Dietary Patterns, Biological Risk Factors and Survival in Elderly European Men and Women

Kim Knoops

Promotoren
Prof. Dr. W.A. van Staveren, Hoogleraar Voeding van de Oudere Mens
Wageningen Universiteit

Prof. Dr. Ir. D. Kromhout, Hoogleraar Volksgezondheidsonderzoek
Wageningen Universiteit

Co-promotor
Prof. Dr. Ir. C.P.G.M. de Groot, Hoogleraar Voedingsfysiologie met bijzondere aandacht voor het Verouderingsproces en de Oudere Mens
Wageningen Universiteit

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Prof. J.C. van Houwelingen, Leiden Universiteit

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Kim T. B. Knoops

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Abstract

Background: The percentage of the population aged 65 and over, which started to rise sharply in the last decades of the past century, is continuing to rise. However, up until now, little is known about dietary factors and diet-related biological factors in elderly in relation to survival in old age.
Aim: The aim of this thesis is to investigate the associations between dietary patterns, diet-related biological risk factors and survival.
Study population: Data from two prospective studies were used, the Healthy Ageing: a longitudinal study in Europe (HALE) and the Normative Aging Study (NAS). The HALE project which involves individuals enrolled in the Survey in Europe on Nutrition and the Elderly: a Concerned Action (SENECA) and the Finland, Italy, the Netherlands, Elderly (FINE) studies, includes 1507 apparently healthy men and 832 women in 11 European Countries, aged 70 to 90 years at baseline and who were followed for 10 years. The NAS is a longitudinal study which started in 1963 by recruiting men, 21 to 80 years of age and free of heart disease and other major health problems, around Boston. Data collected in 1993 and later were used.
Results: We observed that after fortification with folic acid, folate intake and plasma folate concentration increased significantly in three groups with different dietary patterns, derived by cluster analysis. Homocysteine tended to decrease in the group with a relatively high alcohol consumption, low fruit and vegetable dietary pattern in the NAS study.
Dietary patterns defined by three European indexes were significantly associated with all-cause mortality in the HALE project. A Mediterranean type of diet was associated with an approximately 20 % lower mortality rates in both apparently healthy elderly and in post-myocardial infarction patients. Healthy lifestyle factors (moderate alcohol consumption, being physically active and non-smoking) were also inversely associated with all-cause mortality.
Weight loss was significantly associated with increased all-cause mortality in the study centres of Northern Europe while weight gain was weakly associated with increased mortality in these centres in the FINE study. HDL-cholesterol was inversely associated with mortality from cardiovascular diseases and all-causes in the HALE project. Associations between HDL and mortality were strongest in women.
Conclusion: A healthy diet is associated with lower mortality risk in elderly. Besides a healthy diet, it is important for elderly people to be physically active, use alcohol in moderation and quit smoking. It is also important to maintain constant body weight and to keep blood cholesterol at a desirable level, especially to keep HDL-cholesterol high.

Contents

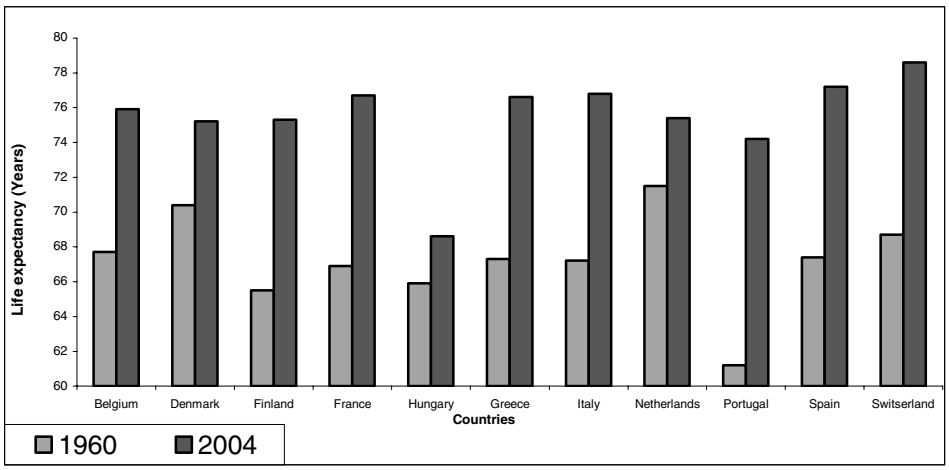
1

Introduction

1.1 Demographical developments in Europe

The world has experienced dramatic improvements in longevity[1]. Life expectancy at birth has increased by 8 years for both sexes over the last 45 years for men and women. In graph 1, the increase in life expectancy for men from 1960-2004 is presented for some countries[1, 2, 3]. For women, the increase in life expectancy during that period was somewhat less (results not shown)[1,2,3]. If the current trend of life expectancy increases continues, the average life expectancy may become 100 years by 2070[1]. The average life expectancy for some European countries in 2005 is given in table 1. The highest life expectancy at birth for men is seen in Switzerland (78.7) and for women in Switzerland (83.9) and Spain (83.8) while Hungary has the lowest life expectancy for men (68.6) and women (76.9)[4].

Graph 1: Life expectancy for men, 1960-2004, by country[3,4].



Not all years of a person's life are lived in perfect health. Chronic diseases, frailty and disability tend to become more prevalent at an older age, so a population with a higher life expectancy may not be healthier[2].

Therefore, besides the life expectancy at 65 years, we presented also the healthy life expectancy (life expectancy without disability) at 65 years[4]. At this age, the average life expectancy for men is 15.7 years, while the average healthy life expectancy for men is only 9.2 years. For women, the average life expectancy at age 65 is 19.2, while the average healthy life expectancy is 10.2 years[4].

Before 1950 most of the gain in life expectancy was due to a reduction in premature deaths. In recent decades it has been due to an improvement in the survival of people over 65.The higher life expectancy together with the low birth rates is transforming the structure of the European Union's age pyramid to an aging population[2]. The percentage of the population aged 65 and over, which started to rise sharply in the last decades of the past century, is continuing to rise.

Table 1: Life expectancy and healthy life expectancy in Europe

	Life expectancy at birth		Life expectancy at 65		Healthy Life expectancy at 65	
Country	Men	Women	Men	Women	Men	Women
Belgium	76.7	82.4	15.8	19.7	11.7	12.6
Denmark	75.6	80.2	15.5	18.6	8.4	9.9
Finland	75.5	82.3	15.8	19.6	6.5	7.1
France	76.7	83.8	-	-	8.2	8.9
Greece	76.6	81.5	16.7	18.7	9.9	10.5
Hungary	68.6	76.9	13.0	16.9	6.1	7.2
Italy	77.6	83.2	16.5	20.4	11.9	14.4
The Netherlands	77.2	81.6	15.8	19.5	9.2	9.5
Portugal	74.9	81.4	15.6	18.9	8.4	7.7
Spain	77.4	83.9	16.8	20.7	11.3	12.5
Switzerland	78.7	83.9	17.5	21.0	-	-

Source: Eurostat, OnLine Database

The countries with the highest proportion of persons aged 65 in 2005 were Italy (19%) and Greece (18%), while the lowest were Ireland (11%), Cyprus and Slovakia (both 12%). Projections for 2050 indicate that, in the European Union, the number of persons aged 65 and over might rise from 75 million in 2005 to nearly 135 million in 2050 (1995: 66 million)[5].

With the increase in life expectancy in the first part of the 20th century, the leading causes of death have shifted dramatically from infectious diseases to noncommunicable diseases and from younger to older individuals. In industrialized countries, about 75% of deaths in persons older than the age of 65 are now from cardiovascular diseases and cancer. Regardless of predisposing factors, diet, lifestyle and biological factors influence morbidity and mortality during the course of life[6]. Because of the cumulative effect of adverse factors throughout life, it is important to advise older persons to adopt diet and lifestyle practices that minimize their risk of death from morbidity and maximize their prospects for healthful aging[6]. However, up till now, little is known about dietary, lifestyle and biological factors in elderly in relation to survival. Therefore, the aim of this thesis was to investigate the associations between dietary patterns, biological risk factors and survival. We used data from two prospective studies in elderly; the Healthy Aging: a longitudinal study in Europe project, and the Normative Aging Study to obtain more insight in these associations. In this chapter, we will first give a short description of the studies we used to investigate the associations between dietary patterns, biological risk factors and survival. In the following paragraphs, we will describe how we evaluated dietary intake in relation to biological risk factors and the associations between

dietary patterns and diet-related biological risk factors in relation to survival in the elderly.

1.2 Study populations

1.2.1 The HALE project

The aim of the HALE project was to study changes in and determinants of usual and healthy aging in terms of morbidity and mortality outcomes as well as in terms of physical, psychological, cognitive, and social functioning in 13 European countries. The HALE project included participants of the Survey in Europe on Nutrition and the Elderly: a Concerted Action (SENECA) and Finland, Italy, the Netherlands, Elderly (FINE) studies who were examined in 1988-1991 and were followed up for 10 years. The SENECA Study started in 1988 and consisted of a random age- and gender stratified sample of inhabitants, born between 1913 and 1918, in 19 European towns. In the HALE project, 13 centers, which carried out a 10-year mortality follow-up, were included[7, 8]. The original participation rate in the centers varied from 37 % to 81 %[9]. At baseline, 1162 men and 1173 women participated in the SENECA study. The surveys were repeated 1993 and 1999. The response rates were 68 % in 1993 and 55 % in 1999. All men and women of the following towns were included: Hamme, Belgium; Roskilde, Denmark; Strasbourg, France; Valence, France; Iraklion, Greece; Monor, Hungary; Padua, Italy; Culemborg, the Netherlands; Vila Franca de Xira, Portugal; Betanzos, Spain; and Yverdon, Burgdorf, and Bellinzona, Switzerland.

The FINE Study consists of the survivors of 5 cohorts of the Seven Countries Study[10, 11]; Ilomantsi, East Finland; Poytya, and Mellila, West Finland; Crevalcore and Montegiorgio, Italy; and Zutphen, the

Netherlands. The FINE Study, which started in 1984 and continued to 2000, recruited men who were born between 1900 and 1920. In the FINE Study, 2284 men were enrolled, with participation rates of 92 % in Finland, 76 % in Italy, and 74 % in the Netherlands[11]. Surveys were repeated in the years 1989-1991, 1994-1995 and 1999-2000. The mortality follow-up is completed till 2000. For the HALE project, we used the 1989-1991 measurements of the FINE Study as baseline measurements.

1.2.2 The Normative Aging Study

The Normative Aging Study (NAS) is a longitudinal study on aging[12, 13]. Between 1961 and 1970, 2280 male volunteers were recruited around Boston; aged 21 till 81 years (mean 42 years). Participants were invited every 3-5 years to complete a health examination and several questionnaires. Almost 98% of subjects were white. Volunteers were excluded if they had initial histories or evidence of hypertension, diabetes mellitus, cardiovascular diseases, cancer or other serious diseases. Dietary intake data have been collected since 1987 and assessment of biomarkers was added in 1993.

1.3 Diet and diet-related health status

1.3.1 Dietary patterns

A substantial increase in people aged 65 years and older is expected[1]. Although the exact mechanisms underlying normal ageing are not fully understood, ageing is generally associated with an increase in chronic diseases, such as cardiovascular diseases, type 2 diabetes, and cancer.

Evidence is accumulating that these diseases are preventable, and that the occurrence of these diseases can be postponed by modifying dietary and lifestyle factors[6, 14, 15]. Traditional analyses in nutritional epidemiology typically examine the associations between a single or a few nutrients or foods and chronic diseases[16, 17]. Although this type of analysis is valuable, it has several conceptual and methodological limitations[16, 17, 18]. First, people do not eat isolated nutrients. Instead, they eat meals consisting of a variety of foods with complex combinations of nutrients that are likely to be interactive or synergistic. The `single nutrient' approach may be inadequate for taking into account complex interactions among nutrients in studies of free-living people (e.g. enhanced iron absorption in the presence of vitamin C)[16]. Second, the high level of intercorrelation among some nutrients (such as potassium and magnesium) makes it difficult to examine their separate effects, because the degree of independent variation of the nutrients is markedly reduced when they entered a model simultaneously. Third, the effect of a single nutrient may be too small to detect but the cumulative effects of multiple nutrients may be sufficiently large to be detectable[18]. Therefore, a current trend is to study dietary patterns that overcome this problem[16-20].

A pattern analysis examines the effects of an overall diet. Conceptually, dietary patterns represent a broader picture of food and nutrient consumption, and may thus improve the prediction of disease risk compared to individual foods or nutrients. Two different approaches have been used to derive dietary patterns: theoretically or ‘a priori’ defined dietary patterns and empirically or ‘a posteriori’ derived dietary patterns. The first involves dietary quality indices to evaluate whether adherence to a certain dietary pattern (e.g. Mediterranean pattern) or current dietary

guidelines lowers the risk of disease. The second approach involved statistical exploratory methods as factor analysis, cluster analysis, principal component analysis, and reduced rank regression to accomplish pattern derivation[16, 19, 20].

1.3.2 Dietary patterns and biological risk factors

Dietary patterns derived by cluster and factor analyses or reduced rank regression have been associated with biological risk factors, morbidity and mortality[21-43]. Studies have shown that dietary patterns derived by cluster or factor analysis were associated with serum carotenoids, folate, serum concentrations of insulin, C-peptide, leptin, homocysteine, blood pressure, plasma glucose, cholesterol concentrations, coronary heart disease, cardiovascular diseases, cancer, and all-cause and cause-specific mortality [22,24,34,36,37,42]. Newby et al. compared factor and cluster analyses in their study. They found that cluster and factor analysis were comparable in relation to plasma lipid biomarkers[34].

As far as we know, no study investigated the effect of an intervention in relation to dietary patterns, derived by cluster or factor analysis.

In 1996, the US Food and Drug Administration issued a regulation requiring that all enriched grain products be fortified with 140 µg of folic acid /100 g of cereal-grain product to reduce the risk of neural-tube defects[44-47]. The prevalence of deficient plasma folate and, to a lesser extent, of elevated total plasma homocysteine, has been reduced after this fortification[46, 47]. However, to our knowledge, the effect of folate fortification has not yet been investigated in relation to dietary patterns. Individuals with certain dietary patterns may differ from others in response to the fortification. For example, individuals with an unhealthy

dietary pattern may have greater benefit from folate fortification than individuals with a healthy dietary pattern, as they are more likely to consume the refined grains that were fortified. Therefore, we investigated the difference in B-vitamin intake, and in plasma B vitamin and homocysteine concentrations before and after folic acid fortification, within dietary patterns derived by cluster analysis, in elderly men participating in the Normative Aging Study.

1.3.3 Dietary patterns and mortality

In the USA and Europe, different dietary quality indices were developed based on dietary guidelines or food-based scores; Diet Quality Index, the Healthy Eating Index, Diet Diversity Score, Dietary Variety Score, Diet Guidelines Index, Food-based quality Index, Healthy Food Index, and the Healthy Diet Indicator[8,48-55]. These dietary scores have been associated with nutrient intake, biomarkers and mortality[8, 48-55]. For example, Huijbregts et al. found an inverse significant relationship between the Healthy Diet Indicator (HDI), which is based on WHO guidelines for the prevention of chronic diseases, and 20-year all-causes mortality in men aged 50–70 years at baseline from Finland, The Netherlands and Italy[53].

In 1995, Trichopoulou et al. proposed a Mediterranean Diet Score (MDS), which measures the adherence to a traditional Greek Mediterranean diet[56]. In this study, the adherence to a Mediterranean diet was associated with a lower mortality risk in elderly of 70 years and older[56]. This diet score or a modified version was used in several studies, to investigate the association between dietary patterns and morbidity or mortality[8, 56-71]. Besides the Mediterranean Diet Score, indexes based on

the Mediterranean Diet Pyramid are proposed by Goulet et al., Panagiotakos et al., Gerber et al., and Ciccarone et al[72-74].

In 1999, Alberti-Fidanza et al. proposed the Mediterranean Adequacy Index (MAI) to assess how close a diet reflects the Italian Reference Mediterranean Diet[75, 76]. The diet consumed in Nicotera in Southern Italy, where a pilot study was carried out for the Seven Countries Study, was used as reference diet. A significant inverse correlation was found between the average population MAI and 25-year population death rates from coronary heart disease in the Seven Countries Study[75].

The relationships between different food indexes and all-cause mortality had not been investigated before. Therefore, in chapter 3, we investigated and compared the relationships between different diet scores and all-cause mortality in elderly men and women from the HALE (Healthy Ageing: a Longitudinal study in Europe) project. Only a few studies had investigated the combined effect of diet and other lifestyle factors. We investigated the association of a Mediterranean type of diet and lifestyle factors (alcohol use, smoking status, and physical activity) on mortality in both apparently healthy elderly men and women, and post-myocardial infarction patients in the HALE project in chapter 4 and 5.

1.4 Diet-related biological risk factors and mortality

Obesity and high blood cholesterol are among the major biological risk factors for noncommunicable diseases, especially cardiovascular diseases[77-79].

Obesity and overweight are a major risk for diet-related chronic diseases, including type 2 diabetes, cardiovascular diseases, and certain types of

cancer[78]. The health consequences range from a higher risk of premature death, to serious chronic conditions that diminish the overall quality of life. The prevalence of overweight and obesity is commonly assessed by using the body mass index (BMI), defined as the weight in kilograms divided by the square of the height in metres (kg/m^2) [78]. A BMI over 25 kg/m^2 is defined as overweight, and a BMI of over 30 kg/m^2 as obesity [78]. Flegal et al. estimated that in 2000 there were about 112,000 deaths in the United States could be attributed to obesity[80]. However, the strength of the association between body weight and mortality varies with age[81]. Conversely, in elderly people, a low body mass index is stronger associated with mortality risk than a high body mass index[80]. Flegal et al found that the majority of excess deaths associated with obesity occurred in individuals younger than 70 years while the majority of excess deaths associated with underweight occurred in individuals aged 70 years and older[80].

Besides underweight, unintentional weight loss is a common problem among elderly people[82]. About 15% -27 % of the elderly persons experience weight loss. Weight loss, both voluntary and involuntary, in older individuals is associated with frailty, functional impairment and even mortality[83-99]. Even small changes of 1 kg/year were found to be predictive of mortality in elderly patients recently discharged from a hospital[84].

In free-living elderly, also associations between body weight loss and mortality were found[100-113]. However, most of these studies have no or limited information at baseline and before the mortality follow-up started or do not adequately use the information about health status. To minimize the possibility that body weight had changed in response to subclinical

diseases, results have to be presented after excluding participants with chronic disease at baseline and those who died during the first period of follow-up[114]. Wannamethee et al found that the increased mortality associated with weight loss in middle-aged and older persons is a direct consequence of ill-health leading to weight loss[110, 112]. Because the associations between body weight changes and mortality are not clear in elderly, we have investigated the association between changes in measured body weight during 5 years and subsequent 10-year mortality follow-up in elderly European men, aged between 70 and 90 years at baseline, in the FINE Study, taking baseline health status into account.

Cholesterol is a key component in the development of atherosclerosis, the accumulation of fatty deposits on the inner lining of arteries[115]. High blood cholesterol causes around one third of all cardiovascular diseases worldwide[116]. In 2000, high cholesterol was estimated to cause 18% of global cerebrovascular diseases (mostly non-fatal events) and 56% of global ischaemic heart diseases. Overall this amounts to about 4.4 million deaths (7.9% of the total)[115]. Studies in adults indicate a direct relationship between total cholesterol, low-density lipoprotein cholesterol (LDL), and cardiovascular diseases while an inverse relationship between high-density lipoprotein cholesterol (HDL) and cardiovascular diseases was found[117-121]. However, the results of studies about the different cholesterol fractions and cardiovascular morbidity and mortality in elderly are inconsistent. Results of several studies showed an inverse relation, or no relation, between serum total cholesterol concentration and risk of death in elderly people[122-125]. Also the associations between the HDL-cholesterol, LDL-cholesterol and VLDL-cholesterol fractions and

cardiovascular morbidity and mortality are not yet clear in elderly. In the study of Sacco et al, increased HDL cholesterol levels were associated with lower risk of ischemic stroke in the elderly[126]. Weverling-Rijnsburger found that HDL cholesterol level but not LDL cholesterol is a risk factor for mortality from coronary artery disease and stroke in old age[127]. Tikhonoff et al also found no significant association between LDL-cholesterol and stroke mortality in subjects aged 65 and older[128]. In a study of Laakso et al, an increased level of the triglyceride-rich VLDL cholesterol was observed to be a powerful risk indicator for coronary heart disease in patients with type 2 diabetes[129].

We investigated the associations between total cholesterol, LDL, VLDL, and HDL cholesterol and mortality from cardiovascular diseases and all-causes in European men and women, aged 70-90 who were followed for 10 years in the HALE project.

1.5 Aim of the thesis

The aim of this thesis was to study the associations between dietary patterns, diet-related biological risk factors and survival in the elderly. In the first part of this thesis, we describe dietary patterns derived by cluster analyses in relation to biomarkers and dietary patterns defined by dietary indexes in relation to survival. In the second part, we report associations between diet-related biological risk factors and mortality. The two biological risk factors investigated are weight changes and blood cholesterol fractions.

