskens, Frans J Kok, Daan Kromhout. Submitted for publication.

Improvement in lifestyle and dietary habits favorably modifies total and high densi lipoprotein (HDL) cholesterol concentrations, but little is known about the cholester lowering effect of these determinants in old age. We investigated the cholester modifying effect of lifestyle and diet in a longitudinal cohort study of elderly men. Tl study population consisted of 234 men born between 1900 and 1920 and participating each dietary and clinical examination of the Zutphen Elderly Study at baseline (1985 and after 5 and 10 years of follow-up. Changes in total and HDL cholester concentrations, body weight, smoking, physical activity, and dietary variables as alcoh use, saturated and trans unsaturated fat, cholesterol and fiber were calculated over five year periods. Repeated measurements were performed using a mixed longitudin model. After adjustment for age, carbohydrate intake and the other parameters, 1% energy increase in polyunsaturated fat intake was associated with 0.03 mmol/L reducti in total cholesterol concentrations. Weight loss was associated with decreased tot cholesterol concentrations, although non-significantly in men without chronic disease One extra glass of alcohol or 5 kg weight loss were both associated with 0.04 mmol increased HDL cholesterol concentrations. Increased intake of saturated fat and dieta cholesterol were significantly related to increased HDL cholesterol concentrations. Tl size of the observed associations is comparable to those from experimental studi performed in younger populations. This study showed that, even in old age, it remai important to change weight and diet to improve the lipid profile.

INTRODUCTION

Despite a fall in serum cholesterol concentrations at older age,[1-6] prospecti studies indicate that high total cholesterol concentrations still convey a increased risk for future coronary heart disease events in elderly persons.[7-10] Th association between high density lipoprotein (HDL) cholesterol concentratio and coronary heart disease among older persons is less clear.[8-10] Meta-analys of trials showed that cholesterol lowering medication remains effective in o age.[11] Improvement in lifestyle or dietary habits in the elderly population cou also favorably modify the total and HDL cholesterol concentrations, and cou therefore be important in the prevention of coronary heart disease. Bod weight,[12] smoking,[13] physical activity,[14] alcohol use[15] and dietary factors a saturated[16-18] and trans fatty acids,[19] dietary cholesterol[16-18] and fiber[20] are a known to influence the cholesterol concentrations in adults. However, there

not much experimental evidence on the cholesterol lowering effect of lifestyle and diet in elderly populations.

Besides trials, longitudinal data can be used to determine whether lifestyle and dietary factors could modify cholesterol concentrations in old age. Furthermore, a longitudinal population-based study provides information about the effectiveness of each of these factors in changing cholesterol concentrations in daily practice. All previous longitudinal studies showed that weight change was a predictor of the change in total and HDL cholesterol.[6,21-24] Changes in physical activity and smoking habits were less consistently associated with changes in cholesterol.[6,22,24] Of these longitudinal studies, only one was performed among elderly people,[6] and others were performed in younger age-groups.[21-24] In addition, one longitudinal study dealt with diet and total cholesterol in a population of elderly men and women, however, only the unadjusted correlation coefficients were reported.[4]

The Zutphen Elderly Study contains longitudinally repeated measurements of total and HDL cholesterol as well as of lifestyle and dietary factors over a 10 year period. Therefore, we were able to investigate the impact of naturally occurring changes in body weight, smoking, physical activity, and changes in intake of dietary factors such as alcohol, fatty acids, cholesterol and fiber on the change in total and HDL cholesterol concentrations in elderly men.

SUBJECTS AND METHODS

Study population

The Zutphen Elderly Study is a longitudinal investigation of chronic disease risk factors in elderly male inhabitants of Zutphen, a town in the eastern part of the Netherlands. It represents a continuation of the Zutphen Study, the Dutch contribution to the Seven Countries Study.[25] In 1985, 367 of the 555 participants of the original cohort still alive, were re-examined. In addition, a random sample of 711 other men from the town of Zutphen in the same age group were asked to participate. This resulted in a total population of 939 men (response rate 74%) born between 1900 and 1919, which formed the cohort of the Zutphen Elderly Study. Dietary and clinical examination including cholesterol determinations took place in 1985, 1990 and 1995. Complete data on

cholesterol, lifestyle and diet were available for 806 men in 1985, 491 men in 1990 and 259 men in 1995.

Data collection

All relevant data were collected according to a standardized protocol in the period between March and June of each examination year. Clinical examinations were conducted by trained medical staff at a local survey site in 1985 and 1990 and by paramedical staff at home in 1995.

Non-fasting venous blood samples were used for cholesterol determinations which were conducted in the same standardized lipid laboratory (Department of Human Nutrition and Epidemiology, Wageningen University, the Netherlands), according to WHO criteria. Cholesterol was determined enzymatically with the CHOD-PAP mono-testkit from Boehringer Mannheim.[26] HDL cholesterol was isolated after precipitation of apo-lipoprotein B containing particles by dextran sulphate-Mg^{2+}.[27] In all three examination rounds the combined within and between variation coefficients for total and HDL cholesterol were below 3%. Mean bias with regard to target values of serum pools provided by the Centers for Disease Control (Atlanta, USA) was +0.2% in 1985, +0.9% in 1990 and -1.1% in 1995 for total cholesterol. For HDL cholesterol the mean bias from the control sera was -0.3% in 1985, +4.2% in 1990, and -0.7 in 1995.

Information about the habitual food consumption was collected using the cross-check dietary history method, adapted to the Dutch situation.[28] Each participant, and if possible also his wife, was interviewed about his average food consumption pattern in the month before the interview. A checklist of foods and quantities of food bought per week were used to calculate and to verify the participant's food consumption pattern. A national database of food composition tables containing information about energy and nutrients including alcohol was specially composed for the analysis of dietary trends.[29] For the present analyses, the edition of 1986-1987, 1989-1990 and 1996 of this database was used. In addition, time-specific tables were compiled with total trans fatty acid contents of consumed foods around 1980, around 1990 and in 1995.[30]

Body weight was measured without shoes and in underwear in 1985 and 1990, and in daily clothing in 1995. In the calculations for 1995, 1.5 kilograms were subtracted from recorded weight to correct for the additional clothes. Information on cigarette smoking was obtained by a standardized questionnaire and categorized into 'no smoking' and 'current smoking'. Minutes of physical

activity (mainly walking, cycling, hobbies, sports, gardening and work) per week were calculated using a self-administered questionnaire originally designed for retired men.[31] Information on cholesterol lowering medication use was obtained through a standardized questionnaire in 1985 and 1990. Cholesterol lowering diet was used as proxy for cholesterol lowering medication use in 1995.

Information on the presence or history of cardiovascular diseases was obtained using the Dutch translation of the Rose questionnaire.[32] Diabetes mellitus or any form of cancer was reported using a standardized questionnaire. Diagnosis of each disease was verified with hospital discharge data and with written information from the subject's general practitioner.

Statistical methods

Statistical analyses were carried out using SAS (version 6.12) statistical analysis computer package. Changes in cholesterol concentrations, body weight and dietary variables from 1985 to 1990 and from 1990 to 1995 were calculated as the 1990 value minus the 1985 value and the 1995 value minus the 1990 value. Changes in cigarette smoking were classified as quit smoking, start smoking or no change in smoking behavior.

Repeated measurements were performed using a mixed longitudinal model with a compound symmetry covariance structure by means of the SAS Proc Mixed Procedure.[33] The dependent variables were 5 years changes in total and HDL cholesterol. The independent variables were quitting smoking, and 5 years change in body weight, physical activity, intake of fiber, dietary cholesterol, intake of energy percentages of saturated fat, monounsaturated fat, polyunsaturated fat and trans fatty acids, and for HDL cholesterol also 5 years change in alcohol consumption. Analyses were performed with or without adjustment for the other determinants. Age at examination year and intake of carbohydrates were additionally included as confounders. Start smoking was not included as a determinant into the models, due to the small numbers of starters (3.4%) during 10 years of follow up. Because change in energy intake was not associated with change in cholesterol concentrations, and additional adjustment for change in energy intake did not alter the results, the results shown were not adjusted for change in energy intake. Additional analyses were performed taking into account the mean bias from the control sera of the total cholesterol and the HDL cholesterol concentrations. Analyses were performed on men who participated in each examination in order to account for selective dropout. A total

of 239 men participated in each of the three examinations; 5 men were omitted from the present analyses because of cholesterol lowering medication in 1985 and 1990 (n=4), or 1995 (n=1). An additional analysis was performed for men with at least 2 out of 3 examinations (1985 and 1990 or 1995) (n=494). Furthermore, analyses were performed for men participating in all 3 examinations but without a history of cardiovascular diseases, cancer or diabetes (n=133).

RESULTS

At baseline, over 95% of the Zutphen Elderly population lived independently. Mean age of the men was 69.4 years (SD=4.3 years) (table 1). Twelve percent of the men had a history of cardiovascular diseases at baseline, which consisted mainly of coronary heart disease. Mean body weight of the participants was 78 kg (SD=11 kg) and a quarter of the men were cigarette smokers. The study population spent on average 99 minutes/day on physical activity.

Table 1. Characteristics (mean ± standard deviation) of participants in the Zutphen Elderly Study 1985-1995 (n=234).

	1985	1990	1995
Total cholesterol (mmol/L)	6.08 ±1.03	6.04 ±1.02	5.51 ±1.04
Adjusted for bias from control sera	6.07 ±1.03	5.98 ±1.01	5.45 ±1.03
HDL cholesterol (mmol/L)	1.13 ±0.26	1.15 ±0.28	1.17 ±0.32
Adjusted for bias from control sera	1.13 ±0.26	1.10 ±0.27	1.18 ±0.32
Age (years)	69.5 ±4.3	74.5 ±4.3	79.5 ±4.3
Body weight (kg)	77.9 ±10.4	77.3 ±10.5	74.4 ±11.2
Physical activity (min/week)	692 ±510	618 ±510	411 ±423
Cigarette smoking (%)	25.6	21.4	17.5
History of cardiovascular diseases (%)	12.0	19.7	28.2
History of diabetes mellitus (%)	4.3	10.3	11.5
History of cancer (%)	2.6	9.4	12.8

During 10 years of follow-up, the mean level of total cholesterol decreased from 6.08 mmol/L to 5.51 mmol/L (table 1). The change in total cholesterol was most pronounced between 1990 and 1995. The mean level of HDL cholesterol increased from 1.13 mmol/L to 1.18 mmol/L. Mean body weight, physical activity and alcohol consumption decreased during follow-up.

The baseline diet was high in saturated fat (about 17% of total energy intake), in trans fat (about 4% of total energy intake) and in fiber (about 26 g/d) (table 2). Mean energy intake decreased during the first 5 year of follow up, and slightly increased during the second 5 year of follow up. Independent of energy intake, mean daily intake of saturated fat, monounsaturated fat, trans fatty acids, dietary cholesterol and fiber decreased during follow-up, whereas the mean daily intake of polyunsaturated fat increased (table 2).

Table 2. Daily nutrient intake (mean ± standard deviation) of participants in the Zutphen Elderly Study 1985-1995 (n=234).

	1985	1990	1995
Energy (MJ)	9.3 ±1.9	8.5 ±1.8	8.6 ±1.8
Alcohol (g)	14.4 ±17.8	11.3 ±14.3	10.6 ±13.8
Carbohydrates (% of energy)	41.3 ±7.1	43.8 ±7.0	43.1 ±6.8
Saturated fat (% of energy)	17.7 ±3.4	15.9 ±3.4	16.7 ±3.5
Monounsaturated fat (% of energy)	14.9 ±3.0	14.5 ±2.5	13.7 ±2.5
Polyunsaturated fat (% of energy)	7.0 ±2.8	7.3 ±3.3	7.1 ±3.1
Trans fatty acids (% of energy)	3.9 ±2.0	2.8 ±1.3	1.9 ±0.6
Dietary cholesterol (mg/MJ)	29.4 ±8.2	27.2 ±7.3	27.7 ±8.3
Dietary fiber (g)	25.9 ±6.8	24.2 ±6.7	23.3 ±6.2

Table 3 shows the unadjusted and adjusted associations of changes in body weight, smoking, physical activity and dietary factors with change in total cholesterol using a repeated measurement model. Unadjusted analyses showed that increase in intake of saturated fat, dietary cholesterol and decrease in polyunsaturated fat intake were significantly associated with increased serum total cholesterol concentrations. After adjustment of age, carbohydrate intake and the other parameters, weight change and change in intake of polyunsaturated fat were significantly associated with the change in total

cholesterol (table 3). The comparable unadjusted and adjusted ß-coefficients (p=0.10) for trans fatty acids suggests that trans fatty acid intake might also modify total cholesterol concentrations.

Table 3. Regression coefficients relating five year changes in serum total cholesterol (mmol/L) to 5-years changes in body weight, smoking and dietary variables in a repeated measurement model: the Zutphen Elderly Study.

	Unadjusted			Adjusted*		
	β	SE	p	β	SE	p
Body weight (kg)	0.014	0.008	0.07	0.015	0.008	0.05
Physical activity (min/week)	-0.00004	0.00006	0.49	-0.00007	0.00006	0.28
Quitting cigarette smoking (yes/no)	0.040	0.132	0.77	-0.005	0.129	0.97
Daily intake of						
Saturated fat (% of energy)	0.021	0.010	0.04	0.012	0.014	0.39
Monounsaturated fat (% of energy)	0.000005	0.012	0.80	-0.026	0.017	0.13
Polyunsaturated fat (% of energy)	-0.031	0.011	0.005	-0.032	0.013	0.02
Trans fatty acids (% of energy)	0.031	0.019	0.12	0.035	0.022	0.10
Dietary cholesterol (mg/MJ)	0.012	0.004	0.006	0.007	0.005	0.14
Dietary fiber (g)	-0.007	0.006	0.20	-0.005	0.006	0.36

β=regression coefficient, SE= standard error, p=p-value
* all parameters plus age at examination and carbohydrate intake (% of energy) were included in the model.

The repeated measurement analyses of determinants of change in HDL cholesterol concentrations showed that a decrease in weight and increase in alcohol consumption were significantly associated with increase in HDL cholesterol in the unadjusted and adjusted analyses (table 4). Increased alcohol consumption with one glass per day or weight loss of 5 kg were both associated with 0.04 mmol/L increased concentrations of HDL cholesterol. Increased intake of saturated fat and dietary cholesterol were also significantly associated with increased HDL cholesterol. The changes in other parameters did not have an impact on the changes in concentrations of HDL cholesterol. After additional adjustment of total and HDL cholesterol concentrations for the bias from the control sera, comparable associations were found (data not shown). Similar

associations were also observed in the total population of men who participated in at least two examinations (n=494) (data not shown).

Among the participants who participated in all three examinations, 43% of the men had a history of diagnosed cardiovascular diseases, cancer or diabetes after 10 year of follow-up. Adjusting for these common chronic diseases and serum albumin levels in the analyses did not alter the results (data not shown). However, subgroup analyses in men free of any common chronic disease in 1995 (n=133) showed no association between changes in body weight and total cholesterol changes (adjusted analyses: ß=0.0004; p=0.97 compared to ß=0.03; p=0.01 for the 101 men with chronic diseases).

Table 4. Regression coefficients relating five year changes in serum HDL cholesterol (mmol/L) to 5-years changes in body weight, smoking, physical activity and dietary variables in a repeated measurement model: the Zutphen Elderly Study.

	Unadjusted			Adjusted*		
	β	SE	p	β	SE	p
Body weight (kg)	-0.007	0.002	0.001	-0.008	0.002	0.001
Physical activity (min/week)	0.00002	0.00002	0.31	0.000007	0.00002	0.63
Quitting cigarette smoking (yes/no)	0.013	0.034	0.70	0.022	0.033	0.50
Daily intake of						
Alcohol (g)	0.003	0.001	0.001	0.004	0.001	0.001
Saturated fat (% of energy)	0.005	0.003	0.05	0.008	0.004	0.04
Monounsaturated fat (% of energy)	0.002	0.003	0.50	0.006	0.005	0.24
Polyunsaturated fat (% of energy)	-0.002	0.003	0.45	0.005	0.004	0.19
Trans fatty acids (% of energy)	-0.008	0.005	0.88	-0.005	0.005	0.40
Dietary cholesterol (mg/MJ)	0.002	0.001	0.08	0.003	0.001	0.05
Dietary fiber (g)	-0.002	0.001	0.14	-0.002	0.001	0.26

β=regression coefficient, SE= standard error, p=p-value
*all parameters plus age at examination and carbohydrate intake (% of energy) were included in the model.

DISCUSSION

The results from our longitudinal analyses showed that changes in body weight and changes in intake of polyunsaturated fat were significantly related to changes in total cholesterol, and that changes in body weight, alcohol use, intake of saturated fat and dietary cholesterol attribute to the changes in HDL cholesterol concentrations at old age.

Change in body weight was a consistent predictor of total and HDL cholesterol in other longitudinal studies among the elderly[6,34] or middle-aged populations.[21-24] Consistent with the results of another elderly population,[6] the associations did not change after adjustment for the presence of common chronic diseases. In the present study, however, subgroup analyses on the men free of common chronic diseases suggest that the observed association between change in weight and changes in total cholesterol depends on disease status. Consistent with other longitudinal studies,[6,22] we showed that change in weight was an independent predictor of change in HDL cholesterol.

As in the present study, change in alcohol consumption was positively associated with change in HDL cholesterol among elderly men and women of the Rancho Bernardo Study.[6] Moreover, in the same study, change in physical activity was not associated with changes in cholesterol concentrations, as in our study. This could be due to the inappropriate intensity of physical activity at older age.[6] In contrast with other longitudinal studies,[6,22,24] we did not observe an association between quitting smoking and cholesterol concentrations. Since the proportion of the men that quit smoking was only 11% in the present study, this might be too small to investigate this issue properly.

Several longitudinal studies have studied the impact of dietary changes on total cholesterol. However, to our knowledge, this is the first longitudinal study to determine whether changes in diet predict changes in HDL cholesterol. In the present study, changes in intake of polyunsaturated fat was associated with changes in total cholesterol and changes in alcohol use, intake of saturated fat and dietary cholesterol were associated with changes in HDL cholesterol. In a population of elderly men and women, the intake of total fat and dietary cholesterol was significantly positively correlated with total cholesterol concentrations.[4] In a longitudinal study among middle-aged men, dietary cholesterol was positively associated with total cholesterol concentrations.[21] Change in Keys score (summarizing the intake of cholesterol, saturated and

polyunsaturated fat) was positively associated with changes in total and LDL cholesterol concentrations in young and middle-aged adults.[23,35]

For a correct interpretation of our results we will address some methodological issues. Habitual food consumption was measured in all three examinations using the cross-check dietary history method, which is acknowledged as a valid method in an epidemiological setting.[28] Intake of dietary components was calculated taking into account changes in food composition in time. To reduce residual confounding we included all dietary and lifestyle factors potentially related to changes in serum cholesterol into one model. Furthermore, the contribution of morbidity to the associations between lifestyle and dietary factors and cholesterol concentrations was determined. We observed that the impact of body weight on total cholesterol was absent in men without cardiovascular diseases, cancer and diabetes mellitus. The interpretation of this finding was hampered, however, because especially among elderly people, disease status is determined by multiple other diseases. Therefore, further studies need to investigate this issue.

Table 5 summarizes our results together with the results of meta-analyses of experimental studies.[12,15-20] Physical activity was not included as the effect of aerobic exercise studied in controlled trials[14] is not comparable with the effect of an increase in overall physical activity in minutes per week as in the present study. For the significant associations in our observational analyses as well as for non-significant associations, e.g. those between trans fatty acids and total cholesterol and between polyunsaturated fat and HDL cholesterol, the size of the estimates is comparable to those from experimental studies (table 5). Concerning the relation of saturated fat and total cholesterol and of trans fatty acids and HDL cholesterol, we observed somewhat smaller estimates compared to the results of three meta-analyses of experimental studies. Also weaker associations were observed for dietary fiber, however, the experimental studies only included soluble fiber while in the present analyses, due to lack of information on these nutrients, total fiber was included.

It is obvious that, because of methodological differences (e.g. the age of the study population, the follow-up time and the control for identifiable differences), comparison of the evidence from randomized, controlled studies and the results of the present observational study is difficult.[36] However, the consistency in these results suggests that the efficacy of weight and dietary changes on lipid

profile under controlled conditions in generally younger populations is comparable to those in daily practice in an elderly population.

Table 5. Results from meta-analyses of intervention trials on the effect of body weight and dietary factors on changes in total and HDL cholesterol in comparison with present results of the Zutphen Elderly Study (ZES).

	Changes in cholesterol (mmol/L)					
	Total cholesterol			HDL cholesterol		
	ZES	Trials	Ref.no	ZES	Trials	Ref.no
Body weight (kg)	0.02†	0.05	12	-0.008	-0.009	12
Alcohol (g)	-			0.004	0.003	15
Saturated fat (% of energy)	0.01*	0.04	16	0.008	0.007	17
		0.05	17, 18		0.012	16
					0.013	18
Monounsaturated fat (% of energy)	-0.03*	0.003	16	0.006*	0.006	18
		0.005*	18		0.009	16
Polyunsaturated fat (% of energy)	-0.03	-0.02	16, 17	0.005*	0.005	18
		-0.03	18		0.007	16
Trans fatty acids (% of energy)	0.04*	0.03	19	-0.005*	-0.02	19
Dietary cholesterol (mg)	0.0008*	0.0006	17	0.0003	0.0001	18
		0.0007	18			
Dietary fiber (g)	-0.005*	-0.05	20	-0.002*	-0.003*	20

ZES=Zuthen Elderly Study, Ref. no= reference number
*not-statistically significant association.
† Change in total cholesterol per kg increase in body weight in total study population (n=234). Total cholesterol changed not-significantly by 0.0004 (p=0.97) per kg increased body weight in subgroup of men without common chronic diseases.

Our results suggest that, even in old age, it remains important to change weight or dietary habits to improve the lipid profile. Increase in polyunsaturated fat intake was the dietary factor that was most consistently associated with decreased total cholesterol concentrations in our elderly men. For HDL cholesterol, decreased body weight and increased alcohol consumption, intake of saturated fat and dietary cholesterol are significantly related to increased HDL cholesterol concentrations. Due to the high risk of coronary heart disease with

age, such beneficial effects on cholesterol concentrations could have a large impact on public health.

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Association between

intake and 10-year risk of coronary heart disease in the Zutphen Elderly Study: a prospective population based study

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Evidence on the relation between trans fatty acid intake and coronary heart disease i limited. We investigated this relation in a Dutch population with a fairly high trans fatt acid intake, including trans fatty acids from partly hydrogenated fish oils. W prospectively studied 667 men of the Zutphen Elderly Study aged 64-84 years and fre of coronary heart disease at baseline. We used dietary surveys to establish th participants' food consumption patterns. Information on risk factors and diet wa obtained in 1985, 1990, and 1995. After 10 years of follow-up from 1985-95, there wer 98 cases of fatal or non-fatal coronary heart disease. Between 1985 and 1995, averag trans fatty acid intake decreased from 4.3% to 1.9% of energy. After adjustment for age body mass index, smoking, and dietary covariates, trans fatty acid intake at baselin was positively associated with the 10-year risk of coronary heart disease. The relativ risk for a difference of 2% of energy in trans fatty acid intake at baseline was 1.28 (95% CI 1.01-1.61). This study shows that a high intake of trans fatty acids (all types o isomers) contributes to the risk of coronary heart disease. The substantial decrease i trans fatty acid intake, mainly due to industrial lowering of trans contents in Dutch edibl fats, could therefore have had a large public-health impact.

INTRODUCTION

Concern about the adverse health effects of trans isomers of unsaturated fatt acids has increased since 1990 after the results of controlled dietary interventio studies.[1-7] Results of these studies showed a detrimental effect of trans fatt acids on LDL and HDL cholesterol. Evidence that intake of trans isomers affect the rate of coronary heart disease is derived from population-based studies don in the USA.[8-11] Until now, a fairly small number of observational studies hav focused on the health effects of trans fatty acids in Europe, with weak o equivocal results.[12-14]

Trans fatty acids are mainly present in solid fats produced by part hydrogenatio of oils, and are naturally found in products originating from ruminant animals The current trans fatty acid intake contributes between 0.5% and 2.1% t energy intake in western Europe,[15] and about 2% of total energy intake in th average US diet.[16] In the Netherlands, because of publicity about adverse effect of trans fatty acids on blood lipoproteins,[17] the amount of trans fatty acids in fat for use in households has decreased substantially. In frequently used foods, suc as hard margarines, the trans fatty acid content has declined from a maximu

of 50% in the 1980s to an average 1-2% nowadays.[18] Consequently, the consumption of trans fatty acids in the Netherlands has decreased greatly.

Most controlled trials[1-4,6] and population-based studies[8-12] have focused on the effect of isomers with 18 carbon atoms (C18:1 trans isomers), since these isomers mainly originate from partly hydrogenated vegetable oils and ruminant fat. In addition, because of different proportions of C18:1 trans isomers, results of some observational studies have made a distinction between manufactured and ruminant trans fatty acids, and suggested more harmful health effects of manufactured trans fatty acids.[8,9,13] Although industry in the USA only uses partly hydrogenated vegetable oils, in European countries foods have also been manufactured with partly hydrogenated fish oils.[19,20] An adverse effect of high amounts of trans fatty acids from hydrogenated fish oil on blood lipids has already been shown.[5] However, the health effects of these isomers in quantities consumed in daily life are unknown.

We investigated the association between trans fatty acid intake and the risk of coronary heart disease in the Zutphen Elderly Study, a population with a fairly high dietary trans fatty acid intake at baseline, including trans fatty acids from partly hydrogenated fish oils.

METHODS

Study population

The study population consisted of men who participated in the Zutphen Elderly Study, an extension of the Zutphen Study. In 1960, the Zutphen Study started with a cohort of 878 men from Zutphen (Netherlands) born between 1900 and 1919, as the Dutch contribution to the Seven Countries Study.[21] In 1985, 367 of 555 participants who were still alive were re-examined. In addition, 711 other men from the town of Zutphen in the same age category were asked to participate. A total of 939 men (response rate 74%) was examined in 1985, 560 in 1990 (response rate 78%), and 343 in 1995 (response rate 74%). Of the 343 men who participated in 1995, a random sample of 280 men took part in the dietary survey. Complete information on diet and risk factors was available for 824 men in 1985. We excluded 157 men with previously diagnosed myocardial infarction or angina pectoris, which left 667 men at baseline in 1985, of whom 435 and 225 participated in the dietary survey in 1990 and 1995, respectively.

Data collection

Dietary surveys and medical examinations were completed between March and June in 1985, 1990, and 1995. We obtained information about the habitual food consumption with the cross-check dietary history method, adapted to the Dutch situation.[22] Each participant, and if possible his partner, was interviewed about his average food consumption pattern in the month before interview. A checklist of foods and quantities of food bought per week was used to calculate and verify the participant's food consumption pattern.

We calculated nutrient intake with corresponding Dutch food tables. Time-specific tables with trans fatty acid content of consumed foods were compiled.[23] National data were available for edible fats analyzed by the Wageningen University, Netherlands, around 1985 and 1990, and by the TRANSFAIR Study[24] in 1995. In 1995, products such as biscuits and pastries (Wageningen University) and dairy products and meats (TRANSFAIR Study) were analyzed. The trans fatty acid contents of the remaining foods were based on analyses from abroad, derived from recipes, or deduced from other foods. Because the gas chromatographic method underestimates measurement of trans fatty acids, contents were adjusted by taking the combination of gas-liquid chromatography of 4,4-dimethyloxazoline derivatives and methyl esters[25] or the infra-red spectrometry as a reference.