Outline of the thesis

In chapter 2, we investigated the associations between dietary patterns and the intake of B-vitamins; and between plasma B-vitamins and homocysteine concentrations before and after folic acid fortification, in the Normative Aging Study. Chapter 3 describes the associations between different dietary indexes and all-cause mortality in the HALE project. We report the associations between a Mediterranean type of diet, lifestyle and mortality in apparently healthy elderly in chapter 4, and in post-myocardial infarction patients in chapter 5. The relations between body weight changes and mortality were investigated in the FINE Study and presented in chapter 6. The results of the associations between different blood cholesterol fractions and mortality from cardiovascular diseases and all-causes in the HALE project are described in chapter 7.

In chapter 8, the main findings of our studies are summarized and discussed.

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2

Do dietary patterns in older men influence change in homocysteine through folate fortification? The Normative Aging Study

Knoops K, Spiro 3rd, de Groot L, Kromhout D, van Staveren W, Tucker K
submitted

Abstract

Background: In 1996, the Food and Drug Administration issued a regulation requiring all enriched grain products to be fortified with folic acid to reduce the risk of neural-tube defects, but it remains unclear how this fortification has affected folate and homocysteine status in subgroups of the population with differing dietary patterns.

Objective: We aimed to describe the difference in B-vitamin intake; and in plasma B vitamin and homocysteine concentrations before and after folic acid fortification, in relation to dietary patterns.

Methods: Homocysteine, folate, vitamin B-12 and pyridoxal-5-phosphate (PLP) were assessed before and after fortification in men participating in the Veterans Affairs Normative Aging Study. We derived dietary patterns from cluster analysis.

Results: Three dietary patterns were identified by cluster analysis: a prudent pattern, with relatively high intake of fruit, vegetables, low-fat milk and breakfast cereals; an unhealthy pattern, with high intake of baked products, sweets and added fats; and a low fruit and vegetable but relatively high alcohol intake pattern. Dietary intake and plasma concentrations of folate increased significantly ($P<0.05$) among all dietary patterns. Homocysteine tended to decrease in non-supplement users and in subjects in the high alcohol, low fruit and vegetable dietary pattern (both, $P=0.08$).

Conclusions: After fortification with folic acid, folate intake and plasma folate concentration increased significantly in all dietary patterns. There was a trend toward greatest homocysteine lowering in the high alcohol and low fruit and vegetable group.

Introduction

Elevated plasma homocysteine has been associated with increased risk of cardiovascular diseases, cognitive decline, hip fractures, and mortality[1-7]. Supplements containing folic acid have been shown to effectively reduce homocysteine in subjects with normal and elevated baseline concentrations[8]. Consumption of fruit and vegetables has been negatively associated, while coffee drinking and alcohol consumption have been positively associated with homocysteine concentration[9-10]. Because of high collinearity among nutrients and food groups, there is increasing interest in investigation of dietary patterns rather than single foods or nutrients in relation to homocysteine[11]. In one study of Chinese adults, a dietary pattern high in refined cereal grains was associated with a high prevalence of elevated plasma homocysteine and low B vitamin concentrations, whereas those following a pattern high in fruit and milk had the lowest plasma homocysteine and highest B vitamin concentrations[11]. In a study of Irish men, a "prudent" dietary pattern, derived by cluster analysis, was associated with a lower homocysteine concentration[12]. Fung at al. found that a prudent dietary pattern, derived by factor analysis, was positively correlated with plasma folate and inversely with homocysteine concentration in a large US cohort[13].

In 1996, the US Food and Drug Administration issued a regulation requiring that all enriched grain products be fortified with 140 µg of folic acid /100 g of cereal-grain product to reduce the risk of neural-tube defects[14-17]. The prevalence of deficient plasma folate and, to a lesser extent, of elevated total plasma homocysteine, has been reduced after this fortification[15,16]. However, to our knowledge, the effect of folate

fortification has not yet been investigated in relation to dietary patterns. Individuals with certain dietary patterns may differ from others in response to the fortification. For example, individuals with an unhealthy dietary pattern may have greater benefit from folate fortification than individuals with a healthy dietary pattern, as they are more likely to consume the refined grains that were fortified. Therefore, we investigated the difference in B-vitamin intake, and in plasma B vitamin and homocysteine concentrations before and after folic acid fortification, within dietary patterns derived by cluster analysis, in elderly men participating in the Normative Aging Study.

Methods

Subjects

The Normative Aging Study (NAS) is a longitudinal study which started in 1963 by recruiting men, 21 to 80 years of age, and free if heart disease and other major health problems, around Boston. Details of the study are described elsewhere[5,18]. Briefly, participants were invited every 3-5 years to complete a health examination and several questionnaires. Dietary intake data have been collected since 1987 and assessment of plasma B vitamins and homocysteine were added in 1993. For this study, we included men who had completed at least one food frequency questionnaire (FFQ) and who had at least one measurement of homocysteine concentrations, both before (prior to October 1, 1996) and after (since August 31, 1997) the fortification period. This resulted in 354 men.

Dietary intake

Dietary intake was assessed with a semi-quantitative FFQ, which requests that participants record the number of times they consume each of 126 food items[5]. The 126 food items were converted into 23 food groups; regular milk and dairy products, low-fat milk and dairy products, fruit, vegetables, sweets, meat, poultry, added fats, legumes, eggs, fish, grains, bread and baked goods, breakfast cereals, potatoes, snacks, tea and coffee, alcohol, other drinks, nuts, and soups.

For subjects with more than one FFQ before or after fortification, the average frequencies of consumption for each food group were calculated, and these averages were used in the cluster analyses to best estimate usual intakes. The average numbers of FFQs for each subject were 2.5 ± 0.8 before and 1.6 ± 0.5 after fortification. To obtain more stable dietary patterns, cluster analyses were performed on all available dietary data, using the average of all available FFQs for each subject in the larger study before and after fortification (n=2263).

Blood collection and analysis

Fasting plasma was drawn at the Veteran's Administration site and stored at -80°C. Samples were transferred on dry ice to the Jean Mayer US Department of Agriculture Human Nutrition Research Center on Aging, where they were again stored at -80°C [5]. Total homocysteine in plasma was measured using an adaptation of the method described by Araki and Sako[19]. The CV for this method in our laboratory is 4.0 %[5]. Plasma folate and vitamin B-12 concentrations were measured by radioassay with a commercially available kit from Bio-Rad (Hercules, CA). The CV's for

these assays are 4.7 % for vitamin B-12 and 4.3 % for folate[5]. PLP was measured enzymatically by tyrosine decarboxylase20. The CV for this assay is 5.0 % in our laboratory[5]. For most of the subjects, only one homocysteine measurement was available before fortification (mean number of measurements was 1.08, with SD=0.23). Therefore, we used the first measurement of blood before (time 1) and after (time 2) fortification for each of the men. The mean time between these two blood collections was 3.1 y (SD 0.5 y).

Assessment of other variables

Date of birth, information about smoking, and education (y) was collected by questionnaire at time 1. Measurement of BMI (Kg/m^2), waist/hip ratio, serum creatinine (mg/dl), and systolic blood pressure were available for all subjects at time 1.

Statistical analysis

Statistical analyses were performed using SAS 9.1 (SAS Institute, Cary, NC). Cluster analysis was used to derive dietary patterns. We removed subjects who consumed any of the food items at ≥ 5 SD from the mean for this item (n= 171, 8 % of total FFQs). Cluster analysis was performed using FASTCLUS in SAS (version 9.1). We ran the clustering procedure with a varying number of clusters (3-6).

Mean age, BMI, waist/hip ratio, waist circumference, creatinine, alcohol intake and energy intake were calculated and compared across the clusters at baseline with the general linear models (GLM) procedure in SAS, using Tukey's adjustment for multiple comparisons. Smoking status was compared across the three clusters with the Chi-Square

statistic. Because there were no strong violations against normality, the untransformed variables were used in the analyses. Differences between pre and post-fortification measures of intake and plasma values of B-vitamins, and of plasma homocysteine, were calculated in the whole group, within and between supplement users and non-supplement users, and within and between dietary patterns and tested with the GLM procedure, with Tukey's adjustment for multiple comparisons. All models were adjusted for age (y), education (y), smoking status (current or former, each vs. never), plasma creatinine (mg/dl), energy intake (MJ), waist/hip ratio, and B-vitamin supplement use. All analyses were repeated for non-supplement users. BMI was tested as an additional confounder, but did not affect the results.

Results

The cluster analysis resulted in three distinct dietary patterns; one characterized by high intake of fruit, vegetables, low fat milk, breakfast cereals and fish (a prudent dietary pattern); a second by high intake of bread and baked products, coffee, added fats, sweets, regular milk and dairy, meat, soft drinks and potatoes (an unhealthy dietary pattern); and a third by low intake of fruit and vegetables and higher intake of alcohol (Table 1). Men in the prudent pattern were significantly older and less likely to smoke compared with the other patterns (Table 2).

Men in the low fruit and vegetable, high alcohol pattern reported significantly lower energy intake relative to men in the other two patterns. Over the observed time period, homocysteine concentrations decreased, although this did not reach significant for the whole group

(P=0.10). The decrease in homocysteine approached significance (P=0.08) in non-supplement users, but was not significant in supplement users. Plasma folate (P<0.0001), vitamin B-12 (P<0.05) and vitamin B-6 (P<0.0001) increased in the full group of men (Table 3) and in non-supplement users.

Table 1: Mean number of servings/d of food groups, by dietary pattern

Food groups	Prudent (n=49)	Unhealthy (n=105)	High alcohol, low fruit & vegetables (n=200)
Full fat milk and dairy	1.0 ± 1.3[1]	1.4 ± 1.3*	1.1 ± 1.1
Low fat milk and dairy	1.1 ± 1.1*	0.7 ± 0.9	0.6 ± 0.8
Fruit	4.1 ± 1.7*	2.3 ± 1.3	1.9 ± 1.0
Vegetables	4.3 ± 1.5*	2.1 ± 0.9	2.0 ± 1.0
Sweets	1.0 ± 1.0	2.8 ± 2.5*	0.8 ± 1.0
Meat	0.7 ± 0.7	1.1 ± 0.7*	0.8 ± 0.5
Poultry	0.4 ± 0.3	0.3 ± 0.3	0.3 ± 0.2
Fats	1.7 ± 0.9	2.8 ± 1.6*	1.6 ± 1.0
Legumes	0.4 ± 0.3*	0.2 ± 0.2	0.2 ± 0.2
Eggs	0.2 ± 0.2	0.3 ± 0.2	0.2 ± 0.2
Fish	0.6 ± 0.4*	0.4 ± 0.3	0.3 ± 0.2
Grains	0.8 ± 0.7*	0.5 ± 0.5	0.5 ± 0.5
Bread and baked products	2.5 ± 1.5	4.6 ± 2.1*	2.0 ± 1.1
Breakfast cereals	0.9 ± 0.6*	0.6 ± 0.5	0.5 ± 0.5
Potatoes	0.5 ± 0.3	0.6 ± 0.3	0.4 ± 0.2
Snacks	0.6 ± 0.5	0.6 ± 0.6	0.5 ± 0.4
Drinks	0.8 ± 0.9	0.9 ± 1.3*	0.7 ± 0.8
Tea and Coffee	2.2 ± 1.4	3.3 ± 1.7*	2.2 ± 1.4
Alcohol	0.8 ± 0.9	1.0 ± 1.4	1.3 ± 1.5*
Nuts	0.4 ± 0.4	0.5 ± 0.6	0.3 ± 0.3
Soups	0.8 ± 1.5*	0.4 ± 0.8	0.3 ± 0.7

[1] Mean ± SD, * ≥ 0.2 serving/d more than other two groups.

Among supplement users, only plasma folate increased significantly. At the same time, dietary intake from foods (including fortified foods) increased significantly only for folate (P<0.0001). Dietary intake of vitamin B-12 decreased significantly in the whole group and in non-supplement users, while intake of B-6 tended to decrease in supplement users (P=0.08). Baseline plasma folate, vitamin B-12 and vitamin B-6 were each significantly higher and homocysteine concentrations lower in supplement users vs. non-supplements users (P <0.05- P<0.0001). Before fortification, homocysteine concentrations appeared to be lower, and plasma B vitamin concentrations higher, in the prudent pattern compared with the "unhealthy" and low fruit and vegetable, high alcohol patterns, although these differences were not significant (Table 4).

Table 2: Characteristics of participants in the Normative Aging Study by cluster at baseline

	Prudent (n=49)	Unhealthy (n=105)	High alcohol, low fruit & vegetables (n=200)
Age (y, mean ± SD)	69.4 ± 5.2	66.4 ± 6.4*	66.0 ± 6.6**
Smoking (%):			
Current	2	7	7
Former	57	69	57
Never	41	25	37
BMI (Kg/m^2, mean ± SD)	27.9 ± 3.9	27.2 ± 3.3	27.9 ± 3.6
Waist/hip ratio (mean ± SD)	0.99 ± 0.06	1.00 ± 0.05	1.00 ± 0.05
Plasma creatinine (µmol/L, mean ± SD)	91.7 ± 17.9	93.0 ± 17.3	90.3 ± 15.1
Energy intake, MJ/d (median, 5th – 95th percentile)	9046**** (5682-13,789)	9389**** (6274-13,590)	6938 (3838-10,091)

Significantly different than the prudent pattern, *P<0.05; **P<0.01, **** Significantly different than the high alcohol, low fruit and vegetable pattern. P<0.0001.

Dietary intakes of folate and B-6 were higher in the healthy pattern compared with the other two ($P<0.05$-$P<0.001$).

Plasma folate increased significantly in all three dietary pattern groups. Plasma vitamin B-6 increased significantly in the prudent and low fruit and vegetable, high alcohol patterns. There were no significant differences in homocysteine, although the decrease in homocysteine concentrations approached significance ($P=0.08$) in the low fruit and vegetable, high alcohol group.

Dietary intake of folate (including from fortified foods) increased significantly in all patterns, dietary (non-supplement) intake of vitamins B-12 decreased significantly in all three groups, and intake of vitamin B6 decreased significantly in the low fruit and vegetable, high alcohol pattern. However, use of vitamin supplements increased significantly over time in all clusters. When we repeated the analyses for non-supplement users, we saw similar results: plasma folate and dietary intake of folate increased significantly in all clusters. We also repeated the analyses for men who remained in the same cluster over time, and for non-supplement users. These results were similar to those described above, despite the lower number of men in each group (data not shown).

Table 3: Plasma homocysteine and B-vitamin concentrations, and B vitamin intakes, pre and post fortification in the NAS population[1].

	All men		Non-supplement users		Supplement users[2]	
n	Pre 354	Post 354	Pre 253	Post 253	Pre 101	Post 101
Plasma	Mean (SE)	Difference (SE)	Mean (SE)	Difference (SE)	Mean (SE)	Difference (SE)
Homocysteine (µmol/L)	10.7 ± 0.16	-0.38 ± 0.23	11.0 ± 0.23	-0.38 ± 0.21†	10.1 ± 0.36^{a}	-0.20 ± 0.34
Folate (nmol/L)	21.7 ± 1.0	+15.5 ± 1.4****	19.8 ± 0.74	+16.2 ± 1.8****	26.6 ± 1.19^{d}	+15.4 ± 2.9****
B-12 (pmol/L)	340 ± 8.5	+26.8 ± 12.0	325 ± 8.9	+24 ± 10.4*	376 ± 14.3^{b}	+21 ± 16.8
B-6 (PLP, nmol/L)	80.6 ± 4.9	+24.0 ± 6.9***	63.2 ± 5.3	+27.6 ± 6.0****	126.9 ± 8.4^{d}	+5.5 ± 9.8
Dietary intake (without supplements)						
Folate (µg/day)	335 ± 6.2	+100 ± 8.9****	330 ± 7.0	+90 ± 10****	348 ± 11	+92 ± 16****
B-12 (µg/day)	7.6 ± 0.2	-0.9 ± 0.3***	7.7 ± 0.3	-1.0 ± 0.3**	7.2 ± 0.5	-0.9 ± 0.5
B-6 (mg/day)	2.2 ± 0.1	+0.1 ± 0.1	2.1 ± 0.1	+0.2 ± 0.1	2.7 ± 0.2^{b}	-0.4 ± 0.2†

Significance of change:† P<0.1; * P<0.05; **P<0.01; ***P<0.001; ****P<0.0001

Significantly different than non-supplement users at baseline: ^{a} P<0.05; ^{b} P<0.01; ^{d} P<0.0001

[1]Adjusted for education, smoking, waist-hip ratio, age, plasma creatinine and (for dietary variables) energy intake.

[2] Use of supplement containing at least one of the B vitamins: folic acid, vitamin B6, vitamin B12.

Table 4: Post-pre fortification differences in plasma homocysteine, B-vitamin concentrations, and B vitamin intakes, by dietary pattern at baseline in the NAS population[1]

n	Prudent 49		Unhealthy 105		High alcohol, low fruit & vegetables 200	
Plasma[1]	Baseline Mean (SE)	Difference (SE)	Baseline Mean (SE)	Difference (SE)	Baseline Mean (SE)	Difference (SE)
Homocysteine (µmol/L)	10.2 (0.5)	-0.02 (0.50)	10.9 (0.4)	-0.22 (0.36)	10.7 (0.3)	-0.46 (0.26)†
Folate (nmol/L)	24.3 (1.8)	+16.4 (4.3)***	21.8 (1.2)	+17.2 (3.0)****	21.2 (0.9)	+15.2 (2.2)****
B-12 (pmol/L)	363 (20)	+3.7 (24.8)	358 (14)	+35.1 (17.3)*	323 (10)	+21.2 (12.7)*
B-6 (PLP, nmol/L)	88.4 (12.2)	+30.1 (14.3)*	79.4 (8.4)	+17.5 (10.1)†	80.2 (6.1)	+21.4 (7.4)***
Intake						
Folate (µg/day)	489 (17)	+82 (24)***	352 (12)^{c}	+88 (17)****	287 (8)^{c}	+94 (12)****
B-12 (µg/day)	9.2 (0.7)	-1.1 (0.8)	8.2 (0.5)	-0.5 (0.2)	6.8 (0.4)	-1.2 (0.4)**
B-6 (mg/day)	3.4 (0.3)	-0.3 ± 0.3	2.3 (0.2)^{a}	+0.2 ± 0.2	1.9 (0.1)^{b}	+0.1 (0.2)
Supplement users (%)	39	+16*	32	+19****	24	+26****

Table 4: Post-pre fortification differences in plasma homocysteine, B-vitamin concentrations, and B vitamin intakes, by dietary pattern at baseline in the NAS population (continued)[1]

Non-supplement users[2] *Plasma*	N=30		N=71		N=152	
Homocysteine (µmol/L)	10.3 (0.7)	0.1 (0.7)	11.5 (0.5)	-0.4 (0.5)	10.9 (0.3)	-0.5 (0.2)
Folate (nmol/L)	20.1 (2.0)	+19.9 (5.7)***	20.7 (1.3)	+17.1 (3.8)****	19.4 (0.9)	+15.7 (2.5)****
B-12 (pmol/L)	336 (25)	+9.1 (29.3)	343 (16)	+36.2 (19.7)†	312 (11)	+22.3 (13.2)†
B-6 (nmol/L)	63.9 (6.5)	29.2 (15.6)†	62.2 (4.2)	+31.7 (10.6)**	62.6 (2.9)	+24.7 (7.2)***
Intake						
Folate (µg/day)	476 (21)	+86.9 (29.7)**	340 (14)	+71.6 (20.5)***	289 (9.5)	+96.4 (13.8)****
B-12 (µg/day)	7.6 (0.9)	-0.44 (0.98)	8.3 (0.6)	-1.4 (0.6)*	7.2 (0.4)	-0.9 (0.4)*
B-6 (mg/day)	2.7 (0.1)	+0.22 (0.15)	2.2 (0.1)	+0.15 (0.10)	1.8 (0.1)	+0.18 (0.07)**

[1]Adjusted for education, smoking, waist-hip ratio, age, plasma creatinine, supplement use, and (for dietary variables) energy intake.
[2]Adjusted for education, smoking, waist-hip ratio, age, plasma creatinine, and (for dietary variables) energy intake.
Significance of change †P<0.1, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.
Significantly different than the prudent pattern: ^{a}P<0.05, ^{b}P<0.01, ^{c}P<0.001.

Discussion

Three dietary patterns were identified by cluster analysis in this group of men: a prudent pattern, with relatively high intake of fruit, vegetables, low-fat milk and breakfast cereals; an unhealthy pattern, with high intake of baked products, sweets and added fats; and a low fruit and vegetable, relatively high alcohol intake pattern. Following men from before to after fortification of the food supply with folic acid, we saw significant increases in dietary folate intake and plasma folate status in the whole group and in all three dietary patterns. In the low fruit and vegetable, high alcohol group, homocysteine concentration showed a tendency to decrease, but did not reach full statistical significance.

Advantages of the present study include the longitudinal design and the availability of information on measurements of dietary intake; supplement use and plasma vitamin and homocysteine measures both before and after folic acid fortification. However, this study also has some limitations. Only men were included, so no conclusions can be made for women. Dietary intake data were obtained by FFQ and patterns were based on reported number of servings per day rather than as percent of total energy. However, we adjust our models for energy intake. The reported energy intake in the low fruit and vegetable, high alcohol cluster was lower compared with the prudent and unhealthy dietary pattern groups. A possible explanation is that individuals in the low fruit and vegetable, higher alcohol cluster underreport their alcohol intake. There is some evidence that heavy drinkers are more likely to underreport their alcohol use than light drinkers[21].

Cluster analysis is a commonly used method for empirically deriving eating patterns, and is based on individual differences in mean dietary

intakes[22]. Similar patterns defined by cluster or factor analysis have been found in other studies[11-13, 23-27]. Villegas et al identified a traditional Irish diet characterized by high intake of saturated fat; a prudent diet, and an alcohol & convenience food pattern, using cluster analysis[12]. In the Health Professionals Follow-up Study, two major dietary patterns were derived by factor analysis; a prudent dietary pattern and a Western dietary[13,23]. Newby et al. found also a "healthy", a "western" and an "alcohol" dietary pattern in the Swedish Mammography Cohort and in the Baltimore Longitudinal Study of Aging[26-27].

Ganji and Kafai found that folate status improved significantly and homocysteine concentrations decreased significantly in NHANES participants, after fortification with folic acid[28]. Consistent with our findings, Jacques et al[15], found a pre-post fortification increase in mean plasma folate concentrations and a decrease in homocysteine concentrations among Framingham Heart Study participants who did not use vitamin supplements. Among vitamin supplement users, mean plasma folate also increased, but no change in homocysteine concentrations were observed. Other studies showed an increase in folate concentrations after fortification, but homocysteine concentrations were not reported in these studies and supplement use was not taken into account[28-30]. Dietary patterns were not investigated in any of these studies.

As we hypothesized, no meaningful change in homocysteine was observed for the prudent dietary pattern. This is likely because folate status was adequate before fortification, leaving little room for improvement. Although it appeared to decrease in the other two dietary pattern groups, this only approached significance in the low fruit and

vegetable, high alcohol pattern. However, because homocysteine tends to increase with age, the observed decreases could be considered conservative, as prevention of an increase may be included. Further, it is likely that the observed decreases would have been significant with larger sample sizes.

The increased use of supplements from before to after folic acid fortification is the most likely explanation for the increases in B12 and B6-vitamin status. After excluding men who were taking vitamin supplements, increases in plasma PLP and plasma B-12 were smaller. In one other study, serum vitamin B12 concentrations were also seen to increase significantly in the US population between the pre and post folic acid fortification periods in elderly persons[31].

Folic acid fortification was undertaken to reduce the risk of neural-tube defects, but it has been hoped that it would also have a beneficial effect on vascular diseases, cognitive function and even mortality, because of the association between inadequate folate intake and higher circulating homocysteine concentrations [1-7, 14, 17]. However, we found that the pre-post fortification decrease in homocysteine was small, despite greater use of supplements as well as fortification over this time period. Andersen et al., saw larger decreases in homocysteine concentrations (from 13.8 to 12.3 μmol/L, $p< 0.001$) after folic acid fortification but the effect on mortality was minor and, likely attributable to other factors[32]. Two recent meta-analyses showed that a 25 % reduction of plasma homocysteine was associated with an 11-16 % decrease in risk of ischemic heart disease and a 19-22 % decrease in risk of stroke[3, 33]. However, thus far, most studies have focused on the homocysteine-lowering effects of folate[5, 25]. Recently, benefits of folate independent of homocysteine lowering have

been reported[32-38]. For example; Voutilainen et al. showed that moderate-to-high serum folate concentrations were associated with a greatly reduced incidence of acute coronary events[37]. More studies are needed to investigate whether this level of fortification will influence cardiovascular morbidity and mortality.

In conclusion, dietary intake of folate and plasma folate increased significantly in this sample of men after fortifying enriched cereal-grain products with 140 µg/100 g, and in all subsets examined, including supplement users and non-users, and all dietary pattern groups. However, improvements in homocysteine were small and approached significance only in the low fruit and vegetable, high alcohol group. Larger studies are needed to get more definitive assessments of the effects of folic acid fortification on differing subsets of the population.

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3

Comparison of Three Dietary Scores in Relation to 10-year Mortality in Elderly European Subjects: The HALE-Project

Knoops K, de Groot L, Fidanza F, Alberti-Fidamza F, Kromhout D, Staveren W
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Abstract

Objective: To investigate and compare the associations between dietary patterns and mortality using different European indexes of overall dietary quality.

Design, Setting and Participants: The HALE (Healthy Ageing: a Longitudinal study in Europe) population includes 2068 men and 1049 women, aged between 70 and 90 years of 10 European countries. Subjects were followed for 10 years. This cohort study was conducted between 1988 and 2000.

Results: During the follow-up period, 1382 people died. The Mediterranean Diet Score (MDS) (HR: 0.82 with 95% CI: 0.75–0.91), the Mediterranean Adequacy Index (MAI) (HR: 0.83 with 95% CI: 0.75–0.92) and the Healthy Diet Indicator (HDI)(HR: 0.89 with 95% CI: 0.81–0.98) were inversely associated with all-causes mortality. Adjustments were made for age, gender, alcohol consumption, physical activity, smoking, number of years of education, body mass index, chronic diseases at baseline and study centre.

Conclusions: The MDS, the MDI and the HDI were significantly inversely related with mortality.

Introduction

During the previous decades most studies on the relationship between diet and mortality investigated the role of single nutrients, foods, or food groups[1-3]. However, it is often difficult to separate the specific effects of nutrients or food groups because of the highly interrelated nature of dietary exposures[4]. A current trend is to study also dietary patterns that overcome this problem. Two different approaches have been used to derive dietary patterns: theoretically or 'a priori' defined dietary patterns and empirically or 'a posteriori' derived dietary patterns. The first involves use of diet-quality scores based on recommended diets or dietary guidelines, while the second approach involved statistical exploratory methods as factor analysis, cluster analysis, principal component analysis, and reduced rank regression to accomplish pattern derivation[4, 5, 6]. In Europe, also different diet-quality scores are used to investigate the association between dietary patterns and mortality risk. Trichopoulou et al. proposed a Mediterranean Diet Score (MDS), which measures the adherence to a traditional Greek Mediterranean type of diet[7-10]. In Greek adults aged between 20 and 86 years, a higher degree of adherence to the Mediterranean diet was associated with a lower total mortality risk[8].

Alberti-Fidanza et al. proposed the Mediterranean Adequacy Index (MAI) to assess how close a diet reflects the Italian Reference Mediterranean Diet[11]. The diet consumed in 1957–1960 by men of the Nicoterra cohort (Calabria, Italy) of the Seven Countries Study (SCS) was selected as reference diet[11-13]. Results of cohort studies on the associations between the MAI and mortality have not been published yet.

However, a significant inverse correlation was found between the MAI and 25-year population death rates from coronary heart disease in the SCS[11].

Huijbregts et al. found an inverse significant relationship between the Healthy Diet Indicator (HDI), which is based on WHO guidelines for the prevention of chronic diseases, and 20-year all-causes mortality in men aged 50–70 years at baseline from Finland, The Netherlands and Italy[1].

Besides these indexes based on the Mediterranean diet or dietary guidelines, two food-based scores were developed in Europe: the Food-based quality Index[14] and the Healthy Food Index[15]. Also, in the USA, different diet-quality scores were proposed; for example, the Diet Quality Index[16], the Healthy Eating Index[17, 18], the Dietary Guidelines Index[19].

Hypothesis-based (i.e. 'a priori') scores seem preferable to assess the adherence to a Mediterranean food pattern or even to be used in future large trials, but 'a posteriori' scores derived from cluster or factor analysis have also been used[15, 20, 21]. However, the relationships between different food indexes and all-causes mortality in the same population have not been investigated.

The aim of the present study is to investigate and compare the relationships between different diet scores and all-causes mortality in elderly men and women from 10 European countries in the HALE (Healthy Ageing: a Longitudinal study in Europe) population. Because we want to evaluate the association between diet scores and mortality in a European population, we only considered European scores, for example, the MDS, the MDI and the HDI. We excluded food-based scores, because different foods are consumed in different parts of Europe.

Material and Methods

3

Study

The HALE project included subjects of the SENECA (Survey in Europe on Nutrition and the Elderly: a Concerned Action) and FINE (Finland, Italy, The Netherlands, Elderly) study who were examined in the period 1988–1991 and who were followed for 10 years[22]. Details about the SENECA and FINE Study have been described elsewhere and are briefly summarized here[22-25].

The SENECA study started in 1988 and consisted of a random age–sex stratified sample of elderly men and women, born between 1913 and 1918, from 19 European towns. Surveys were repeated in 1993 and 1998/1999. In the HALE project, men and women of the 13 centres that carried out a mortality follow-up were included: Hamme (Belgium), Roskilde (Denmark), Strasbourg (France), Valence (France), Iraklion (Greece), Padua (Italy), Culemborg (The Netherlands), Vila Franca de Xira (Portugal), Betanzos (Spain), Yverdon, Burgdorf and Bellinzona (Switzerland).

The FINE study consisted of the survivors of five cohorts of the Seven Countries Study: East Finland (Ilomantsi), West Finland (Pöytyä and Mellilä), Crevalcore and Montegiorgio in Italy and Zutphen in the Netherlands. The FINE study included men who were born between 1900 and 1920. Data of the surveys carried out around 1990, 1995 and 2000 were included in the HALE project.

Data collection

In both studies, food intake data were collected by trained dieticians using a dietary history method[23, 26]. This method provides information

about the usual food consumption of the elderly during 1 month before the interview in SENECA, and 2–4 weeks in FINE. The central part of the dietary history was the same for both studies. The coding was based on frequency tables, which were the same in both studies, but in SENECA, the start of the dietary interview about usual food patterns was based on a food record and on an oral interview in FINE. Both methods were validated[23, 27]. Food consumption data were arranged into food groups following the EUROCODE classification system[28].

Information on alcohol consumption, physical activity, smoking, education (number of years) and the prevalence of myocardial infarction, stroke, diabetes and cancer was collected by questionnaires in a general interview. The prevalence of chronic diseases was confirmed by general practitioners and/or hospital registers in the FINE study but not in the SENECA study. Information on vital status was collected in 1999–2000 and was available for 99.6% of the subjects.

In the SENECA Study, the Voorrips questionnaire was used to obtain information on habitual physical activity, and in FINE, the Morris questionnaire[29, 30]. Both questionnaires were developed for retired elderly subjects and are focused on leisure time activities such as walking, cycling and gardening and included also household activities in the SENECA Study. To divide subjects in an inactive and active group, sex-specific tertiles were composed for the Voorrips and Morris questionnaire. Subjects with a score in the intermediate and the highest tertile were considered as physically active[22].

When alcohol was not included in the diet score, alcohol was divided into two categories: 0 g alcohol a day and more than 0 g alcohol a day[22]. Based on literature, alcohol consumption was initially divided into three

groups: 0 g alcohol a day, 1–29 g alcohol a day and 30 g or more gram alcohol a day[31]. The Kaplan–Meier survival curves of the three alcohol groups showed no difference in survival from all-causes mortality between subjects who consumed between 1–29 g alcohol a day and subjects who consumed 30 g or more alcohol a day. Therefore, we divided alcohol consumption in two groups: abstainers and users[22].

For smoking, subjects were divided into two groups: never smokers or past smokers more than 15 years ago and current smokers or past smokers for 15 years or less because the risk of mortality declines after cessation of smoking and after 10–15 years, it approximates the level of those who have never smoked[32-34].

Body weight and height were measured at baseline[24, 31, 32]. Standing weight was measured to the nearest 0.1 cm and body weight was measured to the nearest 0.5 kg. Calibrated scales were used. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). In the analysis[37], BMI was dichotomized as a BMI index less than or equal to 27 kg/m^2 and a BMI higher than 27 kg/m^2.

Diet scores

Three different diet scores were composed: the MDS, the MAI and the HDI [1, 7, 8, 11-13].

The original MDS of Trichopoulou et al. was composed of the following eight components: ratio of monounsaturated to saturated fatty acids, alcohol, legumes, cereals, fruit, vegetables, meat and meat products, and dairy products[7]. We replaced the group 'legumes' by the group 'legumes/nuts/seeds' and the group 'vegetables' by the group 'vegetables and potatoes' and 'meat and meat products' by 'meat and poultry' because

these food groups were combined in the used European classification system (EUROCODE)[28]. Following the revised MDS of Trichopoulou et al., we added fish to the MDS[8]. Because the component fish was added, the MDS consisted of nine items. Intake values were adjusted to daily intakes of 10.5 MJ (or 2500 kcal) for men and 8.5 MJ (2000 kcal) for women[7]. The sex-specific median intake values were taken as cutoff points. The diet score varied from 0 (low quality diet) to 9 (high quality diet). For the components ratio between monounsaturated fatty acids and saturated fatty acids, fruits and fruit products, vegetables/potatoes, legumes/nuts/seeds, fish and cereals, a value of 1 was assigned to persons whose consumption was equal to or higher than the sex-specific median value, and 0 to the others. For meat and meat products and dairy products, a value 1 was assigned to persons whose consumption was less than the sex-specific median, and 0 to the others[7]. The MDS was also computed without alcohol and consisted of eight components[22].

In the original MAI index, the food groups expressed as percentage of total daily energy intake were divided in Mediterranean (bread, cereals, legumes (raw-dry or raw-fresh), potatoes, vegetables, fruit, fish, vegetable oils and red wine) and non-Mediterranean food groups (milk, cheese, meat, eggs, animal fats and margarines, cakes, pies and cookies, and sugar)[11-13]. The MAI index is computed by dividing the sum of the Mediterranean food groups by the sum of the non-Mediterranean food groups. In the EUROCODE system, the food group vegetable oils and margarines were combined[28]. Therefore, we replaced vegetable oils by the intake of monounsaturated fatty acids and replaced the animal fats and margarines by saturated fatty acids.

The MAI was calculated as

$$MAI = \frac{Cereals + Legumes + Fruit + Vegetables + Potatoes + Fish + mufat + wine}{Milkproducts + Meat + Poultry + Eggs + Sugar + Saturatedfat}$$

These intake values were adjusted to daily intakes of 10.5 MJ (or 2500 kcal) for men and 8.5 MJ (2000 kcal) for women instead of calculating the food groups as the percentage of daily energy because these data were not available for each country[13]. In the MAI without alcohol, the consumption of wine was excluded.

The HDI proposed by Huijbregts et al. was calculated using the dietary guidelines for the prevention of chronic diseases, defined by the World Health Organization[1]. If a person's intake was within the recommended intervals of the guidelines of the WHO, the person scored one point on that item, if the intake was outside this interval, the person scored zero on that item.

The following guidelines were taken into account: <10 percent energy intake from saturated fatty acids, 3–7% energy intake from polyunsaturated fatty acids, 10–15% energy intake of proteins, 50–70% energy intake of complex carbohydrates, 27–40 g of fibers a day, more than 400 g of fruit and vegetables a day, more than 30 g of legumes, nuts and seeds a day, <10% energy from monosaccharides and disaccharides a day, and <300 mg cholesterol a day. In total, a person could obtain nine points on the HDI[1].

The median of each diet score was calculated. Subjects with a score equal or higher than the median were considered as having a healthy diet;

subjects who had a score below the median were considered as having an unhealthy diet[22] (reference group).

Besides the relationship between the three diet scores and mortality, we investigated the effect of the components of each diet score on mortality. For the MDS and the MAI, the median of the components were used as cutoff point whereas for the HDI the WHO guidelines were used. For the healthy items, the subjects who scored zero were used as reference group, whereas for the detrimental items, subjects with a high consumption were used as reference group.

Statistical analysis

Statistical analyses were carried out using the SAS statistical analysis computer package (version 8.0). The Cox proportional hazard model was used to estimate the effect of the diet scores and the different components. The models with a diet score, which included alcohol, were adjusted for gender, age at baseline, smoking, physical activity, BMI, years of education, prevalence of myocardial infarction, stroke, diabetes and cancer at baseline, and study. A random effect of each centre was taken into account in the model[38]. The models with a diet score without alcohol were also adjusted for alcohol consumption.

The interaction of the diet scores and region was tested but was not significant at alpha equal to 0.1. We also stratified our analysis for region because several studies indicated a difference in food patterns between northern and southern Europe[39, 49] and the MAI values in the present study were higher in southern than in the northern Europe before the analyses started. The study centres of Finland, Denmark, The Netherlands, Belgium, the centre Strasbourg in France and the study

centre Burgdorf in Switzerland were considered as northern European. The study centres of Italy, Spain, Portugal and Greece, and the centre Valence in France, Yverdon and Bellinzona in Switzerland were considered as southern Europe.

To minimize the possibility that diet or lifestyle factors had changed in response to subclinical disease or morbidity, the analyses were repeated without subjects with chronic diseases at baseline (myocardial infarction, stroke, diabetes and cancer) and subjects who died during the first 2 years of follow-up[41].

Cohen's Kappa was calculated to measure the agreement between the diet scores in classifying subjects in a group with a healthy diet or unhealthy diet.

Results

Information about diet and vital status was available for 2044 men and 1049 women. In the HALE population, 55% of the men and 44% of the women lived in northern Europe. Baseline characteristics of the HALE population for northern and southern Europe are presented in Table 1. The mean age of the men in the FINE Study was higher than that in the SENECA Study, in both northern and southern Europe. Only 17% of the women in northern and 8% of the women in southern Europe were smokers or had stopped 15 years ago or less, compared with 51% of the men in northern and 48% in southern Europe. The mean Voorrips score was higher in men than in women, in both northern and southern Europe. Men and women in southern Europe were more active than their counterparts in northern Europe. Women consumed less often alcohol

than men, 47% of the women in northern and 57% of the women in southern Europe did not use alcohol compared to 27% of the men in northern and 17% in southern Europe.

Table 1: Baseline characteristics and 10-year mortality data of the HALE population

	Northern Europe			Southern Europe		
	SENECA		FINE	SENECA		FINE
	Women (n=459)	Men (n=462)	Men (n=667)	Women (n=590)	Men (n=554)	Men (N=361)
Age (years) (mean ± sd)	73 ± 2	73 ± 2	76 ± 4	73± 2	73± 2	78± 4
Never smoked or stopped > 15 years (%) (n)	83 (380)	34 (156)	59 (396)	92 (542)	50(276)	55 (198)
Mean activity score (mean ± sd) Voorrips Score Morris Score (minutes/week)	11 ± 9	16 ± 12	560 ±556	12± 10	19± 13	604 ± 651
% alcohol users (n)	53 (241)	78 (359)	70 (467)	43 (254)	80 (445)	87 (314)
Years of education (mean ± sd)	9 ± 2	10 ± 3	8 ± 5	7 ± 4	8 ± 4	5 ± 2
BMI ≤ 27 kg/m^2 (%) (n)	53(241)	64 (295)	68 (453)	59 (349)	58 (321)	60 (218)
Prevalence (%) (n)						
Coronary heart disease	15 (68)	19 (86)	15 (99)	16 (90)	15 (86)	8 (27)
Stroke	2 (10)	4 (20)	4 (29)	1 (8)	3 (16)	7 (25)
Diabetes	7 (36)	5 (25)	9 (61)	10 (59)	9 (50)	10 (36)
Cancer	2(10)	1 (5)	10 (68)	2 (12)	2 (9)	6 (23)
Causes of death, % (N)						
All deaths	30 (137)	55 (256)	60 (403)	25 (147)	47 (261)	50 (180)
Cardiovascular diseases	35 (48)	32 (81)	50 (201)	41 (61)	37 (97)	51 (91)
Cancer	18 (24)	21 (55)	29 (115)	17 (25)	25 (66)	25 (45)
Other causes	11 (16)	14 (36)	18 (73)	15 (21)	16 (41)	9 (17)
Unknown cause	36 (49)	33 (84)	3 (14)	27 (40)	22 (57)	15 (27)

The mean number of years of education was higher in northern Europe than in southern Europe. During the 10-year follow-up period, 659 (58%)

Table 2: Median (p10-p90) of the different diet scores and their components in the HALE population

	Northern Europe			Southern Europe		
	SENECA		FINE	SENECA		FINE
	Women (n=459)	Men (n=462)	Men (n=667)	Women (n=590)	Men (n=554)	Men (N=361)
Mediterranean Diet Score	4 (3-6)	5 (3-6)	4 (2-6)	5 (3-6)	4 (2-6)	5 (3-7)
Monounsaturated/saturated fat ratio[1]	0.86 (0.67-1.09)	0.93 (0.70-1.13)	0.80 (0.54-1.02)	1.12 (0.75-1.96)	1.08 (0.74-1.69)	2.4 (1.55-3.53)
Vegetables/potatoes (g/day)[1]	280 (158-481)	346 (202-529)	314 (167-509)	262 (108-505)	285 (123-528)	183 (81-324)
Fruit (g/day)[1]	214 (50-411)	176 (27-415)	205 (51-441)	302 (101-602)	280 (78-612)	251 (53-524)
Legumes/nuts/seeds (g/day)[1]	2 (0-24)	0 (0-33)	10 (0-50)	5 (0-36)	8 (0-42)	11 (0-33)
Meat and poultry (g/day)[1]	129 (77-195)	160 (108-245)	113 (49-181)	94 (47-168)	116 (55-186)	123 (59-192)
Milk and milk products (g/day)[1]	341 (116-656)	314 (96-666)	574 (185-1063)	316 (72-671)	345 (75-746)	134 (13-483)
Fish (g/day)[1]	17 (0-44)	22 (9-35)	20 (0-94)	31 (7-111)	34 (10-109)	19 (2-52)
Cereals (g/day)[1]	173 (106-255)	217 (125-333)	199 (124-294)	215 (122-317)	275 (167-419)	311 (189-423)
Alcohol (g/day)[1]	1 (0-17)	11(0-41)	3 (0-30)	0 (0-20)	20 (0-61)	36 (0-82)
Mediterranean Diet Score without alcohol	4 (2-6)	4 (2-6)	4 (2-6)	4 (2-6)	4 (2-6)	4 (2-6)
Mediterranean adequacy index	1.36 (0.75-2.57)	1.52 (0.81-2.86)	1.05 (0.52-2.04)	2.04 (0.96-1.34)	2.25 (1.09-5.59)	3.61 (1.51-8.43)
Eggs (g/day)[1]	13 (5-35)	14 (0-40)	18 (5-38)	11 (0-32)	13 (0-37)	7 (0-17)
Saturated fat (g/day) [1]	35 (26-48)	43 (30-57)	46 (34-62)	28 (15-41)	33 (17-51)	20 (14-30)
Monounsaturated fat (g/day)[1]	30 (22-40)	39 (28-51)	36 (27-47)	31 (20-46)	35 (23-50)	49 (36-64)
Sugar/sweets/cookies (g/day)[1]	39 (5-104)	47 (8-123)	74 (22-161)	24 (0-60)	28 (0-73)	30 (6-83)
Wine (g/day)[1]	0 (0-132)	0 (0-275)	0 (0-62)	0 (0-198)	188 (0-710)	328 (0-740)