During medical examinations, we took non-fasting venous blood samples. Serum total cholesterol and HDL cholesterol were determined enzymatically.[26,27] We measured blood pressure in duplicate with a random zero sphygmomanometer while participants were supine. Hypertension was defined as use of antihypertensive medication, a systolic blood pressure of 160 mm Hg or greater, or a diastolic blood pressure of 95 mm Hg or greater. We calculated total minutes of physical activity per week,[28] information on cigarette smoking, and diabetes mellitus, with a questionnaire. We ascertained history of coronary-heart disease with the Dutch translation of the Rose questionnaire.[29]

Follow-up

Incident cases included fatal coronary heart disease plus non-fatal myocardial infarction (whichever arose first) occurring between baseline assessment in 1985 and January, 1995. Three participants were lost to follow-up. We obtained information on vital status of the participants from the municipal registries, and on cause of death between 1985 and June 1990 from Statistics Netherlands. For

deaths thereafter, or if data were not available from Statistics Netherlands, information was obtained from hospital discharge data or general practitioners. We coded causes of death in accordance with the ninth revision of the International Classification of Diseases. Coronary heart disease refers to codes 410-414. Because the underlying cause of death in elderly people is often difficult to establish, we classified coronary heart disease as a primary (n=46) as well as a secondary (n=3) cause of death in the analyses.

We obtained information on non-fatal myocardial infarction by a standardized medical questionnaire, or, in case of non-response, by a short questionnaire completed by the participants or their closest relative. All reported myocardial infarctions were verified with hospital-discharge data. Also, in men who died, information on disease history was obtained from the general practitioner. Diagnosis of myocardial infarction required at least two of the following criteria: a specific medical history, characteristic electrocardiographic changes, and specific increases in concentration of enzymes.

Statistical methods

All statistical analyses were carried out using the SAS (version 6.12) package. Men were divided into tertiles on the basis of the contribution of trans fatty acids to energy intake at baseline. To compare the baseline major risk factors and dietary factors across categories of trans fatty acid intake, we used analysis of variance for normally distributed variables, the Kruskal-Wallis test for skewed variables, and the Chi-square test for categorical variables.

We used Cox's proportional-hazard analysis to calculate relative risks, with the lowest trans fatty acids tertile as the reference group, or including trans fatty acid intake as the continuous variable. In the continuous analyses, we estimated the relative risk associated with a difference of 2% of energy in total trans fatty acid intake. This difference was based on the reports of two prospective studies,[10,11] which is in agreement with the range in trans fatty acid intake at baseline, and the 10-year decrease in trans fatty acid intake in the present study. Adjustments were made for age, intake of energy, body mass index, smoking, alcohol intake, use of vitamin supplements, intake of saturated fatty acids, monosaturated fatty acids, polyunsaturated fatty acids, and cholesterol. We also adjusted for fiber because the association between trans fatty acid intake and coronary heart disease was strongly attenuated after adjustment for fiber in another prospective study.[10] Alcohol intake was used as a categorical

variable (included as two dummies into the model, with non-drinkers as a reference).

RESULTS

The mean daily trans fatty acid intake fell from 1985 to 1990 and 1995 (10.9 g [SD 6.3] *vs.* 6.9 [4.0] *vs.* 4.4 g [1.7]). The mean contribution of trans fatty acid intake to total energy intake decreased from 1985 to 1990 and 1995 (4.3% [SD 2.2] *vs.* 2.9% [1.5] *vs.* 1.9% [0.6]). There was a similar reduction in trans fatty acid intake (-2.1% of energy) in the men who were examined in all three examination years. The intake of manufactured C18:1 trans (a proxy for partly hydrogenated vegetable oils) as well as the manufactured other trans fatty acids (including partly hydrogenated fish oils) decreased substantially between 1985 and 1995, but the intake of ruminant trans fatty acids did not do so (figure 1). The Spearman correlation coefficient between the total trans fatty acid intake expressed in % of energy in 1985 and 1990 was 0.43, and between 1985 and 1995 was 0.24.

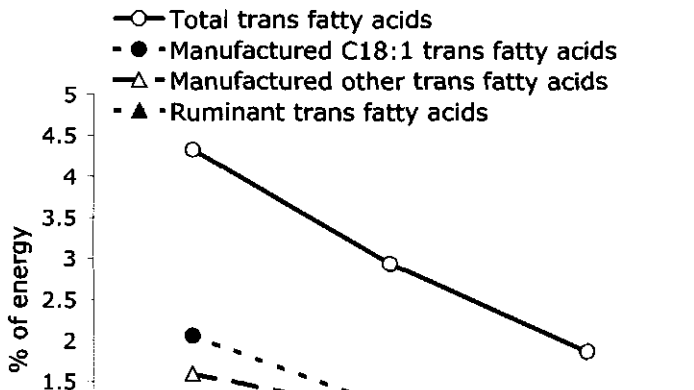

Figure 1. Daily intake of trans fatty acids in the Zutphen Elderly Study between 1985 and 1995.

Table 1. Characteristics at baseline by tertiles of total trans fatty acid intake.

	Total	Trans fatty acid tertile (% of energy)			P value
		<3.11	3.11-4.86	≥4.86	
	(n=667)	(n=222)	(n=223)	(n=222)	
Age (yr)	71.1(5.2)	71.3(5.5)	70.8(5.2)	71.3(5.0)	0.47
Body mass index (kg/m^2)	25.5(3.2)	25.5(3.1)	25.2(3.2)	25.8(3.2)	0.13
Physical activity (min per week)	611(533)	577(467)	601(500)	656(620)	0.91
Serum total cholesterol (mmol/L)	6.08(1.11)	6.14(1.15)	5.94(1.02)	6.16(1.13)	0.07
Serum HDL cholesterol (mmol/L)	1.14(0.30)	1.15(0.31)	1.14(0.29)	1.12(0.30)	0.64
Smoking					
Current	33%	28%	31%	38%	0.08
past	49%	51%	48%	48%	0.73
Use of vitamin supplements	16%	21%	18%	10%	0.003
Hypertension	42%	47%	42%	38%	0.15
Diabetes mellitus	6%	5%	8%	5%	0.25
Daily intake of					
Energy (MJ)	9.2(2.0)	8.6(1.9)	9.4(2.1)	9.5(2.1)	0.0001
Total fat (% of energy)	40.3(6.4)	37.1(6.5)	39.8(5.2)	44.0(5.6)	0.0001
Saturated fat (% of energy)	18.0(3.6)	17.0(3.9)	18.3(3.6)	18.7(3.1)	0.0001
Monounsaturated fat (% of energy)	15.3(3.2)	13.2(2.8)	15.0(2.2)	17.7(2.8)	0.0001
Polyunsaturated fat (% of energy)	7.0(2.8)	6.9(3.5)	6.4(2.4)	7.6(2.1)	0.0001
Cholesterol (mg)	273(97.0)	245(97.4)	280(89.0)	292(98.5)	0.0001
Carbohydrates (% of energy)	41.0(7.3)	42.2(8.0)	42.0(6.6)	38.7(6.8)	0.0001
Protein (% of energy)	14.3(2.6)	14.8(2.8)	14.3(2.4)	13.7(2.5)	0.0001
Alcohol (g)	13.8(17.3)	17.0(20.0)	12.7(16.7)	11.7(14.2)	0.05
non-drinkers	24%	23%	23%	25%	0.85
≥20 g/d	27%	34%	22%	24%	0.01
Fiber (g)	24.9(7.1)	24.4(7.2)	25.2(6.9)	25.1(7.1)	0.46

Values shown as mean (standard deviation) unless otherwise stated.

Table 2. Relative risks of coronary heart disease according to tertiles of trans fatty acid intake at baseline.

	Trans fatty acid tertile (% of energy)			
	<3.11 (n=222)	3.11-4.86 (n=223)	≥4.86 (n=222)	P Value*
Median intake (% of energy)	2.36	3.87	6.38	
No. (%) of cases	24 (11%)	30 (14%)	44 (20%)	
Relative risks (95% CI)				
Crude	1	1.26 (0.74-2.15)	2.03 (1.24-3.34)	0.003
Age + energy adjusted	1	1.36 (0.79-2.34)	2.19 (1.32-3.62)	0.002
Fully adjusted	1	1.34 (0.76-2.37)	2.00 (1.07-3.75)	0.03

*Values were obtained by modeling the median value of each category as a continuous variable.

The total daily intake of trans fatty acids at baseline was positively associated with the daily intake of energy, total fat, saturated and unsaturated fat, and cholesterol, and inversely associated with the daily intake of carbohydrates, protein, alcohol, and the use of vitamin supplements (table 1). No significant associations between total trans fatty acid intake and major risk factors were recorded. However, although not statistically significant, men with a high intake of trans fatty acids were more often smokers and had a higher serum total cholesterol concentration, For manufactured trans fatty acids, similar associations were noted. By contrast, trans fatty acid intake from ruminant sources was inversely associated with the daily intake of energy, polyunsaturated fat, and fiber, and positively associated with the daily intake of protein.

During 10 years of follow-up, we documented 98 (15% of the baseline population) coronary heart disease cases (including 49 cardiac deaths). Table 2 shows the crude relative risks of 10-year coronary heart disease frequency for the different tertiles of trans fatty acid intake at baseline. The relative risks were similar after adjustment for age, body mass index, smoking, use of vitamin supplements, intake of energy, alcohol, specific types of fat, dietary cholesterol, and fiber.

Table 3. Relative risks of coronary heart disease for an increase of 0.5% in energy from trans fatty acids from different sources* at baseline.

	Ruminant trans fatty acids	Manufactured C18:1 trans fatty acids	Other manufactured trans fatty acids
Mean (SD) intake (% of energy)	0.7 (0.2)	2.1 (1.2)	1.6 (1.4)
Relative risks (95% CI)			
Crude	1.11 (0.69-1.78)	1.07 (0.99-1.15)	1.05 (0.99-1.12)
Age + energy adjusted	1.05 (0.66-1.69)	1.08 (1.00-1.17)	1.06 (0.99-1.13)
Fully adjusted	1.17 (0.69-1.98)	1.05 (0.94-1.17)	1.07 (0.99-1.15)

* Intake of ruminant trans fatty acid, manufactured C18:1 trans, and other manufactured trans fatty acids are included simultaneously.

In the continuous analyses we calculated the relative risk associated with a difference of 2% of energy in total trans fatty acid intake at baseline. Adjusted for age and energy intake, this relative risk of 10-year incidence of coronary heart disease was 1.29 (95% CI 1.09-1.52). After additional adjustment for body mass index, smoking, use of vitamin supplements, intake of alcohol, specific types of fat, dietary cholesterol and fiber, the relative risk amounted to 1.28 (1.01-1.61). For fatal coronary heart disease the fully adjusted relative risk for a difference of 2% of energy in trans fatty acid intake was 1.33 (0.96-1.86). Because of different proportions of C18:1 trans isomers in each source, and because of different trans isomers from manufactured sources, we assessed the difference in effect of ruminant trans fatty acids, manufactured C18:1 trans fatty acids, and other manufactured trans fatty acids. We did continuous analyses of baseline intake on coronary heart disease frequency to take into account the difference in range of intake of each type of trans fatty acid. For each 0.5% of energy, the fully adjusted relative risk of coronary heart disease for ruminant trans fatty acids, manufactured C18:1 trans fatty acids, and other manufactured trans fatty acid intake was similar (table 3).

Table 4 summarizes results of previous cohort studies on the association between trans fatty acid intake and the risk of coronary heart disease. Combining the results of the four prospective cohort studies, the pooled variance-weighted relative risk of coronary heart disease associated with a difference of 2% of energy in trans fatty acid intake is 1.25 (95% CI 1.11-1.40; figure 2).

Table 4. Summary of the effect of an increase of 2% of energy in trans fatty acid intake on coronary heart disease* reported in prospective studies.

Study population	N	Sex	Number of events	Follow-up (years)	Age+energy adjusted relative risk (95% CI)	Adjusted† relative risk (95% CI)
Nurses' Health Study[11]	80082	women	939	14		1.62 (1.23-2.13)
Health Professionals follow-up Study[10]	43757	men	734	6	1.59 (1.21-2.08)	1.13 (0.81-1.58)
Alpha-Tocopherol Beta-Carotene Cancer Prevention Study[13]	21930	men	1399	6.1	1.19 (1.00-1.41)	1.14 (0.96-1.35)
Zutphen Elderly Study	667	men	98	10	1.29 (1.09-1.52)	1.28 (1.01-1.61)

*Defined as non-fatal myocardial infarction and fatal coronary heart disease
†For each study, the fully adjusted model is presented here. Details can be found in the original papers.

Discussion

We report that high intake of trans fatty acids at baseline was strongly associated with the risk of coronary heart disease in Dutch elderly men. Our results are similar to those from other prospective studies, such as the 16 population cohorts of the Seven Countries Study,[30] and a US case-control study.[9] However, in the EURAMIC case-control study, no significant overall association was noted between the C18:1 trans fatty acid content of adipose tissue and the risk of first myocardial infarction.[12] In this investigation, however, the trans fatty acid content of adipose tissue was very low in the Spanish centers. After excluding these outlying values, the relative risk for the highest versus the lowest quartile was 1.44 (95% CI 0.94-2.20).

We did not show any actual difference in associations between coronary heart disease and ruminant trans fatty acid intake, intake of C18:1 trans isomers, and other trans isomers from manufactured sources. Human dietary intervention studies on blood lipids that used different sources or trans isomers have similar results.[1,2,4,5] However, in the Nurses' Health Study, a non-significant inverse relative risk of coronary heart disease for ruminant trans fatty-acid intake (highest *vs.* lowest quintile) of 0.59 was recorded.[8] In two other prospective studies, because of the lower intake of trans fatty acids from ruminant sources compared with manufactured sources, differences between ruminant and

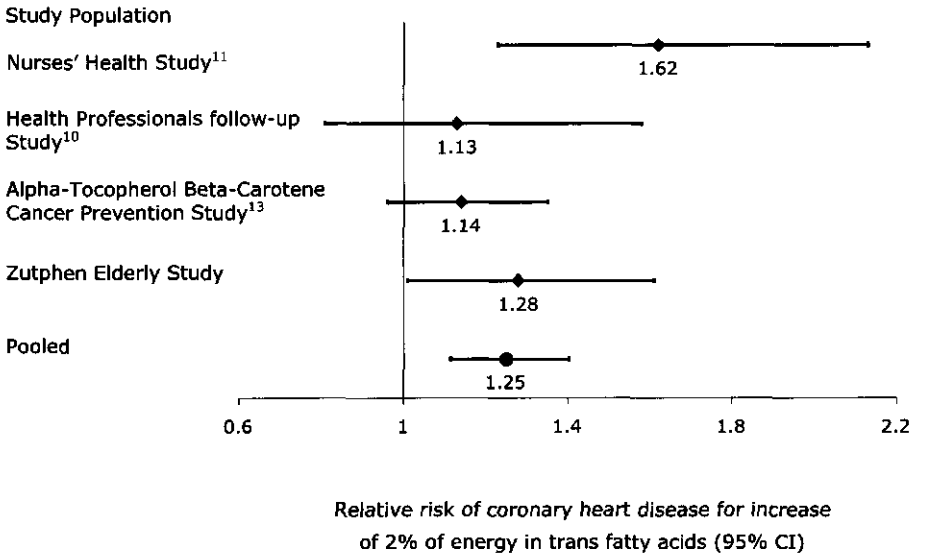

Figure 2. The fully adjusted relative risks of coronary heart disease for an increase of 2% of energy in trans fatty acid intake at baseline according to prospective population based studies, and the pooled variance-weighted relative risk.

manufactured trans fatty acids were less clear.[9,13] We therefore conclude that the health effect of trans fatty acids from ruminant sources and from manufactured sources is similar.

Is the association between trans fatty acid intake and coronary heart disease estimated adequately with a baseline measurement in a cohort with a declining trans fatty acid intake? In the Nurses' Health Study, a stronger association between trans fatty acids and coronary heart disease was reported when cumulative average diets were used rather than baseline or only the most recent diet.[31] Taking into account changes in food composition and dietary habits among participants in our study, use of cumulative average diets or the most recent diet for the second 5 years of follow-up gave weaker results than those for the baseline diet (data not shown). When the intake of trans fatty acids is fairly stable, as in the Nurses' Health Study,[31] the cumulative average intake probably best indicates the long-term intake. However, for our population of men aged 64-84 years who changed their trans fatty acid intake only recently, the baseline measurement probably better shows the long-term intake.

Keeping misclassification to a minimum is essential to adequately detect associations with disease or to control for confounding. In our study, habitual food consumption was measured by the cross-check dietary history method, which is acknowledged as a valid method in an epidemiological setting.[22] Trans fatty acid contents of mainly Dutch foods were available to calculate trans fatty acid intake. Adjustments were made for systematic differences due to different analytical methods. Furthermore, the effect of trans fatty acid intake on coronary heart disease could be confounded by other dietary or risk factors that were not included in our analyses. To lower residual confounding, we adjusted for many dietary and lifestyle factors. All had minor effects on the relative risks. Our results for fatal coronary heart disease, including non-fatal myocardial infarction, were considered. Because of power, we focused on the association of fatal plus non-fatal coronary heart disease.

The reduction in consumption of trans fatty acids in the Netherlands and in the use of both partly hydrogenated vegetable and fish oils explains the decline in the contribution of trans fatty acids to total energy intake. In 1996, a further decrease in trans fatty acid content of edible fats was recorded in the Netherlands.[15,18] Also, in other European countries, a fall in the trans fatty-acid content of margarines[19] contributed to a decline in trans fatty acid intake.[15] The trans fatty acid intake at baseline was much higher than the 2% of energy reported in previous studies done in the USA.[8,10,11] However, in the USA, the trans fatty acid intake remained stable, because a decrease in trans fatty acids from margarines was counterbalanced by an increase in trans fatty acids from commercially baked products and fast foods.[16]

We did not record a clear cross-sectional association between trans fatty acid intake and total or HDL cholesterol at baseline. However, by use of longitudinal analyses of both trans fatty acids and cholesterol concentrations, there was an association in accordance with the results of controlled dietary intervention studies (Chapter 2). Also other mechanisms might be implicated in increasing the risk for coronary heart disease, since relative risk is higher than can be predicted from the effects of trans fatty acids on cholesterol concentrations alone.[16] Several studies have shown effects of trans fatty acids on triglycerides[1,4,6,7] and lipoprotein (a) concentrations.[5,7] Trans fatty acids might have other adverse physiological effects on e.g. thrombotic mechanisms[32] or insulin resistance.[33]

Evidence from observational and dietary intervention studies suggests that a decrease in trans fatty acid intake has a role in lowering coronary heart disease mortality.[17] The number of coronary heart disease deaths attributable to trans fatty acids in the USA is thought to be substantial.[34] The decrease in trans fatty acid intake of 2.4% of energy we report could have contributed to about 23% less coronary deaths (i.e., about 4600 of 20000 coronary deaths in the Netherlands per year).

Possibilities for further industrial reductions in trans fatty acid contents are restricted nowadays to bakery products[21] and fast foods.[18,35] Also, the substitution of trans fatty acids requires further attention, because in the current manufacturing process trans fatty acids are partly replaced by saturated fatty acids.[15,17-19]

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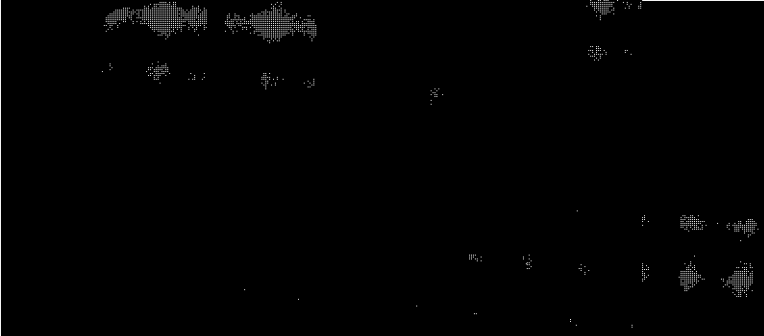

consumption and coronary heart disease mortality in Finland, Italy and the Netherlands

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this association is due to n-3 polyunsaturated fatty acids, especially fatty fish may b responsible for this protective effect. The association between total, lean and fatty fis consumption and the risk of CHD mortality was examined in 1,088 Finnish, 1,097 Italia and 553 Dutch men participants in the Seven Countries Study who were aged 50-6 years and free of CHD around 1970. After 20 years of follow-up, 242 (22.4%) men i Finland, 116 (10.6%) men in Italy and 105 (19.0%) men in the Netherlands had died CHD. Cox proportional hazard analysis showed no association between total fis consumption and CHD mortality. After adjustments were made for age, body mass inde smoking, energy intake and relevant dietary variables, the pooled relative risk for th highest quartile of total fish compared with no fish consumption in the three countrie was 1.08 (95% confidence interval 0.76-1.53). Lean fish consumption also was n associated with CHD mortality in any country. Fatty fish compared with non-fatty fis consumption was associated with lower CHD mortality; the adjusted, pooled relative ris for fatty fish consumers was 0.66 (95% confidence interval 0.49-0.90). These da suggest that especially fatty fish is protective against CHD mortality.

INTRODUCTION

Since Bang et al. suggested that the low mortality rate from coronary hea disease (CHD) among Eskimo compared with Danes may be due to the consumption of large quantities of seafood,[1] the health effects of fish hav attracted considerable scientific interest. Results from several cohort studie have suggested that consumption of a small amount of fish is inverse associated with CHD mortality.[2-7] However, in some studies that examined th impact of fish consumption on nonfatal CHD, no association was observed.[8,9] I populations whose average fish intake was high, such as those in Finlan Norway and Hawaii, also no inverse association between fish consumption an CHD risk was observed.[10-13] Among the people in these studies, almost everyon eats fish regularly; therefore, these populations probably are not suited t studying the impact of the consumption of a small amount of fish on CH mortality.

A difference in the type of fish consumed, for example, fatty fish or lean fis might explain further inconsistency in the relation between fish intake and CH mortality across populations. In the diet and reinfarction trial, carried out amon

cardiac patients, a modest intake of fatty fish was found to reduce all-cause mortality by about 29%, which was entirely attributable to a reduction in the number of deaths from coronary heart disease.[14] Consumption of fatty fish may be protective because this type of fish contains high levels of the n-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid. N-3 fatty acids have important metabolic effects, such as inhibiting the platelet aggregation and lowering serum triglyceride levels, which could play a role in the prevention of CHD.[15] In addition, instead of the inconsistent results found in some prospective studies that examined the dose-response relation between fish consumption and CHD mortality, one study observed a dose-response relation between n-3 fatty acids, quantified in both the diet and red blood cell membranes, and the risk of primary cardiac arrest.[16]

We hypothesized that because of its higher n-3 fatty acid content, especially fatty fish may be responsible for the protective effect of fish consumption. We analyzed the association between total, lean and fatty fish consumption and 20-year CHD mortality in the Finnish, Italian, and Dutch cohorts of the Seven Countries Study.

MATERIALS AND METHODS

Study population

Between 1958 and 1964, 16 population samples of men aged 40-59 years from seven countries were enrolled in and were examined for the Seven Countries Study.[17] In five population samples from Finland, Italy, and the Netherlands, individual dietary information was collected during follow-up. The two Finnish cohorts from two geographically defined areas, Ilomantsi in east Finland and Pöytyä and Mellilä in west Finland, were enrolled in 1959, and participation rates were high (99.3% and 97.0%, respectively). The cohorts from two small, rural villages in Italy, Crevalcore and Montegiorgio, and from a small town in the Netherlands, Zutphen, were enrolled in 1960 (participation rates: Italy, 98.5% and 99.0%; the Netherlands, 84.3%). Baseline dietary information used in this study was gathered in 1969 for participants in east Finland (N=608) and west Finland (N=694) and in 1970 for participants in the Netherlands (N=615) and in Crevalcore (N=592). For Montegiorgio, dietary information collected in 1965 was used for the men still alive in 1970 (N=627), because the 1970 dietary data were collected for only a subset of men.

Data collection

Experienced dieticians and nutritionists conducted dietary interviews with all cohorts in Finland from September to November and in the Netherlands and Italy from March to June. Food consumption data were collected by using the cross-check dietary history method,[18] which was adapted to each specific country; the methodology used was comparable across cohorts. This method provides information about the habitual food consumption during the 6-12 months preceding the interview. First, the habitual food consumption pattern of a person was assessed during the week and weekends. This part of the interview contained questions about the foods used at breakfast, lunch, dinner and between meals. Second, a checklist with an extensive number of foods was used, and the frequencies and quantitaties of the different foods consumed were recorded. The information about the food consumption pattern was then compared with the information from the checklist. Total fish consumption was computed by adding the number of grams of all fish consumed per day per subject. The following subgroups of types of fish were discerned: 1) lean (unprepared, ≤ 10% fat; prepared, ≤ 12% fat; e.g. plaice, cod-fish, bream, perch, pike); 2) fatty (e.g. mackerel, (salted) herring, eel); and 3) canned (e.g. sardines, salmon). Local food tables were used to convert food intake data into intake of energy and nutrients, including alcohol, for participant in the three different countries.[19-21]

Information about the number of cigarettes smoked was collected by using a standardized questionnaire; participants were categorized as men who had never smoked, had stopped smoking, or currently smoked fewer or more than 20 cigarettes a day. Other risk factors such as serum total cholesterol, blood pressure and anthropometric measures were determined according to a standardized protocol.[18] Body mass index (kg/m^2) was calculated from weight and height measurements.

Ascertainment of mortality and causes of death was complete for all men in the subsequent 20 years. None of the men was lost to follow up. All mortality data collected from death certificates, hospital records or information from the general practitioner, family members and other witnesses of the death were coded by one reviewer according to the World Health Organization's International Classification of Diseases, Eighth revision by using standard criteria for interpretation and coding. In case of multiple causes of death, priority was given to accidents, followed by advanced-stage cancer, CHD and stroke. For the present analyses, CHD referred to the primary or secondary cause of death

based on International Classification of Diseases codes 410-414 (Finland, 223; Italy, 81; the Netherlands, 88) and, when a cardiac origin was mentioned, to the primary cause of sudden cardiac death based on code 795 (Finland, 19; Italy, 35; the Netherlands, 17).

Statistical methods

All statistical analyses were conducted by using the SAS statistical analysis computer package (version 6.11; SAS Institute, Inc., Cary, North Carolina). Men with a field diagnosis of CHD based on the standardized criteria were excluded from analysis (Finland, n=214; Italy, n=122; the Netherlands, n=62) leaving a total of 2,738 men. For each country, the men were divided into categories based on the number of grams of fish they consumed per day (g/day). Categories of 0, 1-19, 20-39 and ≥40 g/day were used for total and lean fish consumption and 0, >0 g/day for fatty fish consumption, since only a small proportion consumed fatty fish. Canned fish was assigned to neither the lean nor the fatty fish category and, because of a low level of consumption, was not found to be related to CHD mortality. Because of small numbers, Finnish men who consumed 0 g/day of total or lean fish (no fish. n=33; no lean fish, n=48) were grouped with men who consumed 1-19 g/day. For the Netherlands, the total and lean fish consumption category of 20-39 g/day was merged with the category of ≥40 g/day of total fish (n=63) or lean fish (n=41). However, excluding these men from the analyses did not change the results.