Table 2: Median (p10-p90) of the different diet scores and their components in the HALE population (continued)

Mediterranean Adequacy Index without alcohol	1.30 (0.74-2.48)	1.42 (0.75-2.42)	1.00 (0.48-1.94)	1.89 (0.90-4.02)	1.83 (0.91-4.34)	2.55 (1.14-5.39)
Healthy Diet Indicator	2 (1-4)	3 (1-4)	3 (1-4)	3 (1-5)	3 (2-5)	4 (2-5)
Saturated fatty acids (% energy intake)	16 (12-22)	16 (11-21)	17 (13-23)	13 (7-19)	13 (7-19)	8 (6-12)
Polyunsaturated fatty acids (% energy intake)	6 (4-12)	7 (4-12)	6 (3-11)	5 (3-11)	4 (2-9)	3 (2-6)
Protein (% energy intake)	15 (11-20)	14 (11-18)	15 (12-19)	15 (12-20)	15 (13-20)	14 (12-17)
Complex carbohydrates (% energy intake)	22 (16-29)	23 (17-31)	24 (17-39)	26 (16-42)	28 (18-45)	39 (29-48)
Monosaccharide and disaccharides (% energy intake)	21 (14-29)	19 (13-28)	20 (8-31)	20 (11-30)	19 (11-28)	14 (8-22)
Dietary fibre (g/day)	20 (12-30)	23 (14-35)	25 (17-38)	19 (9-29)	22 (12-35)	20 (14-29)
Fruit and vegetables/potatoes (g/day)	461 (255-738)	508 (278-806)	468 (260-745)	487 (229-900)	522 (262-887)	446 (207-670)
Legumes, nuts, seeds (g/day)	2 (0-22)	0 (0-29)	9 (0-43)	4 (0-29)	7 (0-38)	10 (0-34)
Cholesterol (mg/day)	288 (176-440)	326 (206-534)	294 (169-554)	222 (124-401)	280 (148-475)	182 (108-270)

[1]grams/day, corrected for 2500 kcal/day in men, 2000 kcal in women

men and 137 (30%) women died in northern and 441 (48%) men and 147 (25%) women in southern Europe.

Table 3: Cox proportional hazard ratios (HR) of the different diet scores for 10-year mortality in elderly European men and women.

Diet scores	Total population HR (95 % CI)	Northern Europe HR (95 % CI)	Southern Europe HR (95 % CI)
Mediterranean Diet Score[2]	0.82 (0.75-0.91)[1]	0.83 (0.74-0.93)[1]	0.88 (0.78-0.98)[1]
Mediterranean Diet Score without alcohol[3]	0.78 (0.71-0.87)[2]	0.89 (0.77-1.02)[2]	0.92 (0.84-1.02)[2]
Mediterranean Adequacy Index[2]	0.83 (0.75-0.92)[1]	0.79 (0.74-0.85)[1]	0.96 (0.86-1.08)[1]
Mediterranean Adequacy Index without alcohol[3]	0.87 (0.79-0.97)[2]	0.83 (0.74-0.92)[2]	0.97 (0.86-1.10)[2]
Healthy Diet Indicator[2]	0.89 (0.81-0.98)[2]	0.93 (0.85-1.02)[2]	0.93 (0.84-1.02)[2]

[1] Subjects with a score equal or higher than the median were considered as having a healthy diet; subjects who had a score below the median were considered as having an unhealthy diet (reference group)[2] Model adjusted for age, gender, physical activity, smoking, number of years of education, BMI, chronic disease at baseline, and study centre [3] Model additionally adjusted for alcohol

In Table 2 the median for the diet scores and median intake for their different components are presented. The median for the MDS without alcohol was 4 for men and women in northern and southern Europe. The medians of the MAI with and without alcohol were higher for both men and women in southern than in northern Europe. The HDI is 1 point higher for men in FINE in southern Europe, compared with northern Europe. For SENECA men, there is no difference in HDI between northern and southern Europe. The monounsaturated/saturated fatty acids ratio, the consumption of fruit, legumes/nuts/seeds, cereals, complex carbohydrates and the alcohol consumption were higher for men and women in southern than in northern Europe. However, the consumption

of vegetables/potatoes, eggs, saturated fatty acids and sugar/sweets/cookies is higher in northern than in southern Europe.

Table 4: Cox proportional hazard ratios (HR) for the components of the different diet scores for 10-year mortality in elderly European men and women.

Component of the diet score	Total population HR (95 CI)[1]	Northern Europe HR (95 CI)[1]	Southern Europe HR (95 CI)[1]
Mediterranean Diet Score[2]			
Monounsaturated/satured fat ratio	0.89 (0.81-1.00)	1.03 (0.92-1.15)	0.77 (0.58-1.02)
Vegetables/potatoes g/day	0.99 (0.90-1.09)	0.99 (0.89-1.12)	1.05 (0.91-1.22)
Fruit g/day	0.86 (0.78-0.94)	0.98 (0.89-1.08)	0.87 (0.77-0.99)
Legumes/nuts/seeds g/day	0.95 (0.86-1.04)	1.00 (0.91-1.12)	0.99 (0.90-1.09)
Meat and poultry g/day	0.97(0.87-1.09)	1.07 (0.94-1.21)	0.92 (0.78-1.08)
Milk and milk products g/day	1.10 (1.00-1.21)	1.10 (0.94-1.29)	1.06 (0.87-1.28)
Fish g/day	0.89 (0.82-0.97)	0.89 (0.75-1.04)	0.88 (0.78-0.99)
Grains g/day	0.84 (0.77-0.92)	0.90 (0.84-0.97)	1.07 (0.92-1.25)
Alcohol	0.84 (0.77-0.92)	0.82 (0.67-0.99)	0.77 (0.64-0.94)
Mediterranean Adequacy Index without alcohol[2]			
Mediterranean components	0.86 (0.79-0.93)	0.95 (0.85-1.07)	0.83 (0.73-0.94)
Non-Mediterranean components	0.91 (0.82-1.01)	0.91(0.82-1.02)	1.00 (0.83-1.22)
Healthy diet indicator[2]			
Saturated fatty acids	1.25 (1.10-1.41)	0.98 (0.61-1.56)	1.19 (0.95-1.48)
Polyunsaturated fatty acids	1.03 (0.94-1.13)	1.04 (0.96-1.13)	0.98 (0.82-1.17)
Protein	1.00 (0.93-1.08)	0.90 (0.82-0.99)	1.13 (1.02-1.24)
Complex carbohydrates	0.93 (0.81-1.08)	1.38 (0.60-3.19)	1.02 (0.87-1.20)
Dietary fibre (g/day)	0.90 (0.81-0.99)	0.86 (0.75-0.99)	0.93 (083.-1.05)
Fruit & vegetables/potatoes	0.88 (0.78-0.99)	0.89 (0.76-1.03)	0.84 (0.68-1.05)
Legumes, nuts, seeds	1.10 (0.99-1.21)	1.19 (1.07-1.34)	1.07 (0.90-1.27)
Monosachharides and disaccharides	0.97 (0.87-1.08)	0.99 (0.89-1.11)	0.90 (0.79-1.03)
Cholesterol	0.99 (0.91-1.07)	0.89 (0.80-1.00)	0.96 (0.84-1.09)

[1]Model adjusted for age, gender, physical activity, smoking, alcohol use, number of years of education, BMI, chronic disease at baseline, and study centre. [2] The median of the items was used as cut-off point. For the healthy items, the subjects who scored zero were used as reference group, while for the detrimental items; subjects with a high consumption were used as reference group.

Table 3 presents the proportional hazard rates for the different diet scores in relation to mortality. In the total population, all diet scores were significantly related to mortality and varied between 0.78 and 0.89. In northern and southern Europe separately, all diet scores were also inversely associated with mortality, but only the MDS was significantly related to mortality in northern and southern Europe, and the MAI and MAI without alcohol in northern Europe. The association between the MAI and mortality was stronger in northern than in southern Europe. Similar results were obtained in the total population after excluding subjects with chronic diseases at baseline and subjects who died during the first 2 years of follow-up (prevalence of myocardial infarction: 454; stroke: 112; diabetes: 267, cancer: 127, number that died during the first 2 years of follow-up: 230, in total 977 subjects were excluded); MDS (HR: 0.87 with 95% CI: 0.74–1.02), MDS without alcohol (HR: 0.77 with 95% CI: 0.68–0.88), MAI (HR: 0.77 with 95% CI: 0.65–0.91), MAI without alcohol (HR: 0.85 with 95% CI: 0.72–1.01) and the HDI (HR: 0.92 with 95% CI: 0.81–1.05).

In Table 4, the Cox proportional hazard rates for the different components of the diet scores are presented. The consumption of fruit, fish, cereals, dietary fibers and the Mediterranean component of the MAI were significantly inversely related to mortality. The intake of saturated fatty acids was positively associated with mortality in the total population.

A moderate agreement was found between the MDS and the MAI (Kappa=0.45, 95% CI: 0.41–0.46) and the MDS without alcohol and MAI without alcohol (Kappa=0.49; 95% CI: 0.45–0.51). The agreement between the scores, which measure the agreement with the Mediterranean diet, and

the HDI was fair; for the HDI and the MDS, the Kappa was 0.19 (95% CI: 0.15–0.22) and 0.22 (95% CI: 0.18–0.25) for the HDI and the MAI.

Discussion

Men and women aged 70 and 90 years at baseline in the HALE population, adhering to a Mediterranean type of diet or following the dietary guidelines for preventing chronic diseases proposed by the WHO, had a significantly lower risk of all-causes mortality. Because several studies have shown that alcohol consumption is an independent factor of mortality, the MDS and the MAI were also calculated without taking alcohol consumption into account. The results for the MDS and the MAI without alcohol were similar.

In previous studies in European elderly, the lower risk in overall mortality associated with increased adherence to the Mediterranean diet was similar to that found in the present study[7, 8, 25]. Excluding subjects with chronic diseases at baseline did not change the association between the diet scores and mortality. The results of the MDS and mortality in healthy subjects in the HALE population have, recently, been published[22]. Trichopoulou et al. found similar associations between the MDS and all-causes mortality and cardiac mortality in patients with coronary heart disease[9]. This suggests that the Mediterranean diet is beneficial for elderly subjects with and without baseline chronic disease, and that the results could not be explained by residual confounding from health status at baseline.

The MDS relies on epidemiological evidence concerning the individual dietary components[7, 8]. The components of the diet score have been validated[42]. However, Trichopoulou et al. did not find strong associations for the individual components of the MDS[7-10]. Investigating the individual components of the MDS in our population, we found that the ratio of monounsaturated versus saturated fatty acids, cereals, fruit and fish were inversely associated with the risk of mortality in the whole population, but the effect was only borderline significant for the ratio of monounsaturated versus saturated fatty acids. The association between the ratio of monounsaturated versus saturated fat and mortality was stronger in southern compared with northern Europe. This may be because of a more favourable fatty acid pattern in the Mediterranean countries[43]. A protective effect of alcohol consumption was found in this study. A similar effect of alcohol was found after excluding subjects with chronic diseases at baseline, those with a low self-perceived and those who were disabled in basic activities of daily living[22]. The protective effect of moderate alcohol consumption on mortality is also confirmed in other studies[44-46].

Alberti-Fidanza et al., proposed the MAI and calculated that MAI of the Reference Italian-Mediterranean Diet of Nicoterra in 1957 was 7.5 [11-13]. In Crevalcore, the MAI was 2.9 in 1965 and 2.2 in 1991; and in Montegiorgio, 5.6 in 1965 and 3.9 in 1991, indicating that in Italy the MAI values differ in time and place. In the present study carried out around 1990, similar MAI values were found for southern Europe (ranging from 2.04 to 4.90). As expected, the MAI values were lower in northern Europe, indicating that subjects in southern Europe eat a more

Mediterranean type of diet compared with northern Europe. The association between the MAI and all-causes mortality has not been investigated earlier, but a significant inverse association was found between MAI and population mortality rates from coronary heart disease in the SCS[12]. In the present study, the MAI and the MAI without alcohol were both inversely associated with mortality.

In a previous study, a significant inverse relationship between the HDI and mortality was found in elderly men although most of the individual components of the HDI were not statistically significantly related to mortality[1]. Besides an association between the HDI and mortality, we found a significant inverse association between fibers and between fruit and vegetable intake and mortality.

Advantages of the present study carried out in 10 European countries are its great diversity in dietary patterns and lifestyle factors, its prospective nature, its large sample size, its reliance on a sample of the general population in all countries and the measurement of many potential confounders. SENECA and FINE could be pooled because the estimates of the separate models were similar and there were no important differences in study population and measurements. To be sure that the relationships between diet and mortality could not be explained by a study effect, this variable was included as potential confounder in the analyses.

The present study had also several limitations. Because the European classification system (EUROCODE)[28] was used, it was not possible to investigate the separate effects of vegetables and potatoes, of oils and margarines and of legumes, nuts and seeds. In the MDS, cereals were included as beneficially item and no distinction could be made between

highly refined cereals and unrefined cereals[47]. Dietary patterns are highly determined by cultural and genetic differences between populations in the different research centres and between northern and southern Europe. In a multi-centre study, adjustment for study centre is needed to account for factors related to the outcome, which vary from centre to centre but do not adjust for instance for unmeasurable factors as the quality of the centres' staff[38]. For this reason, a random intercept for each centre is taken into account in the adjusted models.

Although national heritage could be a confounder in our analyses, as suggested by Craighead, we did not adjust for region (northern or southern Europe) in the analysis because the variable region has a strong cultural component, which could be responsible for (a part of) the variation in dietary patterns[48, 49]. Adjustment for region would result in an overadjustment and an underestimation of the true association between diet scores and mortality.

To be sure that the associations between diet and mortality could not be explained by region, we repeated our analyses for northern and southern Europe separately. The strength of the associations between the MDS, the HDI and mortality were generally similar after stratification, although the absolute mortality risk was higher in northern than in southern Europe (Table 2). However, we found stronger associations between the MAI in relation to all-causes mortality in northern than in southern Europe. A possible explanation could be the 'arbitrary' choice of the median as cutoff point. The medians of the MAI and the MAI without alcohol were higher in southern Europe compared with northern Europe; for southern Europe, the medians were 2.33 for the MAI and 1.98 for the MAI without alcohol, while in northern Europe the medians were, respectively, 1.27

and 1.21. If we apply the cutoff points of northern Europe (respectively, 1.27 and 1.21) also in southern Europe, then the hazard rates of 0.90 with 95% CI: 0.81–1.00 for the MAI and 0.87 with 95% CI: 0.78–0.96 for the MAI without alcohol are quite similar to those in northern Europe. The medians of the MDS and the MDS without alcohol were similar for northern and southern Europe, respectively, 5 and 4. Applying these medians in northern and southern Europe gave similar results in northern and southern Europe, suggesting that this diet score is more generally applicable than the MAI.

In conclusion, the MDS, the MDI and the HDI were significantly inversely related with mortality.

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Mediterranean Diet, Lifestyle Factors, and 10-Year Mortallity in Elderly European Men and Women: The HALE-Project

Knoops K, de Groot L, Kromhout D, Perrin A, Moreiras-Varela O, Menotti A, van Staveren W

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Abstract

Context Dietary patterns and lifestyle factors are associated with mortality from all causes, coronary heart disease, cardiovascular diseases, and cancer, but few studies have investigated these factors in combination.

Objective To investigate the single and combined effect of Mediterranean diet, being physically active, moderate alcohol use, and non-smoking on all-cause and cause-specific mortality in European elderly individuals.

Design, Setting, and Participants The Healthy Ageing: a longitudinal study in Europe (HALE) population, comprising individuals enrolled in the Survey in Europe on Nutrition and the Elderly: a Concerned Action (SENECA) and the Finland, Italy, the Netherlands, Elderly (FINE) studies, includes 1507 apparently healthy men and 832 women, aged 70 to 90 years in 11 European countries. This cohort study was conducted between 1988 and 2000.

Main Outcome Measures Ten-year mortality from all causes, coronary heart disease, cardiovascular diseases, and cancer.

Results During follow-up, 935 participants died: 371 from cardiovascular diseases, 233 from cancer, and 145 from other causes; for 186, the cause of death was unknown Adhering to a Mediterranean diet (hazard ratio [HR], 0.77; 95% confidence interval [CI], 0.68-0.88), moderate alcohol use (HR, 0.78; 95% CI, 0.67-0.91), physical activity (HR, 0.63; 95% CI, 0.55-0.72), and non-smoking (HR, 0.65; 95% CI, 0.57- 0.75) were associated with a lower risk of all-cause mortality (HR's controlled for age, sex, years of education, body mass index, study, and other factors). Similar results were observed for mortality from coronary heart disease, cardiovascular diseases, and cancer. The combination of 4 low risk factors lowered the all-cause mortality rate to 0.35 (95% CI, 0.28-0.44). In total, lack of adherence to this low-risk pattern was associated with a population attributable risk of 60% of all deaths, 64% of deaths from coronary heart disease, 61% from cardiovascular diseases, and 60% from cancer.

Conclusion Among individuals aged 70 to 90 years, adherence to a Mediterranean diet and healthful lifestyle is associated with a more than 50% lower rate of all-causes and cause-specific mortality.

Introduction

The number of older people is growing rapidly worldwide. More than 580 million people are older than 60 years, and the number is projected to rise to 1000 million by 2020[1]. With the increase in life expectancy, the leading causes of death have shifted dramatically from infectious diseases to non-communicable diseases and from younger to older individuals. In industrialized countries, about 75% of deaths in persons older than the age of 65 are now from cardiovascular diseases and cancer[2].

Regardless of predisposing factors, diet and lifestyle influence morbidity and mortality during the course of life[2]. Because of the cumulative effect of adverse factors throughout life, it is particularly important for older persons to adopt diet and lifestyle practices that minimize their risk of death from morbidity and maximize their prospects for healthful aging [2].

Dietary patterns and other modifiable lifestyle factors are associated with mortality from all causes, coronary heart disease (CHD), cardiovascular diseases (CVD) and cancer[3-8]. As yet, few studies have investigated the combined effect of diet and other lifestyle factor[7, 9]. In the current study, we investigated the association of individual and combined dietary patterns and lifestyle factors (alcohol use, smoking status, and physical activity) with mortality from all causes, CHD, CVD, and cancer in elderly men and women from[11] European countries in the Healthy Ageing: a Longitudinal study in Europe (HALE) population.

Methods

Study Population

The HALE project included participants of the Survey in Europe on Nutrition and the Elderly: a Concerned Action (SENECA) and Finland, Italy, the Netherlands, Elderly (FINE) studies who were examined in 1988-1991 and were followed up for 10 years. Details about the SENECA and FINE studies have been described elsewhere[10, 11] and are briefly summarized herein.

The SENECA study started in 1988 and consisted of a random age- and sex-stratified sample of inhabitants, born between 1913 and 1918, of 19 European towns. In the HALE project, 13 centres that carried out mortality follow-up were included. The original participation rate in the centres varied from 37% to 81%[12]. Surveys were repeated in 1993 and 1999. The response rates for SENECA were 68% in 1993 and 55% in 1999, and for FINE they were 86% in 1993 and 85% in 1993. All men and women of the following towns were included: Hamme, Belgium; Roskilde, Denmark; Strasbourg, France; Valence, France; Iraklion, Greece; Monor, Hungary; Padua, Italy; Culemborg, the Netherlands; Vila Franca de Xira, Portugal; Betanzos, Spain; and Yverdon, Burgdorf, and Bellinzona, Switzerland[10]. The FINE study consists of the survivors of 5 cohorts of the Seven Countries Study: Ilomantsi, East Finland; Poytyä, and Mellilä, West Finland; Crevalcore and Montegiorgio, Italy; and Zutphen, the Netherlands.

The FINE study, which started in 1984 and continued to 2000, recruited men who were born between 1900 and 1920. For our study, we used the 1989-1991 baseline measurements of men aged 70 to 90 years.11 Surveys were repeated in the years 1994-1995 and 1999-2000. The

response rates in 1989-1991 were 92% for the Finnish cohorts, 74% for the Dutch cohort, and 76% for the Italian cohorts[13]. Because CHD, CVD, cancer, and diabetes increase the risk of mortality and can induce changes in diet and lifestyle, participants who had these diseases were excluded at baseline. The average follow-up time was 10 years in both SENECA and FINE.

In both studies, food consumption data were collected by trained dieticians using a dietary history method[10, 11]. This method provides information about the usual food consumption of the participants from the month before the interview in SENECA and from 2 to 4 weeks before the interview in FINE. The central part of the dietary history was the same for both studies. The coding was based on frequency tables, which were the same in both studies, but the start of the dietary interview about usual food consumption patterns was based on a food record and by an oral interview in FINE. Both dietary histories were validated[10-13]. Information on smoking status; physical activity level; educational achievement (number of years); the prevalence of CHD, stroke, diabetes, and cancer; the use of antihypertensive medication (only in FINE); and occupation (only in FINE) was collected using questionnaires. The prevalence of chronic diseases was confirmed by general practitioners, hospital registrants, or both in the FINE study only. Information on physical habitual activity was obtained using the Voorrips questionnaire in SENECA and the Morris questionnaire in FINE[14,15]. Both questionnaires were developed for retired elderly individuals and focus on leisure-time activities, such as walking, cycling, and gardening. SENECA also included household activities[14, 15]. Weight, height, and waist circumference (SENECA only) measurements have been described

in detail elsewhere[10, 11]. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. In the analyses, BMI was dichotomized as 25 or less vs greater than 25. The HALE study comprised 98.6% of individuals who lived independently.

Definition of Low-Risk Groups

To assess the association of diet and the lifestyle factors with mortality, a low risk group was defined for diet and lifestyle factors. For dietary intake, the low risk group was defined as those who had a score of at least 4 on a modified version of the Mediterranean diet score proposed by Trichopoulou et al [4]. The modified Mediterranean diet score comprised 8 components: ratio of monounsaturated to saturated fat; legumes, nuts, and seeds; grains; fruit; vegetables and potatoes; meat and meat products; dairy products; and fish. Intake of each component was adjusted to daily intakes of 2500 kcal (10.5 MJ) for men and 2000 kcal (8.5 MJ) for women. The sex-specific median intake values were taken as cut off points. The diet score varied from 0 (low-quality diet) to 8 (high quality diet). For the components monounsaturated fatty acids to saturated fatty acids (MUFA to SAFA) ratio; fruits and fruit products; vegetables and potatoes; legumes, nuts, and seeds; fish; and grains, a value of 1 was assigned to persons whose consumption was at least as high as the sex-specific median value, and 0 to the others. The vegetables group of the original Mediterranean diet score was replaced by the vegetables and potatoes group because the European classification system (EUROCODE) was used when the 2 food groups were assessed together[16]. For meat and meat products and dairy products, a value of 1

was assigned to persons whose consumption was less than the sex-specific median and 0 to the others.

The low-risk group for alcohol was defined as those who consumed more than 0 g of alcohol per day. Alcohol consumption was initially divided into 3 groups: 0 g, 1 to 29 g, and 30 g or more of alcohol per day. However, the Kaplan-Meier survival curves of the 3 alcohol groups showed no difference in survival between participants who consumed between 1 g to 29 g of alcohol per day and those who consumed 30 g or more alcohol per day.

For smoking, individuals were considered to be at low risk if they had never smoked or had stopped smoking more than 15 years ago[17-19]. Individuals with a score in the intermediate and the highest tertile on the Voorrips or Morris questionnaire were considered the low-risk group for physical activity[14, 15].

A lifestyle score was calculated by adding the individual scores for diet, physical activity level, smoking status, and alcohol intake. Individuals scored 1 point if they belonged to the low-risk group for diet or a particular lifestyle factor and 0 if they belonged to the high-risk group. In total, an individual could obtain 4 points: 1 point for a Mediterranean diet and 3 points for the healthful lifestyle factors.

Health Status

Information on vital status and causes of death was collected every 5 years in FINE and in 1999-2000 for SENECA and was available for 99.7% of participants. Causes of death were coded by an experienced physician and classified according to the *International Classification of*

Diseases, Ninth Revision (ICD-9) coding system[20]. Codes 410- 414 were used for defining CHD, codes 390-459 for CVD, and codes 140-240 for cancer. Causes of death were available for 92% of the participants in FINE and 72% of the participants in SENECA. Mortality from other causes was defined as total mortality minus the deaths from unknown causes, CHD, CVD, and cancer.

Statistical Analysis

Statistical analyses were carried out using the SAS statistical software version 8.2 (SAS Institute, Cary, NC). Before pooling the data for SENECA and FINE, a test for heterogeneity was performed, which was not significant (*P*=.93); no significant interactions between diet, lifestyle factors, and study were found. Cox proportional hazards models were used to estimate the single and the combined effect of diet, smoking status, alcohol consumption, and physical activity level on mortality[21]. In the analyses for cause-specific mortality and mortality from other causes, individuals with a missing cause of death were excluded. For all models, the Cox proportional hazards assumption was tested and met. Dummy variables were created for the number of healthful dietary and lifestyle factors; a score of 0 or 1 for the healthful dietary and lifestyle factor was used as reference category. All models were adjusted for the other diet and lifestyle factors, sex, age at baseline, BMI, and study population (SENECA vs FINE). To correct the estimates for socioeconomic status, the models were adjusted for the number of years of education.

Region (northern or southern Europe), centre, occupation, waist circumference (continuous), marital status, and use of antihypertensive medication were tested as potential confounders but did not affect our

estimates. Because several studies indicate that exposure to pesticides and industrialized environment increases the risk of mortality[22]. We classified participants' occupation as farmers (an indicator of pesticide exposure) vs non-farmers and whether individuals were exposed or not exposed to an industrialized environment. Occupation as farmer and exposure to an industrialized environment also did not affect our results.

To minimize the possibility that diet or lifestyle factors had changed in response to sub-clinical disease, the analyses were repeated after excluding the first 2 years of mortality follow-up. [9] Interactions between all dietary and lifestyle factors and confounders were tested, but none of them was statistically significant at α=.1. To ensure that the estimates were not biased by multi-co linearity, the crude hazard ratios (HR's) for the dietary and lifestyle factors were also calculated and compared with the adjusted HR's. The crude HR's and the adjusted HR's were similar, indicating that the estimates were not biased by multi-co linearity.

Population attributable risk, an estimate of the percentage of mortality in this population that would not have occurred if all participants were in the low-risk group, was calculated [9, 23]. The unadjusted Kaplan-Meier curves for the number of protective factors in relation to all-cause mortality were fitted and the log-rank test was calculated. $P < .05$ was considered to be statistically significant for all possible predictors in the model, but to investigate interaction between all predictors, we considered $P < .01$ to be statistically significant.

Results

Information about diet, lifestyle factors, and vital status was available for 1507 men (781 from the SENECA study, 726 from the FINE study) and 832 women without CHD, CVD, diabetes, and cancer at baseline (Table 1).

Table 1: Baseline characteristics and 10-year mortality data of the SENECA and FINE study in persons without chronic diseases.

	SENECA		FINE
	Women (n=832)	Men (n=781)	Men (n=726)
Age (years) , mean ± sd	73 ± 1.8	73 ± 2.0	77 ± 4
Northern Europe, % (n)	43 (355)	45 (350)	64 (462)
Years of education, mean ± sd	8 ± 3.5	9 ± 4.0	7 ± 4.5
Mediterranean diet score, median (p10-p90)	4 (2-6)	4 (2-6)	4 (2-6)
Components of the diet score[1], median (p10-p90)			
Monounsaturated/satured fat ratio	1 (0.70-1.63)	1 (0.73-1.55)	1 (0.58-2.90)
Vegetables/potatoes	272 (136-484)	306 (148-504)	248 (113-456)
Fruit	262 (74-519)	228 (43-528)	209 (47-457)
Legumes/nuts/seeds	5 (0-31)	7 (0-38)	10 (0-46)
Meat and poultry	107 (53-181)	130 (66-217)	113 (58-181)
Milk and milk products	317 (90-624)	313 (80-683)	392 (28-969)
Fish	23 (0-73)	26 (2-90)	20 (0-57)
Grains	194 (116-302)	248 (144-389)	231 (133-379)
Alcohol users, % (n)	56 (466)	83 (647)	76 (555)
Voorrips Score (Mean ± sd)	12 ± 9	19 ± 13	
Lowest tertile Voorrips Score	5.5	9.5	
Morris Score (Mean ± sd)			598 ± 605
Lowest tertile Morris Score			200
Never smoked or stopped > 15 years , % (n)	88 (731)	43 (337)	58 (444)
BMI ≤ 25 kg/m^2, % (n)	41 (343)	39 (307)	42 (305)
Causes of death, % (n)			
All deaths	24 (197)	47 (366)	51 (372)
Coronary heart disease	9 (18)	12(45)	16 (59)
Cardiovascular diseases	39 (76)	32 (117)	48 (178)
Cancer	18(35)	26 (96)	27 (102)
Other causes	13 (27)	15 (56)	17 (62)
Unknown causes	30 (59)	27 (97)	8 (30)

[1]grams/day, corrected for 2500 kcal/day in men, 2000 kcal in women.

Men in the SENECA study were 7 years younger on average than men in the FINE study. The median of the diet score in the total population was 4; 3 in northern Europe vs 5 in southern Europe. The mean alcohol intake was 21 g/d for men and 6 g/d for women. In northern Europe, the mean alcohol consumption was 17.5 g/d for men and 5.5 g/d for women compared with 31 g/d for men and 6 g/d for women in southern Europe. The cut off point for the lowest percentile for activity in the FINE study was 200 min/wk, which means approximately 30 minutes of activity per day, with an intensity higher than 2.0 kcal/kg per hour each day.

In the SENECA study, there were almost 5 times as many male smokers as there were female smokers. The mean follow- up time was 10 years, with a range of 8.9 to 10.5 years. Twice as many men died during the 10-year follow-up as did women.

Table 2: Cox proportional hazard rates (HR) for dietary pattern and three lifestyle factors for 10-year all-causes and cause-specific mortality in elderly European men and women without chronic diseases at baseline.

Protective factors HR (95 % CI)[2]	All-causes Risk =2339 Events=935	CHD[1] Risk=2152 Events=122	CVD [1] Risk=2152 Events=371	Cancer Risk=2152 Events=233	Other causes Risk=2152 Events=145
Mediterranean diet	0.77 (0.68-0.88)	0.61 (0.43-0.88)	0.71 (0.58-0.88)	0.90 (0.70-1.17)	0.61 (0.44-0.85)
Alcohol	0.78 (0.67-0.91)	0.60 (0.40-0.88)	0.74 (0.59-0.93)	0.73 (0.54-0.98)	0.63 (0.44-0.90)
Physical activity	0.63 (0.55-0.72)	0.72 (0.48-1.07)	0.65 (0.52-0.81)	0.64 (0.48-0.84)	0.52 (0.37-0.74)
Non-smoking	0.65 (0.57-0.75)	0.80 (0.54-1.17)	0.68 (0.54-0.85)	0.47 (0.36-0.62)	0.92 (0.59-1.24)

[1]CHD=coronary heart disease, CVD=cardiovascular diseases

[2] Model adjusted for age, gender, number of years of education, BMI, and study.

Table 2 shows that a Mediterranean diet, moderate alcohol consumption, moderate to high physical activity levels, and non-smoking were associated with lower mortality rates from all causes, CHD, CVD, cancer, and other causes during the 10-year follow up. The models were adjusted for sex, age, years of education, study, and BMI. Individuals with 2, 3, or 4 low-risk factors had a significantly lower risk of all-cause and cause-specific mortality compared with individuals with 0 or 1 low-risk factor during 10 years of follow-up (Table 3).

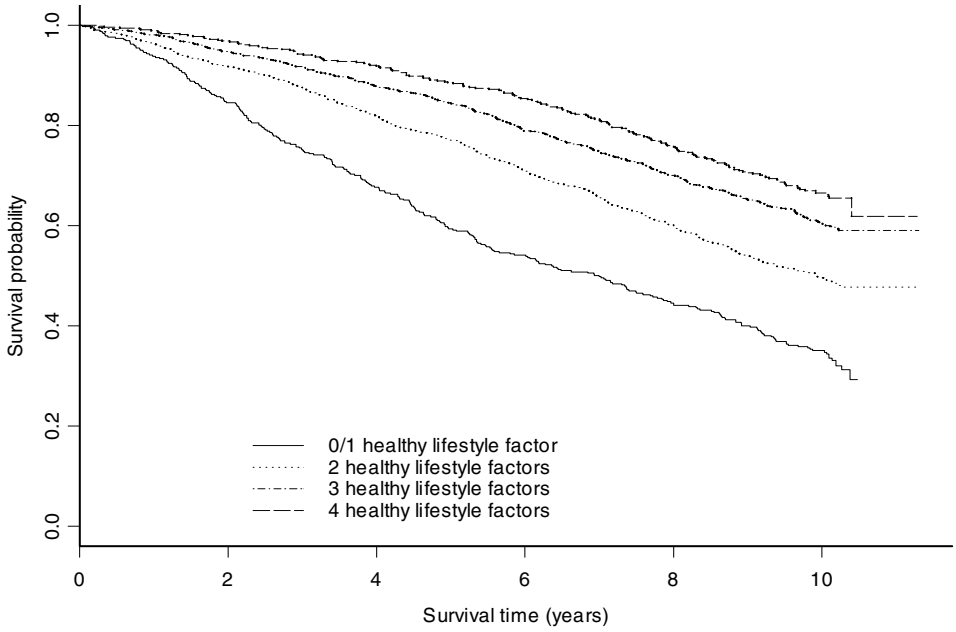
Table 3: Cox proportional hazard rates (HR) and population attributable risks (PAR) of the combined diet and lifestyle factors for 10-year all-causes and cause-specific mortality in elderly European men and women

Mortality	Number of protective factors			
	0/1 (n=246)	2 (n=702)	3 (n=954)	4 (n=437)
All-causes				
HR (95% CI)	1.00	0.62 (0.51-0.75)	0.45 (0.37-0.54)	0.35 (0.28-0.44)
PAR (%)	-	14	37	60
Coronary heart disease				
HR (95% CI)	1.00	0.50 (0.29-0.85)	0.43 (0.26-0.71)	0.27 (0.14-0.53)
PAR (%)	-	18	38	64
Cardiovascular diseases				
HR (95% CI)	1.00	0.60 (0.44-0.82)	0.44 (0.33-0.60)	0.33 (0.22-0.47)
PAR (%)	-	13	38	61
Cancer				
HR (95% CI)	1.00	0.65 (0.45-0.96)	0.42 (0.28-0.62)	0.31 (0.19-0.50)
PAR (%)	-	14	38	60
Other causes				
HR (95% CI)	1.00	0.55 (0.34-0.90)	0.38 (0.23-0.61)	0.33 (0.19-0.58)
PAR (%)	-	20	40	61

Sixty percent of all deaths were associated with not adhering to this low-risk pattern. For cause specific mortality, 64% of deaths due to CHD; 61% due to CVD; 60% due to cancer, and 61% due to other causes during the 10-year follow-up period were associated with not adhering to this low-risk pattern. Including individuals with CHD, CVD, cancer, or

diabetes at baseline in the analysis and adjusting for prevalence of these long term diseases at baseline did not change our estimates.

Figure 1: Kaplan-Meier curves for the number of healthful lifestyle factors



Similar results were found after excluding from the analysis the 140 persons who died during the first 2 years of follow-up: adhering to a Mediterranean diet (HR, 0.77; 95% confidence interval [CI], 0.67-0.89), moderate alcohol consumption (HR, 0.83;95%CI, 0.71-0.91), engaging in physical activity (HR, 0.65; 95% CI, 0.56-0.76), and non-smoking (HR, 0.67, 95% CI, 0.57-0.78) were associated with a significantly lower risk of all-cause mortality. The combination of 4 low-risk factors lowered the all-cause mortality rate to 0.37 (95% CI, 0.29-0.47). For cause-specific mortality, the HR's for the single and the combined effects from dietary and lifestyle factors were also similar after excluding the first 2 years of follow-up.

The figure shows the Kaplan- Meier curves for the number of healthful dietary and lifestyle factors. The corresponding log-rank test statistic was 101.9, *P*<.001.

Discussion

During the 10-year follow-up, men and women between the ages of 70 and 90 years who had adhered to a Mediterranean diet, were non-smokers or had stopped smoking more than 15 years ago, were physically active, and used alcohol moderately had less than half the mortality rate from all causes, CHD, CVD, and cancer, and mortality from other causes than those who did not.

Advantages of this Europe-wide study were its great diversity in dietary patterns and lifestyle factors, its prospective nature, its large sample size, and its measurements of many potential confounders. SENECA and FINE could be pooled because the estimates of the separate models were similar and there were no important differences in study population and measurement of risk factors. To ensure that the relationships between diet, lifestyle factors, and mortality could not be explained by a study effect, this variable was included as a potential confounder in all pooled analyses.

This study also had several limitations. Despite the large number of participants and long follow-up, few cases had no healthful diet or lifestyle factors. Therefore, we had to combine those who had scores of 0 or 1 in the healthful diet and lifestyle factor ranking to have a stable reference category. The initial response rate in the SENECA centres was lower (37%- 81%) than in the FINE centres (74%- 92%)[12, 13]. In

prospective cohort studies, distributions of dietary and lifestyle factors in the study population may have been affected by selective participation in the different cohorts. However, we had almost 100% mortality follow-up, so the relationships between dietary factors and mortality should not be distorted[24]. Another limitation is the low number of deaths due to cause specific mortality; for example, only 122 deaths were from CHD. Due to these low numbers, it is possible that the power was too low to find a significant effect of all dietary and lifestyle factors in the cause-specific analysis.

A Mediterranean diet score of 4 or more points was associated with a lower risk of all-cause and cause-specific mortality; the strongest association was observed for CHD. The Mediterranean diet scale relies on generally strong epidemiological evidence concerning the individual dietary components[4, 6.] The components of the diet score have been validated[25]. In previous studies [4, 6, 7] involving elderly persons in which a Mediterranean diet score similar to ours was used, the reduction in overall mortality associated with increased adherence to the Mediterranean diet was also similar to that found in our study. Similar results were also noted for mortality from CHD and cancer [4]. Our results are also compatible with those of 2 randomized trials of secondary prevention of CHD through the use of variants of the Mediterranean diet[26, 27]. In the Mediterranean diet score proposed by Trichopoulou et al[4], alcohol was also included. In our study, alcohol was considered as a separate lifestyle factor because many studies observed an independent effect of alcohol on survival[28-30]. When we included alcohol in our diet score, we found that individuals scoring 4 or more points on the original Mediterranean diet score have a 14% lower risk of mortality compared

with 23% in the present study. Participants who were physically active had a lower risk of all-cause and cause-specific mortality. Other studies found also that a sedentary lifestyle was associated with a significantly higher risk of all-cause and cause-specific mortality compared with being moderately active[17,31] Davis et al[17] reported that non-recreational physical activity was an even better predictor of survival time in older persons (aged 65-74 years) than in middle-aged persons (aged 45-54 years)[17].

Non- smoking was associated with lower risk from all-cause and cause specific mortality. Other studies also found that smokers had a higher mortality risk although the relative risk was lower in older persons than in middle-aged individuals[17, 18].

Although diet and lifestyle habits can change over time, generally they are characteristic of a person's way of living and reflect lifelong health habits[32, 33]. For smoking, we contrasted persons who never smoked or stopped smoking more than 15 years ago with those who still smoked or stopped less than 15 years ago. This definition of the smoking variable takes a long exposure period into account. In SENECA, dietary and lifestyle factors were measured 5 years apart. The Spearman correlation coefficient for alcohol consumption, diet score, and physical activity between 1988-1989 and 1993 was highly significant ($P<.01$), suggesting that diet and lifestyle factors were stable in older individuals. Several other studies also found that smoking habits and activity patterns in middle-aged and many elderly men were stable over the years[32, 33].

A multiplicative model was used to assess the combined effect of diet and lifestyle factors on all-cause and cause specific mortality. The more healthful dietary and lifestyle factors a participant had, the lower the risk for all cause mortality and cause-specific mortality. In the current study,

60% to 64% of mortality was associated with lack of adherence to this low-risk pattern. This supports the hypothesis that participants who follow a Mediterranean type of diet and maintain a healthful lifestyle are less likely to die from all cause and cause-specific mortality even at ages 70 to 90 years. Establishing a causal relationship would require an intervention study, and the number of years an individual needs to maintain such a lifestyle to realize a benefit is unknown. However, a Mediterranean diet, rich in plant foods in combination with non-smoking, moderate alcohol consumption, and at least 30 minutes of physical activity per day is associated with a significantly lower mortality rate, even in old age.