To compare the baseline risk factors and dietary variables across categories of fish consumption, we used analysis of variance for normally distributed variables, the Kruskal-Wallis test for skewed variables, and the chi-square test for categorical variables. Cox proportional hazard analyses were performed by using SAS procedure PHREG, and the analyses were stratified by cohort using the STRATA statement.[22] Relative risks, 95% confidence intervals and p values for linear trend were calculated to investigate the association between fish consumption categories and CHD mortality for each country. Fish consumption was not used as a continuous variable, since none of the tests for linear trend supported a linear relation. For total fish consumption, men who consumed no fish (Italy, the Netherlands) or the lowest amount of fish (Finland) were considered the reference group. For lean fish consumption, participants who consumed the lowest amount of lean fish (irrespective of their fatty fish intake) were taken as the reference group, and intake of fatty fish was included in the model as a confounder. For fatty fish consumption, the reference group

consumed no fatty fish. Additional adjustments were made for age, cigarette smoking, body mass index, intake of energy and alcohol, and consumption of vegetables, fruit, meat, margarine and butter -the food products associated with fish consumption in our data and potentially associated with CHD mortality. Alcohol intake (0, 1-39, 40-59, ≥60 g/day) was used as a categorical variable. If the association of fish consumption with CHD mortality was similar between the countries, data from the three countries were pooled and analyses were stratified by cohort. Two-tailed significance levels of 0.05 were used.

RESULTS

The average age of the men was 58.0 years at baseline. The mean daily fish intake was 39 g (standard deviation (SD), 47) in Finland, 20 g (SD, 21) in Italy and 18 g (SD, 20) in the Netherlands. In Finland, 77% of the fish consumed was lean and 23% was fatty. In Italy, 86% was lean and 14% was fatty; in the Netherlands, 80% was lean and 11% was fatty. Less than 1% of the fish consumed in Finland and Italy was canned compared with 9% in the Netherlands. We found that total fish consumption was positively associated with cigarette smoking and with serum cholesterol levels in Finland and inversely associated with age in Italy (table 1). No significant association between total fish consumption and major risk factors was observed for the Netherlands.

Total fish consumption was positively associated with vegetable and fruit consumption in Finland and the Netherlands (table 2). In contrast, fish consumption was inversely associated with fruit consumption in Italy. Alcohol intake was positively associated with fish consumption, especially in Italy. Meat intake and butter intake were positively associated with fish consumption in Finland, whereas these associations were inverse in Italy. Total fish consumption was positively associated with energy intake in all three countries, with monounsaturated fatty acid intake in Italy, and with polyunsaturated fatty acid intake in the Netherlands. An inverse association was observed for saturated fatty acid intake in Italy and the Netherlands. For lean and fatty fish consumption, similar associations for dietary variables were observed (data not shown).

During 20 years of follow-up, 242 (22.2%) men died of CHD in Finland, 116 (10.6%) in Italy and 105 (19.0%) in the Netherlands. In Finland, for men with the highest compared with the lowest levels of fish consumption, the crude

Table 1. Baseline level of major risk factors for coronary heart disease, according to categories of fish consumption, for men aged 50-69 years in Finland, Italy and the Netherlands, 1970-1990.

Country and fish consumption (g/day)	No. of men	Risk factor*				
		Age (years)	Body mass index (kg/m^2)	Systolic blood pressure (mmHg)	Serum total cholesterol (mmol/liter)	Cigarette smoking (%)
Finland						
0-19	476	58.2 (5.7)	24.5 (3.6)	145 (23)	6.77 (1.20)	43.9
20-39	263	57.4 (5.4)	24.6 (3.5)	146 (21)	6.96 (1.33)	51.7
≥40	349	57.7 (5.4)	24.9 (4.1)	148 (23)	7.18 (1.40)†	56.2†
Italy						
0	264	59.1 (5.1)	25.9 (3.7)	153 (21)	5.64 (1.22)	51.5
1-19	347	58.5 (4.7)	25.4 (3.9)	154 (22)	5.67 (1.24)	53.6
20-39	323	57.6 (4.8)	26.0 (4.0)	153 (23)	5.75 (1.13)	48.0
≥40	163	57.7 (5.2)†	26.1 (4.3)	151 (22)	5.66 (1.13)	50.9
The Netherlands						
0	157	58.3 (5.4)	25.4 (2.9)	145 (21)	6.23 (1.15)	55.8
1-19	169	58.6 (5.3)	24.9 (2.3)	145 (19)	6.06 (1.02)	54.4
≥20	227	58.2 (5.3)	25.1 (2.8)	149 (22)	6.18 (1.08)	50.2

*Values in the first four columns are expressed as mean (standard deviation).
†Statistically significant different ($p<0.05$) between fish consumption categories (analysis of variance for normally distributed variables, chi-square for dichotomous variables).

relative risk for CHD mortality was 1.39 (95% confidence interval (CI) 1.00-1.92) (table 3). Adjustment for age, energy intake, body mass index, and cigarette smoking, and further adjustment for dietary variables, attenuated this relative risk to 1.25 (95% CI 0.89-1.76). In Italy, men who consumed ≥40 g/day fish had a relative risk for CHD mortality of 0.67 (95% CI 0.33-1.39) compared with men who consumed no fish, after adjustment for potential confounders. In the Netherlands, no association -neither crude nor after adjustment for potential confounders- between total fish consumption and CHD mortality was observed. The overall estimated relative risks for total fish consumption compared with no fish consumption in the three countries, pooled after stratification by cohort,

Table 2. Baseline daily intake of various foods and other nutrients (mean and standard deviation), according to categories of fish consumption, in men aged 50-69 years in Finland, Italy and the Netherlands, 1970-1990.

	Fish consumption (g/day)									
Item (unit of intake)	Finland			Italy				The Netherlands		
	0-19	20-39	≥40	0	1-19	20-39	≥40	0	1-19	≥ 20
Lean fish (g)	8 (6)	24 (8)	61 (47)†	0	10 (5)	23 (9)	50 (25)†	0	9 (6)	29 (19)†
Fatty fish (g)	1 (2)	5 (8)	23 (29)†	0	2 (4)	4 (8)	7 (18)†	0	2 (4)	3 (8)†
Meat (g)	136 (77)	150 (76)	161 (89)†	151 (72)	108 (77)	119 (74)	118 (81)†	138 (44)	139 (44)	143 (41)
Vegetables (g)	66 (48)	84 (66)	94 (68)†	60 (54)	63 (51)	72 (50)	80 (60)†	180 (61)	178 (59)	184 (57)
Fruit (g)	158 (164)	191 (233)	184 (173)†	190 (204)	138 (143)	134 (152)	144 (171)†	153 (116)	156 (104)	191 (153)
Alcohol (g)	6 (11)	8 (18)	7 (11)†	77 (53)	81 (60)	87 (57)	91 (66)†	9 (17)	8 (10)	11 (13)
Margarine (g)	3 (8)	3 (7)	4 (10)	8 (13)	16 (16)	16 (16)	16 (15)†	48 (21)	53 (22)	56 (25)†
Butter (g)	67 (39)	75 (43)	85 (54)†	15 (14)	8 (14)	7 (12)	6 (12)†	8 (16)	8 (15)	6 (14)
Energy (MJ)	14.9 (4.1)	16.1 (4.8)	16.6 (5.0)†	12.3 (3.3)	11.9 (3.2)	12.6 (3.1)	12.9 (3.3)†	10.6 (2.3)	11.0 (2.1)	11.2 (2.2)†
Total protein‡	12.9 (1.7)	13.0 (1.8)	13.7 (2.0)†	12.0 (2.7)	10.3 (2.8)	10.7 (2.5)	11.1 (2.5)†	12.4 (2.0)	12.4 (1.7)	12.9 (2.0)†
Total fat‡	37.4 (6.7)	36.6 (6.4)	37.9 (7.2)	28.6 (7.6)	27.9 (7.8)	27.4 (7.6)	27.2 (6.8)	40.5 (5.6)	41.7 (4.8)	40.7 (5.3)
Saturated fatty acids‡	21.8 (4.5)	21.4 (4.4)	22.1 (5.0)	11.0 (3.6)	9.2 (3.5)	8.8 (3.4)	8.4 (3.4)†	16.9 (3.1)	17.2 (2.8)	16.2 (2.6)†
Monounsaturated fatty acids‡	11.5 (2.2)	11.3 (2.1)	11.8 (2.3)†	13.8 (4.3)	15.1 (4.7)	15.3 (4.8)	15.3 (4.2)†	17.1 (3.0)	17.6 (2.6)	17.2 (2.8)
Polyunsaturated fatty acids‡	2.9 (0.4)	2.8 (0.4)	2.9 (0.5)	3.9 (1.9)	3.6 (2.0)	3.3 (1.4)	3.5 (1.6)†	6.2 (2.0)	6.7 (2.0)	7.1 (2.3)†
Dietary cholesterol (mg)	641 (248)	685 (256)	762 (285)†	348 (159)	261 (158)	294 (153)	303 (144)†	392 (178)	413 (150)	438 (176)†

†Statistically significant different ($p<0.05$) between fish consumption categories (analysis of variance for normally distributed variables, Kruskal-Wallis test for skewed variables).

‡In percentage of energy intake

Table 3. Relative risks (95% confidence intervals) for 20-year CHD* mortality according to categories of fish consumption, for men aged 50-69 years in Finland, Italy and the Netherlands, 1970-1990.

Country and fish consumption (g/day)	No. of men	No. of CHD deaths (%)	Mortality rate†	Relative risk (95% confidence interval)		
				Crude	Adjusted ‡	Adjusted §
Finland						
0-19	476	100 (21.0)	13.9	1.00	1.00	1.00
20-39	263	52 (19.8)	13.1	0.95 (0.67-1.34)	0.98 (0.69-1.40)	0.97 (0.68-1.38)
≥40	349	90 (25.8)	18.5	1.39 (1.00-1.92)	1.31 (0.94-1.84)	1.25 (0.89-1.76)
P for trend¶				0.05	0.12	0.20
Italy						
0	264	32 (12.1)	8.2	1.00	1.00	1.00
1-19	347	37 (10.7)	7.1	0.87 (0.52-1.46)	0.93 (0.55-1.57)	0.94 (0.55-1.59)
20-39	323	34 (10.5)	6.7	0.81 (0.47-1.38)	0.99 (0.57-1.72)	0.93 (0.53-1.63)
≥40	163	13 (8.0)	5.0	0.56 (0.27-1.13)	0.69 (0.34-1.42)	0.67 (0.33-1.39)
P for trend¶				0.11	0.38	0.33
The Netherlands						
0	157	29 (18.5)	11.7	1.00	1.00	1.00
1-19	169	30 (17.8)	11.6	1.00 (0.60-1.66)	1.01 (0.60-1.69)	1.00 (0.59-1.68)
≥20	227	46 (20.3)	13.1	1.13 (0.71-1.80)	1.16 (0.72-1.86)	1.10 (0.68-1.79)
P for trend¶				*0.60*	*0.55*	*0.69*

* CHD, coronary heart disease.
† Per 1,000 person-years.
‡ Adjusted for age, body mass index (kg/m^2), cigarette smoking and energy intake.
§ Adjusted for age, body mass index (kg/m^2), cigarette smoking, intake of energy, vegetables, fruit, alcohol, meat, butter and margarine.
¶ Values for linear trend across categories of total fish consumption.

were 0.93 (95% CI 0.68-1.27) for 1-19 g/day, 0.95 (95% CI 0.69-1.31) for 20-39 g/day and 1.08 (95% CI 0.76-1.53) for ≥40 g/day.

Lean fish was consumed by 96% of the men in Finland, 71% in Italy and 61% in the Netherlands; the proportions of fatty fish consumers were 36%, 16%, and

18%, respectively. The average fatty fish consumption for those who consumed fatty fish was 25 (SD, 28) g/day in Finland, 17 (SD, 16) g/day in Italy and 11 (SD, 9) g/day in the Netherlands. Crude inverse associations were observed between fatty fish consumption and CHD mortality for all three countries; adjustment for potential confounders, including age, body mass index, cigarette smoking, energy intake and relevant dietary variables, did not substantially change the relative risks. In Italy, fatty fish consumption was most strongly associated with a reduced risk for CHD (adjusted relative risk=0.40, 95% CI 0.19-0.84) (table 4). For fatty fish consumers in Finland and the Netherlands, the adjusted relative risks were 0.80 (95% CI 0.51-1.26) and 0.70 (95% CI 0.38-1.27), respectively, compared with non-fatty-fish consumers. For the three countries, the overall estimated relative risks for fatty fish consumption, pooled after stratification by cohort, were 0.57 (95% CI 0.40-0.80) for 1-19 g/day, and 0.87 (95% CI 0.59-1.27) for ≥20 g/day compared with no fatty fish consumption. The pooled estimated relative risk for fatty fish consumers in the three countries was 0.66 (95% CI 0.49-0.90). Lean fish intake was not associated with CHD risk in any of the three countries; neither the relative risks, nor the tests for linear trend were statistically significant (table 4).

Discussion

This prospective study in three European countries showed an inverse association of fatty fish consumption, but not of lean or total fish consumption, with 20-year CHD mortality. The pooled results for fatty fish were consistent with a 34% (95% CI 10-51%) reduction in CHD mortality.

Our results suggest that n-3 fatty acids are responsible for the protective effect of fish. Consumption of 15 g/day of lean fish (e.g., plaice or codfish), as consumed by our populations, results in a daily intake of about 50 mg of n-3 fatty acids; in contrast, 15 g/day of fatty fish (e.g., mackerel or herring) provides about 400 mg of n-3 fatty acids a day.[23] In this study, the direct relation between n-3 fatty acids and CHD mortality was not analyzed because of rather limited information about the type of fish consumed by our cohorts, which could have introduced misclassification regarding intake of this nutrient.

Several earlier studies showed an inverse relation between n-3 fatty acids from seafood and (sudden) cardiac mortality.[5,16,24] However, some prospective studies observed a stronger beneficial effect of fish consumption than of intake of n-3

Table 4. Adjusted* relative risks (95% confidence intervals) for 20-year CHD† mortality, according to categories of lean and fatty fish consumption, for men aged 50-69 years in Finland, Italy and the Netherlands, 1970-1990.

Country and fish consumption (g/day)	No. of men	No. of CHD deaths	Adjusted relative risk
Finland			
Fatty fish			
0	697	155	1.00
>0	391	87	0.80 (0.51-1.26)
Lean fish			
0-19	568	124	1.00
≥ 20	253	51	0.95 (0.68-1.33)
≥ 40	267	67	1.08 (0.78-1.50)
p for trend‡			0.63
Italy			
Fatty fish			
0	923	106	1.00
>0	174	10	0.40 (0.19-0.84)
Lean fish			
0	318	34	1.00
< 20	365	41	1.09 (0.66-1.81)
≥ 20	281	30	0.97 (0.55-1.69)
≥ 40	133	11	0.80 (0.38-1.66)
p for trend‡			0.57
The Netherlands			
Fatty fish			
0	451	92	1.00
>0	102	13	0.70 (0.38-1.27)
Lean fish			
0	216	38	1.00
< 20	146	24	0.93 (0.55-1.55)
≥ 20	191	43	1.29 (0.82-2.03)
p for trend‡			0.27

* Adjusted for age, body mass index (kg/m^2), cigarette smoking, intake of energy, vegetables, fruit, alcohol, meat, butter and margarine, with fatty and lean fish consumption as dummy-variables in one model.

† CHD, coronary heart disease.

‡ Values for linear trend across categories of lean fish consumption

fatty acids.[8,24] This observation may be partly explained by a random misclassification of dietary exposure due to error in the quantification of n-3 fatty acids in food tables, which tends to attenuate existing associations. Another inconsistency in previous results concerned the dose-response relation between fish consumption and CHD mortality. Our results suggest that differences in lean and fatty fish consumption could also explain this inconsistency, since the n-3 fatty acid content of lean and fatty fish is dissimilar.

A variety of actions could explain the beneficial effects of n-3 fatty acids on CHD mortality. Experimentally, n-3 fatty acids in the form of fish oil supplements lower triglyceride and very low density lipoprotein levels in animals and humans[25] and inhibit platelet aggregation as a result of reduced synthesis of thromboxane A_2.[26] N-3 fatty acids also influence antiarrhythmic pathways because they have been shown in in vitro studies to synchronize the beating rate of the heart,[27] and they have been shown to reduce the incidence of ventricular fibrillation in rats[28] as well as in patients with frequent ventricular arrhythmias.[29]

The physiologic effects of consuming small amounts of fish have been investigated in observational studies. Men from Zutphen who habitually consumed about 30 g/day of fish for 26 years had lower serum triglyceride levels and lower triglyceride concentrations in the intermediate density lipoprotein fraction than the control group.[30] In this study, however, small amounts of fish were not associated with platelet function.[31] On the other hand, in the ARIC-Study, one serving of fish per day was inversely associated with hemostatic factors such as fibrinogen and factor VII.[32] Furthermore, consumption of one fish meal per week versus no fish was associated with an increase in heart rate variability as large as that observed for fish oil supplementation.[33,34] Arterial compliance was better in healthy subjects and diabetic patients who consumed one serving of fish per week than in those who consumed no fish.[35] Finally, fish consumption at least two times per week was inversely associated with small myocardial lesions.[35] Thus, our results are consistent with experimental and observational evidence on the beneficial physiologic effects of a small quantity of n-3 fatty acids.

A recent epidemiological study suggests that fish consumption may have an especially beneficial effect on sudden cardiac death.[24] In the present study, sudden cardiac death was included in the outcome variable coronary heart disease mortality; however, because the number of sudden cardiac death was small, it was not possible to separate them from nonsudden cardiac deaths.

In our analyses of the Zutphen cohort from the Netherlands, a small decrease in CHD mortality was observed for fatty fish but not for lean or total fish consumers. Previous results from this cohort showed that consumption of (total) fish once or twice a week in 1960 was associated with a 50% reduction in CHD mortality during 20 years of follow-up.[2] One explanation could be that the coronary events that occurred during the first years of follow-up, when men were younger, were more severe and were strongly associated with fish consumption.[24]

For Finnish men, we observed a small increase in CHD mortality for those who consumed ≥40 g/day of fish (mostly lean fish) and a small decrease for those who consumed fatty fish. A possible harmful effect of fish consumption was observed in two other studies from Finland.[10,11] Several methodological issues may account for the difference in the effect of (type of) fish consumption observed in Finland compared with Italy and the Netherlands. First, the mercury content of fish may explain the more harmful effect of fish in Finland. In the eastern Finnish study, lean fish but not fatty fish was associated with mercury intake and with an excess CHD risk.[10] In general, the mercury content in fish depends on its size, its age and the concentration of mercury in the area.[37,38] Thus, besides the difference in the n-3 fatty acid content of lean fish and fatty fish, in Finland the mercury content of the type of fish consumed may also differ. Second, the high level of fish consumption observed in all Finnish studies, including our cohorts, may limit the possibility of studying the effect of consuming small amounts of fish (especially of total and lean fish). This issue does not apply to the strength of this association between fatty fish intake and CHD risk, since the majority of the Finnish men in our study consumed no fatty fish. Third, we cannot exclude the possibility that the associations between consumption of lean and fatty fish and CHD risk observed in the Finnish cohorts were partly affected by random misclassification due to rather limited information about the type of fish consumed by each man in our Finnish population.

Although an inverse association was observed between fatty fish consumption and CHD mortality in the three countries, no linear trend was found in the pooled analyses of fatty fish consumption. However, the quantity of fatty fish consumption in the three countries overlapped only to a small extent, and the highest consumption was in Finland. Therefore, by categorizing the fatty fish consumption in the pooled analysis, the difference in the effect of fatty fish consumption between the countries was examined rather than a dose-response

relation of fatty fish consumption. In addition, the effects of total and lean fish consumption in Finland, Italy and the Netherlands were rather heterogeneous. In general, this finding might partly be explained by cultural influences, since fish consumption itself, as well as its association with dietary and lifestyle factors, varied among the three countries.

Our results could have been influenced by residual confounding. Inadequate measurement of dietary habitual intake seems unlikely, since the cross-check dietary history method used in all three countries is acknowledged to be valid in an epidemiological setting.[38,39] Additionally, the results could have been biased because of a healthier lifestyle practiced by (fatty) fish consumers. In our data, consumption of fish was associated with a lower intake of saturated fatty acids in the Netherlands and Italy, probably because fish-substitute foods, for example, meat, contain a relatively high level of saturated fatty acids. Furthermore, in all three countries, consumption of vegetables and fruit was positively associated with the amount of fish consumed. In the present study, no reliable measure for physical activity was available. Alternatively, an approximation of physical activity was calculated (energy intake per kg body weight); in all three countries, this variable was positively associated with fish consumption (data not shown). Because these associations were observed for fatty fish as well as for lean fish consumers, and no protective effect of lean fish consumption was observed, the more healthy lifestyle of fish consumers may not have biased the specific effect of fatty fish consumption. Also, when we adjusted our analysis for dietary variables such as intake of vegetables, fruit, and saturated fatty acids or included the proxy of physical activity instead of energy intake, the estimated relative risks did not change appreciably.

In conclusion, our data suggest that especially fatty fish is protective against CHD mortality. Independent of lean fish consumption and potential confounders, we found that consumption of fatty fish was associated with a reduction of 34% (95% CI 10-51%) in the CHD mortality in three different European countries.

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intake is not beneficially associated with 10-year risk of coronary heart disease, The Zutphen Elderly Study

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Evidence on the relation between α-linolenic acid intake and coronary heart diseas (CHD) is limited. Other dietary components appear to modify the reported relations. W examined whether dietary α-linolenic acid was inversely associated with the risk of CH We prospectively studied 667 men of the Zutphen Elderly Study aged 64-84 years o and free of CHD at baseline. Dietary intake was assessed by the use of the cross-che dietary history method, and detailed data on different fatty acids in foods. During 1 years of follow-up, we documented 98 cases of CHD. After adjustment for age, standa coronary risk factors, and intake of trans fatty acids and other nutrients, α-linolenic ac intake was not statistically significantly associated with CHD risk. The relative risk (RI of CHD for the highest compared with the lowest tertile of α-linolenic acid intake wa 1.68 (95% CI: 0.86, 3.29). α-Linolenic acid intake from sources with trans fatty acid was also non-significantly positively associated with CHD risk. α-Linolenic acid intak from foods without trans fatty acids was not associated with the CHD risk, the RR of CH for the highest compared with the lowest tertile was 1.15 (95% CI: 0.63, 2.11). conclusion, we did not observe a beneficial effect of dietary α-linolenic acid intake on th risk of 10-year CHD. The association between intake of α-linolenic acid and trans fat acids complicated studying this hypothesis. Given the results of current prospecti studies, a protective cardiac effect of α-linolenic acid is questionable.

INTRODUCTION

Diets enriched with α-linolenic acid (C18:3 n-3) have been reported to increas the blood concentrations of α-linolenic acid as well as n-3 long-cha polyunsaturated fatty acids, especially eicosapentaenoic acid (EPA), in humans. [8] N-3 long-chain polyunsaturated fatty acids were considered to have a variety favorable physiological cardiac effects.[9] Although the efficiency of conversion α-linolenic acid to EPA is relatively low,[1,2,10,11] dietary intervention trials reporte beneficial effects of α-linolenic acid intake on eicosanoid metabolism and platel aggregation[2-4,12] or on arterial compliance.[7] In contrast, no consensus exists c the effect of α-linolenic acid on serum lipid concentrations[12-14] and bloc pressure.[3,7,8,12,13]

Case-control studies on the association between markers for α-linolenic ac intake and risk of myocardial infarction,[15-17] angina pectoris,[18] or sudden cardia death[19] yield conflicting results. Also, platelet or plasma α-linolenic acid conten

of nested-case control studies were inconsistently associated with the risk of coronary heart disease (CHD).[20-22]

Until now, only a few prospective studies focused on the association between the intake of α-linolenic acid and CHD. In two dietary intervention trials, a remarkable reduction in the risk of cardiac events was achieved in survivors of myocardial infarction who took an α-linolenic-acid-enriched Mediterranean-type diet,[23] or mustard oil (containing α-linolenic acid).[24] Previously reported cohort studies have suggested that a higher intake of α-linolenic acid may reduce the CHD risk.[25-28] However, in most population based studies, other dietary factors such as total or trans fat were (potentially) associated with α-linolenic acid and (could) have influenced the results.[17,23,25-28]

Accordingly, insight is needed into the association between the α-linolenic acid intake and the CHD risk in different populations with characteristic dietary habits. We previously reported the intake and sources of α-linolenic acid for men participating in the Zutphen Elderly Study.[29] We have now examined the relation between α-linolenic acid intake and CHD incidence, carefully taking into account the intake of several other fatty acids.

SUBJECTS AND METHODS

Study population

The study population consisted of men who participated in the Zutphen Elderly Study, an extension of the Zutphen Study. In 1960, the Zutphen Study started with a cohort of 878 men from the town of Zutphen (the Netherlands) born between 1900 and 1919, as the Dutch contribution to the Seven Countries Study.[30] In 1985, 367 of the 555 participants who were still alive were re-examined. In addition, 711 other men from Zutphen in the same age category were asked to participate. A total of 939 men (response rate 74%) was examined in 1985,[31] and complete information on diet and risk factors was available for 824 men. Men with previously diagnosed CHD were excluded from the present analyses (n=157), leaving 667 men at baseline in 1985.

Data collection

Dietary and medical examinations were completed between March and June in 1985. Information about the habitual food consumption was collected using the

cross-check dietary history method, adapted to the Dutch situation.[32] Each participant, and if possible also his wife, was interviewed about his average food consumption pattern in the month before the interview. A checklist of foods and quantities of food bought per week were used to calculate and verify the participant's food consumption pattern. Nutrient intake data were calculated using the corresponding Dutch food table,[33] that was partly updated[34] and completed with data of α-linolenic acid,[29] trans fatty acids,[35] linoleic acid, EPA, docosahexaenoic acid (DHA),[36] β-carotene and vitamin E.[37]

During medical examinations non-fasting venous blood samples were taken. Serum total cholesterol and high-density lipoprotein (HDL) cholesterol were determined enzymatically.[38,39] Weight and height were measured in light clothing without shoes, and body mass index (BMI) was calculated (weight/height^2). Information on cigarette smoking (never, former, current) was obtained by a standardized questionnaire. The total minutes of physical activity per week were calculated using information from a self-administered questionnaire designed for retired men.[40]

Follow-up

Incident cases included fatal CHD plus non-fatal myocardial infarction (whatever occurred first) occurring between the baseline assessment in 1985 and January 1995. Information on vital status of the participants was obtained from the municipal registries. Three participants were lost to follow-up. Information on cause of death was obtained between 1985 and June 1990 from Statistics Netherlands. For deaths occurring thereafter, or if not available from Statistics Netherlands, information was obtained from hospital discharge data and/or general practitioners. Causes of death were coded following the ninth revision of the International Classification of Diseases. CHD refers to codes 410-414. Because the underlying cause of death in the elderly is often difficult to establish, both CHD as a primary (n=46) as well as a secondary (n=3) cause of death were considered in the analyses.

Data on the prevalence of CHD was obtained by the Dutch translation of the Rose questionnaire,[41] and after 1990 by a standardized medical questionnaire. In case of non-response, information of major chronic diseases was obtained from a short questionnaire filled out by the subjects themselves or their closest relative or caretaker. Diagnosis of each disease was confirmed with hospital discharge data. Also, for men who died, information on disease history was

obtained from the general practitioner. Coronary heart disease at baseline in 1985 was considered to be present when either myocardial infarction or angina pectoris was diagnosed. For myocardial infarction (between baseline and January 1995) the final diagnosis required at least two of the following criteria: a specific medical history, characteristic electrocardiographic changes or specific enzyme elevations. During 10 years of follow-up, we documented 98 CHD cases in the men without a previously diagnosed CHD (14.7% of the baseline population), of which 49 were fatal.

Statistical methods

Men were divided into tertiles based on the contribution of α-linolenic acid to energy intake at baseline. To test for differences in baseline major risk and dietary factors across categories of α-linolenic acid intake, we used analysis of variance for normally distributed variables, the Kruskal-Wallis test for skewed distributed variables, and the chi-square test for categorical variables. Spearman correlation coefficients (r_s) were calculated between α-linolenic acid and other dietary fatty acids. Cox's proportional-hazard analysis was performed to calculate relative risks (RRs), with the lowest α-linolenic acid tertile as the reference group, or using α-linolenic acid intake as continuous variable. Additional analyses were conducted to examine associations with CHD incidence for intake of α-linolenic acid from sources with and without trans fatty acids separately, and for consumption of oil and salad dressing plus mayonnaise (α-linolenic-rich foods). Adjustments were made for age, energy intake, BMI, smoking, alcohol consumption, use of vitamin supplements, and dietary factors (in the analyses on α-linolenic acid) or food groups (in the food analyses) potentially associated with CHD. Other risk factors were not included into the model because they were viewed as intermediate variable (as cholesterol or blood pressure) or were not associated with α-linolenic acid intake (physical activity, history of diabetes mellitus and history of hypertension). All statistical analyses were carried out using SAS (version 6.12) statistical analysis computer package.

Results

The mean (± SD) daily intake of α-linolenic acid was 1.32 ± 0.47 g, which contributes 0.53 ± 0.15 % to total energy intake. The main sources of α-linolenic

Table 1. Characteristics (mean value and standard deviation) at baseline by tertiles of α-linolenic acid intake in energy percent. The Zutphen Elderly Study.

	Total group	α-linolenic acid tertile			
Range (% of energy)		<0.45	0.45-0.58	≥0.58	P
N	667	222	223	222	values[1]
Age (yr)	71.1(5.2)	71.3	71.4	70.8	0.48
Body mass index (kg/m^2)	25.5(3.2)	25.3	25.3	25.8	0.13
Physical activity (min/week)	611(533)	581	633	620	0.85
Systolic blood pressure (mm Hg)	151(21)	154	149	150	0.02
Serum total cholesterol (mmol/L)	6.08(1.11)	6.05	6.00	6.19	0.18
Serum HDL cholesterol (mmol/L)	1.14(0.30)	1.15	1.12	1.14	0.58
Smoking					
current (%)	32.4	26.6	34.1	36.5	0.07
past (%)	48.7	55.4	42.6	48.2	0.03
Use of vitamin supplements (%)	15.9	22.3	12.6	13.1	0.007
Daily intake of					
Energy (MJ)	9.2(2.0)	9.1	9.4	9.1	0.24
Total fat (% of energy)	40.3(6.4)	37.2	40.9	42.9	0.0001
Saturated fat (% of energy)	18.0(3.6)	17.3	18.8	17.9	0.0001
Trans fatty acids (% of energy)	4.3(2.2)	2.8	4.4	5.8	0.0001
Cis unsaturated fat (% of energy)	18.0(3.9)	17.1	17.7	19.2	0.0001
Linoleic acid (% of energy)	5.0(2.4)	5.1	4.6	5.4	0.0001
EPA + DHA (% of energy)	0.08(0.14)	0.05	0.09	0.09	0.22
Cholesterol (mg)	273(97.0)	253	289	274	0.0004
Carbohydrates (% of energy)	41.0(7.3)	42.5	41.0	39.4	0.0001
Protein (% of energy)	14.3(2.6)	14.3	14.2	14.3	0.92
Alcohol – g	13.8(17.3)	18.3	12.5	10.7	0.001
non-drinkers (%)	23.5	20.3	23.8	26.6	0.29
≥20 g/d (%)	26.7	34.7	24.2	21.2	0.003
Fiber (g)	24.9(7.1)	23.8	25.7	25.1	0.01
Vitamin E (mg)	8.5(2.6)	8.0	8.7	8.9	0.0001
Vitamin C (mg)	90.3(39.5)	92.9	89.7	88.2	0.54
β-carotene (mg)	1.4(0.6)	1.3	1.4	1.4	0.02

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid

[1]p-value for any difference between α-linolenic acid categories (analysis of variance for normally distributed variables, Kruskal-Wallis test for skewed variables, chi-square test for dichotomous variables).

Table 2. Relative risks and 95% CIs of fatal plus non-fatal coronary heart disease and fatal coronary heart disease according to tertiles of α-linolenic acid intake.

	Tertile			p
	1	2	3	trend
Range (% of energy)	<0.45	0.45-0.58	≥0.58	
Median intake (% of energy)	0.40	0.51	0.67	
Fatal plus non-fatal coronary heart disease				
No. (%) of cases	21 (9.5%)	34 (15.3%)	43 (19.4%)	
Relative risks				
Crude	1	1.68 (0.97-2.89)	2.24 (1.33-3.77)	0.003
Age + energy adjusted	1	1.69 (0.98-2.92)	2.23 (1.32-3.76)	0.003
Fully adjusted[1]	1	1.49 (0.82-2.70)	1.68 (0.86-3.29)	0.17
Fatal coronary heart disease				
No. (%) of cases	12 (5.4%)	15 (6.7%)	22 (9.9%)	
Relative risks				
Crude	1	1.27 (0.59-2.71)	1.97 (0.97-3.98)	0.05
Age + energy adjusted	1	1.26 (0.59-2.69)	1.95 (0.96-3.94)	0.05
Fully adjusted[1]	1	0.99 (0.43-2.28)	1.59 (0.62-4.08)	0.26

[1] 95% CI in parentheses. Models included the following variables: age, intake of energy, BMI, ex-smoking (yes/no), current smoking (yes/no), alcohol intake, use of vitamin supplements (yes, no), intake of saturated fatty acids, trans fatty acids, linoleic acid, eicosapentaenoic+ docosahexaenoic acid, other cis unsaturated fatty acids, protein (all in energypercentages) intake of dietary cholesterol, fiber, vitamin E, vitamin C and β-carotene. Alcohol intake (0, 1-19, ≥20 g/d) was used as a categorical variable (included as two dummies into the model, with the non-drinkers as a reference).

acid in the diet of these elderly men were margarines, meat, bread and vegetables, contributing together over 50% of the total intake of α-linolenic acid.

The daily intake of α-linolenic acid at baseline expressed as a percentage of total energy was positively associated with cigarette smoking, and the daily intake of total, saturated and unsaturated fat, cholesterol, fiber, vitamin E and β-carotene and inversely with systolic blood pressure, the use of vitamin supplements, the daily intake of carbohydrates and alcohol (table 1). Concerning the correlation with other fatty acids, α-linolenic acid intake strongly correlated with intakes of total fat (r_s =0.40), trans fatty acid (r_s =0.61) and cis monounsaturated fatty acids (r_s =0.44), and weakly with linoleic acid (r_s =0.19) and saturated fat

(r_s=0.08). The correlation coefficient between the intake of α-linolenic acid and that of n-3 fatty acids from fish was not statistically significant (r_s =0.03).

The crude relative risk of 10-year fatal plus non-fatal CHD was 2.24 (95% CI 1.33-3.77) for the highest compared with the lowest tertile of α-linolenic acid intake (table 2). After adjustment for age, BMI, smoking, use of vitamin supplements and dietary vitamin intake, intake of energy, alcohol, dietary cholesterol, fiber and specific fatty acids including trans fatty acids, the association betweenα-linolenic acid intake and CHD was reduced, and no longer statistically significant. The adjusted relative risk of incident CHD for the highest compared with the lowest tertile of α-linolenic acid intake was 1.68 (95% CI 0.86-3.29, p-trend=0.17) (table 2). In addition, the adjusted relative risk of CHD for a 0.5 energy percent increase in intake of α-linolenic acid was 1.58 (95% CI 0.67-3.74). The association was similar for fatal CHD. The adjusted relative risk of fatal CHD for the highest compared to the lowest tertile of α-linolenic acid intake was 1.59 (95% CI 0.62-4.08, p-trend=0.26) (table 2). In addition, for an increase of 0.5% of energy, the adjusted relative risk of fatal CHD was 1.40 (95% CI 0.36-5.41).

In the present study, the largest contribution to the intake of α-linolenic acid is provided by foods containing also trans fatty acids, e.g. margarines and meat, that may be connected to the increased risk. We therefore examined α-linolenic-acid-sources with trans fatty acids (e.g. margarines, cooking fat, butter, cookies, pastries, meat, dairy products and bread) and without trans fatty acids (e.g. cereals, legumes, vegetables and fruit) separately in relation to CHD risk. There was a significant positive association between α-linolenic acid intake from sources with trans fatty acids and CHD risk, which became non-significant after additional adjustment for trans fatty acid intake (table 3). In contrast, the intake of α-linolenic acid from foods without trans fatty acids was not associated with the CHD risk.

The main sources of α-linolenic acid in the present study differed from those in other studies, i.e. vegetable oils,[42,43] or salad dressing and mayonnaise.[28] We also examined the relations of oil consumption and consumption of creamy salad dressing plus mayonnaise, with the CHD risk. The most important oils consumed by this elderly population were sunflower oil (n=50; 41% of the total oil consumption), soybean oil (n=46; 33% of the total oil consumption), olive oil (8%) and safflower oil (8%). The men consuming oils were younger, consumed more alcohol and vegetables, and had a lower trans fatty acids intake.

Table 3. Relative risks and 95%CIs of fatal plus non-fatal coronary heart disease according to tertiles of α-linolenic acid intake from sources with and without trans fatty acids.

	Tertile			p
	1	2	3	trend
α-Linolenic acid from sources with trans fatty acids				
Range (% of energy)	<0.40	0.40-0.52	>0.52	
Median intake (% of energy)	0.35	0.46	0.61	
No. (%) of cases	21 (9.5%)	35 (15.7%)	42 (18.9%)	
Relative risks				
Crude	1	1.71 (0.99-2.94)	2.18 (1.29-3.68)	0.004
Age + energy adjusted	1	1.71 (1.00-2.95)	2.20 (1.30-3.71)	0.004
Adjusted[1]	1	1.56 (0.88-2.77)	1.90 (1.06-3.40)	0.04
Fully adjusted[2]	1	1.42 (0.78-2.57)	1.51 (0.75-3.04)	0.31
α-Linolenic acid from sources without trans fatty acids				
Range (% of energy)	<0.04	0.04-0.06	>0.06	
Median intake (% of energy)	0.03	0.05	0.07	
No. (%) of cases	32 (14.4%)	31 (13.9%)	35 (15.8%)	
Relative risks				
Crude	1	0.93 (0.57-1.52)	1.08 (0.67-1.75)	0.77
Age + energy adjusted	1	0.90 (0.55-1.48)	0.97 (0.58-1.63)	0.90
Adjusted[1]	1	1.06 (0.62-1.81)	1.17 (0.63-2.15)	0.63
Fully adjusted[2]	1	1.06 (0.62-1.81)	1.15 (0.63-2.11)	0.67

[1] 95% CI in parentheses. Models included the following variables: age, intake of energy, BMI, ex-smoking (yes/no), current smoking (yes/no), alcohol intake, use of vitamin supplements (yes, no), intake of saturated fatty acids, linoleic acid, eicosapentaenoic+ docosahexaenoic acid, other cis unsaturated fatty acids, protein (all in energypercentages) intake of dietary cholesterol, fiber, vitamin E, vitamin C, β-carotene and intake of α-linolenic acid from sources with (in case of α-linolenic acid from sources without trans fatty acids) or without (in case of α-linolenic acid from sources with trans fatty acids) trans fatty acids. Alcohol intake (0, 1-19, ≥20 g/d) was used as a categorical variable (included as two dummies into the model, with the non-drinkers as a reference).
[2] Additional adjustment for trans fatty acid intake.

A crude significant inverse association was observed between oil consumption and CHD incidence (table 4). After adjustment for potential confounders, the relative risk for oil consumers compared with no oil consumers was 0.53 (95% CI 0.26-1.06). Additional adjustment for intake of α-linolenic acid, linoleic acid,

Table 4. Relative risks and 95% CIs of fatal plus non-fatal coronary heart disease according to consumption of oil and salad dressing.

	No	Yes
Oil		
N	552	115
Median intake (g/d)	0	2
No. (%) of cases	89 (16.1%)	9 (7.8%)
Relative risks		
Crude	1	0.47 (0.23-0.92)
Fully adjusted[1]	1	0.53 (0.26-1.06)
Mayonnaise and other creamy dressing		
N	442	225
Median intake (g/d)	0	3
No. (%) of cases	63 (14.3%)	35 (15.6%)
Relative risks		
Crude	1	1.00 (0.66-1.51)
Fully adjusted[1]	1	1.09 (0.71-1.66)

[1] 95% CI in parentheses. Models included the following variables: age, intake of energy, BMI, ex-smoking (yes/no), current smoking (yes/no), alcohol intake, use of vitamin supplements (yes/no), intake of vegetables, fruit, meat, fish, and fats for household use (e.g. margarine, butter, cooking fat, frying fat). Alcohol intake (0, 1-19, ≥20 g/d) was used as a categorical variable (included as two dummies into the model, with the non-drinkers as a reference).

trans fatty acids or vitamin E did not appreciably change the results (data not shown). Furthermore, no association was observed between the intake of creamy salad dressing and mayonnaise and CHD (table 4).

Discussion

In the present study, we observed a non-significant positive association between α-linolenic acid intake and CHD risk. This seems to be due to the strong association between intake of α-linolenic acid and trans fatty acids. It is likely

that also in other populations with comparable sources of α-linolenic acid as in the present study, intake of α-linolenic acid is (strongly) associated with intake of trans fatty acids. Moreover, in a Norwegian case-control study, contents of trans fatty acid and α-linolenic acid in adipose tissue were also intercorrelated and associated with increased risk.[17] This emphasizes the importance to adjust for other dietary factors and the difficulty to pursue this hypothesis epidemiologically or to generalize the epidemiological findings to other populations.

Imprecision in the estimate of α-linolenic acid could have obscured an association with CHD. Habitual food composition was measured by use of the cross-check dietary history method, that is acknowledged as a valid method in an epidemiological setting.[32] The α-linolenic acid content of approximately 1000 products that were consumed by the participants of the Zutphen Elderly Study was used to calculate the intake of α-linolenic acid.[29] Random misclassification of dietary exposure, due to error in the quantification of food composition data including α-linolenic acid, cannot be excluded. However, values in the nutrient database were updated as much as possible, taking into account improvements in the quality of analytical methods and changes in food composition in time.[34,44] Intercorrelation between α-linolenic acid and other dietary factors, mainly trans fatty acids, complicated the estimation of the independent effect of α-linolenic acid. We confirmed the results of our analyses by relating the α-linolenic acid intake of foods with and without trans fatty acids to CHD risk. However, due to the strong association between intake of α-linolenic acid and trans fatty acids, residual confounding cannot be totally excluded. It might be that the effects of α-linolenic acid on CHD are especially present when larger amounts of α-linolenic acid from sources without trans fatty acids are consumed.

A limitation of the present study was that it included only men aged 64-84 years at baseline. The etiology of CHD in elderly people may be altered, due to advanced coronary atherosclerosis. The beneficial effects of α-linolenic acid on platelet aggregation or arterial compliance might be larger in younger populations, however, there are no data available on the effect of age on the association between α-linolenic acid and CHD risk or risk factors. Our results were consistent using fatal CHD as well as including non-fatal myocardial infarction. Because of power, we mainly focused on the association of fatal plus non-fatal CHD.

A few prospective cohort studies have previously reported on the association between α-linolenic acid intake and CHD.[25-28] A strong inverse association was observed in the Nurses' Health Study.[28] In the other cohort studies, however, the results were less clear.[25-27] Firstly, the results of the other cohort studies were strongly affected by adjustment for other dietary factors. Adjustment for total fat in the Health Professional Study,[26] or adjustment for trans-, cis-monounsaturated and saturated fatty acids in the ATBC Prevention Study,[27] strengthened the associations. In the MRFIT Study, the association may have been confounded by other dietary factors, because such adjustments were not made.[25] Secondly, there was no suggestion of a linear dose-response relationship for quintiles of intake of α-linolenic acid in data of the MRFIT and the Health Professional Study. In the MRFIT Study, the adjusted relative risks for the quintiles of α-linolenic acid intake were respectively 1, 0.98, 0.57, 0.98 and 0.68.[25] In the Health Professionals Study, the relative risk on fatal CHD was not reduced in the highest quintile, however, a reduced risk of fatal CHD was observed in the analyses using α-linolenic acid as a continuous variable.[26] Thus, prospective studies provide not enough evidence to support the hypothesis that a higher intake of α-linolenic acid will reduce the risk of CHD.

Our results for α-linolenic acid are not consistent with those observed in the Nurses' Health Study. The range in α-linolenic acid intake in our cohort is comparable with that in the Nurses' Health Study. However, in the Nurses' Health Study, approximately 70% of the α-linolenic acid intake was derived from vegetable or plant sources, of which salad dressings were the most important food group (30%).[28] In the present study, a borderline-significant inverse association was observed between the intake of oils and CHD incidence. In addition, no association was observed between the intake of salad dressing and CHD. Neither α-linolenic acid, nor linoleic acid or vitamin E, also abundantly present with these oils, seems to be responsible for the protective effect of oil, because including these components into the model yield similar results. The results could have been biased, because oil consumption was limited in these Dutch Elderly men and may be a marker for healthier lifestyle. However, adjusting for potential confounders, or additionally adjusting for physical activity, history of hypertension or history of diabetes mellitus (data not shown) did not change the relative risks appreciably. Therefore, the potential protective effect of oil consumption, including the responsible components, deserves further research.

In a secondary prevention trial, recurrence of cardiac events was substantially lower among patients randomly assigned to a Mediterranean diet enriched with α-linolenic acid compared with those in the control group.[23] However, other dietary changes occurred simultaneously in this trial. In another secondary prevention trial, cardiac events were significantly lower after 1 year treatment with mustard oil compared with the placebo group.[24] However, the experimental and control group differed in other characteristics relevant to cardiovascular health (e.g. smoking habit), whereas these were not taken into account in the final risk estimates. Therefore, also based on these trials it cannot be concluded that the protective effect was solely due to α-linolenic acid.

In conclusion, we observed no beneficial association between dietary α-linolenic acid intake and the risk of 10-year CHD in Dutch elderly men. The substantial differences between crude and adjusted relative risks of CHD for α-linolenic acid intake in prospective studies, together with the limited evidence on the mechanisms, indicates that the protective cardiac effect of α-linolenic acid is questionable.

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intake, serum total homocysteine concentrations and 10-year risk of coronary heart disease in the Zutphen Elderly Study

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B-vitamin intake may be associated with the risk of coronary heart disease (CHD through their role in homocysteine metabolism. However, data from prospective studie relating B-vitamin intake to CHD risk are limited. We investigated whether dietary folate vitamins B2, B6 and B12 are associated with serum homocysteine concentrations an CHD risk in 815 men of the Zutphen Elderly Study aged 64-84 years. Information on die and risk factors including serum homocysteine was obtained at baseline. After 10 year of follow-up, 140 CHD cases had occurred. Only intake of folate and vitamin B6 wer independently inversely associated with homocysteine. After adjustment for age, intak of other B-vitamins and other potential confounders, the mean homocystein concentration for the men in the lowest tertile of folate was 15.3 μmol/L compared t 13.9 μmol/L in the highest tertile of folate. The homocysteine concentration was 15. μmol/L in the lowest vitamin B6 tertile compared to 13.9 μmol/L in the highest vitami B6 tertile. However, folate and vitamin B6 intake were not associated with CHD risk i these elderly men. Vitamin B2 was non-significantly inversely associated with CH (relative risk=0.61 for the highest compared to the lowest vitamin B2 tertile; p for tren 0.08). Vitamin B12 was positively associated with CHD risk (p for trend=0.03). Th present study did provide evidence that dietary folate and vitamin B6 are related t serum homocysteine. B-vitamin intake was not protective for CHD, although there was suggestion of a protective effect of vitamin B2.

INTRODUCTION

An elevated blood concentration of total homocysteine (tHcy) is suggested to b an independent risk factor for cardiovascular diseases.[1] Because the results fro prospective studies are weaker than those from case-control studies, an because there is lack of certainty about underlying mechanisms, causality in th association between tHcy and vascular diseases is still in dispute.[2,3] B-vitamin (folate, vitamins B2, B6 and B12) are involved in the tHcy metabolism as co enzymes and cofactors.[4] Several dietary intervention trials have provide evidence for a tHcy lowering effect of supplemental B-vitamins, especially of fol acid.[5]

Until now, a number of case-control studies[6-12] and prospective studies[13- relating blood levels of folate, vitamin B6 and vitamin B12 to the risk of coronar heart disease (CHD) were performed, showing inconsistent results. Howeve

circulating levels of B-vitamins are known to change within days, and may therefore not be satisfactory as a marker of long-term intake.

As underlying diseases or short-term variations in nutrient intake may influence plasma vitamin levels, prospective studies on associations between dietary B-vitamins intake and CHD are most informative. Until now, two prospective studies have investigated the association between dietary intake of B-vitamins and CHD. In the ARIC study, neither folate, vitamin B6 nor vitamin B12 was associated with CHD.[16] In contrast, in the Nurses' Health Study an inverse association was noted between both folate and vitamin B6 intake and CHD risk.[20] In the Nurses' Health Study, however, due to the high intake of B-vitamins by supplements, the independent effect of folate and vitamin B6 could not be investigated.

To provide additional prospective data on the association of B-vitamin intake with CHD, we analyzed data of the Zutphen Elderly Study. Among this cohort of elderly men, we previously reported that tHcy levels are positively associated with 10-year CHD mortality. The association between tHcy and CHD incidence was less consistent.[21] The present study provides information on the association between dietary intake of folate, vitamins B2, B6, B12 and both CHD incidence and CHD mortality. Also the associations between B-vitamin intake and serum tHcy levels are studied.

METHODS

Study population

The Zutphen Elderly Study is a longitudinal investigation of chronic disease risk factors in elderly male inhabitants of Zutphen, a town in the eastern part of the Netherlands. It represents a continuation of the Zutphen Study, the Dutch contribution to the Seven Countries Study.[22] In 1985, 367 of the 555 participants of the original cohort still alive, were re-examined. In addition, a random sample of 711 other men from the town of Zutphen in the same age group were asked to participate. This resulted in a total population of 939 men (response rate 74%) born between 1900 and 1919, which formed the cohort of the Zutphen Elderly Study. All participants signed an informed consent form. Complete information on diet and risk factors was available for 815 men.

Data collection

Dietary and medical examinations were completed between March and June in 1985. We obtained information about the habitual food consumption with the cross-check dietary history method, adapted to the Dutch situation.[23] Each participant, and if possible his partner, was interviewed about his average food consumption pattern in the month before interview. A checklist of foods and quantities of food bought per week was used to calculate and verify the participant's usual food consumption pattern.

We calculated nutrient intake with the Dutch food table, completed with data of folate,[24] β-carotene, vitamin E,[25] and trans fatty acids.[26]

During medical examinations we took non-fasting venous blood samples. After about 1 hour, samples were centrifuged and the serum was separated and stored at -20°C until tHcy determination in 1995.[21] We measured weight and height in light clothing without shoes, and calculated body mass index (BMI; weight/height^2). We calculated total minutes of physical activity per week with a self-administered questionnaire designed for retired men.[27] We ascertained information on cigarette smoking, history of hypertension and diabetes mellitus with a standardized questionnaire.

Incident cases included fatal CHD plus non-fatal myocardial infarction (whichever arose first) occurring between baseline assessment in 1985 and January 1995. Three participants were lost to follow-up and censored at 1991. We obtained information on vital status of the participants from the municipal registries and on cause of death between 1985 and June 1990 from Statistics Netherlands. For deaths occurring thereafter, or if data were not available from Statistics Netherlands, information was obtained from hospital discharge data and/or general practitioners. We coded causes of death in accordance with the International Classification of Diseases (ICD). CHD refers to ICD-9 codes 410-414. Because the underlying cause of death in the elderly is often difficult to establish, we classified CHD as a primary (n=81) and secondary (n=6) cause of death in the analyses.

We obtained information about myocardial infarction and angina pectoris between 1985 and 1990 by the Dutch translation of the Rose questionnaire,[28] and after 1990 by a standardized medical questionnaire. In case of non-response, a short questionnaire was used completed by the subjects themselves or their closest relative. Diagnosis of each disease was confirmed with hospital

discharge data. Also, for men who died, information on disease history was obtained from the general practitioner. CHD at baseline in 1985 was considered to be present when either myocardial infarction or angina pectoris was diagnosed. Diagnosis of myocardial infarction (between baseline and January 1995) required at least two of the following criteria: a specific medical history, characteristic electrocardiographic changes or specific enzyme elevations.

Statistical methods

To improve normality of the distributions of the different B-vitamin intakes and tHcy levels, we performed logarithmic transformations. Thereafter, B-vitamin intakes were energy-adjusted by regressing B-vitamin intake on total energy intake.[29] Men were divided into tertiles on the basis of energy-adjusted intake of folate, vitamin B2, B6 or B12. To study the associations between intake of B-vitamins and tHcy, we calculated the mean tHcy level per tertile of B-vitamin intake with analysis of covariance. Trends were evaluated with linear regression, by using the median value of each category and modeling this as a continuous variable. To study the association between intake of B-vitamins and CHD incidence, we used Cox proportional hazard (survival) analysis to calculate relative risks with the lowest tertile of each B-vitamin intake as a reference group.

Results

At baseline, the mean age of the Zutphen Elderly men was 71.3 years (SD=5.2 years). Thirty percent of the men were cigarette smokers and about 19% had a previous diagnosed CHD. The mean intakes were 200 μg/d (SD=61 μg/d) for folate, 1.70 mg/d (SD=0.50 mg/d) for vitamin B2, 1.62 mg/d (SD=0.38 mg/d) for vitamin B6 and 5.29 μg/d (SD=3.27 μg/d) for vitamin B12. Intake of folate, vitamin B2, B6 and B12 were positively correlated. The correlation coefficient was highest between vitamin B2 and vitamin B12 (r_s=0.57). Other correlation coefficients were between r_s=0.18 (between intake of vitamins B6 and B12) and 0.50 (between intake of folate and vitamin B2).

Table 1. Baseline level of major risk factors for coronary heart disease, according to energy-adjusted tertiles of B-vitamins intake in 824 men aged 64-84 years old.