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5

Lifestyle, Mediterranean Diet and Survival in European post-Myocardial Infarction Patients: The HALE-Project

Iestra J, Knoops K, Kromhout D, de Groot L, Grobbee D, van Staveren W
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Abstract

Background: The extend and benefits of adherence to lifestyle and dietary recommendations in secondary prevention are largely unknown.
Design: We examined the frequency of healthy dietary and lifestyle behaviors and their impact on survival in post-myocardial infarction (MI) patients in a prospective cohort study of European elderly.
Methods: Adherence to a Mediterranean-type diet was measured with a modified Mediterranean Diet Score (MDS) on an eight-point scale.
Results: Participants were 426 men and women, aged 70 years or more, from ten European countries, with a history of MI. During ten years of follow-up mortality was 53%. Frequency of non-smoking behavior (85%), moderate to vigorous physical activity (54%), moderate alcohol consumption (45%) and a Mediterranean-type diet (63%) in patients differed only marginally as compared with 'healthy' elderly. The median MDS in patients from Northern Europe was two points lower than in Southern Europe. Non-smoking (HR 0.62 (95%CI 0.44-0.88), physical activity (HR 0.69 (95%CI 0.53-0.90), moderate alcohol consumption (HR 0.77 (95%CI 0.58-1.02) and a Mediterranean-type diet (HR 0.75 (95%CI 0.57-0.97) were associated with lower all-causes mortality. Presence of at least three healthy behaviors was associated with 40% lower mortality.
Conclusion: There is a strong relationship between lifestyle and dietary habits and mortality in post-MI patients. The findings implicate that substantial health gain can be obtained by better adherence to dietary and lifestyle recommendations.

Introduction

Diet and lifestyle factors (smoking habits, physical activity and alcohol consumption) have been shown to play an important role in prognosis of patients with symptomatic Coronary Artery Disease[1]. European Guidelines for Secondary Prevention of Cardiovascular Diseases[2] have translated research findings into practical recommendations for the individual patient. Research on the implementation of these guidelines has generally focused on drugs prescription and prevalence of the classical risk factors[3]. While some have described the proportion of patients receiving dietary or lifestyle advices[4], adherence to these recommendations and its impact on survival in cardiac patients has only scarcely been addressed.

An alternative approach to evaluating an individual's diet by analyzing the nutrient content has been proposed by Trichopoulou et al[5]. As many of the recommended dietary components are integral parts of the Mediterranean diet and substantial prognostic benefits are associated with a Mediterranean–type diet, they developed an instrument to measure the resemblance of an individual's diet with the traditional Greek Mediterranean diet. This Mediterranean Diet Score (MDS) has been shown to be associated with survival in several populations[6-9].

The prognostic value of the MDS and other lifestyle factors has been demonstrated in the 'healthy' population of the HALE (Healthy Ageing: a Longitudinal study in Europe) project[10]. In the present study the frequency of healthy dietary and lifestyle behaviors and the associations with mortality were evaluated in HALE-participants with a history of myocardial infarction (MI). We compared the frequency of healthy

behaviors between post MI-patients and 'healthy' study participants as well as between subgroups of patients by gender or region.

Methods

Study population

We selected patients with a history of MI from the HALE-project. This project consists of participants from two European cohort studies: the Survey in Europe on Nutrition and the Elderly: a Concerted Action (SENECA) and the Finland-Italy and the Netherlands Elderly (FINE) study. The HALE-project and both cohort studies have been described elsewhere[10-12].

Briefly, the SENECA study started in 1988 and consisted of a random age- and gender-stratified sample of inhabitants, born between 1913 and 1918. Of the 19 participating centres, 13 carried out a mortality follow-up in 1993 and 1999. Participation rates varied between the SENECA centres from 37-81%[13]. The FINE cohort is an extension of the Seven Countries Study beyond the 25 years follow-up. It started in 1984 including men aged 65-85 years. Participation rates were between 74% and 92%[14]. For the HALE-project participants were selected with a baseline age of 70 or older in the 1989-1991 surveys and a mortality follow-up until 2000.

The diagnosis of MI in our study population was self reported (SENECA) or medically confirmed (FINE). We excluded patients who reported a history of cancer, as this might have changed their diet, lifestyle and mortality risk. Included patients originated from 20 study centres. Ten study centres in Northern Europe were: Ilomantsi (FINE),

East Finland; Pöytyä and Mellilä (FINE), West Finland; Roskilde (SENECA), Denmark; Zutphen (FINE) and Culemborg (SENECA), the Netherlands; Hamme (SENECA), Belgium; Strasbourg (SENECA), France; Burgdorf (SENECA), Switzerland. Ten centres located in Southern Europe were: Vila Franca de Xira (SENECA), Portugal; Betanzos (SENECA), Spain; Valence (SENECA), France; Yverdon (SENECA) and Bellinzona (SENECA), Switzerland; Padua (SENECA), Crevalcore (FINE) and Montegiorgio (FINE), Italy; Iraklion (SENECA), Greece.

Demographic, lifestyle, anthropometric and medical variables

Baseline information was collected using a questionnaire on chronic disease (Myocardial Infarction, Stroke, Diabetes Mellitus or Cancer), educational achievement (number of years), marital status, smoking habits, physical activity level, and alcohol consumption. For FINE-participants the presence of chronic disease was confirmed by information from general practitioners or hospital registers.

We classified smoking habits in current smokers and non-smokers (including ex-smokers). The questionnaires on habitual physical activity differed between the two studies: in SENECA the Voorrips questionnaire was used, in FINE the Morris questionnaire[15,16]. Both validated questionnaires were developed for retired elderly and focused on activities, such as walking, cycling, and gardening. We calculated gender- and study specific tertiles of the distribution. Subjects with a score in the lowest tertile were defined as physically inactive; those in the second or third tertile as moderately to vigorously active.

In contrast to the studies of Trichopoulou et al. we defined alcohol consumption as a lifestyle factor rather than considering this as part of the Mediterranean diet. Categories were abstainers (alcohol intake<1 gram/day), moderate drinkers (1-20 gram/day for women and 1-30 gram/day for men) and excessive drinkers (>20 gram/day for women and >30 gram/day for men). In the Cox proportional hazards models we used only 2 groups for alcohol consumption (abstainers versus alcohol users), because the Kaplan Meier survival curves of the moderate and more excessive drinkers showed no difference.

Anthropometric measurements were obtained by trained study personnel using standard procedures[14, 17]. Body Mass Index (BMI) was calculated as weight in kilograms divided by height in squared meters. Obesity was defined as a BMI > 30 kg/m^2. Non-fasting blood samples were collected and analyzed for total- and HDL-serum cholesterol and fasting blood samples for triglycerides, using standardized enzymatic methods[14, 18].

Food consumption data

In both studies, food consumption data were collected by trained dieticians using a dietary history method[19]. This method provides information about the usual food intake. The main part of the interview was the same in both studies, but the reference period was one month in SENECA and two to four weeks in FINE. The interview data from both studies were coded using the EUROCODE-system[20]. Nutrient intakes were calculated using the food composition tables of each country. Validation of the methods used in both studies is described elsewhere[11, 21].

Each dietary component was energy adjusted by dividing the daily intake (in grams) through the individual's total energy intake (in kcal) and

multiplying it with the gender and study population specific median of energy intake: 2150 kcal for men and 1750 kcal for women.

Mediterranean diet score

The original MD-score was constructed in 1995 by Trichopoulou et al[5] and revised several times[6-8, 10] to include fish and to change the lipid ratio from monounsaturated/saturated to unsaturated/saturated (as this makes it more applicable to the Northern European countries as well). In the current study the score components were defined as follows:

- the ratio between unsaturated fats and saturated fats in the diet,
- vegetables, including potatoes (in EUROCODE potatoes were coded as vegetables),
- fruit and fruit products (including canned fruit, but not fruit juices),
- cereals (including bread, breakfast cereals, pasta, rice etc),
- legumes, nuts and seeds,
- fish,
- dairy (including milk, milk products and cheese),
- meat, poultry and derived products

Values 0 or 1 were assigned to each of the eight components, using as cut-off the gender-specific median for the energy adjusted daily intake of the component in the chronic-disease-free HALE-participants (excluded were those with a history of Cancer, Diabetes Mellitus, Myocardial Infarction or Stroke at baseline). Persons whose energy adjusted consumption of the 5 beneficial components (vegetables, fruits, cereals, legumes or fish) was below the median consumption were assigned a value of 0, whereas for individuals with consumption above the median, a value of 1 was given. In contrast, persons with below-the-median

consumption of presumed detrimental components (meat and dairy) were assigned a value 1, whereas individuals whose consumption of these 2 components was above the corresponding median were given a value 0. Finally, for lipid intake, the ratio of unsaturated to saturated fats was calculated. A ratio below the chronic-disease-free participant's median was rewarded with 0 and a ratio above the median with 1. The MD-score was calculated by adding up the scores for the 8 items. Beside this European-wide MD-score also a regional MD-score was calculated. For the regional MD-score value 0 or 1 were assigned for each dietary component using the region- and gender-specific median of the chronic-disease-free HALE population as cut-off point.

Follow-up

Information on vital status and causes of death was collected every five year in FINE. For SENECA this was done after ten years of follow-up. Vital status was available for 99.7% of the HALE-participants.

Data analysis

Data were analyzed using SAS statistical software version 8.2 (SAS Institute, Cary NC). Before pooling, mortality data from the SENECA- and FINE-study were tested for heterogeneity (p=.93).

Baseline data and healthy behaviors were compared and tested for differences between the post MI-patients and the reference group of healthy HALE-participants and between patient subgroups. The Chi-Square test was used for the dichotomous variables, the Student t-test for normally distributed continuous variables (presented as mean ± SD) and

the Wilcoxon test for data that were not normally distributed (presented as median and 25- and 75-percentiles).

To address the mortality risk associated with diet and lifestyle in the total patient population and subgroups by gender and region, we used the Cox proportional hazards model.

One model was used to assess the association between mortality and each of the single dietary components (0/1). This model was adjusted for study (FINE, SENECA), gender, age (continuously), years of education (continuously), BMI (continuously), history of diabetes (Y/N) or stroke (Y/N), smoking (Y/N), physical activity (Y/N), alcohol consumption (Y/N). In a second Cox proportional hazards model we assessed the association between mortality and each of the four healthy habits (non-smoking (Y/N), physical active (Y/N), alcohol consumption (Y/N), Mediterranean diet (Score ≥4 versus <4)), while adjusting for the same variables (except lifestyle and dietary factors). A third model assessed the association between mortality and the number of healthy behaviors (0-2 versus 3-4), while adjusting for the same variables (except lifestyle and dietary factors). The proportional hazards assumptions for each model were tested and met. The interaction of gender and region was tested for each model but was not significant at alpha equal to 0.1.

Results

Information about diet, lifestyle factors and vital status was available for 2954 HALE-participants without cancer at baseline (1955 men and 999 women; 2002 from SENECA and 952 from FINE). Mean follow-up time

Table 1. Baseline characteristics of post-MI patients compared to 'healthy' participants in the HALE-project

	Males		p-value[1]	Females		p-value[2]	p-value[3]
	Post-MI patients n= 284	Other participants n= 1671		Post-MI Patients n= 142	Other participants n=857		
Age[4] yrs	75 ± 4	75 ± 4	ns	73 ± 2	73 ± 2	ns	<0.01
Educational achievement [5] yrs	8 (5-10)	8 (5-10)	ns	7 (4-8)	8 (6-9)	<0.05	<0.05
Marital status married %	79	79	ns	43	43	ns	<0.01
Region North %	62	55	<0.05	46	43	ns	<0.01
TCHOL/HDL ratio[5]	5.5 (4.5-6.8)	4.8 (3.9-5.9)	<0.01	5.2 (4.2-6.6)	4.6 (3.7-5.7)	<0.01	ns
Triglycerides[5] mmol/l	1.4 (1.0-2.0)	1.2 (0.9-1.7)	<0.01	1.4 (1.0-1.2)	1.2 (0.9-1.7)	<0.01	ns
Obesity (BMI>30 kg/m^2) %	15	14	ns	32	25	ns	<0.01
Diabetes Mellitus %	10	8	ns	14	8	<0.05	ns
Stroke %	8	3	<0.01	5	1	<0.01	ns
Energy intake [5] kcal/day	2186 (1793-2529)	2272 (1915-2698)	<0.01	1758 (1356-2032)	1782 (1491-2081)	ns	<0.01
Mediterranean diet (score ≥4)[6]	60%	62%	ns	70%	61%	<0.05	<0.05
Non-smokers %	80	75	ns	95	93	ns	<0.01
Physically active %	53	66	<0.01	60	66	ns	ns
Alcohol pattern abstainers%	29	21	<0.01	66	49	<0.01	<0.01
moderate drinkers%	54	53		28	42		
excessive drinkers%	17	26		6	9		
Number of lifestyle factors: 0-1	15%	14%	ns	13%	11%	ns	ns
2	35%	32%		32%	31%		
3	36%	37%		47%	42%		
4	14%	17%		8%	16%		
Mortality rate	67	50	<0.01	40	25	<0.01	<0.01

[1]testing males: patients vs other participants; [2]testing females: patients versus other participants; [3]testing patients: all males vs all females;
[4]Average ± SD , t-test; [5]median (interquartile limits), Wilcoxon test; [6]based on gender specific cut-of points of the healthy part of the HALE-population

Table 2: Patient characteristics of 426 European post-Myocardial Infarction patients by gender and region

Characteristics	Males n=284			Females n=142		
	Northern Europe n=175	Southern Europe n=109	p-value[1]	Northern Europe n=66	Southern Europe n=76	p-value[1]
Age ≥ 75 years	35%	30%	ns	15%	24%	ns
Education ≥ 6 years	84%	51%	<0.01	98%	42%	<0.01
Chol/HDL ratio > 5	67%	48%	<0.01	53%	43%	ns
Serum triglycerides > 2 mmol/l	25%	21%	ns	29%	13%	<0.05
Obesity (BMI>30 kg/m^2)	11%	20%	<0.05	26%	38%	ns
Diabetes Mellitus	8%	13%	ns	14%	14%	ns
Stroke	10%	4%	<0.05	5%	5%	ns
Median intakes[2] for						
Fat ratio[3]	1.46	1.92	<0.01	1.63	2.24	<0.01
Vegetables (g)	303	244	<0.01	235	242	ns
Fruit (g)	172	236	<0.01	189	244	<0.01
Cereals (g)	170	256	<0.01	150	201	ns
Legumes, seeds,	4	5	ns	0	1	ns
nuts(g)	19	33	<0.01	22	41	<0.01
Fish (g)	383	258	<0.01	267	242	ns
Dairy (g)	122	103	<0.01	118	78	<0.01
Meat & poultry (g)	3 (2-4)	5 (4-6)	<0.01	3 (2-4)	5 (4-6)	<0.01
Mediterranean Diet (score ≥4)	45%	83%	<0.01	45%	92%	<0.01
Non-smoker	79%	82%	ns	91%	99%	<0.05
Physically active	53%	52%	ns	48%	66%	<0.05
Alcohol habits abstainer	35%	18%	<0.01	52%	79%	<0.01
moderate drinker	55%	53%		41%	16%	
excessive drinker	9%	29%		7%	5%	
Number of lifestyle factors 0-1	21%	5%	<0.01	24%	3%	<0.01
2	36%	35%		32%	32%	
3	30%	46%		36%	57%	
4	13%	15%		8%	9%	
Mortality rate	71%	59%	<0.05	47%	33%	ns

1. Proportions tested by chi-square test; Medians tested by Wilcoxon test; 2. Standardized for energy intake; 3. Unsaturated fats / saturated fats

Table 3: Cox proportional Hazards Ratio (HR) for all-causes mortality for dietary components, diet score and lifestyle factors in 426 European post-Myocardial Infarction patients

	HR All causes Mortality (95% CI)[1]				
	All[2] n=426	Men[2] n=284	Women[2] n=142	Northern Europe[3] n=241	Southern Europe[3] n=185
Dietary components[4]					
Unsaturated / Saturated Fat	0.92 (0.70-1.20)	0.85 (0.62-1.15)	1.21 (0.66-2.21)	0.84 (0.58-1.21)	0.88 (0.51-1.53)
Vegetables (incl. potatoes)	1.06 (0.82-1.38)	1.15 (0.85-1.55)	0.75 (0.42-1.32)	0.91 (0.95-1.28)	1.36 (0.85-2.18)
Fruits	0.74 (0.57-0.96)*	0.79 (0.59-1.06)	0.57 (0.32-1.02)	0.72 (0.52-1.00)	0.85 (0.54-1.36)
Cereals	0.83 (0.63-1.08)	0.86 (0.63-1.16)	0.75 (0.43-1.30)	0.83 (0.57-1.20)	1.14 (0.69-1.89)
Legumes, nuts, seeds	0.91 (0.70-1.18)	0.91 (0.67-1.23)	0.90 (0.52-1.55)	1.03 (0.74-1.43)	0.74 (0.48-1.14)
Fish	0.80 (0.70-1.18)	0.79 (0.59-1.06)	1.04 (0.57-1.89)	0.81 (0.58-1.13)	1.20 (0.74-1.97)
Dairy	0.83 (0.64-1.09)	0.73 (0.53-10.7)	1.29 (0.74-1.34)	0.68 (0.47-0.97)	1.07 (0.68-1.67)
Meat & poultry	0.99 (0.76-1.29)	0.95 (0.70-1.29)	1.30 (0.72-2.33)	1.32 (0.92-1.88)	1.07 (0.68-1.68)
Mediterranean Diet[5]					
MD-Score ≥4	0.75 (0.57-0.97)*	0.74 (0.55-1.00)	0.86 (0.46-1.61)	0.68 (0.49-0.95)*	1.33 (0.82-2.17)
Lifestyle[5]					
Non-smoking	0.62 (0.44-0.88)*	0.67 (0.47-0.97)*	0.38 (0.12-1.24)	0.72 (0.47-1.10)	0.51 (0.28-0.96)*
Physically active	0.69 (0.53-0.90)*	0.72 (0.53-0.97)*	0.62 (0.36-1.10)	0.70 (0.50-0.98)*	0.72 (0.46-1.13)
Moderate alcohol consumption	0.77 (0.58-1.02)	0.77 (0.56-1.07)	0.77 (0.42-1.42)	0.62 (0.44-0.89)*	1.01 (0.58-1.76)
Number of healthy lifestyle habits[6]					
3 or more out of 4	0.58 (0.45-0.75)*	0.59 (0.44-0.78)*	0.71 (0.41-1.21)	0.54 (0.39-0.74)*	0.83 (0.53-1.28)

* $p<0.05$; [1.] comparing the healthy to the unhealthy behaviour (reference group); [2]. Cut-off points for dietary components were based on sex-specific medians of the healthy population. [3.] Cut-off points for dietary components were based on sex- and region- specific medians of the healthy population. [4.] Model 1, this model was adjusted for study (FINE, SENECA), gender, age (continuously), years of education (continuously), BMI (continuously), history of diabetes (Y/N) or stroke (Y/N), smoking (Y/N), physical activity (Y/N), alcohol consumption (Y/N). [5.] Model 2, this model was adjusted for the same variables as in model 1 except the lifestyle factors. [6.] Model 3, this model was adjusted for the same variables as in model 1 except the lifestyle factors.

was 10 years (range of 8.9 to 10.5 years). Of these 2954 participants 426 reported a history of MI of whom 285 (67%) were males and 314 (74%) came from the SENECA study.

The post-MI patients had fewer years of education (females only), a worse serum lipid profile and a higher prevalence of a history of stroke compared to the other study participants (Table 1). Dietary and lifestyle differences between post-MI patients and other participants were small. In the patients the frequency of a Mediterranean-type diet was higher (in female patients only), while the frequency of moderate to vigorous physical activity was lower. Patients were more often alcohol abstainers. The distribution of the number of healthy behaviors was not significantly different between patients and other participants. Mortality rate was 1.5 times higher among patients. Between male and female patients differences were seen in age and educational level (both lower in females). Obesity, non-smoking behavior and a Mediterranean-type diet were more frequent in females, while moderate alcohol consumption was more frequent in men. No difference between the genders was found in the median number of healthy behaviors. Female patients had a lower mortality.

Male patients from Northern Europe (Table 2) had a higher frequency of ≥6 years of education and a higher proportion of patients with dyslipidemia, obesity or a history of stroke than those from the Southern region. The percentage of alcohol consumers and adherence to the Mediterranean diet was higher in Southern patients. There were a higher proportion of patients in the Southern region adhering to three or more healthy behaviors. Mortality was lower in the Southern European male patients. Female patients from both regions differed with respect to

education (more frequently ≥ 6 years in the North), serum lipid profile (worse in the North), MD-score (two points higher in the South), smoking behavior (more non-smokers in the South), physical activity (more moderate to vigorously active in the South) and alcohol consumption (more abstainers in the South). The proportion of female patients adhering to three or more healthy behaviors was significantly higher in the South. Mortality in Southern females was lower, although not statistically significant (p=0.09).

Table 3 shows the Cox proportional hazard ratio's for all-causes mortality, comparing patients with a healthy behavior to those with an unhealthy behavior. Of the individual dietary components only the high fruit consumption was significantly associated with a lower mortality in the total group. A Mediterranean diet (MD Score ≥4) was significantly associated with a lower mortality risk (HR 0.75 (95% CI 0.57-0.97), in the total group as well as in the subgroup from Northern Europe.

Protective effects were also shown for non-smoking (HR 0.62 (95% CI 0.44-0.88)), a higher level of physical activity (HR 0.69 (95%CI 0.53-0.90) and moderate alcohol consumption, although the latter relationship was not statistically significant (HR 0.77 (95%CI 0.58-1.02) in the total patient population.

A combination of at least three healthy habits was associated with a 42% lower mortality rate (95%CI: 22% - 56%) in the total patient group. All subgroups showed a lower mortality associated with a higher level of adherence to the four lifestyle recommendations, although not always statistically significant.

Discussion

5

Our data show that post-MI patients do not live healthier than elderly without a history of cardiac disease. Adherence to healthy behaviors differs between subgroups by gender and European region. Patients with a healthier lifestyle or dietary habits had a better prognosis than those with more unhealthy behavior. Mortality differences associated with the individual diet and lifestyle factors varied between 20-40%.

Advantages of this study include its prospective nature, its reliance on a European-wide sample, the population of free-living post-MI patients outside the hospital setting and it's almost 100% mortality follow-up. A possible limitation of the study is that the data could have been affected by selective participation (more health conscious people included) or self-reporting of diet and lifestyle (social expectation bias), resulting in an overestimation of the frequency of the healthy behaviors.

The definition of the components of the MD-score was influenced by the EUROCODE data-coding system used. In contrast to the original Greek study[5], in the present study potatoes were grouped with vegetables, poultry with meat and nuts and seeds with legumes. These combinations of foods hamper the interpretation of the differences for vegetables consumption between the regions. They possibly also attenuated the observed relationship with mortality, as it is unlikely that potatoes have the same protective effect as vegetables. Also the original definitions[5] of some score-components (e.g. a negative value for all milk products, without distinction between low or high fat milk products; a positive value for all cereals, both refined and whole meal cereals) might have attenuated the observed associations.

Our definition of physical inactivity was based on a relative criterion: the tertile-distribution of the healthy population. Prevalence of this behavior is therefore difficult to compare with other studies. Due to the small sample size and the low mortality rate in some of the subgroups (females and Southern European patients), the power of our study was limited and the association between the lifestyle determinants and mortality could not be determined for all subgroups. Finally, we cannot rule out the possibility of unmeasured and residual confounding.

With respect to the frequency of healthy behaviors in post MI-patients, other studies[9, 22-24] show various outcomes. Panagiotakos et al[23] described a significantly lower frequency of healthy lifestyle factors and dietary characteristics in 800 Greek post MI-patients in the year preceding their first event compared to healthy controls. Barzi et al.[22] found in a longitudinal study of 13,000 Italian post-MI patients a substantial improvement of dietary quality between time of event and 6 months follow-up, which was maintained for 3 years. Spencer et al[24] in an Australian cohort found a better adherence to a 'healthy lifestyle' in post-MI patients than in healthy controls. The absence of a difference in healthy habits between patients and healthy participants in our study should be interpreted in the light of these studies. It might have been that the post-MI patients initially had worse habits (which placed them at high risk) than their healthy counterparts and made already a substantial change in the desired direction after their first MI.

The strength of the associations between healthy habits and mortality in the present study (20 to 40% lower mortality) is comparable with those observed in the healthy participants in the HALE-project[10]. Other studies in patients with cardiovascular diseases also showed prognostic benefits

associated with healthy dietary behaviors (resp. 27%[9] and 50%[22] lower mortality) or a combination of healthy dietary and lifestyle habits (25% lower mortality)[24].

Promoting longstanding dietary and lifestyle changes in post-MI patients is evidence –based, relevant[1] and achievable[22], yet this knowledge is not fully implemented in many hospitals[4]. Many patients are not appropriately informed on the potential benefits of a healthy diet and lifestyle. The MD-score may offer a useful tool to promote adherence to the dietary recommendations in patients. Compared with dietary recommendations in nutrients the MD-score has the advantage of comprehensibility as most of its components are formulated in terms of foods. The cut-off points used are medians of intake in a specific population and not an absolute minimum (or maximum) amount as defined in nutritional guidelines (e.g. 400 grams of fruits and vegetables). Aiming at an intake of at least the median of the local population has the advantage of feasibility in the local situation.

Research might further improve the applicability of the MD-score for patient education. Definitions of some of the score-components need further reflection for application in different countries. Another research topic is the development of effective organizational and educational strategies to enhance longstanding compliance with dietary and lifestyle recommendations. A tailored approach is required as the frequency of health behaviors differs substantially between subgroups by gender and region.

In conclusion, there is a strong relationship between lifestyle and dietary behaviors and survival in post-MI patients. Post-MI patients do not live healthier than elderly without a history of MI. There is a wide variation in

the frequency of healthy behaviors between subgroups of the European patient population. The findings implicate that considerable health gain can be expected from efforts to promote a healthy diet and lifestyle in post-MI patients.

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6

Changes in Body weight over 5 Years and 10-year Mortality in European Elderly Men: The FINE-Project

Knoops K, de Groot L, van Staveren W, Giampaoli S, Nissinen A, Kromhout D
submitted

Abstract

Objective: To investigate the relationship between 5-year changes in body weight and mortality during 10 subsequent years of follow-up.
Design, Setting, and Participants: The FINE (Finland, Italy, and Netherlands Elderly) Study is a prospective cohort study, which includes men from Finland, Italy, and the Netherlands, aged 70-90 years at baseline.
Methods: Body weight was measured in 1985 and 1990. Mortality follow-up was completed between 1990 and 2000. The Cox proportional hazards model was used to investigate the association between body weight changes and mortality.
Results: Between 1985 and 1990, 39 % of the men lost at least 2 kg of body weight while 27 % of the men gained at least 2 kg of body weight. During the subsequent 10 years follow-up, 673 of the 1185 participants died. After excluding men with chronic diseases in 1985 and 1990, men with a BMI < 18.5 kg/m ^{2} and men who died during the first 2 years of follow-up, men who had lost more than 5 kg (adjusted HR: 1.94; 95% CI: 1.43-2.63) had a higher mortality risk compared to men with stable weight. Weight gain was not related to mortality. However, analyses stratified for region showed that weight loss of more than 5 kg was strongly significantly related to mortality in Northern Europe (HR: 2.26; 95 % CI: 1.59-3.22) but not in Southern Europe. Weight gain of more than 5 kg was weakly associated with mortality in centers in Northern Europe (HR: 1.40; 95% CI: 0.90-2.17) but not in Southern Europe.
Conclusions: Weight changes are stronger associated with mortality in centers in Northern Europe compared to centers in Southern Europe.

Introduction

The association between body weight and mortality varies with age. In younger adults, a high body mass index is associated with an increased risk of type 2 diabetes, coronary heart disease, and a number of other health conditions[1]. Flegal et al. estimated that there were about 112,000 obesity-attributable deaths in the United States in 2000[2]. Conversely, in elderly people, a low body mass index is more strongly associated with a higher mortality risk[2]. Flegal et al found that the majority of excess deaths associated with obesity occurred in individuals younger than 70 years while the majority of excess deaths associated with underweight occurred in individuals aged 70 years and older[2].

Besides underweight, unintentional weight loss is a common problem among elderly people[3]. About 15 -27 % of the elderly subjects experience weight loss. Weight loss, both voluntary and involuntary, in older individuals is associated with frailty, functional impairment and mortality[4-10]. Even small changes of 1 kg/year were found to be predictive of mortality in elderly patients recently discharged from a hospital[7]. In free-living elderly, also associations between body weight loss and mortality were found[8-21]. However, most of these studies had no or limited information on body weight at baseline and before mortality follow-up started or did not address baseline information about health status in data analysis[6, 8]. To minimize the possibility that body weight had changed in response to (sub) clinical diseases, results should be presented after excluding participants with chronic diseases at baseline and those who died during the first years of follow-up.[22] Wannamethee et al found that the higher mortality associated with weight loss in middle-

aged and older elderly is a direct consequence of ill-health leading to weight loss[18, 21]. In the study of Newman et al., an association between weight loss and mortality was found after adjustments for chronic diseases[7]. After excluding subjects with chronic diseases in the interim period, from baseline to the point at which the follow-up started, survival curves showed that the slope for mortality decreased gradually in all groups but steeper in the weight loss group. However, they did not exclude subjects who died during the first year(s) of follow-up to minimize the possibility that body weight had changed in response to subclinical diseases[8]. Therefore, we investigated the association between 5-years body weight changes and subsequent 10-year mortality follow-up in elderly European men, aged 70 till 90 years at baseline, in the FINE study. In our analyses, we excluded prevalent cases of chronic diseases and men with a low BMI. Furthermore, we also investigated if a linear trend in body weight loss or body weight gain was present and stratified to region.

Methods

Study

The FINE Study started in 1985 and consisted of the survivors of five cohorts of the Seven Countries Study: East Finland (Ilomantsi), West Finland (Pöytyä and Mellilä), Crevalcore and Montegiorgio in Italy and Zutphen in the Netherlands. Details about the FINE Study have been described elsewhere[23]. The FINE Study included men who were born between 1900 and 1920. Surveys were carried out around 1985, 1990 and mortality data were collected between 1990 and 2000. The response rates

in 1985 were for Finland 92 %, for the Netherlands 74 % and for Italy 76 %. Information about 5-years changes in body weight and vital status was available for 1196 men (464 from the Netherlands, 385 from Finland and 347 men from Italy).

Data collection

Body weight and height were measured at baseline in 1985 and 1990[23, 24]. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m2). Information on smoking status (never smokers, former smokers and current smokers), education (number of years), and the prevalence of myocardial infarction, stroke, diabetes and cancer was collected by questionnaires in a general interview. The prevalence of these chronic diseases was confirmed by information from general practitioners and/or hospital registers.

Information on vital status was collected till 2000 and was available for all the subjects.

Definition of body weight changes

Changes in body weight were calculated by subtracting body weight measured in 1985 from body weight measured in 1990. A difference of 2.0 kg or more was defined as a change in body weight. Five body weight change categories were created: weight stable (-2.0 kg < body weight < + 2.0 kg), weight loss between 2 and 5 kg, weight loss of 5 kg or more, weight gain between 2 and 5 kg, and weight gain of 5 kg or more .

Statistical analysis

Statistical analyses were carried out using the SAS statistical analysis computer package (version 9.1). The Cox proportional hazards model was used to estimate the association between the five groups of body weight change and mortality. Dummy variables were created for the different body weight groups; the weight stable group (-2.0 kg < body weight < + 2.0 kg) was used as reference group. For all models, the Cox proportional hazards assumption was tested and met.

Adjustments were made for age at baseline (continuous), number of years of education (continuous), baseline BMI (categories were created following the WHO-criteria: <18.5 kg/m^2, between 18.5 kg/m^2 and 25 kg/m^2, between 25 kg/m^2 and 30 kg/m^2, and 30 kg/m^2 or more; the category between 18.5 and 25 is used as reference category), smoking (3 categories: never, former and current smokers; the category never smokers is used as reference category), chronic diseases at baseline (myocardial infarction, stroke, diabetes, and cancer), and study center. Subjects with a body mass index <18.5 kg/m^2 were excluded from the analyses. To minimize the possibility that body weight had changed in response to subclinical diseases, the analyses were repeated after excluding participants with chronic disease in 1985 or in 1990 and those who died during the first 2 years of follow-up[22]. Interactions between baseline BMI and body weight groups, between BMI and smoking, groups of body weight changes and smoking, between region and BMI and between region and groups of body weight changes were tested, but none of them was statistically significant at α=0.05.

The results were also stratified for region; where Finland and the Netherlands were called Northern Europe and Italy Southern Europe.

Results

Between 1985 and 1990, 36 % of the men remained stable in body weight (less than 2 kg body weight change) while 18 % experienced weight loss of at least 5 kg, and 20 % of the men experienced between 2 and 5 kg body weight loss while 16 % of the men gained between the 2 and 5 kg and 10 % gained at least 5 kg of body weight.

Table 1: Characteristics of the FINE population in 1990.

Variable	The Netherlands (n=462)	Finland (381)	Italy (n=342)
Age (years) (Mean ± Sd)	76 ± 4	77 ± 5	76 ± 4
Education (years) (Mean ±Sd)	10 ± 4	4 ± 3	5 ± 2
Smoking status			
Non-smoker, N (%)	82 (18)	111 (29)	115 (34)
Former smoker, N (%)	278 (60)	227 (60)	174 (51)
Current smoker, N (%)	102 (22)	43 (11)	52 (15)
Body weight (kg) (Mean ±Sd)	76 ± 11	75± 12	72 ± 11
Body height (cm)(Mean ±Sd)	173 ± 6	170 ± 6	167 ± 7
BMI (kg/m^2)	26 ± 3	26 ± 4	26 ± 3
Baseline disease, N (%):			
Myocardial infarction	66 (14)	67 (18)	23 (7)
Stroke	17 (4)	28 (7)	19 (6)
Diabetes	45 (10)	31 (8)	33 (10)
Cancer	45 (10)	42 (11)	23 (7)

The baseline risk characteristics of the countries participating in the FINE Study are presented in table 1. The average age of the men in the FINE Study at baseline was 71 years. Men in the Netherlands received more years of education compared to those from Finland and Italy (respectively 10, 4 vs 5). The average BMI at baseline was 26 kg/m ^{2} in all three countries. In the Netherlands and Finland, the prevalence of myocardial infarction was higher compared to Italy (respectively 14, 18

vs 7 %). The 10-year mortality rate was also higher in the Netherlands and Finland, compared to Italy (respectively 60, 62, vs 47 %).

Table 2: Body weight changes between 1985 and 1990 and mortality follow-up between 1990 and 2000 in the FINE study.

Weight changes	Hazard rates (95 % CI)	
N at risk: 1185 N deaths: 682	Crude model	Multivariate model[1]
≥ 5 kg weight loss (n=240)	2.26 (1.84-2.77)	2.16 (1.75-2.67)
Between 2 and 5 kg weight loss (n=220)	1.27 (1.03-1.57)	1.25 (1.01-1.55)
Stable weight (< 2 kg weight change) (reference) (n=430)	-	-
Between 2 and 5 kg weight gain (n=198)	0.98 (0.77-1.24)	1.01 (0.79-1.29)
≥ 5 kg weight gain (n=119)	1.27 (0.97-1.68)	1.20 (0.91-1.58)
p-value trend test for weight loss	<0.001	<0.001
p-value trend test for weight gain	0.17	0.26
Age	-	1.09 (1.08-1.11)
Number of years of education	-	0.98 (0.96-0.99)
Myocardial infarction (reference=no)	-	1.35 (1.09-1.66)
Stroke (reference=no)	-	1.50 (1.10-2.05)
Diabetes (reference=no)	-	1.27 (0.99-1.61)
Cancer (reference=no)	-	1.33 (1.03-1.70)
Smoking: Nonsmokers Former smokers Smokers	- - -	reference 1.04 (0.86-1.27) 1.53 (1.23-1.91)
BMI: Between 18.5 and 25 Between 25 and 30 > 30	- - -	reference 0.89 (0.75-1.04) 0.76 (0.58 -1.00)

[1]Model adjusted for study centre

The results of the Cox proportional hazards model for the association between changes in body weight and mortality in the FINE Study are presented in table 2. Losing at least 5 kg of body weight (HR: 2.26, 95 % CI: 1.84-2.77) and losing between 2 and 5 kg of body weight (HR: 1.27,

95 % CI: 1.03-1.57) between 1985 and 1990 was significantly associated with a higher mortality risk, compared to those who maintained their weight. Adjusting for age, chronic diseases, number of years of education, smoking, BMI at baseline and study centre did not change these conclusions.

Table 3: Body weight changes between 1985 and 1990 and 10-year mortality in apparently healthy elderly men.

Weight changes	Hazard rates (95 % CI)	
N at risk=749 N deaths =355	Crude model	Adjusted model[2]
≥ 5 kg weight loss (n=104)	2.01 (1.48-2.71)	1.94 (1.43-2.63)
Between 2 and 5 kg weight loss (n=157)	1.37 (1.03-1.81)	1.37 (1.03-1.83)
Stable weight (< 2 kg weight change) (reference) (n=287)	-	-
Between 2 and 5 kg weight gain (n=133)	0.92 (0.67-1.28)	1.01 (0.72-1.41)
≥ 5 kg weight gain (n=75)	1.29 (0.89-1.87)	1.29 (0.88-1.88)
p-value trend test for weight loss	<0.001	<0.001
p-value trend test for weight gain	0.3136	0.3864

[1]Participants with BMI< 18.5, prevalent cases of chronic diseases in 1985 and 1990, and those who died during the first 2 years of mortality follow-up were excluded. [2]Model also adjusted for age, education, smoking, BMI, and study centre.

In the crude and the adjusted proportional hazards models, no significant association was observed between weight gain and mortality risk. Age, myocardial infarction, stroke, cancer, and current smoking were positively associated with mortality. Number of years of education was inversely associated with mortality risk.

Similar results were obtained after excluding men with chronic diseases in 1985 and 1990, men with a BMI < 18.5 kg/m ^{2} at 1990 and those who

died during the first 2 years of follow-up (table 3). Losing at least 5 kg of body weight (HR: 1.94, 95 % CI: 1.43-2.63) and losing between 2 and 5 kg of body weight (HR: 1.37, 95 % CI: 1.03-1.81) during the 10 year follow-up was significantly associated with a higher mortality risk, compared to stable body weight, after adjustments for age, number of years of education, smoking, BMI at baseline and study centre.

Table 4: Body weight changes between 1985 and 1990 and 10-year mortality in apparently healthy men, stratified by region

	Hazard rates (95 % CI)[2]	
Weight changes	Northern Europe (N at risk = 511) (N deaths = 267)	Southern Europe (N at Risk =238) (N deaths=88)
≥ 5 kg weight loss	2.26 (1.59-3.22)	1.38 (0.73 -2.62)
Between 2 and 5 kg weight loss	1.48 (1.07-2.04)	0.96 (0.51-1.79)
Stable weight (< 2 kg weight change) (reference)	-	-
Between 2 and 5 kg weight gain	0.90 (0.60-1.35)	1.17 (0.63-2.15)
≥ 5 kg weight gain	1.40 (0.90-2.17)	1.02 (0.46-2.23)
p-value trend test for weight loss	<0.001	0.06
p-value trend test for weight gain	0.49	0.75

[1]Participants with BMI< 18.5, prevalent cases of chronic diseases in 1985 and 1990, and those who died during the first 2 years of mortality follow-up were excluded. [2]Model also adjusted for age, education, smoking, BMI, and study centre .

When we stratified for region, we observed that weight loss was stronger associated with mortality in the centers of Northern compared to the centers of Southern Europe (Table 4). In the centers of Northern Europe, losing at least 5 kg of body weight was strongly related to mortality (HR: 2.26, 95 % CI: 1.59-3.22). In the centers of Southern Europe, the

association between losing at least 5 kg of body weight and mortality was not statistically significant. Weight gain of more than 5 kg was associated with mortality in the centers of Northern Europe, although the association was only weakly significant (HR: 1.40, 95% CI: 0.90-2.17). In the centers of Southern Europe, body weight gain was not associated with mortality.

Discussion

In this study, weight loss was strongly inversely associated with mortality during 10 years of follow-up in European men free from chronic diseases and with a BMI > 18.5 kg/m^2. This association was statistically significant in Northern European men only. Weight gain of more than 5 kg was weakly significantly associated with increased mortality also in Northern European study centers only.

Advantages of this study were its standardized measurements of body weight within the different European countries, its prospective nature, long follow-up period, its complete mortality follow-up and its measurements of many potential confounders. However, this study has also limitations. We are not able to differentiate between intentional and unintentional body weight loss. The differentiation between intentional and unintentional weight loss may have some important implications in the evaluation of weight loss effects on mortality because it is known that unintentional weight loss may reflect illness leading to increased mortality[21,25]. However, the results of the few epidemiological studies on weight loss that have been able to differentiate between intentional and

unintentional weight loss are inconsistent and the review of Sorensen suggested that also intentional weight loss is associated with mortality.[26]

The prevalence of weight loss in the present study is in line with the estimates of other studies. Also other epidemiological studies have shown that most elderly people maintain their weight over 5-10 years, while 15-20 % experienced weight loss. In these studies, weight loss was defined as either 5 kg or more or 5 % of usual body weight over 5-10 years[3, 7, 27, 28]. Weight loss in elderly over 70 years old is often seen because of ageing related processes such as anorexia of aging and sarcopenia. Normal-weight healthy elderly decrease their energy intake below their energy expenditure and consequently loose weight and because of loss of lean body mass (sarcopenia)[29]. Anorexia of aging has multiple causes such as changes in taste and smell, increased gastrointestinal satiation signals, increased leptin levels, and a decline in central feeding drive[30]. This physiological anorexia of aging makes older persons more vulnerable to develop severe anorexia and muscle wasting when they develop disease[30].

We observed a strong association between weight loss and mortality, especially in Northern European men. Other studies found similar associations between weight loss and mortality in elderly, aged 65 or older. Weight loss of 4-5 % or more of body weight within 1 year or 10 % or more, over 5-10 years or longer is associated with increased morbidity or mortality or both[8, 10, 15, 27, 31-35].

Weight gain of more than 5 kg was weakly significantly associated with a higher risk of mortality in Northern Europe. In a Swedish cohort, with an average age of 70 years, Dey et al. found that body weight gain of ≥ 5% between age 70 and 75 resulted in a numerically lower 5 y mortality risk

for the males (RR: 0.62, 95% CI 0.38 -1.01), and a numerically higher risk for females (RR: 2.18, 95% CI 0.95 - 4.99) of the same weight change group. However, both RR's did not reached statistically significance[10].

The present study shows that in elderly with a BMI ≥ 18.5 kg/m^2 at baseline in Northern Europe weight loss of more than 2 kg in 5 years was strongly related with increased mortality even after exclusion of mortality during the first 2 years of follow-up. In these persons, there was also a weak association between weight gain of more than 5 kg in 10 years and increased mortality. Significant associations between either weight loss or weight gain were not seen in Southern Europe, although the p-trend for weight loss and mortality was borderline significant. An explanation for this may be the superiority of the Mediterranean diet in relation to survival as observed in this project[36].