B-vitamins	N	Age (years)	Body mass index (kg/m^2)	Physical activity (min/week)	Smoking Current (%)	Smoking never (%)	Use of vitamin supplements (%)	Prevalence of CHD (%)
Folate intake								
< 173 µg/d	271	71.9	25.5	540	34.0	17.7	14.8	18.1
173-215 µg/d	272	71.5	25.3	634	30.2	19.5	14.3	19.9
>215 µg/d	272	70.6*	25.6	624	27.6	17.3	16.9	18.4
Vitamin B2 intake								
< 1.5 mg/d	271	71.9	25.4	546	33.2	15.7	11.8	18.5
1.5-1.8 mg/d	272	70.9	25.5	673	32.0	20.6	15.1	18.8
>1.8 mg/d	272	71.1	25.5	581*	26.5	18.2	19.1	19.2
Vitamin B6 intake								
< 1.5 mg/d	271	71.8	25.0	591	34.0	17.0	17.3	19.6
1.5-1.7 mg/d	272	71.6	25.6	559	31.3	22.1	15.4	18.8
>1.7 mg/d	272	70.5*	25.8*	649	26.5	15.4	13.2	18.0
Vitamin B12 intake								
< 3.6 µg/d	271	71.9	25.0	596	27.7	17.3	12.9	19.6
3.6-5.0 µg/d	272	71.2	25.6	583	30.5	19.5	16.2	19.5
>5.0 µg/d	272	70.8	25.8*	620	33.5	17.7	16.9	17.3

CHD=coronary heart disease
* statistically significantly different ($p<0.05$) between B-vitamin intake tertiles (analyses of variance for normally distributed variables, Kruskal-Wallis test for skewed variables, chi-square test for dichotomous variables)

Relation between B-vitamin intake and possible confounders

To determine potential confounders in the relation between B-vitamin intake, tHcy and CHD, differences in characteristics were tested across B-vitamin tertiles. The men in the highest tertiles of B-vitamin intake were younger (statistically significant for folate and vitamin B6), and had a higher BMI (statistically significant for vitamin B6 and B12) compared to the lowest tertiles of B-vitamin intake (table 1). In addition, levels of physical activity significantly

differed between the tertiles of vitamin B2 intake. B-vitamin intake was significantly positively associated with intake of protein (including methionine) and dietary fiber (data not shown). Furthermore, intake of folate, vitamin B2 and B6 was significantly inversely associated with intake of saturated fat and trans fatty acids, and significantly positively with intake of vitamin C and beta-carotene. Also, there was a significant positive association between intake of folate and vitamin E, and a significant inverse one between vitamin B2 intake and alcohol consumption.

Table 2. Geometric means of serum homocysteine concentrations (μmol/L) according to energy-adjusted tertiles of B-vitamin intake.

	Tertiles			
	T 1 (n=271)	T 2 (n=272)	T 3 (n=272)	p-trend
	Folate intake			
Median intake (μg/d)	153	192	246	
Unadjusted tHcy	15.9	14.4	13.4	0.0001
Adjusted* tHcy	15.3	14.5	13.9	0.007
	Vitamin B2 intake			
Median intake (mg/d)	1.3	1.6	2.1	
Unadjusted tHcy	15.4	14.2	14.0	0.002
Adjusted* tHcy	14.3	14.3	15.0	0.16
	Vitamin B6 intake			
Median intake (mg/d)	1.3	1.6	1.9	
Unadjusted tHcy	15.8	14.7	13.2	0.0001
Adjusted* tHcy	15.0	14.7	13.9	0.02
	Vitamin B12 intake			
Median intake (μg/d)	2.9	4.1	6.9	
Unadjusted tHcy	15.2	14.6	13.9	0.002
Adjusted* tHcy	14.4	14.6	14.6	0.80

T=tertile, tHcy=total homocysteine concentration
*Adjusted for age, prevalence of coronary heart disease, smoking, body mass index, intake of energy, other B-vitamins, alcohol, vitamin supplements, methionine and coffee.

Cross-sectional relation between B-vitamin intake and tHcy

The intake of all B-vitamins was inversely associated with tHcy (table 2). After adjustment for other B-vitamin intake and major determinants of tHcy, including age, prevalence of CHD, smoking, BMI, use of vitamin supplements, intake of energy, alcohol, methionine and coffee, the associations between intake of all B-vitamins and tHcy became weaker. Significant decreased tHcy levels were observed for higher intake of folate and vitamin B6 (table 2). Intake of vitamin B2 and vitamin B12 were both not significantly associated with tHcy in the adjusted models.

Relation between B-vitamin intake and 10-year CHD risk

During 10 years of follow up, 140 men out of 815 men had developed CHD, of which 87 were fatal. Folate intake was not associated with the 10-year risk of CHD, neither crude not after adjustment for determinants of CHD (table 3). Vitamin B2 intake was borderline significantly inversely associated with CHD risk (adjusted p-trend=0.08). For vitamin B6 intake, we observed a small non-significant reduction in CHD risk for the men in the medium and highest tertile compared to those in the lowest tertile of vitamin B6, but there was no significant trend. Furthermore, we noted a significant positive association between vitamin B12 intake and CHD incidence, which became stronger after adjustment for major coronary risk factors and dietary factors. Additional adjustment for tHcy levels in the analyses on B-vitamin intake showed similar relative risks (table 3). Additional adjustment for prevalence of diabetes mellitus, prevalence of hypertension, or physical activity, or excluding men with a previous diagnosed CHD at baseline from the analyses (n=153), did not substantially changed the results (data not shown).

Due to the intercorrelation between intake of vitamin B12 and vitamin B2, adjustment for vitamin B12 in the analyses of vitamin B2 intake and CHD risk substantially affected the results. After adjustment for all major risk and dietary factors except for vitamin B12 intake, the relative risk of the highest versus the lowest tertile of vitamin B2 was 0.80 (95% confidence interval (CI) 0.50-1.34; P for trend=0.45), compared to the relative risk of 0.61 after additional adjustment for vitamin B12 (table 3). Other associations between B-vitamin intake and CHD, respectively tHcy, remained similar before and after adjustment for other B-vitamin intakes (data not shown).

Table 3. Relative risks (RR) and 95% confidence intervals of 10-year fatal plus non-fatal coronary heart disease according to energy-adjusted tertiles of B-vitamin intake.

	Tertiles			
	T 1 (n=271)	T 2 (n=272)	T 3 (n=272)	p-trend
		Folate intake		
Median intake (µg/d)	153	192	246	
No. (%) of cases	42 (15.5%)	51 (18.8%)	47 (17.3%)	
Unadjusted RR	1	1.26 (0.84-1.90)	1.16 (0.76-1.75)	0.55
Adjusted* RR	1	1.29 (0.82-2.01)	1.12 (0.64-1.96)	0.77
Adjusted* + tHcy RR	1	1.31 (0.83-2.06)	1.13 (0.65-1.98)	0.75
		Vitamin B2 intake		
Median intake (mg/d)	1.3	1.6	2.1	
No. (%) of cases	56 (20.7%)	42 (15.4%)	42 (15.4%)	
Unadjusted RR	1	0.74 (0.50-1.11)	0.73 (0.49-1.09)	0.15
Adjusted* RR	1	0.73 (0.47-1.12)	0.61 (0.36-1.03)	0.08
Adjusted* + tHcy RR	1	0.73 (0.47-1.13)	0.60 (0.35-1.02)	0.07
		Vitamin B6 intake		
Median intake (mg/d)	1.3	1.6	1.9	
No. (%) of cases	52 (19.2%)	42 (15.4%)	46 (16.9%)	
Unadjusted RR	1	0.79 (0.53-1.19)	0.83 (0.56-1.24)	0.35
Adjusted* RR	1	0.82 (0.53-1.28)	0.82 (0.48-1.40)	0.45
Adjusted* + tHcy RR	1	0.83 (0.53-1.30)	0.82 (0.48-1.40)	0.45
		Vitamin B12 intake		
Median intake (µg/d)	2.9	4.1	6.9	
No. (%) of cases	42 (15.5%)	47 (17.3%)	51 (18.8%)	
Unadjusted RR	1	1.12 (0.74-1.70)	1.26 (0.84-1.89)	0.28
Adjusted* RR	1	1.33 (0.85-2.09)	1.82 (1.09-3.05)	0.03
Adjusted* + tHcy RR	1	1.34 (0.85-2.10)	1.83 (1.09-3.08)	0.03

T=tertile, tHcy=total homocysteine

*Adjusted for age, prevalence of coronary heart disease, smoking, body mass index, intake of energy, other B-vitamins, alcohol, vitamin supplements, saturated fat, trans fatty acids, fiber, vitamin E, vitamin C and β-carotene.

A total of 125 men (15.3%) used vitamin supplements, of which 69 men used B-vitamin containing supplements. There was no significant difference in CHD risk for B-vitamin supplements users compared to no B-vitamin supplement users, the relative risk was 0.90 (95% CI 0.47-1.73) after adjustment for age, smoking, BMI, energy intake and alcohol consumption. However, due to the lack of information on the frequency of taking B-vitamin supplements and their vitamin content, we could not assess the B-vitamins intake from supplements quantitatively. After excluding men using vitamin supplements (n=125), the results remained similar (data not shown).

We repeated our analyses including fatal events only. Similar results were observed for all B-vitamins. In addition, the relative risks of CHD mortality did also not change after adjustment for tHcy (data not shown).

DISCUSSION

In the present study among elderly men, only intake of folate and vitamin B6 were independently inversely associated with tHcy. We observed no clear association of intake of folate and vitamin B6 with the risk of CHD. Furthermore, we observed a non-significant inverse relation between vitamin B2 intake and CHD risk, which was suggested to be independent of tHcy. Vitamin B12 intake was positively associated with CHD risk in elderly men.

The major advantages of the present study are its prospective design, and the detailed data on dietary intake. Habitual food consumption was measured using the cross-check dietary history method, which is acknowledged as a valid method in an epidemiological setting.[23] The independent effect of each B-vitamin could be evaluated since adjustment was made for the intake of other B-vitamins. However, we could not study the B-vitamin intake from supplements quantitatively. Nevertheless, the results remained similar after excluding the vitamin supplement users.

Consistent with the results of the present study, an inverse association between intake of folate and tHcy was also observed in other cross-sectional studies among adults or the elderly.[30-32] An inverse association between dietary vitamin B6 and tHcy as shown in the present study is observed in some[30] but not all[31,32] observational studies. Consistent with the present findings, in general, no

association between the intake of vitamin B12[30-32] or vitamin B2[32] with tHcy was observed.

Until now, only two other prospective studies investigated the association between dietary intake of B-vitamins and the risk of CHD. In the ARIC study, no significant associations between the intake of folate, vitamin B6 or B12 and CHD were observed.[16] In the Nurses' Health Study, an inverse association was observed between folate and vitamin B6 intake and CHD, while there was no association for either vitamin B2 or B12.[20] This is inconsistent with our findings of a positive association of vitamin B12 intake, a non-significant inverse association of vitamin B2, and no association of folate and vitamin B6 with the risk of CHD.

The differences in intake of the three prospective studies are large. In the Nurses' Health Study,[20] the mean intake of folate (366 μg/d) and vitamin B6 (3 mg/d) was about twice as high as the intake of the participants of the Zutphen Elderly Study. This was due to the high intake of folate and vitamin B6 from vitamin supplements and fortified breakfast cereals. Also in the ARIC study,[16] use of vitamin supplementation was higher (about 27%) compared to the supplement use in the Zutphen Elderly Study. In our study, despite a lack of association with CHD, an inverse association between the intake of folate and vitamin B6 and tHcy was observed. This indicates that the level of intake of folate and vitamin B6 from foods was high enough to influence tHcy. This suggest that other determinants of tHcy than intake of folate and vitamin B6, or other risk factors (i.e. blood cholesterol[33] or blood pressure[34]) might play a more important role in CHD in this population of elderly men.

In the present study, vitamin B12 intake was positively associated with the risk of CHD. Because vitamin B12 is derived from animal products (meat 52%, milk and milk product 25%), other dietary factors occurring in the same foods or lifestyle and dietary factors associated with high animal food consumption could have affected the association between vitamin B12 intake and CHD. However, after adjustment for confounders the direct relationship remained. A positive association with CHD was also observed in two previous observational studies using blood levels of vitamin B12.[6,15] Other studies observed no[7-10,12,16,20] or an inverse[11] association between vitamin B12 and CHD. Based on this evidence, a detrimental effect of vitamin B12 itself, e.g. due to enzyme induction associated with long-life vitamin B12 accumulation in the liver,[35] can not be totally excluded.

This is one of the first studies that performed analyses on the association between vitamin B2 intake and the risk of CHD. After adjustment for risk and

dietary factors, vitamin B2 intake was borderline significantly inversely associated with the risk of CHD. This association was independent of tHcy. In the data of the Nurses' Health study, no association between the intake of vitamin B2 and CHD was observed.[20] Besides the role of vitamin B2 as a cofactor for the enzyme 5,10-methylenetetrahydrofolate reductase, vitamin B2 has a vital role in cellular oxidation. However, its biochemical functions do not easily explain the role in CHD. Other dietary factors in the same foods could have affected the results for vitamin B2. The underlying mechanism for a possible inverse association between vitamin B2 and CHD deserves further investigation.

Our findings indicate that, of the B-vitamins, folate and vitamin B6 intake are independent determinants of tHcy, while only vitamin B2 intake is non-significantly inversely associated with risk of CHD in a population of elderly men. The positive association between vitamin B12 intake and CHD risk requires further investigation. Randomized trials are needed to better clarify the potential protective effect on CHD of folate, vitamin B2 or B6.

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intake and 25-year coronary heart disease mortality: the Seven Countries Study

Based on:

Edith JM Feskens, Claudia M Oomen, Elbert Hogendoorn, Alessandro Menotti, Daan Krombout. Eur Heart J 2001;22:611-2.

coronary heart disease, as it is a precursor of nitric oxide. Results from huma and animal experiments showed that arginine treatment was antiatherogenic.[2] We have investigated the relationship between arginine intake and risk coronary heart disease within the cohort of the Zutphen Elderly Study, amon men aged 64-84 years old (chapter 8).[4] In this study, we observed n association between arginine intake and coronary heart disease mortalit possibly because of the limited range of variation in arginine intake (mean 4.3 g/d, SD 1.07 g/d)

Recently, we completed the investigation of arginine and coronary heart diseas in the Seven Countries Study. This study has been designed to investigate th diet-heart issue using a broad range of variation in dietary intake. It started i between 1958 and 1964, and involved 12,763 men aged 40-59 from 16 cohort The vital status of the men was checked at regular intervals and after 25 yea of follow-up. Primary cause of death was established centrally. Dieta information was collected at baseline in small random samples using th 'weighed record' method. In 1985 and 1986 the original dietary data wer recoded and the average food intake of the men was calculated. In 1987, th foods representing the baseline diet were bought locally and sent to th Netherlands. Foods were combined into representative food equivale composites. These were homogenized, freeze-dried and stored (-20°C) for lat use. The arginine content of the food composites was analysed twice in 199 using an HPLC-method with fluorescence detection and precolumn fluorgen labelling with 9-flurorenylmethylchloroformate (OPA).

Arginine intake varied from 5.4 g/d in Corfu (Greece) to 13.8 g/d in Ushibuk (Japan). The correlation between arginine intake and coronary heart diseas mortality amounted to -0.35 (p=0.17). High intake of arginine was associate with relatively low levels of coronary heart disease mortality (such as in Japa (Figure 1), but at low levels of arginine intake high (East-Finland) as well as lo coronary heart disease rates (Corfu) were observed. In an earlier study w observed that 90% of the variation in coronary heart disease mortality could b explained by three factors: saturated fat intake, flavonol intake and smoking Arginine intake was strongly associated with flavonol intake (r=0.66, p=0.006 with smoking (r=0.46, p=0.07) and inversely with the energy-percentage saturated fatty acids (r=-0.49, p=0.05). In the unadjusted regression analysi

an increase in arginine intake of 1 g/d was associated with a coronary heart disease risk reduction of 1.11% (p=0.17). However, when adjusted for the three main explaining factors, separately or combined, the association between arginine intake and coronary heart disease mortality was reduced towards zero (e.g. a reduction of -0.26% coronary heart disease risk per 1 g/d of arginine, p=0.53, adjusted for saturated fatty acids and smoking; additional adjustment for flavonols was hampered by multicollinearity).

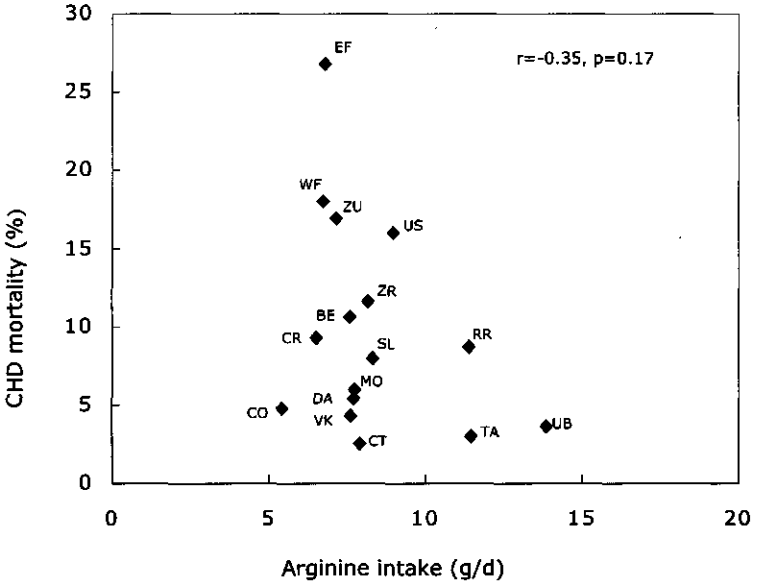

Figure 1. Association between population average of arginine intake (g/d) at baseline and 25-year age-adjusted mortality rates from coronary heart disease (%) in the Seven Countries Study.
EF=East Finland, WF=West Finland, ZU=Zutphen, the Netherlands, US=US Railroad, BE=Belgrade, Serbia (former Yugoslavia), ZR=Zrenjanin, Serbia (former Yugoslavia), VK=Velika Krsna, Serbia (former Yugoslavia), SL=Slavonia, Croatia (former Yugoslavia), DA=Dalmatia, Croatia (former Yugoslavia), RR=Rome Railroad, Italy, CR=Crevalcore, Italy, MO=Montegiorgio, Italy, CT=Crete, Greece, CO=Corfu, Greece, UB=Ushibuka, Japan, TA=Tanushimaru, Japan.

These results indicate that arginine is not clearly associated with coronary heart disease mortality at the population level. This is in agreement with our findings at the individual level (chapter 8).[4] In both study designs, arginine intake was strongly associated with the intake of energy and other nutrients through its widespread presence in animal and vegetable foods. The potential impact of arginine is therefore difficult to disentangle. Additional observational studies,

preferably in populations with a relatively high range of intake, and including e.g. biomarkers for arginine intake, should be carried out to confirm the hypothesis of a protective effect of arginine on coronary heart disease as suggested by recent human and animal experiments.

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intake and risk of coronary heart disease mortality in elderly men

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From experimental studies, the hypothesis is derived that the amino acid arginine, th precursor of nitric oxide, could restore the impaired endothelial function and increase platelet activation observed in atherosclerosis. We investigated whether dietary intake arginine is associated with reduced coronary heart disease risk in elderly persons. Th study population consisted of 806 men aged 64-84 years at baseline who participated the Zutphen Elderly Study, a population-based cohort followed up for 10 year Information about habitual food consumption was collected by use of the cross-che dietary history method. Ninety men (11.2%) died from coronary heart disease. Mea baseline arginine intake was 4.35 g/d (SD=1.07). Meat was the main source of arginir intake (37.1%), followed by bread (13.1%) and milk and milkproducts (12.1%). Arginir intake was not associated with coronary heart disease mortality. After adjustment f age, the relative risk (RR) for the medium tertile of arginine intake was 0.72 (95 confidence interval (CI): 0.44-1.18) and the RR for the highest tertile was 0.71 (95% C 0.43-1.19; p for trend=0.19) compared to the lowest tertile of arginine intake. Aft additional adjustment for history of coronary heart disease and diabetes mellitus, energ intake, body mass index, smoking habit, physical activity and other relevant dietary ar biological risk factors the RR was 1.86 (95% CI: 1.06-3.27) for the medium intake ar 1.56 (95% CI: 0.83-2.93) for the highest intake (p for trend=0.17). These results do n support the hypothesis that dietary arginine intake lowers the risk of coronary hea disease mortality.

INTRODUCTION

The discovery of nitric oxide as a signaling molecule in the cardiovascular syste was rewarded with the 1998 Nobel Prize in Medicine. The semi-essential amir acid L-arginine is the precursor of nitric oxide. It has been demonstrated tha administration of L-arginine can improve endothelium-dependent vascul relaxation through the release of nitric oxide.[1] Chronic dietary supplementatic of L-arginine in hypercholesterolemic animals has been associated with reduce atherosclerosis and anti-atherogenic effects such as reduction in surface are and intimal thickness of atheromatous lesions, decrease of platelet aggregatic and attenuation of cell proliferation and of vascular monocyte accumulation.[2-8]

In humans, endothelial dysfunction could be restored after dieta supplementation[9-12] and intravenous administration of L-arginine[13-15] in patien

with hypercholesterolemia,[9,13,14] heart failure,[12] and nonobstructive or advanced coronary artery disease.[10,11,15] Reduction in platelet aggregation by L-arginine has also been observed.[16-18]

Consequently, arginine was recently presented as a possible new pharmacological therapy for the atherogenic process leading to coronary heart disease.[19] Antiatherogenic effects of arginine are especially demonstrated in human subjects with more or less advanced atherosclerosis. Coronary atherosclerosis is increasingly present in the elderly. Possibly, arginine is associated with reduced coronary heart disease risk in elderly persons. Therefore, we investigated the hypothesis that dietary arginine intake is inversely associated with coronary heart disease mortality among participants in the Zutphen Elderly Study.

METHODS

Study population

The study population consisted of men who participated in the Zutphen Elderly Study, an extension of the Zutphen Study, the Dutch contribution to the Seven Countries Study.[20] In 1960, the Zutphen Study started with a cohort of 878 men from Zutphen (the Netherlands) born between 1900 and 1919. In 1985, 367 of the 555 participants who were still alive were re-examined. In addition, 711 other men from the town of Zutphen in the same age category were asked to participate. A total of 939 men (response rate 74%) were examined in 1985 and complete information on diet and risk factors was available for 806 men. In 1990, 781 of these 939 men were still alive, and 560 took part in a follow-up examination. Dietary intake in 1990, together with complete information on diet and risk factors at baseline (1985) was available for 508 men.

Data collection

Dietary and medical examinations were completed between March and June 1985 and after 5 years of follow-up, between March and June 1990. Information about the habitual food consumption was collected by use of the cross-check dietary history method, adapted to the Dutch situation.[21] Participants, together with the person who prepared the meals, were interviewed about their usual food consumption pattern during weekdays and weekends for the 2-4 weeks

preceding the interview. Quantities of food bought per week were used to calculate and verify the food consumption of a participant on an average weekday.

Nutrient intake data, including ethanol, were based on the Dutch food table.[22] Because of lack of information on the amino acid composition of Dutch foods, arginine contents of chemical analysis were used, preferably from the United Kingdom,[23] and subsequently from the United States.[24] The arginine contents were converted by use of the protein contents from the Dutch food table.[22]

During medical examinations non-fasting venous blood samples were taken. Serum total cholesterol and high density lipoprotein (HDL) cholesterol were determined enzymatically.[25,26] In 1995, serum total homocysteine was measured in serum stored at -20°C as described previously.[27] Weight and height were measured while participants were wearing underwear only, and body mass index was calculated (kg/m^2). At the end of the physical examinations blood pressure was measured twice with participants in the supine position. Information on smoking status (nonsmoker, exsmoker, current smoker), medication use, and history of hypertension and diabetes mellitus was obtained by a standardized questionnaire. Patients with insulin-dependent and non-insulin-dependent diabetes mellitus were considered in the present study. Minutes of physical activity (mainly walking, cycling, hobbies, sports, gardening and work) per week were calculated by use of a self-administered questionnaire originally designed for retired men.[28] Information regarding a history of coronary heart disease was obtained by using the Dutch translation of the Rose questionnaire.[29]

Follow-up

Information involving vital status of all participants was obtained from the municipal registries until January 1995. Three participants were lost to follow-up. Information about the cause of death was obtained from the Dutch Central Bureau of Statistics for deaths occurring between baseline assessment and June 1990, after verification with hospital discharge data and information from the deceased's general practitioners, and for deaths occurring thereafter from the hospital discharge data and/or participants' general practitioners and if such information was not available from the Dutch Central Bureau of Statistics. Causes of death were coded according to the ninth revision of the International Classification of Diseases (ICD). Coronary heart disease refers to ICD codes 410-414. Because the underlying cause of death in the elderly is often difficult to

determine, the primary (n=84) and the secondary (n=6) causes of death were both considered in the analysis.

Statistical methods

Differences in baseline characteristics of the participants between tertiles of arginine intake were evaluated by analysis of variance for normally distributed variables, by Kruskal-Wallis test for skewed variables and by Chi-square test for categorical variables. Pearson correlation coefficients (r_p) were calculated between the intake of arginine and other dietary factors.

Energy-adjusted nutrient intake (i.e. arginine) was computed as residuals from the regression model with energy intake as the independent variable and nutrient intake as the dependent variable.[30] Before computing the residuals of nutrient intake, the logarithmic transformation of each nutrient variable including arginine was calculated to improve the normality. Cox proportional hazard (survival) analysis was used to calculate crude and adjusted relative risks (RRs), 95% confidence intervals (CIs) and p-values for linear trend. The lowest tertile of arginine intake was taken as a reference group. Several multivariate analyses were performed to take into account potential confounding by major risk factors such as age, history of coronary heart disease, smoking status, body mass index and other factors associated with arginine intake and potentially associated with coronary heart disease mortality. To evaluate confounding a p-value smaller then 0.2 was considered. Energy-adjusted analysis included the energy-adjusted tertiles of arginine intake plus a term for energy intake. Interactions between arginine intake and relevant factors, such as age, history of coronary heart disease and smoking status were explored to determine whether the association between arginine intake and coronary heart disease mortality differed among subgroups of the population. Proportional hazard analyses including arginine intake and all the covariables as time-dependent covariates were also performed. The baseline measurement (1985) for the first 5 years of follow-up and the measurement for the second 5 years of follow-up (1990) were used. When the measurement for 1990 was not available, only the baseline measurement was used for the total follow-up period.

RESULTS

Baseline

Mean (±SD) baseline arginine intake of the 806 participants was 4.35 (±1.07) g/d. Meat provided the largest contribution to the total arginine intake (37.1%), followed by bread (13.1%) and milk and milk products (12.1%). A relatively large amount of arginine was also provided by peanuts (2.5%); this was due to the high percentage of arginine in the protein of peanuts (13.0%).

Table 1. The Zutphen Elderly Study: Baseline (1985) characteristics according to tertiles of arginine intake in 806 men.

	tertiles of arginine intake (g/d)			
	0 to 3.85	3.86 to 4.65	more than 4.65	P*
	(low)	(medium)	(high)	
Characteristic	(n=268)	(n=269)	(n=269)	
Age (y.)	72.3 ± 5.4	71.7 ± 5.3	70.0 ± 4.8	0.0001
Body mass index (kg/m^2)	25.6 ± 3.2	25.4 ± 2.6	25.5 ± 3.5	0.57
Smoking - current (%)	29.9	29.0	31.2	0.85
- past (%)	51.9	52.8	49.8	0.78
Alcohol users (%)	66.4	77.3	75.5	0.01
Physical activity (min./week)	490 ± 484	642 ± 562	660 ± 559	0.0001
Systolic blood pressure (mmHg)	151 ± 22	152 ± 21	149 ± 20	0.25
Serum total cholesterol (mmol/L)	6.12 ± 1.18	6.01 ± 1.08	6.21 ± 1.07	0.12
Serum HDL cholesterol (mmol/L)	1.11 ± 0.33	1.13 ± 0.29	1.13 ± 0.26	0.16
Serum homocysteine (µmol/l)	17.8 ± 10.5	15.2 ± 6.8	13.9 ± 6.0	0.0001
Medication use - hypertension (%)	11.9	14.5	9.3	0.18
- cholesterol lowering (%)	2.6	1.1	0.8	0.17
History of coronary heart disease (%)	23.9	16.4	16.0	0.03
History of diabetes mellitus (%)	3.0	6.7	7.8	0.02
History of hypertension (%)	22.8	21.2	17.1	0.24

* Analysis of variance for normal distributed variables, Kruskal-Wallis test for skewed variables, chi square for dichotomous variables.

We examined the distribution of risk factors within categories of arginine intake. Arginine intake was inversely associated with age, history of coronary heart disease and serum homocysteine levels and positively associated with physical activity, alcohol use and history of diabetes mellitus (table 1). However, the distributions of other risk factors did not differ appreciably across the arginine categories. Concerning dietary factors, arginine intake was positively associated with the intake of (saturated and unsaturated) fat, carbohydrates, protein, fiber, cholesterol and total energy (table 2).

Daily intake of arginine was strongly correlated with the intake of energy (r_p =0.65, P=0.001). After adjustment for energy, the associations between arginine intake and dietary factors were weakened (table 3). Energy-adjusted

Table 2. The Zutphen Elderly Study: Baseline (1985) mean daily nutrient intake according to tertiles of arginine intake in 806 men.

	tertiles of arginine intake (g/d)			
Nutrient	0 to 3.85 (low) (n=268)	3.86 to 4.65 (medium) (n=269)	More than 4.65 (high) (n=269)	P*
Arginine (g)	3.31 ± 0.42	4.22 ± 0.23	5.50 ± 0.90	
Energy (MJ)	8.0 ± 1.7	9.4 ± 1.6	11.0 ± 2.0	0.0001
Total fat (g)	83.2 ± 24.3	101.6 ± 27.8	121.3 ± 33.6	0.0001
Saturated fatty acids (g)	36.3 ± 11.6	43.7 ± 12.7	51.1 ± 15.2	0.0001
Monounsaturated fatty acids (g)	31.1 ± 10.5	37.9 ± 12.2	46.0 ± 14.8	0.0001
Polyunsaturated fatty acids (g)	12.6 ± 6.2	16.2 ± 7.7	19.5 ± 8.8	0.0001
Carbohydrates (g)	206 ± 60	228 ± 56	265 ± 65	0.0001
Total protein (g)	62.9 ± 8.7	78.8 ± 6.6	98.5 ± 14.5	0.0001
Vegetable protein (g)	20.2 ± 5.1	23.9 ± 5.4	29.6 ± 8.2	0.0001
Animal protein (g)	42.9 ± 8.4	55.1 ± 8.5	69.1 ± 14.4	0.0001
Alcohol (g)	12.1 ± 16.1	14.7 ± 18.6	12.8 ± 16.2	0.07
Fiber (g)	21.2 ± 5.6	25.1 ± 6.4	29.9 ± 7.9	0.0001
Cholesterol (mg)	275 ± 95	340 ± 102	399 ± 123	0.0001

* Analysis of variance for normal distributed variables, Kruskal-Wallis test for skewed variables, chi square for dichotomous variables.

arginine intake remained significantly positively associated with protein, dietary cholesterol and fiber. In addition, energy-adjusted arginine intake was positively associated with history of diabetes mellitus and body mass index, and inversely associated with age and serum homocysteine levels (data not shown).

Table 3. The Zutphen Elderly Study: Baseline (1985) mean daily nutrient intake according to tertiles of energy-adjusted arginine intake in 806 men.

	Tertiles of energy-adjusted arginine intake (g/d)			
	0 to 3.91	3.92 to 4.53	more than 4.53	P*
	(low)	(medium)	(high)	
Nutrient	(n=268)	(n=269)	(n=269)	
Energy (MJ)	9.5 ± 2.3	9.5 ± 2.1	9.4 ± 2.1	0.67
Total fat (g)	103.4 ± 34.5	102.2 ± 31.6	100.6 ± 32.2	0.63
Saturated fatty acids (g)	44.8 ± 15.8	44.2 ± 14.1	42.1 ± 13.6	0.07
Monounsaturated fatty acids (g)	38.4 ± 14.8	38.5 ± 12.9	38.1 ± 14.3	0.94
Polyunsaturated fatty acids (g)	15.8 ± 8.3	15.6 ± 7.4	17.0 ± 8.6	0.11
Carbohydrates (g)	243 ± 67	236 ± 65	220 ± 61	0.0001
Total protein (g)	68.5 ± 8.7	79.5 ± 13.1	92.2 ± 17.4	0.0001
Vegetable protein (g)	23.0 ± 6.4	24.5 ± 6.6	26.3 ± 8.8	0.0001
Animal protein (g)	45.7 ± 12.1	55.3 ± 10.7	66.2 ± 14.9	0.0001
Alcohol (g)	14.8 ± 18.2	12.5 ± 17.8	12.3 ± 14.9	0.47
Fiber (g)	23.3 ± 6.9	25.6 ± 6.4	27.3 ± 8.3	0.0001
Cholesterol (mg)	316 ± 122	338 ± 107	360 ± 122	0.0001

* Analysis of variance for normal distributed variables, Kruskal-Wallis test for skewed variables, chi square for dichotomous variables.

Follow-up

During ten years of follow-up 374 participants died, of which 90 (11.2%) died from coronary heart disease. In the crude analysis, arginine intake was inversely associated with the risk of coronary heart disease, with a RR of 0.63 (95% CI: 0.38-1.04) for the highest intake (p for trend=0.07, table 4). In different multivariate models, with potential confounding by factors associated with arginine intake and potentially associated with coronary heart disease mortality

taken into account, no inverse association between arginine intake and coronary heart disease mortality was observed. Especially because of adjustment for energy intake, the RR adjusted for age, history of coronary heart disease, history of diabetes mellitus and energy intake were higher, 1.55 (95% CI: 0.93-2.58) for the medium tertile and 1.19 (95% CI: 0.68-2.07) for the highest tertile of arginine intake. After adjustment for body mass index, cigarette smoking, physical activity, alcohol consumption and energy-adjusted intake of saturated fatty acids, polyunsaturated fatty acids, cholesterol and fiber, the RRs for the medium and high tertiles compared to the lowest were 1.68 (95% CI: 0.97-2.90) and 1.48 (95% CI: 0.80-2.72), respectively. Tests for linear trend were not statistically significant. The inclusion of serum total and HDL cholesterol, blood pressure and serum homocysteine as potential confounders in the analysis increased the RRs further (table 4).

Table 4. Relative risks and 95% CIs for mortality from coronary heart disease in 806 men during 10-year follow-up according to tertiles of arginine intake.

	tertiles of arginine intake*			
Coronary heart disease mortality	Low (n=268)	Medium (n=269)	High (n=269)	p for trend
No. of deaths	36	28	26	
Mortality rate, per 1000 person-years	18.8	13.6	12.0	
Relative risks				
Crude	1.0	0.72	0.63	0.07
95% CI		0.44-1.19	0.38-1.04	
Age adjusted	1.0	0.72	0.71	0.19
95% CI		0.44-1.18	0.43-1.19	
Multivariate†	1.0	1.87	1.58	0.16
95% CI		1.06-3.29	0.84-2.96	

* Cut-off points 3.85 g/d and 4.66 g/d for the tertiles unadjusted for energy intake, 3.91 g/d and 4.53 g/d for tertiles adjusted for energy intake (residual from regression analysis with energy intake).

† Factors include age, history of coronary heart disease and diabetes mellitus, energy intake, body mass index, smoking, alcohol, physical activity, intake of saturated and polyunsaturated fatty acids, cholesterol, fiber (all as residuals from the regression model with energy intake), systolic blood pressure, total and HDL cholesterol, homocysteine.

It may be questioned whether energy intake is a confounder in this population, because the association between energy intake and coronary heart disease mortality was not statistically significant in the adjusted analysis. Therefore, we repeated the multivariate analysis (the full model, with systolic blood pressure, cholesterol and homocysteine) without adjustment for energy intake. The RR was 0.88 (95% CI: 0.49-1.56) for the medium intake and 0.99 (95% CI: 0.50-1.98) for the highest intake (p for trend=0.98).

We further examined whether the association between arginine intake and coronary heart disease mortality was modified by other risk factors, with use of an interaction term in the multivariate analysis. Only for age was a significant interaction observed. The association was significant and more strongly positive in men below the median age of 71 years and inverse, but not significant, in men aged > 71 years.

Finally, to reduce the likelihood that our results were biased by changes in dietary intake and lifestyle, analyses were also performed with the use of time-dependent covariates. This resulted in significantly increased risks; in the full multivariate model the RR was 2.19 (95% CI: 1.22-3.95) for medium intake and 2.44 (95% CI: 1.30-4.59) for the highest intake (p for trend=0.01).

Discussion

This is the first observational study investigating the association between dietary arginine intake and coronary heart disease mortality. We observed no beneficial effect of dietary arginine intake on the risk of coronary heart disease in elderly men.

Our results regarding arginine intake and coronary heart disease mortality are not consistent with the anti-atherogenic effects of arginine experimentally demonstrated in human subjects with more or less advanced atherosclerosis.[9-18] In healthy subjects, some experimental studies observed no effect on endothelial function after infusion or supplementation of arginine.[31,32] Plasma arginine levels in these subjects were also unaffected after supplementation.[32] This finding suggests that, at least under normal conditions, sufficient endogenous arginine can be synthesized to maintain the rate of nitric oxide-synthesis.[33] However, some findings in healthy young subjects showed that infusion of arginine could increase vasodilatation and blood viscosity and reduce platelet aggregation.[34]

Studies using a tracer for arginine also have shown that dietary arginine can affect the endogenous arginine metabolism and plasma arginine levels in humans.[33,35] These findings, and those from experiments in humans with a high risk profile for coronary heart disease, suggest a relative lack of endogenous arginine for the synthesis of nitric oxide, which could be restored by arginine administration. Uncertainty remains regarding the exact conditions in which insufficient endogenous arginine is present.

An important limitation of the present study was that it included only men aged 64-84 years at baseline. The beneficial effects of arginine on vasodilatation might be higher in younger populations, because stiff arteries are not as prevalent in the young. However, in the elderly, atherosclerosis is increasingly present, just as in the subjects of experiments in which anti-atherogenic effects of oral L-arginine supplementation were demonstrated. Subgroup analysis indicated that in the oldest men arginine was inversely associated with coronary heart disease mortality, albeit non-significantly. On the basis of these data, however, generalizability with respect to other age-groups and women is uncertain.

In experiments in which anti-atherogenic effects of oral L-arginine supplementation were demonstrated, subjects were supplemented with 3 to 21 g L-arginine per day during a short[10,17] or long period.[9,11,12] The dose used in those experiments was generally much higher than the average arginine intake in the present study. Therefore, it cannot be excluded that the effects of arginine on atherosclerosis are especially present when large amounts of arginine are supplemented, and that the range of dietary arginine intake in our cohort was not wide enough to observe an association.

A true inverse association between arginine intake and coronary heart disease mortality could also be missed if the measurement of the arginine content of the diet is imprecise. Habitual food composition was measured by use of the cross check dietary history method, which is acknowledged as a valid method in an epidemiological setting.[21] Random misclassification of dietary exposure, due to error in the quantification of arginine in food tables, cannot be excluded. Plasma arginine levels were not measured; however, these might reflect short-term rather than long-term dietary intake, because of endogenous synthesis and conversion of arginine.[35] The estimate of daily average arginine intake of 4.4 g of the Zutphen elderly population was comparable to the estimated arginine intake of 4.2 g/d after 5 years of follow-up (r_p=0.50, P=0.001) and to the estimated average arginine intake of the American diet of 5.4 g/d, which is based on the

arginine contents of foods available in 1970.[36] In addition, using the baseline measurement of arginine and the measurement after 5 years of follow-up, arginine intake was even more strongly positively associated with coronary heart disease mortality compared with the measurement using arginine intake at baseline only. Thus, it is unlikely that misclassification of dietary exposure explains our findings.

Because higher arginine intake was associated with a healthier lifestyle and diet, including a high energy intake, in our study population, we used several multivariate models to reduce residual confounding. A higher risk of coronary heart disease mortality with arginine intake was observed with the multivariate model. This apparent positive association is probably due to chance, because there is no biological explanation for this finding. Furthermore, the effect of L-arginine on coronary heart disease mortality could be confounded by other dietary factors that were not included in our analysis. Since arginine is derived from different foods, other dietary factors occurring in the same foods could have affected the results for arginine. For instance, in addition to arginine, nuts also contain other potentially protective constituents.[37] Furthermore, arginine intake is highly associated with the intake of other amino acids, such as methionine (r_p=0.89, P=0.001). However, our results are adjusted for serum homocysteine, the intermediate in the potential association between methionine and coronary heart disease. Also, other dietary factors influencing the arginine-nitric oxide pathway, such as L-glutamine[38,39] or vitamin C,[40] could have interfered with the role of arginine. Vitamin C could inhibit the inactivation of nitric oxide by oxygen-derived free radicals. However, adjustment for intake of vegetables, fruit, or vitamin C in the analysis did not appreciably change the results (data not shown).

In conclusion, we observed no association between dietary arginine intake and coronary heart disease mortality in a cohort of elderly men. However, this result can not be generalized, because of the elderly population studied and unmeasured dietary factors (potentially) associated with arginine. Additional observational studies are needed, preferably in other age groups in populations with a relatively high arginine intake or on more specific parameters, such as endothelial function. Furthermore, other lines of research, including studies involving biomarkers for arginine intake, should be carried out to confirm or reject the beneficial impact of arginine on coronary heart disease suggested by human and animal experiments.

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General discussion

prospective studies on dietary factors,
disease incidence and mortality in men
lifestyle were studied in relation to cha
(HDL) cholesterol (chapter 2). Further
of dietary factors in relation to the ri
(trans fatty acids, (n-3 fatty acids fro
respectively), B-vitamins (chapter 6) a

The results presented are mainly bas
study of elderly men, detailed infor
biological risk factors, incidence of
causes of death between 1985 and 19
investigate the diet-heart hypotheses
data of 5 cohorts from Finland, Italy a
16 cohorts of the Seven Countries Stu

MAIN FINDINGS

Using repeatedly collected data of seru

intake and the 10-year risk of coronary heart disease among the participants in the Zutphen Elderly Study (chapter 5).

Of the B-vitamins that are involved in the metabolism of the intracellular amino acid homocysteine, only intake of folate and vitamin B6 were inversely associated with homocysteine concentrations (chapter 6). However, both vitamins were not associated with the risk of coronary heart disease. Independent of homocysteine, vitamin B12 intake was significantly positively associated with the risk of coronary heart disease, and vitamin B2 intake was non-significantly inversely associated with the risk of coronary heart disease.

Finally, intake of the amino acid arginine was not beneficially associated with the risk of coronary heart disease mortality, neither at the population level (chapter 7) nor at the individual level (chapter 8).

A overview of the main findings of this thesis is presented, according to the endpoint studied, in table 1.

Table 1. Main findings of the studies described in this thesis.

Endpoint	Association?	Exposure: effect estimate	Ch.
Total cholesterol	Yes	PUFA: -0.03 mmol/L per 1 energy%	2
		Weight: 0.02 mmol/L per 1 kg	2
	No	SFA, MUFA, trans fatty acids, cholesterol, fiber, alcohol, quit smoking, physical activity	2
HDL cholesterol	Yes	SFA: 0.008 mmol/L per 1 energy%	2
		Cholesterol: 0.0008 mmol/L per 1 mg	2
		Alcohol: 0.004 mmol/L per 1 g	2
		Weight: -0.008 mmol/L per 1 kg	2
	No	MUFA, PUFA, trans fatty acids, fiber, quit smoking, physical act.	2
Homocysteine	Yes	Folate: -1.4 μmol/L in T3 vs. T1	6
		Vitamin B6: -1.1 μmol/L in T3 vs. T1	6
	No	Vitamin B2, vitamin B12	6
Coronary heart disease	Yes	Trans fatty acids: RR =1.28 (95% CI 1.01-1.61) per 2 energy%	3
		Fatty fish: pooled RR=0.66 (95% CI 0.49-0.90) (yes vs. no)	4
		Vitamin B2: RR=0.61 (95% CI 0.36-1.03) in T3 vs. T1	6
		Vitamin B12: RR=1.82 (95% CI 1.09-3.05) in T3 vs. T1	6
	No	Total fish, lean fish	3
		α-Linolenic acid	5
		Folate, vitamin B6	6
		Arginine	7, 8

Ch= chapter, SFA= saturated fatty acids, MUFA=monounsaturated fatty acids, PUFA=polyunsaturated fatty acids, T=tertile, RR= relative risk, CI= confidence interval

METHODOLOGICAL CONSIDERATIONS

Several methodological issues deserve attention in this final chapter. We will discuss the precision and validity (internal and external) of the results of the study.

Precision

In the Zutphen Elderly Study, after 10 years of follow up, there were 98 coronary heart disease cases out of 667 men without previously diagnosed coronary heart disease and having complete data on risk and dietary factors. For detecting a relative risk for coronary heart disease of 0.6 for the highest tertile of dietary exposure compared to the lowest tertile of the Zutphen Elderly Study at conventional level of statistical significance ($p<0.05$), the power is about 80%.[1] This power calculation indicates that it is not possible to go into subgroup analyses or analyses of interaction. Lack of power due to the relatively small sample size will primarily have resulted in rather wide confidence intervals and in the case of a weak true association in the absence of statistical significance.

Internal validity

Internal validity is the extent to which the results are valid for the target population. Internal validity is mainly determined by selection bias, information bias and bias due to confounding. Selection bias results from procedures used to select subjects, that lead to an effect estimate among participants of the study that differs from the estimate obtainable from the entire study population. Information bias occurs whenever there are errors in the classification of the diet, outcome measurement or confounding variables of subjects. Differential and non-differential error may lead to misclassification of a variable. An error is regarded as differential when the value of a variable (i.e. exposure) tends to vary across the true values of other relevant variables (i.e. disease status). Differential error can either exaggerate or underestimate an effect.[2] When the proportion of subjects misclassified on exposure does not depend on disease status or when the proportion of the subjects misclassified on disease does not depend on exposure, the error is non-differential.[2] Random non-differential error will generally weaken the results (dilution towards the null). However, systematic non-differential misclassification may also bias measures of the exposure-disease association away from the null, e.g. for exposure variables with more than two categories, when there is misclassification in two of the categories and not in the

others.[3,4] Furthermore, non-differential misclassification of a confounding variable will usually reduce the degree to which confounding can be adjusted for and thus can cause a bias in either direction, depending on the direction of the confounding.

Internal validity of our results is discussed with regard to selection bias, information bias of dietary intake and outcome measurement, and bias due to confounding.

Selection or response bias

When selecting a cohort at risk for disease, selection bias will usually not threaten validity because disease outcome has not yet manifested itself. In that case, loss to follow up can lead to selection bias. However, follow-up data were (almost) complete in the studies described in this thesis. Furthermore, in a population-based study, selection bias may have occurred if the respondents differed systematically from the non-respondents (non-response bias). However, since the response rate was very high for the examinations of the Seven Counties study in the sixties and the seventies, no major impact of non-response on the results is expected. For the Zutphen Elderly Study, the participation rate was 74% in 1985. Results from a non-response survey during the 1993 examination period of the Zutphen Elderly Study, with a similar response rate as in 1985, showed that participants had a lower prevalence of myocardial infarction than non-respondents.[5] Selective non-response may lead to bias in the assessment of the impact of diet on coronary heart disease. For example, systematic overestimation could have occurred if men with a low trans fatty acid intake and the healthiest risk profile for coronary heart disease and men with a high trans fatty acid intake and a high risk for coronary heart disease are systematically more frequently included than low trans fatty acid consumers with high coronary heart disease risk or high trans fatty acid consumers with low coronary heart disease risk. However, it is unlikely that this has occurred in the Zutphen Elderly Study.

Information bias in dietary intake

A major concern in nutritional epidemiology is the quality of dietary intake data. The cross-check dietary history method, which was used in the Zutphen Elderly Study, is, like all dietary assessment methods, difficult to validate. The reproducibility, an important part of the validation process, provides information about random error in the dietary measurement. The reproducibility of the cross-check dietary history method was judged by repeated dietary surveys carried out

3 and 12 months after the initial survey of the Zutphen Elderly Study in 1985.[6] Correlation coefficients were higher than 0.70 for bread, milk products and alcohol beverages, while for meat and for vegetables correlation coefficients ranged from 0.44 to 0.51. This may have adversely influenced the reproducibility of nutrients for which vegetables and meat are the main source e.g. folate. For energy, saturated and unsaturated fat, cholesterol, protein and vitamin B2, attenuation factors between 0.65 and 0.90 are found. This implies that the nutrient-disease association will be lowered only slightly because of error in the dietary measurement. Relative validity of the cross-check dietary history method has been investigated by several authors, using e.g. the diet record method or the fatty acid composition of lipid fractions as a reference.[7,8] In these studies, correlation coefficients between the dietary history method and the reference method ranged from 0.4 to 0.9. If these differences in ranking individuals with regard to dietary intake between the two methods are the result of random error, this indicates that dietary measurement error will weaken the results to some extent.

The within-person error which occurs when the cross-check dietary history method is used may be either random or systematic. However, systematic within-person error is difficult to measure in validation studies since, for most food components, no current method has so far been able to measure 'true' long-term dietary intake. Persons may consciously or unconsciously tend either to underestimate or to exaggerate their food intake. Nutrients that are often underreported are e.g. fat and alcohol. Consumption of fruit and vegetables, and consequently intake of vitamins from these foods, is often overreported. Systematic incorrect reporting of food intake could have lead to differential error because it may depends on persons' risk profile for coronary heart disease, e.g. age or body mass index. However, besides persons' risk profile, the subject's ability to report food consumption accurately depends on many other factors e.g. memory, awareness of dietary risk factors and the variety of foods consumed.[9] Probably some misclassification of dietary exposure may therefore have occurred due to both differential and non-differential error. Expression as a proportion of total energy[9] or adjustment for these risk factors in the analyses will reduce, but not totally exclude, misclassification of dietary exposure.

In the chapters 2, 3, 5, 6 and 8 of this thesis, food composition data for approximately 1000 foods that were consumed by the participants of the Zutphen Elderly Study were used to calculate the nutrient intake of interest. A lot of effort was put to limit measurement error in nutrient intake due to errors

and missings in the food composition data. We used a nutrient database that was improved on inconsistencies, new analytical methods and missing data.[10] Furthermore, the database was updated with changes of nutrient contents in foods over time.[10,11] This was particularly important for the intake of fatty acids, because meat products and many prepared foods changed considerably in their fat content and fatty acid composition between 1985 and 1995, the examination period of the Zutphen Elderly Study. The percentage of trans fatty acids in fats for use in households decreased substantially in the Netherlands during that period. In addition, as a result of selective breeding, a reduction in fat content of meat took place already before 1985. However, due to a delay in registration inherent to a nutrient database, the fat intake from meat calculated with the 1985 database would have resulted in an overestimation, while the updated database took these changes into account.[10] Consequently, improving food composition data to calculate nutrient intake would have reduced misclassification, however, residual non-differential error of nutrient intake cannot totally be excluded.

For trans fatty acids, differences in the analytical methods may have introduced systematic measurement error. Therefore, in the compilation of the trans fatty acids food composition table, we adjusted for differences in analytical methods used for the determination of the trans fatty acids content. However, these adjustment for differences in analytical methods were shown to have only a small effect on ranking persons based on their trans fatty acids intake.[11]

Another source of variation with respect to food composition data that is more difficult to estimate is the variation with respect to type, brand or preparation of the food.[12] For example, processing, cooking or storage of foods can lead to large differences in the food content of many vitamins. In our assessment of folate intake used in chapter 8, we used detailed analyzed data about the food folate composition, taking into account the great variability in the folate content of foods.[13] Therefore, bias in our calculation of folate intake due to non-random sampling for chemical analysis is limited. However, due to lack of information of e.g. brand names in our consumption data, we could not capture all variation in nutrient intake at the individual level.

A single measurement of dietary intake may not represent long-term exposure, especially if dietary exposure tends to change over a period of time relevant to the risk of disease. In addition, it is important to know for which dietary component what period of time before diagnosis is relevant. Therefore, baseline

as well as time-dependent or cumulative analyses were performed in all chapters to take exposure at different periods of time into account.

In addition to methodological aspects of measuring dietary intake, bioavailability of nutrients (the variation in utilization by the body of a nutrient present in different foods) could have influenced the fraction of nutrients absorbed and used, and consequently the association between nutrient intake and disease. It is known that bioavailability of folate may differ in vegetables and fruits, depending on the food matrix and the chain length of folate in food.[14] Homocysteine is a functional parameter of folate status. In chapter 6 we observed that a mean difference in the highest and lowest tertile of folate intake of 93 μg/d was associated with a mean decrease in homocysteine concentrations of 1.4 μmol/L. A reduction of 25% or about 3 μmol/L in homocysteine concentrations can be achieved with folic acid supplements in randomized trials.[15] However, these data are difficult to compare with dietary intake data because supplementation trials used a much higher dosage (0.5-5 mg/d) of folic acid, and folic acid from supplements has about twice the bioavailability of food folate.[14] The association between homocysteine and dietary folate as observed in chapter 6 was comparable with that observed in other observational studies (about -1 μmol/L homocysteine per 100 μg/d folate).[16,17] This suggests that the measurement of folate intake provides a good index of the bioefficacy of folate. Besides folate, bioavailability is applicable to virtually all nutrients.[12] However, until now, it has been scarcely studied.

Other methodological limitations in dietary intake may have occurred in the observational studies on arginine intake and coronary heart disease mortality. We reported no beneficial association with coronary heart disease mortality in two studies described in chapter 7 and 8. First, this could be due to the amount of arginine in the diet found in observational studies. Beneficial effects of arginine were observed in experimental studies, however, using a much higher dose (6-21 g/d) compared to the intake of arginine from habitual food consumption.[18] Second, in chapter 7, cross-cultural data of the Seven Countries Study were used to establish this relationship. An advantage of ecological studies is the much larger variation in dietary intake between-populations than within-populations. In contrast, no causal inferences can be made about individual-level phenomena.[19,20] Third, the degree of variation in arginine intake might not be adequate to study associations between arginine and coronary heart disease mortality in the individual data of the Zutphen Elderly Study (chapter 8). In the Zutphen Elderly Study, the variation coefficient (=standard deviation/mean) was

25% for arginine intake. This is much lower compared to e.g. the variation coefficient of 58% for trans fatty acid intake, which was significantly associated with coronary heart disease risk in this cohort.

In conclusion, in the Zutphen Elderly Study, the dietary intake was assessed accurately, taking into account limitations of the use of a food composition table as much as possible. However, misclassification of dietary exposure will be present to some extent. This is especially a problem when true associations are weak and contrasts in the intake of dietary factors are small. Methodological limitations with respect to dietary intake may partly explain the absence of an association between coronary heart disease risk and the intake of different B-vitamins (chapter 6) and arginine (chapter 8).

Information bias in outcome measurement

Information bias in the ascertainment of coronary heart disease (mortality) could have introduced measurement error in the outcome variable. Municipal registries were used to obtain the date of death, which are known to provide valid data. Cause of death was obtained from Statistics Netherlands, or collected through hospital discharge data and/or general practitioners. For some dietary factors, i.e. arginine and fish, we focused only on coronary heart disease mortality because experimental evidence showed that coronary heart disease mortality appeared to be of primary importance. However, it is important to realize that when only coronary heart disease mortality was chosen as outcome, men with a non-fatal myocardial infarction were classified as non-case. It may be questioned whether this decision was always supported by etiological evidence. Similarly, other diseases associated with coronary heart disease, i.e. non-identified silent infarctions, could have influenced the results if associated with diet. This could have weakened our findings on e.g. arginine intake. Concerning the other relations between dietary factors and coronary heart disease (chapters 3, 5, 6), we studied associations between different dietary factors and fatal coronary heart disease as well as non-fatal myocardial infarction. Because of power, we focused on the association of fatal plus non-fatal coronary heart disease. The notion that the results on both outcome measurements were consistent makes it unlikely that misclassification in the outcome variable has affected our results.

Confounding

Inadequate adjustment for confounding is another major threat to the internal validity of the results. In studying relations between dietary factors and coronary heart disease, all currently known potential confounders including age, smoking,

alcohol consumption, physical activity and other dietary factors were adjusted for. However, in case of misclassification, residual confounding may still occur after adjustment for confounding, leading to underestimation or overestimation of the strength of an association.[2] As addressed in chapter 5, the non-significant positive association between α-linolenic acid intake and coronary heart disease may have occurred due to the strong correlation between intake of α-linolenic acid and trans fatty acids. Even when adjustment for trans fatty acids is performed, the potential for residual confounding by trans fatty acids remains. This was excluded by studying α-linolenic acid from sources without trans fatty acids. However, due to the small amount of α-linolenic acid from sources without trans fatty acids, the relationship between α-linolenic acid intake and coronary heart disease incidence could not be studied adequately. Arginine intake was also strongly associated with the intake of other dietary factors, including energy, methionine and flavonols through its widespread presence in animal and vegetable foods. The potential protective effect of arginine on coronary heart disease is therefore difficult to disentangle. No such strong confounders have been known in case of the significant associations observed in this thesis, i.e. the inverse association between fish consumption and coronary heart disease mortality, and the positive association between trans fatty acid intake and coronary heart disease. The crude and adjusted results were similar and support the large body of experimental and observational evidence on these associations. This convinced us that the reported associations are probably causal.

We performed analyses on energy-adjusted nutrient intakes in relation to the occurrence of coronary heart disease. This was done in order to compare nutrient intakes of subjects independent of variations in energy intake, to adjust for confounding and to reduce extraneous variation.[21] In addition, from a public health point of view, changes in energy intake cannot be made unless changes in weight or physical activity also occur, therefore, changes in nutrient intake must be accomplished by changes in the composition of the diet. Due to their contribution to energy, intake of fat, protein and carbohydrate are difficult to isolate from energy. Other nutrients, however, were also correlated with total energy intake even though they did not contribute to energy intake. For example, the correlation coefficients between intake of energy and the individual B-vitamins studied in chapter 6 were between 0.28-0.59. These correlations highlight the need to consider total energy intake when interpreting associations between specific nutrients and disease occurrence in epidemiological studies. In each of the studies reported in this thesis, we considered several approaches to adjust appropriately for energy intake.[21] In the data of the Zutphen Elderly

Study, there was an inverse association between energy intake and coronary heart disease (9% reduction in coronary heart disease ($p=0.03$) for each increase in energy of 1 MJ), which became weaker and non-significant after adjustment for age, smoking, body mass index, physical activity and prevalence of coronary heart disease. This crude inverse association between energy intake and coronary heart disease may not be assigned to energy intake, but to a general healthier (including more physically active) lifestyle which is associated with energy intake. For this reason, in epidemiological studies nutrient intakes adjusted for energy intake are of primary interest in relation to disease risk.[21]

Another important confounder in the relation between dietary factors and risk of coronary heart disease is the baseline prevalence of coronary heart disease. In chapter 3, 4 and 5, the men with a history of coronary heart disease were excluded from the analyses. Restriction to men without previously diagnosed coronary heart disease was particularly important in the Zutphen Elderly Study, due to the high proportion of men with a history of coronary heart disease (19%) having a high 10-year recurrence rate of coronary heart disease (29%; coronary heart disease mortality 26%). In addition to the results described in chapter 3, we observed no association between trans fatty acid intake and coronary heart disease in prevalent cases of coronary heart disease (data not shown). Probably the recurrence of coronary heart disease is not influenced by trans fatty acid intake. An alternative explanation is that coronary heart disease cases could have changed their food and nutrient intake. Individuals who suffer from coronary heart disease may follow the advice to reduce their intake of hard margarines rich in trans fatty acids. For other dietary factors studied in the Zutphen Elderly Study, i.e. B-vitamins, arginine, and α-linolenic acid, no clear association was observed with coronary heart disease incidence neither in the total population, nor after excluding the men with a history of coronary heart disease.

In addition to the confounders that were taken into account, residual confounding may occur for possible known or unknown confounders that have not been included in the analyses. It is important to be sure that the beneficial effect of fatty fish consumption on coronary heart disease as well as the detrimental effect of trans fatty acids intake on coronary heart disease cannot be explained by confounders. On the other hand, some true associations might have been obscured due to residual confounding. Known risk factors, but difficult to operationalize, may have influenced the results. Persons who choose to consume fish are generally more health conscious (chapter 4). We included

several variables into the analyses to adjust for indicators of a healthy diet and lifestyle e.g. use of vitamin supplements or physical activity. However, since energy intake per kg body weight was used as an approximation of physical activity, the degree of adjustment for physical activity in the association between fish consumption and coronary heart disease mortality might be limited. Also, other factors such as drug use or self-rated health may be more appropriate health indicators. Socio-economic status is another parameter associated with both dietary intake and coronary heart disease. In the Zutphen Elderly Study, associations between dietary factors and coronary heart disease remained similar after additional adjustment for occupational history of the men. However, one may question whether socio-economic status is a risk factor for coronary heart disease, or a marker for lifestyle or dietary habits including trans fatty acids intake or fish consumption. In case of the latter, adjusting for socio-economic status would have overadjusted the results. Finally, there may also be unknown risk factors which are both associated with dietary intake (including trans fatty acids intake and fish consumption) and coronary heart disease. We adjusted for the intake of antioxidant vitamins in the Zutphen Elderly Study, and, in the study on fish consumption, for potential protective foods such as vegetables and fruits. However, intake of other bioactive compounds in foods e.g. polyphenols could not be included in the analyses, therefore, residual confounding cannot be totally excluded.

In conclusion, residual confounding will not have a great impact on the association between coronary heart disease risk and fatty fish consumption or trans fatty acid intake. Residual confounding may still exist in the relation between α-linolenic acid and coronary heart disease risk, and between arginine intake and coronary heart disease risk, due to the strong correlation with other dietary and/or other risk factors.

External validity

External validity of the studies refers to the generalizability of the findings with respect to relations between dietary factors and coronary heart disease risk. For the interpretation of our findings, the biological relevance i.e. the use of an elderly male population has to be considered. Specific features of elderly populations with regard to methodology and etiology are discussed in this paragraph. Furthermore, generalizability of our findings with regard to women is considered.

The prevalence of diagnosed and undiagnosed disease at baseline is high in a population as the Zutphen Elderly Study. This could have influenced the results if the underlying disease is associated with diet as well as with coronary heart disease occurrence and is no intermediate in the relation between diet and disease. Adjustment or stratification for prevalence of diabetes mellitus or hypertension did not influence the results. In chapter 2, history of common chronic diseases (cardiovascular diseases, cancer and diabetes mellitus) after 10 years of follow-up was an effect modifier in the relation between changes in body weight and changes in total cholesterol. This suggests that morbidity could have contributed to the positive association between body weight and total cholesterol in this population of elderly men. However, in men without chronic diseases, other (undiagnosed) diseases like infections, physical health problems or depression may also have (differently) modified body weight or total cholesterol concentrations. This indicates that the proportion of men without any disease relevant to studying the effect modification of disease in the association between body weight and total cholesterol is expected to be low. Therefore, in this population of elderly men, it was not possible to study the interaction with disease status in detail.

Other methodological issues with respect to elderly populations need to be considered for interpretation of the results. Most considerations have already been extensively discussed previously.[22] It is, for example, possible that the associations between diet and coronary heart disease are weaker in an elderly population, since the susceptible individuals with unhealthy dietary habits have already been eliminated from the population (selective survival). Besides, an elderly population has a high risk for coronary heart disease, which is reflected by the high incidence rate and the high prevalence of risk factors. This positively influences the power of the study, making it possible to study associations between dietary factors and coronary heart disease incidence in relatively small sample sizes. Associations between dietary factors and disease occurrence e.g. fish consumption and coronary heart disease mortality will have a large absolute impact in a high-risk population as the elderly. However, because the magnitude of a relative risk depends on the incidence rate in the reference group, the same absolute risk can correspond to smaller relative risks in a high-risk compared to a low-risk population.[2]

Alterations in etiology of coronary heart disease in elderly people may also have an impact on the association between the dietary factors and coronary heart disease risk. Although we observed no association between arginine intake and

coronary heart disease mortality in the elderly (chapter 8), we cannot exclude the possibility that arginine intake is beneficially associated with coronary heart disease mortality in younger populations. Experimental studies demonstrated the anti-atherogenic effects of high dosage of arginine mainly in human subjects with atherosclerosis. The beneficial effects of arginine intake might be weaker in the elderly, e.g. if arginine could improve especially the early stages of atherosclerosis.[23] In addition, the requirements for essential amino acids and total protein of elderly people are higher than for younger persons, because of the higher prevalence of disease in the elderly.[24] Until now, except for the observational studies described in chapter 7 and 8, no other prospective studies on arginine intake and the risk of coronary heart disease were reported.

In concurrence with the high prevalence of atherosclerosis in the elderly, dietary factors potentially affecting the progression of coronary heart disease including e.g. thrombosis may become stronger associated with the risk of coronary heart disease. An elevated blood concentration of homocysteine may be an independent risk factor for coronary heart disease in prospective studies, as shown in the Zutphen Elderly Study,[25] due to promotion of both atherogenesis and thrombosis. On the other hand, although in the Zutphen Elderly Study folate and vitamin B6 intakes were associated with lower homocysteine concentrations, they were not associated with coronary heart disease risk. In addition, α-linolenic acid, which is considered to have effects on thrombosis and arterial compliance,[26-30] was not associated with the risk of coronary heart disease in the Zutphen Elderly Study.

It is also possible that the severity of the manifestation of coronary heart disease has changed over time, and consequently has influenced the impact of diet on coronary heart disease. The elderly population is becoming older: the number of persons over 80 years of age is growing rapidly in many countries including the Netherlands.[31] There has been a substantial reduction in coronary heart disease mortality among older persons in the past decades. This reduction may be due to a lower incidence (fewer new events), and to a substantial reduction in in-hospital case-fatality rates for acute myocardial infarction (i.e. a higher survival rate).[31] Manifestation of coronary heart disease might has become less severe as a result of already improved lifestyles and better management of risk factors. This is supported by e.g. the decline in total cholesterol levels in the Netherlands during the last decade.[32,33]

In conclusion, methodological and etiological issues with respect to the use of an elderly population in epidemiological research could have influenced the strength of the associations in either direction. Therefore, and because of lack of previous observational studies on the relation between arginine intake and coronary heart disease, the results of chapter 7 and 8 should not be generalized. However, for the other dietary factors studied in this thesis, there is no indication of effect modification by age from other prospective studies in large populations with a wide range in age.[34-38] In addition, chapter 2 of this thesis shows that the strength of the associations between changes in diet and weight and changes in cholesterol in an elderly population is comparable with to those obtained in younger populations. Therefore, the associations observed in this thesis are not necessarily different from those in younger populations.

Finally, in the Seven Countries Study, women were excluded during the baseline survey because coronary heart disease was viewed as a major health problem of middle-aged men only. Gender-differences in coronary heart disease rates worldwide[31] suggest differences in the manifestation of coronary heart disease by gender. Other prospective studies among populations that included women, however, do not suggest that the associations between dietary factors and coronary heart disease are modified by gender.[37,39] Therefore, it is unlikely that the results of this thesis are only applicable to men.

CONCLUSIONS

This thesis focused on the role of dietary factors in coronary heart disease occurrence.

We observed that changes in intake of saturated fat, polyunsaturated fat, dietary cholesterol and alcohol, and changes in body weight were significantly related to changes in total and/or HDL cholesterol concentrations (chapter 2).

In chapter 3 of this thesis, we showed a positive association between trans fatty acid intake and coronary heart disease risk in a prospective cohort study of Dutch elderly men. These results are comparable with those from other prospective studies. Combining the observational evidence, the pooled relative risk of coronary heart disease associated with an increase of 2 energy percent in trans fatty acid intake is 1.25.

In chapter 4 of this thesis we showed that men who consumed fatty fish experienced a 34% lower risk of coronary heart disease mortality compared to no fatty fish consumers after 20 years of follow up (chapter 4). These data, together with the results of studies on the effect of n-3 fatty acids, suggest that n-3 fatty acids are responsible for the protective effect of fish.

Intake of α-linolenic acid (chapter 5), B-vitamins (chapter 6), and arginine (chapter 7 & 8) were not related to the risk of coronary heart disease in the Zutphen Elderly Study or Seven Countries Study.

Interpretation of the findings

Concerning the interpretation of these results, some methodological issues have to be taken into account (table 2).

Table 2. Summary of the interpretation of the results concerning the relation between dietary factors and coronary heart disease studied in this thesis.

Chapter	Dietary factor	Impact on CHD proven?		Comment (from this thesis)
		Yes	No	
3	Trans fatty acids	X		Consistent
4	Marine n-3 fatty acids	X		Consistent
5	α-linolenic acid		X	Residual confounding trans fatty acids
6	Folate, vitamins B2, B6, B12		X	Methodological limitations (see text)
7, 8	Arginine		X	Methodological limitations (see text)

First, the relation between α-linolenic acid and coronary heart disease could not be studied independently from trans fatty acid intake because the intake of α-linolenic acid and that of trans fatty acids was strongly correlated in the Zutphen Elderly Study. In addition, the true associations between the intake of different B-vitamins and coronary heart disease may have been too weak to observe due to the limited power of the present study or to a relatively small variation in the intake of B-vitamins from only foods (information about the intake of B-vitamins

from supplements was not available). For arginine, methodological limitations refer to a limited variation in arginine intake or the failure of ecological-level associations to reflect individual-level associations. Therefore, the impact of α-linolenic acid, arginine and B-vitamins on coronary heart disease is evaluated as inconclusive (table 2).

PUBLIC HEALTH RELEVANCE

In chapter 2, we noted that the strength of the associations between changes in body weight, consumption of alcohol, saturated, polyunsaturated, trans fatty acids and dietary cholesterol and changes in total or HDL cholesterol are comparable with those from observational studies in different age-groups and to those observed in experimental studies performed in younger populations. Prevention strategies to improve cholesterol concentrations are therefore relevant also at old age. The benefits and risks of weight loss, however, are difficult to determine from observational studies because it is not possible to distinguish voluntary from involuntary or disease-induced weight loss. However, the similarity of observed associations with the results from intervention studies makes it likely that weight reduction in general results in improvement of cholesterol concentrations. Further research is needed to determine whether, under what circumstances, and by what means weight loss is beneficial in the elderly.

The inconsistent results in the literature on α-linolenic acid, arginine and B-vitamins make it impossible to propose public health recommendations yet. We will discuss the public health recommendations of trans and marine n-3 fatty acids in the following paragraphs.

Trans fatty acids

The positive association between trans fatty acid intake and the risk of coronary heart disease as described in chapter 3 is supported by the results from dietary intervention studies which have shown that trans fatty acid intake increases LDL cholesterol and decreases HDL cholesterol.[40] Insufficient and inconsistent data are available about the effect of trans fatty acids on insulin sensitivity[41,42] or hemostatic factors.[43,44] There are no prevention trials conducted that investigated the effect of trans fatty acids intake on coronary heart disease. However, the available evidence from both observational and dietary

intervention studies on serum total and HDL cholesterol indicates a causal relation between trans fatty acid intake and coronary heart disease risk.

The industrial lowering of the trans fatty acid content in Dutch edible fats was suggested to be important from a public health perspective.[45] In chapter 3, we estimated that a decrease in trans fatty acid intake of 2.4% of energy as shown in our cohort could have contributed to about 23% less coronary deaths. In Dutch margarines trans fatty acids were partly replaced by saturated fatty acids. However, the sum of trans and saturated fatty acids in margarines decreased by about 10-15% in favor of cis unsaturated fatty acids.[46] In addition, the studies on the replacement of trans fatty acids in margarine with saturated fatty acids suggest a favorable effect on plasma lipid levels.[47]

A reduction in trans fatty acid intake from both manufactured and ruminant sources may be important from a public health perspective. In chapter 3, we did not find any difference in associations between coronary heart disease and ruminant trans fatty acid intake, intake of C18:1 trans isomers and other trans isomers from manufactured sources. The difference in effect of trans fatty acids from ruminant sources and manufactured sources as observed in some population-based studies may be attributable to a small between-person variability in ruminant rather than manufactured trans fatty acid intake.[36,48,49] Dietary intervention studies on blood lipids that used different trans isomers have shown identical results.[50-53] However, there are no studies addressing specifically the effects of trans-vaccenic acid from ruminant fat. In addition, some differences in metabolism of different trans fatty acids are suggested.[54] A study using an in-vitro model of the human intestinal cell suggests that, of the trans isomers, the effects of elaidic acid may be more pronounced on serum triglyceride and HDL cholesterol, while the effect of vaccenic acid or palmitelaidic acid may be more pronounced on LDL cholesterol.[55] The multiple mechanisms which are suggested to underlie the effects of the individual trans fatty acids on lipid profiles need further investigation. So far, the issue about the health effects of trans fatty acids from ruminant sources is not settled yet.

In the Netherlands, age-adjusted coronary heart disease incidence, mortality and case-fatality decreased between 1972 and 1994.[56] Several registration data, standardized for the size and composition of the population, were used to quantify this decline. The number of hospitalizations for acute myocardial infarction decreased by 19% in men and 5% in women between 1972 and 1994. Some more specific data were available on the decline in coronary heart disease

incidence during the examination period of the Zutphen Elderly Study. Between 1988 and 1994, incidence of a first-ever myocardial infarction decreased about 30% in men and women in the Netherlands. In addition, the decrease in suspected myocardial infarction between 1983 and 1994 was about 21% in men, whereas no such decrease was observed in women during this period.[56] This decline may be explained by the better management of risk factors and improved lifestyles, including the decline in trans fatty acid intake. However, since other changes may also have occurred, e.g. changes in prescription guidelines and drug use with improved efficacy, no direct conclusions can be derived on these trend data on coronary heart disease.

Our study showed that food manufacturers can play an important role in population-based strategies to reduce coronary heart disease risk. Individualized dietary counseling has primarily been proposed as a method of achieving population goals for reducing coronary heart disease. However, when dietary interventions are implemented in community settings the effectiveness is generally moderate.[57,58] Especially the effectiveness of simple low fat and low cholesterol diets is small, probably due to difficulties in complying with the prescribed dietary advice. Larger effects, however, were seen when studies have focused on promoting fatty fish consumption[59] or on promoting a Mediterranean diet combined with changes in the composition of margarine.[60]

For public health purposes, changes in food composition together with individual dietary counseling may improve the effectiveness of preventive strategies. Recently, a decrease in total fat intake of 2.8% of energy in the Netherlands was achieved due to changes in consumption patterns (e.g. shift to skimmed milk products) and new products with low fat content that became available in the past 10 years.[10] Besides a lower content of trans fatty acids, the current margarines contain also α-linolenic acid. More specific changes in food composition may support individual dietary counseling. Recently new margarines containing plant stanols or sterols were launched that are useful for more effective low density lipoprotein cholesterol lowering in high-risk individuals or cardiac patients.[60] The compliance to the consumption of these products, their long-term side-effects and, consequently, their effectiveness in prevention strategies, however, needs to be evaluated.

Fish, n-3 fatty acids

Many studies have investigated the effect of fish consumption or n-3 fatty acids and coronary heart disease. Combining the results of these studies, conclusions can be drawn about the public health impact of fish consumption.

Most, but not all, observational studies observed an association between fish or marine n-3 fatty acids and the risk of coronary heart disease mortality. Several explanations for the inconsistency in results can be mentioned. In a review on all prospective cohort studies on the association between fish intake and coronary heart disease mortality, fish consumption was suggested not to be associated with coronary heart disease mortality in low-risk populations.[62] Furthermore, a high mercury content in fish could counteract the protective metabolic effects of fatty acids in fish. The mercury content of fish from Finnish lakes is known to be high. In the Finnish data of chapter 4, a smaller protective effect of fatty fish was observed compared to the results in the two other countries. A recent prospective study from Finland showed that the protective effect of fish was dependent on its mercury content.[63] Finally, in chapter 4 of this thesis, we suggest that further inconsistencies could be explained by the n-3 fatty acid content in fish. An inverse association between n-3 fatty acids intake and (sudden) cardiac death is observed in most,[34,38,64] although not all[65] observational studies. Non-differential misclassification of n-3 fatty acids intake may partly explain this, due to measurement error in the food tables.

The beneficial biochemical effects of marine n-3 fatty acids were studied in many human intervention as well as observational studies. Marine n-3 fatty acids have been shown to decrease triglyceride and very low density lipoprotein levels in dietary intervention studies.[66] In an observational study, besides a significant reduction in serum total triglycerides also a significant reduction in the triglycerides of the very atherogenic intermediate density lipoprotein faction was observed of the men who consumed fish once a week compared to a control group.[67] Results of intervention studies on the impact of marine n-3 fatty acids on hemostatic factors are insufficient and inconsistent. There is some evidence, however, for a beneficial effect on bleeding time and erythrocyte flexibility from studies using a high dose (≥1.8 g/d) of n-3 fatty acids.[68] No differences between thromboxane formation, bleeding time and platelet number were observed among habitual fish consumers compared with controls.[69] An inverse dose-response effect on blood pressure for a high n-3 fatty acid intake (≥3 g/d) is observed in a meta-analyses of controlled trials.[70] In epidemiological studies no relation could be detected between fish consumption and blood pressure.[71]

Marine n-3 polyunsaturated fatty acids may also have an effect on the elasticity of the arterial wall. In humans, marine n-3 fatty acids may improve endothelial function by increased synthesis of nitric oxide.[72] Another index of arterial function, compliance, was better in healthy subjects and diabetic patients who consumed fish compared with no-fish consumers.[73]

Until now, three secondary prevention trials studied the protective effect of fish or n-3 fatty acids on cardiac events. In the Diet and Reinfarction trial (DART), cardiac patients received either or not the advice to use fatty fish two or three times per week.[59] The difference in fatty fish consumption was associated with a significant reduction of 29% in all cause mortality during 2 years of follow up, which was entirely attributable to coronary heart disease mortality. In a trial from India (IEIS-4), patients with suspected myocardial infarction were randomized to daily supplementation with fish oil, mustard oil or placebo.[74] Cardiac events were significantly reduced after 1 year in the fish oil group. However, the interpretation of this finding is problematic because no adjustments were made for differences in other characteristics (e.g. smoking habits) between the experimental and control groups. In the GISSI-prevention trial, survivors of a myocardial infarction were randomly assigned to one of four groups receiving dietary supplementation of marine n-3 fatty acids of 850 mg/d, vitamin E, a combination or no supplements, and were followed for 3.5 years.[75] In a four-way analysis investigating each group, there was a significant relative reduction of 35% in cardiac death (combined coronary heart disease death and sudden death) in patients assigned to n-3 fatty acid treatment. In the two-way factorial analysis, the relative reduction in risk for cardiac death was 28% and also significant.

The reductions in cardiac events in the DART and GISSI trial could have partly been explained by the effects of n-3 fatty acids on triglyceride levels and hemostatic profile. In addition, since there was no significant reduction in non-fatal myocardial infarction in the experimental groups of these trials, benefits may also have resulted from an antiarrhythmic effect of n-3 fatty acids. Also in the observational data of the Physicians' Health Study, an inverse association was observed between fish consumption and the risk of sudden cardiac death,[38] while no association was observed on nonfatal coronary heart disease.[76] However, the Western Electric Study reported an inverse association between fish consumption and the risk of non-sudden but not sudden myocardial infarction.[77] Differences in ascertainment of the endpoint sudden death may explain these inconsistencies. The antiarrhrythmic effects of marine n-3 fatty

acids were supported by a retrospective, population-based case-control study on primary cardiac arrest.[64] Until now, antiarrhythmic properties of marine n-3 fatty acids have primarily been shown in in-vitro and animal studies.[78,79] The antiarrythmic effect of n-3 fatty acids investigated dietary human intervention trials by the means of heart rate variability is suggested in patients as well as healthy individuals.[80] In a cross-sectional study, a modest intake of n-3 fatty acids from fish was also positively associated with heart rate variability among patients referred for coronary angiography.[81]

Combining the evidence of observational and experimental studies on the effect of fish or n-3 fatty acids, the optimal intake to prevent coronary heart disease mortality could be assessed. In chapter 4, we observed a reduced risk of coronary heart disease mortality for the men consuming fatty fish compared to those who consumed no fatty fish. The mean intake of those who consumed fatty fish ranged from 11 in the Netherlands to 25 g/d in Finland, or between about 250-500 mg/d marine n-3 fatty acids. Most observational studies (including our study described in chapter 4) did not observe additional protection for coronary heart disease with a higher intake of (fatty) fish. In the DART trial, the difference in average intake of eicosapentaenoic acid (EPA) between two groups was reported to amount 230 mg/d.[59] The total intake of n-3 fatty acids (including docosahexaenoic acid=DHA) in the DART trial, however, may be at least about 500 mg/d (given that the content of EPA and DHA in fish is at least similar, and that the fish advice was at least 28 g/d). In the GISSI trial, although a n-3 fatty acid supplementation of 850 mg/d was prescribed, about 30% of the patients permanently stopped taking n-3 fatty acids after 1 year.[75] Therefore, the average intake of about 600 mg/d n-3 fatty acids may have been responsible for the reduction in cardiac deaths in this trial.

High consumption of n-3 fatty acids could be associated with higher susceptibility to oxidation of LDL cholesterol, and consequently to increased atherosclerosis. Whether an increased intake of n-3 fatty acids leads to a clinically relevant enhanced in vivo oxidation of LDL cholesterol, however, is debatable.[82] Data from the GISSI trial do not suggest that the protective effect of n-3 fatty acids depends on the intake of vitamin E, an antioxidant which could protect LDL cholesterol from lipid peroxidation.[75] In addition, supplementation of n-3 fatty acids with 1.5 g/d for 2 years did not suggest to increase atherosclerosis.[83]

We support the recommendations of a published expert workshop summary.[71] Results obtained so far from epidemiological studies, animal experiments and

controlled trials in humans suggest that the risk of coronary heart disease mortality and sudden cardiac death may be reduced by intake of marine n-3 fatty acids. This effect may only occur in high-risk individuals, i.e. in cardiac patients or in those with unhealthy lifestyles including smoking, a high saturated fat intake and a low fish intake. People at risk for coronary heart disease are therefore advised to eat fish, preferably fatty fish at least once a week. Since in the Netherlands only 32% of the men and 29% of the women consumed fish at least once a week in 1996, and about 40% of the fish consumed is fatty fish,[84] this suggest that the public health impact of an advice on (fatty) fish consumption could be substantial.

FUTURE RESEARCH

The studies presented in this thesis emphasize the importance of diet in the occurrence of coronary heart disease. Changes in food composition with respect to the trans fatty acid content had probably an important impact on public health. Nowadays, attention has to be paid to lower the trans fatty acid content of other industrial derived foods and to the substitution of trans fatty acids by cis unsaturated fatty acids.

Despite the large number of studies completed on the beneficial effect of fish and marine n-3 fatty acids, the quantification of risk reduction for a regular consumption of fish remains relatively imprecise. Intervention studies of marine n-3 fatty acids on sudden cardiac death could support, and more accurately estimate, the intake of n-3 fatty acids needed for maximal health benefit. With respect to the feasibility of such intervention, high-risk individuals should be recruited, e.g. cardiac patients, to limit the number of participants, and consequently the costs, needed to study this relationship.

The number of prospective studies on the association between coronary heart disease risk and intake of α-linolenic acid, folate, vitamins B2, B6 and B12 and arginine is limited. Because of some methodological shortcomings in our studies, additional large population-based studies with detailed data on dietary intake and the use of vitamin supplement may be useful.

Regarding prospective studies on the association between α-linolenic acid and coronary heart disease, the interaction between α-linolenic acid and trans fatty acids, and additionally, the relationship between α-linolenic acid and coronary

heart disease in a population with a low intake of trans fatty acids, needs special attention.

Dietary intervention studies may be useful in further clarifying biological mechanisms underlying a possible beneficial effect of α-linolenic acid. Long-term dietary intervention studies of α-linolenic acid supplementation should be carried out especially with respect to coronary heart disease intermediates such as ventricular fibrillation, heart rate variability and endothelial function. Studies showing a beneficial effect of α-linolenic acid could not distinguish between a direct or indirect effect (through the synthesis of EPA) of α-linolenic acid. Supplementation trials of α-linolenic acid should be conducted to clarify determinants (e.g. other fatty acids in the diet or time period) of the conversion of dietary α-linolenic acid into plasma or platelet EPA. Finally, intervention studies which estimate the impact of α-linolenic acid supplementation on coronary heart disease should be conducted.

Regarding the association between B-vitamins and coronary heart disease, the independent impact of each B-vitamin, and, more specifically, the possible inverse relationship of vitamin B2 with coronary heart disease are of interest. Several planned and ongoing trials of B-vitamin supplements on clinical cardiovascular endpoints[85] will give more insight on the effect of B-vitamin intake on coronary heart disease. Recently, two intervention trials studied the relationship between B-vitamins (folate, vitamin B6 and in one trial also vitamin B12) and atherosclerosis.[86,87] However, methodological problems, e.g. the lack of a control group[86] or inconsistent effects on different measures of atherosclerosis[87] limited the interpretation of these findings. Furthermore, future trials should be able to distinguish the relative importance of the different B-vitamins.

With respect to arginine, prospective studies on the association between arginine intake and coronary heart disease risk should be performed in populations with a wide range in arginine intake, e.g. populations with a high variation in consumption of animal products, or multi-center studies with detailed dietary data, such as EPIC Heart. Also prospective studies performed in younger age groups may provide new insights in the association between arginine and coronary heart disease.

Long-term interventions with different dosages (including practical dose) of arginine, on endothelial function or atherosclerosis are of interest. Dietary intervention trials should also be directed toward the use of biomarkers, e.g. blood concentrations of arginine, as a reflection of (short-term) arginine intake.

Finally, also dietary intervention trials in high risk groups are needed to improve insight in the risk profile of individuals who could benefit from arginine supplementation.

In the near future, more insight in the mechanisms that are involved in the relation between diet and coronary heart disease may be useful to identify persons who could particularly benefit from dietary therapy to reduce their risk of coronary heart disease. In addition, future dietary intervention studies should investigate the applicability of 'functional foods' (foods that, by virtue of physiologically active components, provide health benefits beyond basic nutrition)[88] to support individual dietary counseling. This may improve the effectiveness of dietary interventions in the reduction of coronary heart disease risk.

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SUMMARY

In this thesis, the results of prospective studies on fatty acids, B-vitamins and arginine and the occurrence of coronary heart disease have been described. Coronary heart disease is the major cause of mortality in industrialized countries such as the Netherlands. In the coming decades, there will be an increase in the number of older people. This is expected to be accompanied by an increase in the prevalence of coronary heart disease. Therefore, it is important to get more insight in dietary factors related to coronary heart disease.

For the results presented in this thesis, data were used of cohorts from the Seven Countries Study. This population-based study started between 1958 and 1964, involving 12,763 men aged 40-59 years from 16 cohorts. Cross-cultural data of the Seven Countries Study were used to investigate the association between dietary intake and 25-year coronary heart disease mortality. Individual dietary information collected around 1970 in 3136 men aged 50-69 years from the Finnish, Italian and Dutch cohorts of the Seven Countries Study allowed us to study the relation between diet and 20-year coronary heart disease in an international setting. One of these cohorts, a cohort of 878 men from Zutphen, was selected as the Dutch contribution to the Seven Countries Study. In 1985, this cohort of men has continued to be examined within the framework of the Zutphen Elderly Study. The former cohort was therefore extended with other men from the town of Zutphen in the same age category, resulting in a total population of 939 men aged 64-84 years. Detailed information was available on dietary intake, biological risk factors, incidence of major chronic diseases, vital status and cause of death between 1985 and 1995. This allowed us to study the association between nutrient intake, serum cholesterol response and the risk of coronary heart disease, taking into account e.g. intake of other nutrients.

In chapter 2 of this thesis, we have described the association between changes in lifestyle and dietary factors on changes in serum total and high density lipoprotein (HDL) cholesterol in the Zutphen Elderly Study. Data of 234 men were used who participated in each dietary and clinical examination at baseline (1985), and after 5 and 10 years of follow-up. Dietary factors studied were saturated, monounsaturated, polyunsaturated and trans fatty acids, cholesterol and fiber, lifestyle related factors studied were body weight, smoking, alcohol use and physical activity. After adjustment for age, carbohydrate intake and the other parameters, a 1 energy percent increase in polyunsaturated fat intake was

associated with a 0.03 mmol/L reduction in total cholesterol concentrations. Weight loss was associated with decreased total cholesterol concentrations. One extra glass of alcohol or 5 kg weight loss were both associated with 0.04 mmol/L increased HDL cholesterol concentrations. Increased intake of saturated fat and dietary cholesterol were significantly related to increased HDL cholesterol concentrations. For these statistically significant associations as well as for non-significant associations, e.g. those between trans fatty acids and total cholesterol and between polyunsaturated fatty acids and HDL cholesterol, the strength of the estimates is comparable with those from experimental studies performed in younger populations. This study suggested that, even in old age, it remains important to change weight and diet to improve the lipid profile.

Using again the data of the Zutphen Elderly Study, we estimated the association between trans fatty acid intake and the 10-year risk of coronary heart disease in 667 men free of coronary heart disease at baseline in 1985 (chapter 3). During 10 years of follow up, we documented 98 cases of coronary heart disease. Between 1985 and 1995, average trans fatty acid intake of the elderly men decreased from 4.3 to 1.9% of energy. Trans fatty acid intake at baseline was positively associated with the 10-year risk of coronary heart disease. The relative risk for a difference of 2 energy percent in trans fatty acid intake at baseline was 1.28 (95% confidence interval (CI) 1.01 to 1.61). The association was observed for trans fatty acid intake from ruminant sources as well as different trans isomers from manufactured sources. Based on our results and those from previous prospective cohort studies, we estimated that the observed decrease in trans fatty acid intake, mainly due to industrial lowering of trans contents in Dutch edible fats, is compatible with a 23% reduction in risk of coronary heart disease.

The association between total, lean and fatty fish consumption and the risk of coronary heart disease mortality was examined in 1,088 Finnish, 1,097 Italian and 553 Dutch men of the Seven Countries Study free of coronary heart disease around 1970 (chapter 4). After 20 years of follow-up, 242 men died of coronary heart disease in Finland, 116 in Italy and 105 in the Netherlands. Total and lean fish consumption were not associated with coronary heart disease mortality in any of the three countries. Fatty fish consumption, however, was associated with lower coronary heart disease mortality compared with non-fatty fish consumption. The adjusted pooled relative risk for fatty fish consumers was 0.66 (95% CI 0.49-0.90) compared to no fatty fish consumption. These data suggest that especially fatty fish is protective against coronary heart disease mortality.

The association between α-linolenic acid intake and the 10-year risk of coronary heart disease was investigated in 667 men free of coronary heart disease of Zutphen Elderly Study (chapter 5). Intake of α-linolenic acid and trans fatty acids were strongly correlated (r_s =0.61). This complicated studying the association between α-linolenic acid intake and coronary heart disease. α-Linolenic acid intake from sources with trans fatty acids was positively associated with coronary heart disease risk (adjusted relative risk 1.51; 95% CI 0.75-3.04 for the highest compared to the lowest tertile). α-Linolenic acid intake from foods without trans fatty acids was not associated with the risk of coronary heart disease. The adjusted relative risk was 1.15 (95% CI 0.63-2.11) for the highest compared to the lowest tertile. These results indicate that α-linolenic acid was not beneficially associated with the risk of coronary heart disease in this cohort of elderly men.

We investigated whether intake of folate, vitamins B2, B6 and B12 are associated with homocysteine concentrations and 10-year coronary heart disease incidence in 815 men of the Zutphen Elderly Study (chapter 6). After 10 years of follow-up, 140 cases of coronary heart disease had occurred. Only intake of folate and vitamin B6 were independently inversely associated with homocysteine. However, both vitamins were not associated with the risk of coronary heart disease. Independent of homocysteine, vitamin B2 intake was non-significantly inversely associated with coronary heart disease risk. The adjusted relative risk for the highest compared to the lowest tertile of vitamin B2 was 0.61 (95% CI 0.36-1.03; p for trend 0.08). Vitamin B12 intake was significantly positively associated with coronary heart disease risk (p for trend=0.03). This study did provide no evidence that B-vitamins are protective for coronary heart disease, although there was a suggestion for a protective effect of vitamin B2.

Cross-cultural analyses of the 16 population cohorts of the Seven Countries Study were performed to estimate the association between arginine intake and 25-year coronary heart disease mortality in middle-aged men at the population level (chapter 7). After 25 years of follow up, the coronary heart disease mortality rate varied from 2.5% in Crete (Greece) to 26.8% in East-Finland. Arginine intake was not significantly associated with coronary heart disease mortality on the population level. In the unadjusted regression analysis an increase in arginine intake of 1 g/d was associated with a reduction in the risk of coronary heart disease mortality of 1.11% (p=0.17). In the adjusted analyses, this association was reduced towards zero. We also investigated this association

among 806 men aged 64-84 years at baseline who participated in the Zutphen Elderly Study (chapter 8). During 10 years of follow up, 90 men died from coronary heart disease. Arginine intake was not associated with the 10-year coronary heart disease mortality on the individual level. The adjusted relative risk for the men in the highest tertile of arginine intake was 1.56 (95% CI 0.83-2.93) compared to the men in the lowest tertile of arginine intake. The results of both prospective studies do not support the hypothesis that dietary arginine intake lowers the risk of coronary heart disease mortality.

In chapter 9, our results are discussed with regard to methodological considerations and public health implications. The consistency in strength of the associations between changes in diet and weight and changes in total and HDL cholesterol of our study described in chapter 2 and in the literature suggest that public health strategies on dietary interventions and weight reduction in order to improve cholesterol concentrations are relevant also at old age. Fatty fish consumption was estimated to reduce coronary heart disease mortality with 34%. Based on epidemiological studies, animal experiments and controlled trials in humans, it is concluded in chapter 9 that (sudden) cardiac death risk will be reduced by an increased intake of n-3 fatty acids from (fatty) fish. People at risk for coronary heart disease are therefore advised to eat fish, preferably fatty fish at least once a week, to reduce their risk for coronary heart disease. Trans fatty acid intake was positively associated with coronary heart disease risk. The substantial decrease in trans fatty acid intake, mainly due to the industrial lowering of the trans fatty acid content in Dutch edible fats, is therefore important from a public health perspective. We did not find evidence for a protective effect of intake of α-linolenic acid, however, the strong association with trans fatty acids complicated studying this relationship. Of the B-vitamins, only the intake of folate and vitamin B6 were independently inversely associated with homocysteine in a population of elderly men. In our cohort, intake of different B-vitamins did not protect against coronary heart disease, although there is a suggestion for a protective effect of vitamin B2. Arginine intake was not associated with the risk of coronary heart disease mortality in two prospective studies. Results of large prospective and intervention studies are needed to before definite statements can be made about the potential protective effect on coronary heart disease of B-vitamins, arginine and α-linolenic acid.

SAMENVATTING

In dit proefschrift zijn de verbanden beschreven tussen enerzijds de inname van vetzuren, B-vitamines en arginine en anderzijds het optreden van coronaire hartziekten. Coronaire hartziekten vormen de belangrijkste doodsoorzaak in geïndustrialiseerde landen zoals Nederland. In de komende decennia zal het percentage ouderen toenemen. Naar verwachting zal dit gepaard gaan met een toename van het totaal aantal personen met coronaire hartziekten. Het is daarom van groot belang inzicht te krijgen in de mogelijke verbanden tussen voedingsfactoren en coronaire hartziekten.

De resultaten zijn gebaseerd op longitudinale gegevens afkomstig uit de Zeven Landen Studie. Tussen 1958 en 1964 werden 16 cohorten bestaande uit in totaal 12.763 mannen van 40-59 jaar oud uit 7 landen onderzocht. De informatie over het gemiddelde voedselpatroon van elk van de 16 cohorten werd gebruikt om het verband tussen voeding en de 25-jaars sterfte aan coronaire hartziekten te bestuderen. In de Zeven Landen Studie werden rond 1970 de voedingsgewoonten nagevraagd bij 3.136 Finse, Italiaanse en Nederlandse mannen van 50-69 jaar. Gedurende 20 jaar zijn de doodsoorzaken van de overleden deelnemers geregistreerd. Deze gegevens zijn gebruikt om in een internationale context het verband te bestuderen tussen voeding en de 20-jaars sterfte aan coronaire hartziekten. Het Nederlandse cohort van de Zeven Landen Studie werd gevormd door een onderzoekspopulatie bestaande uit 878 mannen die in 1960 in Zutphen woonden. In 1985 werd het onderzoek in Zutphen voortgezet. De oorspronkelijke onderzoekspopulatie werd hiervoor aangevuld met een nieuwe populatie van mannen uit dezelfde leeftijdscategorie, eveneens woonachtig in Zutphen. In 1985 werden in totaal 939 mannen van 64-84 jaar oud uitgenodigd voor dit onderzoek, dat bekend staat als de Zutphen Ouderen Studie. Gedetailleerde informatie was beschikbaar over voedingsgewoonten, risicofactoren, incidentie van belangrijke chronische ziekten, vitale status en doodsoorzaken verzameld tussen 1985 en 1995. Hiermee konden we verbanden tussen enerzijds de inname van voedingsstoffen en anderzijds veranderingen in serumcholesterol en het risico op coronaire hartziekten bestuderen.

In hoofdstuk 2 van dit proefschrift bestudeerden we het verband tussen veranderingen in voeding en leefstijlfactoren en veranderingen in serumtotaal- en hoge-dichtheidslipoproteïnen (HDL)-cholesterol in de Zutphen Ouderen

Studie. Van 234 mannen zijn gegevens gebruikt. Deze namen deel aan zowel het voedselconsumptie- als het lichamelijke onderzoek aan het begin van de studie in 1985 en aan de vervolgonderzoeken respectievelijk 5 en 10 jaar later. De onderzochte leefstijlfactoren waren: lichaamsgewicht, roken, alcoholgebruik en lichamelijke activiteit. Als voedingsfactoren werden verzadigde, enkelvoudig onverzadigde en meervoudig onverzadigde vetzuren, transvetzuren, voedingscholesterol en vezel bestudeerd. Onafhankelijk van leeftijd, inname van koolhydraten en de andere onderzochte leefstijl- en voedingsdeterminanten ging een verhoging van 1 energieprocent aan meervoudig onverzadigde vetzuren gepaard met een daling in het totaalcholesterolgehalte van 0,03 mmol/l. Gewichtsverandering hing significant samen met een verandering in het totaalcholesterolgehalte. Toename van de alcoholconsumptie met 1 glas per dag, of een gewichtsafname van 5 kg gingen beide gepaard met een verhoogd HDL-cholesterolgehalte van 0,04 mmol/l. Een verhoogde inname van verzadigde vetzuren en voedingscholesterol verlaagden het totaalcholesterolgehalte significant. Deze associaties zijn qua omvang vergelijkbaar met de resultaten van experimentele onderzoeken die bij jongere populaties zijn uitgevoerd. Dit gold ook voor de niet-significante verbanden, zoals de relaties tussen transvetzuren en totaalcholesterol en tussen meervoudig onverzadigde vetzuren en HDL-cholesterol. Dit onderzoek laat zien dat het op hoge leeftijd belangrijk blijft om het serumcholesterolgehalte te verbeteren door het gewicht en/of de voeding te veranderen.

De gegevens van de Zutphen Ouderen Studie werden ook gebruikt om het verband tussen transvetzuurinname en het risico op coronaire hartziekten te bestuderen (hoofdstuk 3). De onderzoekpopulatie bestond uit 667 mannen van 64-84 jaar zonder coronaire hartziekten aan het begin van de studie in 1985. Gedurende 10 jaar kregen 98 mannen een coronaire hartziekte. Tussen 1985 en 1995 nam de transvetzuurinname af van 4,3% naar 1,9% van de totale energie-inname. Deze daling in transvetzuurinname is voornamelijk een gevolg van een veranderd productieproces van voedingsmiddelen die bereid worden met geharde plantaardige oliën. Transvetzuurinname bleek het 10-jaars risico op de ontwikkeling van coronaire hartziekten te verhogen. Het relatieve risico voor een verschil van 2 energieprocent in transvetzuurinname bedroeg 1,28 (95% betrouwbaarheidsinterval 1,01-1,61). Dit verband was aanwezig voor transvetzuren uit dierlijke producten en voor verschillende transvetzuren uit voedingsvetten van geharde plantaardige oliën. Wanneer we onze bevindingen combineren met die van eerder uitgevoerde longitudinale cohortonderzoeken

blijkt dat, naar schatting, de daling in transvetzuren in Nederland het risico op coronaire hartziekten met ongeveer 23% kan hebben verminderd.

We bestudeerden de dagelijkse consumptie van magere vis, vette vis en de visconsumptie in totaal in relatie tot sterfte aan coronaire hartziekten in de Finse, Italiaanse en Nederlandse cohorten van de Zeven Landen Studie (hoofdstuk 4). Gegevens waren beschikbaar van 1.088 Finse, 1.097 Italiaanse en 553 Nederlandse mannen die aan het begin van de studie rond 1970 50-69 jaar waren en geen coronaire hartziekte hadden. Gedurende 20 jaar vervolgonderzoek stierven 242 Finse mannen aan coronaire hartziekten, 116 Italianen en 105 Nederlanders. In de drie landen werd geen duidelijk verband waargenomen tussen enerzijds de consumptie van vis in totaal en magere vis en anderzijds de sterfte aan coronaire hartziekten. Consumptie van vette vis bleek wel gerelateerd te zijn aan een verminderd risico op sterfte aan coronaire hartziekten. Indien de gegevens van de drie landen samengevoegd werden, bedroeg het gecorrigeerde relatieve risico voor gebruikers van vette vis 0,66 (95% betrouwbaarheidsinterval 0,49-0,90) vergeleken met degene die geen vette vis gebruikten. Deze gegevens suggereren dat vooral de consumptie van vette vis van belang is bij de preventie van fatale coronaire aandoeningen.

Het verband tussen inname van α-linoleenzuur en het 10-jaars risico op de ontwikkeling van coronaire hartziekten is bestudeerd bij de 667 deelnemers van de Zutphen Ouderen Studie die vrij waren van coronaire hartziekten (hoofdstuk 5). De inname van α-linoleenzuur en transvetzuren hingen sterk samen (r_s =0,61). Dit bemoeilijkte het onderzoek naar de relatie tussen α-linoleenzuur inname en coronaire hartziekten. De inname van α-linoleenzuur uit transvetzuurrijke producten bleek positief geassocieerd te zijn met het risico op coronaire hartziekten (gecorrigeerde relatieve risico 1,51; 95% betrouwbaarheidsinterval 0,75-3,04 voor de hoogste tertiel ten opzichte van de laagste tertiel). Er werd geen verband gevonden tussen de inname van α-linoleenzuur uit voedingsmiddelen zonder transvetzuren en het risico op coronaire hartziekten. Het gecorrigeerde relatieve risico bedroeg 1,15 (95% betrouwbaarheidinterval 0,63-2,11) voor de hoogste tertiel ten opzichte van de laagste tertiel. Hieruit kunnen we concluderen dat in de Zutphen Ouderen Studie de α-linoleenzuur inname niet duidelijk samenhangt met de ontwikkeling van coronaire hartziekten.

Het verband tussen de inname van folaat, vitamines B2, B6 en B12 enerzijds en homocysteïneniveaus en de 10-jaars ontwikkeling van coronaire hartziekten

anderzijds werd eveneens onderzocht in de Zutphen Ouderen Studie (hoofdstuk 6). Gedurende 10 jaar vervolgonderzoek kregen 140 van de 815 mannen een coronaire hartziekte. Alleen de inname van folaat en vitamin B6 vertoonden een onafhankelijke inverse relatie met het homocysteïneniveau. Beide vitamines bleken echter geen verband te vertonen met het risico op coronaire hartziekten. Er bestond een niet-significant invers verband tussen vitamine B2 inname en het risico op coronaire hartziekten, onafhankelijk van het homocysteïneniveau. Het gecorrigeerde relatieve risico voor de hoogste ten opzichte van de laagste tertiel van vitamine B2 bedroeg 0,61 (95% betrouwbaarheidsinterval 0,36-1,03; p voor trend 0,08). De vitamine B12 inname bleek het risico op coronaire hartziekten significant te verhogen (p voor trend=0,03). Dit onderzoek levert geen bewijs voor een mogelijke beschermende rol van B-vitamines op de ontwikkeling van coronaire hartziekten, al is een mogelijk beschermend effect van vitamine B2 niet uitgesloten.

In een cross-cultureel onderzoek met gegevens van de 16 cohorten van de Zeven Landen Studie werd de relatie tussen arginine inname en 25-jaars sterfte aan coronaire hartziekten onderzocht (hoofdstuk 7). Na 25 jaar varieerde het sterftecijfer voor coronaire hartziekten van 2,5% op Kreta (Griekenland) tot 26,8% in Oost-Finland. De gemiddelde arginine inname vertoonde op populatieniveau geen verband met sterfte aan coronaire hartziekten. In de ongecorrigeerde regressieanalyse bleek een toename van arginine inname van 1 g/dag gerelateerd te zijn aan een afname van het risico op coronaire hartziekten van 1,11% (p=0,17). Dit verband werd zwakker na correctie voor mogelijke verstorende variabelen. We bestudeerde deze relatie ook met behulp van de gegevens van 806 deelnemers van de Zutphen Ouderen Studie (hoofdstuk 8). Gedurende 10 jaar vervolgonderzoek overleden 90 mannen aan coronaire hartziekten. De arginine inname vertoonde geen relatie met de 10-jaars sterfte aan coronaire hartziekten. Het gecorrigeerde relatieve risico voor mannen in de hoogste tertiel van arginine inname bedroeg 1,56 (95% betrouwbaarheidsinterval 0,83-2,93) ten opzichte van mannen in de laagste tertiel van arginine inname. De resultaten van beide longitudinale onderzoeken leveren geen ondersteuning op voor de hypothese dat arginine inname het risico op coronaire hartziekten verlaagt.

In hoofdstuk 9 bespreken we de in dit proefschrift gepresenteerde resultaten met betrekking tot methodologische tekortkomingen en volksgezondheidsimplicaties. De sterkte van de verbanden tussen veranderingen in de inname van voedingsstoffen en gewicht en veranderingen in totaal- en

HDL-cholesterol zoals beschreven in hoofstuk 2 zijn vergelijkbaar met hetgeen gerapporteerd is in de literatuur. De uitkomsten hiervan suggereren dat strategieën gericht op veranderen van voedingsgewoonten of gewicht ter verbetering van totaal- en HDL-cholesterol ook relevant zijn op oudere leeftijd. Consumptie van vette vis kan volgens onze berekening de sterfte in coronaire hartziekten met 34% verlagen. Op basis van epidemiologische studies, dierexperimenten en gecontroleerde interventiestudies bij proefpersonen werd in hoofdstuk 9 geconcludeerd dat het aantal (acute) overlijdensgevallen ten gevolge van coronaire hartziekten kan afnemen door de inname van n-3 vetzuren uit (vette) vis te verhogen. Personen met een hoog risico op het krijgen van coronaire hartziekten krijgen daarom het advies om vis te eten, bij voorkeur minimaal één keer per week vette vis, om hun risico op coronaire hartziekten te verminderen. De inname van transvetzuren is positief geassocieerd met het risico op coronaire hartziekten. De daling in de inname van transvetzuren door de betere samenstelling van voedingsvetten in Nederland kan daarom een belangrijke bijdrage hebben geleverd aan het verbeteren van de volksgezondheid. Onze resultaten ondersteunen een beschermend effect van de inname van α-linoleenzuur op coronaire hartziekten niet. De sterke associatie tussen α-linoleenzuur en transvetzuren bemoeilijkte echter de interpretatie van ons onderzoek. Van de B-vitamines bleken alleen de inname van folaat en vitamine B6 invers samen te hangen met homocysteineniveaus in de Zutphen Ouderen Studie. De inname van verschillende B-vitamines bleek geen bescherming te bieden tegen het ontstaan van coronaire hartziekten, hoewel een beschermend effect van vitamine B2 niet kon worden uitgesloten. De arginine inname bleek niet samen te hangen met het risico op coronaire hartziekten in twee prospectieve studies. Grote prospectieve en interventiestudies zijn nodig om het mogelijk beschermende effect van α-linoleenzuur, B-vitamines en arginine op coronaire hartziekten aan te tonen.

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ABOUT THE AUTHOR

Claudia Maria Oomen was born on October 20, 1972 in Heeze, the Netherlands. In 1991, she passed secondary school at 'Strabrecht College' in Geldrop. In the same year, she started the study 'Human Nutrition' at what is now called the Wageningen University. As part of that study she conducted an epidemiological research project on the relation between physical activity and other lifestyle factors at the Robert Koch Institute, Federal Institute for infectious and non-infectious diseases in Berlin (May-November 1995). Furthermore, she conducted a research project on simple muscle strength measurements to assess functional ability in the elderly at the former department of Human Nutrition at the Wageningen University (January-May 1996). In September 1996, she received her MSc degree.

From October 1996 to February 2001, she was appointed as a Ph.D fellow to the division of Human Nutrition and Epidemiology at the Wageningen University. During this period, she carried out the research described in this thesis at the department of Chronic Diseases Epidemiology of the National Institute of Public Health and the Environment (RIVM) in Bilthoven. She jointed the education program of the Graduate School VLAG (advanced courses in Food Technology, Agrobiotechnology, Nutrition and Health Sciences). In June 1997, she attended the Annual New England Epidemiology Summer Program at Tufts University, Boston, USA. In September 1998, she conducted a feasibility study for a population based-trial on the effect of α-linolenic acid to clinical cardiovascular endpoints on behalf of the Wageningen Centre for Food Sciences, Wageningen.

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