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7
Different Cholesterol Fractions and 10-year Cardiovascular and All-cause Mortality in European Elderly Men and Women: The HALE-Project

Knoops K, de Groot L, van Staveren W, Kromhout D
submitted

Abstract

Context Associations between different cholesterol fractions and mortality from cardiovascular diseases and all-causes have been investigated in elderly, but the results are inconsistent.

Objective To investigate the association between total cholesterol, HDL, LDL and VLDL -cholesterol and 10-year mortality from cardiovascular diseases and all-causes in European elderly men and women.

Design, Setting, and Participants The Healthy Ageing: a Longitudinal study in Europe (HALE) population includes 1344 apparently healthy men and 643 women, aged 70 to 90 years in 11 European countries, who were followed for 10 years.

Main Outcome Measures Ten-year mortality from cardiovascular diseases and all-causes.

Results During follow-up, 718 participants died and 302 from cardiovascular diseases. In men and women, a significant inverse association was found between HDL-cholesterol and cardiovascular and all-cause mortality. Men and women in the highest tertile versus the lowest tertile had a significant lower risk of mortality from cardiovascular diseases (HR: 0.72, 95% CI: 0.54-0.95) and all-causes (HR: 0.80, 95% CI: 0.66-0.96). These associations remained significant after adjustments for gender, age, education, BMI, smoking, study centre and LDL and VLDL-cholesterol. The associations between HDL-cholesterol and mortality were strongest in women. Total cholesterol, LDL and VLDL-cholesterol were not significantly related to mortality.

Conclusion Among individuals aged 70 to 90 years, HDL-cholesterol is inversely associated with mortality from cardiovascular diseases and all-causes. These associations were strongest in women.

Introduction

In adults, a direct relationship between serum total cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides , and indicator of VLDL, and coronary heart disease and an inverse relationship between serum high-density lipoprotein (HDL) cholesterol and coronary heart disease exist[1,2]. However, the results of studies concerning serum total cholesterol and cardiovascular morbidity and mortality and mortality from all-causes in elderly are inconsistent[3-24]. Results of several studies showed an inverse relation, or no relation, between serum total cholesterol level and risk of death in elderly people[6, 9, 10, 18]. A U-shaped relationship has also been observed, in which both low and high concentrations of serum total cholesterol in elderly people are associated with increased mortality[3, 17]. Other studies found that low serum total cholesterol levels were associated with increased mortality in the oldest old[11, 16, 18, 20, 24].

Also the associations between different cholesterol fractions such as LDL and VLDL, and cardiovascular morbidity and mortality are not consistent in elderly[3-9, 11, 19, 24]. In the study of Kronmal et al., and Schupf et al., LDL-cholesterol was inversely associated with mortality, while Weverling-Rijnsburger et al. found no significant association between LDL-cholesterol and mortality[17, 19, 24]. Triglycerides were significantly inversely associated with coronary heart disease in older Japanese-American men, while other studies did not found an association between triglycerides and mortality[7, 11, 24]. Also the studies which investigated the association between HDL-cholesterol and cardiovascular mortality and mortality from all-causes were inconsistent; some studies indicated an

inverse association between HDL and mortality, while other studies found no association[2, 3, 6, 11, 24].

Because the associations between serum total cholesterol and cardiovascular mortality and between LDL, VLDL, and HDL-cholesterol and cardiovascular mortality in elderly are not yet clear, we investigated these associations. The aim of the present study was to investigate the associations between total cholesterol, LDL, VLDL, HDL-cholesterol and mortality from cardiovascular diseases and all-causes in elderly European men and women, aged 70-90 who were followed for 10 years in the HALE project.

Methods

Study population

The HALE project included all subjects of the SENECA (Survey in Europe on Nutrition and the Elderly: a Concerned Action) and FINE (Finland, Italy, Netherlands, Elderly) Study who participated in the survey of 1988-1991 and were followed for 10 years[25,26]. Details about the SENECA and FINE Study have been described elsewhere[27, 28], and are briefly summarized here.

The SENECA Study is a prospective follow-up study investigating whether diet, lifestyle and biological risk factors are associated with health status in elderly people. The SENECA Study started in 1988 and consisted of a random age- and sex-stratified sample of European elderly aged 70–75 years. Surveys were repeated around 1995 and 1999[27]. In the HALE project, all men and women of the following towns were included: Hamme (Belgium), Roskilde (Denmark), Strasbourg (France), Valence

(France), Iraklion (Greece), Monor (Hungary), Padua (Italy), Culemborg (The Netherlands), Vila Franca de Xira (Portugal), Betanzos (Spain), Yverdon, Burgdorf and Bellinzona (Switzerland)[25].

The FINE Study consists of the survivors of five cohorts of the Seven Countries Study: East Finland (Ilomantsi), West Finland (Pöytyä and Mellilä), Crevalcore and Montegiorgio in Italy and Zutphen in the Netherlands. The FINE Study started in 1984 among men who were born between 1900 and 1920. For the present study, the data collected around 1990 in men aged 70-90, were used[25].

Information on smoking (never, former and current smoking), education (number of years), and the prevalence of coronary heart disease, stroke, diabetes and cancer was collected by questionnaires. The prevalence of chronic diseases was confirmed by information from general practitioners and/or hospital registers in the FINE Study. Blood pressure was measured only in the FINE Study[28].

Body Mass Index (BMI) was calculated as measured weight (kg) divided by height squared (m^2). Weight and height measurements are described in detail elsewhere[27, 28]. In the analyses, BMI was dichotomised in BMI less or equal than 25 or a BMI higher than 25 kg/m^2.

Blood lipids

In SENECA, blood samples were collected by venipuncture after an overnight fast[24]. Serum was obtained by low-speed centrifugation, stored at -80^{o}C and sent on dry ice to the Department of Human Nutrition at Wageningen University in the Netherlands. Total and HDL cholesterol and triglycerides were measured by using enzymatic calorimetric methods[29].

For the FINE Study[21], fasting blood samples were taken. In each country, total and HDL cholesterol, and triglycerides were enzymatically determined. The lipid laboratories participating in the HALE project were standardized according to the criteria of the World Health Organization Lipid Reference Laboratories in Prague, Czech Republic, and the Centers of Disease Control, Atlanta, Georgia, USA[21].

VLDL cholesterol was calculated using the Friedewald formula[30-31]:

VLDL-cholesterol (mmol/L)= 0.456* triglycerides (mmol/L)

LDL-cholesterol was calculated as follows[30]:

LDL-cholesterol (mmol/L) = total cholesterol (mmol/L) – (HDL-cholesterol (mmol/L) - 0.456 *triglycerides (mmol/L).

When plasma triglyceride concentrations exceeded 4.52 mmol/L, LDL-cholesterol was not calculated, because the Friedewald formula is not valid for such high values (n=10).

Health status

Information on vital status was collected in 1999-2000 and was available for 99.7 % of the subjects. Causes of death were available for 92 % of the subjects in FINE and 74 % of the subjects in SENECA study. Causes of death were coded by an experienced clinical epidemiologist according to the 9th revision of the WHO-ICD coding system[32]: cardiovascular mortality was defined as ICD-codes 390-459.

Statistical analysis

Statistical analyses were carried out using the SAS statistical analysis computer package (version 9.1). The Cox proportional hazards model was used to estimate total cholesterol and the different cholesterol

fractions in relation to cardiovascular and all-cause mortality. Total cholesterol and the cholesterol fractions were divided in tertiles. Dummy variables were created, with the first tertile as reference category.

Subjects with myocardial infarction, stroke, diabetes, or cancer, and those who died the first two years of follow-up were excluded[33]. We also excluded subjects with a BMI < 18.5 kg/m^2 at baseline.

Besides the crude models, the models for cardiovascular mortality and mortality from all-causes were repeated after adjustments for age, gender, number of years of education, smoking, BMI, and study centre.

Interactions were tested for the different cholesterol fractions in relation to gender, BMI, and region. In the FINE Study, blood pressure was added to the model as potential confounder, but did not affect our effect estimates. The interaction between cholesterol fractions and body weight changes (stable body weight, 2-5 kg body weight gain or body weight loss, > 5 kg body weight gain or body weight loss) was tested but also hardly affect our estimates.

The study centres of Finland, Denmark, The Netherlands, Belgium, the centre Strasbourg in France and the study centre Burgdorf in Switzerland were considered as Northern Europe. The study centres of Italy, Spain, Portugal, and Greece and the centre Valence (France), Yverdon and Bellinzona in Switzerland were considered as Southern Europe. There was no interaction observed between region and the different cholesterol fractions.

This study used data of the HALE project (Healthy Ageing: Longitudinal study in Europe) and was supported by a grant from the European Union (QLK6-CT-2000-00211).

Results

Information on serum total cholesterol, LDL, VLDL, HDL-cholesterol, and mortality was available for 1334 men and 630 women with a BMI > 18.5 kg/m^2 and without myocardial infarction, stroke, diabetes or cancer at baseline (table 1).

Table 1: Baseline characteristics and 10-year mortality data in elderly men and women: the HALE project[1].

Characteristics	SENECA		FINE
	Women (n=630)	Men (n=604)	Men (n=730)
Age (years), mean (sd)	73 (2)	73 (2)	76 (4)
Years of education, mean (sd)	7 (4)	9 (4)	7 (4)
Northern Europe, N (%)	279 (44)	2787(46)	493 (68)
Smokers, N (%)	51 (8)	189 (31)	121 (17)
Body Mass Index, mean (sd)	27.0 (4.3)	26.6 (3.7)	26.1 (3.4)
Blood lipids (mmol/L), mean (sd)			
Total cholesterol	6.70 (1.26)	6.05 (1.05)	5.77 (1.09)
LDL cholesterol	4.58 (1.13)	4.16 (0.97)	3.87 (1.03)
VLDL cholesterol	0.63 (0.31)	0.62 (0.31)	0.70 (0.32)
HDL cholesterol	1.47 (0.38)	1.27 (0.34)	1.23 (0.32)
Causes of death, N (%)			
All-causes	127 (20)	248 (41)	343 (47)
Cardiovascular diseases	51 (40)	86 (35)	165 (48)
Coronary heart disease	14 (11)	36 (15)	51 (15)
Cancer	21 (17)	70 (28)	91 (27)
Unknown	37 (29)	59 (24)	26 (8)

[1]Participants with chronic disease and/ or with a BMI < 18.5 were excluded.

Men in the SENECA Study were on average 3 years younger than men in the FINE Study. In the SENECA Study, there were almost 5 times as many male smokers as there were female smokers. The average follow-up period was 10 years, with a range of 8.9 to 10.5 years. Twice as many men died during the 10-year follow-up as did women. Total cholesterol, LDL- cholesterol, and HDL-cholesterol were higher in men in SENECA

compared to men in the FINE Study. VLDL-cholesterol was lower in men in the SENECA compared to men in the FINE Study. In women, total cholesterol, LDL- cholesterol, and HDL-cholesterol were higher compared to men. VLDL-cholesterol was higher in FINE compared to the SENECA Study.

Table 2 presents the results of the Cox proportional hazards models for total cholesterol, the different cholesterol fractions and mortality from cardiovascular diseases and all-causes. Total cholesterol, LDL, and VLDL-cholesterol were not associated with mortality from cardiovascular diseases. HDL-cholesterol was significantly inversely associated with mortality from cardiovascular diseases. Elderly in the third tertile of HDL-cholesterol had a significantly lower risk of mortality from cardiovascular diseases, even after adjustments for age, gender, education, BMI, smoking status, and study centre. The trend between HDL cholesterol and mortality from cardiovascular diseases was statistically significant. High total and LDL cholesterol were significantly associated with lower all-cause mortality (table 2). However, after adjustments for age, gender, education, BMI, smoking and study centre, the association was not longer statistically significant. VLDL-cholesterol was not associated with all-cause mortality.

The association between HDL-cholesterol and all-cause mortality was inverse and statistically significant, even after adjustments. However, there was a strong interaction between HDL and gender in the crude model ($p=0.0006$) and after adjustments for age, education, BMI, smoking and study centre ($p=0.0006$).

Table 2: Cox proportional hazards model for total, LDL, VLDL, HDL-cholesterol, and 10-year mortality in the HALE population.

Cholesterol and cholesterol fractions	Mortality from cardiovascular diseases N at risk=1841 N deaths= 302		All-cause mortality N at risk =1964 N deaths = 718	
	Crude HR (95% CI)	Adjusted HR (95% CI)	Crude HR (95% CI)	Adjusted HR (95% CI)
Total				
1st Tertile (ref)	1.0	1.0	1.0	1.0
2nd Tertile	0.76 (0.58-1.00)	0.81 (0.62-1.07)	0.87 (0.73-1.04)	0.90 (0.75-1.07)
3rd Tertile	0.78 (0.59-1.02)	0.92 (0.70-1.21)	0.76 (0.63-0.90)	0.84 (0.70-1.01)
	p for trend = 0.08	p for trend = 0.55	p for trend=0.002	p for trend = 0.052
LDL	N=1833	N=1833	N=1954	N=1954
1st Tertile (ref)	1.0	1.0	1.0	1.0
2nd Tertile	0.84 (0.64-1.11)	0.92 (0.70-1.22)	0.88 (0.73-1.05)	0.92 (0.77-1.10)
3rd Tertile	0.78 (0.59-1.03)	0.91 (0.69-1.21)	0.77 (0.64-0.92)	0.84 (0.70-1.01)
	p for trend = 0.08	p for trend = 0.53	p for trend=0.003	p for trend =0.051
VLDL				
1st Tertile (ref)	1.0	1.0	1.0	1.0
2nd Tertile	1.37 (1.03-1.81)	1.29 (0.97-1.71)	1.00 (0.83-1.20)	0.96 (0.80-1.15)
3rd Tertile	1.16 (0.87-1.55)	1.11 (0.83-1.50)	1.03 (0.87-1.24)	1.01 (0.84-1.22)
	p for trend = 0.53	p for trend = 0.72	p for trend =0.74	p for trend=0.87
HDL				
1st Tertile (ref)	1.0	1.0	1.0	1.0
2nd Tertile	0.82 (0.63-1.07)	0.79 (0.60-1.04)	0.94 (0.79-1.12)	0.90 (0.75-1.07)
3rd Tertile	0.72 (0.55-0.95)	0.72 (0.54-0.95)	0.84 (0.70-1.00)	0.80 (0.66-0.96)
	p for trend = 0.02	p for trend = 0.022	p for trend =0.058	p for trend =0.021

[1]Model adjusted for age, sex, smoking, BMI, education and study centre

Table 3: Cox proportional hazard model for total, LDL, VLDL, and HDL-cholesterol and 10-year mortality in men and women in the HALE project.

Biological risk factor	All-causes mortality[1]			Mortality from cardiovascular diseases[1]		
	Men N at risk =1327 N deaths= 588	Women N at risk =627 N deaths= 125	Men and women N at risk =1954 N deaths= 713	Men N at risk =1243 N deaths=251	Women N at risk =590 N deaths= 50	Men and women N at risk =1833 N deaths= 301
Total cholesterol[1]	1.0 0.93 (0.77-1.13) 0.85 (0.70-1.04)	1.0 0.78 (0.52-1.18) 0.74 (0.48-1.13)	1.0 0.90 (0.75-1.07) 0.84 (0.70-1.01)	1.0 0.85 (0.62-1.15) 0.98 (0.73-1.34)	1.0 0.78 (0.40-1.52) 0.74 (0.37-1.48)	1.0 0.81 (0.62-1.07) 0.92 (0.70-1.21)
Blood cholesterol fractions[2]:						
LDL						
1st Tertile (ref)	1.0	1.0	1.0	1.0	1.0	1.0
2nd Tertile	0.95 (0.78-1.16)	0.80 (0.51-1.25)	0.91 (0.76-1.10)	0.95 (0.70-1.30)	0.74 (0.36-1.53)	0.89 (0.67-1.17)
3rd Tertile	0.84 (0.69-1.04)	0.84 (0.53-1.33)	0.84 (0.69-1.01)	0.90 (0.65-1.23)	0.96 (0.47-1.98)	0.86 (0.65-1.16)
VLDL						
1st Tertile (ref)	1.0	1.0	1.0	1.0	1.0	1.0
2nd Tertile	1.05 (0.55-1.29)	0.67 (0.42-1.08)	0.97 (0.80-1.17)	1.46 (1.06-2.02)	0.74 (0.36-1.53)	1.28 (0.96-1.71)
3rd Tertile	1.04 (0.84-1.30)	0.68 (0.42-1.10)	0.97 (0.80-1.19)	1.17 (0.82-1.66)	0.53 (0.25-1.14)	1.02 (0.74-1.40)
HDL						
1st Tertile (ref)	1.0	1.0	1.0	1.0	1.0	1.0
2nd Tertile	1.03 (0.84-1.26)	0.55 (0.35-0.85)	0.91 (0.76-1.09)	0.86 (0.63-1.16)	0.53 (0.26-1.06)	0.79 (0.60-1.04)
3rd Tertile	0.91 (0.73-1.14)	0.41 (0.25-0.68)	0.79 (0.65-0.97)	0.80 (0.57-1.10)	0.38 (0.17-0.85)	0.70 (0.52-0.95)

[1]Model adjusted for age, sex, smoking, BMI, education and study centre, [2]Model adjusted for age, sex, smoking, BMI, education and study centre and the other blood cholesterol fractions

In table 3, the associations between serum total cholesterol and different cholesterol fractions in relation to mortality cardiovascular diseases and all-causes is presented for men and women separately. The strongest associations were observed between HDL and mortality in women. In men, the association between HDL and mortality from cardiovascular diseases and all-causes did not reached significance.

Discussion

Among individuals aged 70 to 90 years, men and women in the highest tertile versus the lowest tertile of HDL-cholesterol had a significantly lower risk of mortality from cardiovascular diseases and all-causes. These associations remained significant after adjustments for age, gender, education, BMI, smoking, study centre and LDL and VLDL-cholesterol. The associations between HDL-cholesterol and mortality from cardiovascular diseases and from all-causes were strongest for women. Total, LDL and VLDL-cholesterol were not significantly related to mortality.

Advantages of this Europe-wide study are its great diversity in cholesterol fractions, its prospective nature, its relatively large sample size, and its measurements of many potential confounders. The SENECA and FINE Study could be pooled because the estimates of the separate models were similar and there were no important differences in study population and measurement of blood lipids. This study has also limitations. First, although vital status follow-up was nearly complete, the cause of death could not be ascertained in the SENECA Study for 26

% of the subjects. This limited our power to study cause-specific associations. Second, the number of women enrolled in this study was relatively small and that limited gender specific analysis.

In this study, HDL-cholesterol was inversely associated with mortality from cardiovascular diseases and all-causes, especially in women. Adjustments for the other cholesterol fractions LDL and VLDL-cholesterol did not change these conclusions. Other studies also found an inverse association between HDL and mortality from cardiovascular diseases and all-causes[9, 11, 19]. Weverling-Rijnsburger found that HDL was inversely associated with mortality from coronary artery disease and stroke in Dutch elderly of 85 years and older[19]. HDL-cholesterol was associated with mortality from coronary heart diseases and the occurrence of new coronary heart disease events in persons older than 70 years in the study of Corti et al.[9]. In the study of Chyou et al., a high level of HDL-cholesterol was also associated with a lower mortality risk from all-causes in men aged 65-74 years[11]. Not all studies support these findings[6, 24]. However, the studies that did not found an association between HDL-cholesterol and mortality, had a relative short follow-up period (4 year and less) or included also participants with baseline diseases who may confound the results[6, 24].

In the present study, the association between HDL-cholesterol and mortality was strongest for women. This is in line with the study of Nikkila et al[3]. In that study, the relative risk for mortality in women with a HDL-cholesterol < 0.80 mmol/L was 2.58 ($p< 0.01$) compared to women with a HDL-cholesterol of ≥ 1.80 mmol/L. The relative risks for women with a HDL-cholesterol between 0.80 and 1.29 mmol/L and between 1.30 and 1.79 mmol/L were respectively 2.35 ($P< 0.01$) and 2.21

($p<0.01$)[3]. In men no significant association was found between HDL-cholesterol and mortality[3]. In the study of Weverling-Rijsburger et al., the relative risks for low HDL-cholesterol in relation to cardiovascular mortality were also stronger for women (HR: 2.2 (95% CI: 1.1-4.4)) compared to men (HR: 1.6 (95% CI: 0.8-3.4))[19]. However, the study of Corti et al, reported no difference in relative risk for HDL-cholesterol in relation to mortality for men and women[9]. In the study of Chyou et al., HDL-cholesterol was only associated with all-cause mortality in men. This study included also participants with history of coronary heart disease who may confound the results[11].

The crude models suggested that total cholesterol and LDL-cholesterol were inversely associated with mortality, but these associations were not statistically significant after adjustments for age, gender, education, BMI, smoking and study centre. Total cholesterol and LDL-cholesterol were strongly correlated (Pearson correlation Coefficient= 0.95), which explains similar results for total cholesterol and LDL-cholesterol in our study. Other studies found also no association between serum total cholesterol or LDL-cholesterol and mortality[6, 9, 18, 19, 23]. Some other studies however also suggested that low serum cholesterol levels were associated with increased mortality in the oldest old[17, 18, 20, 34]. There is some evidence that cholesterol levels decline with age[18] and with chronic disease, inflammation, malnutrition, or poor health status in elderly persons[13-15]. Lower cholesterol levels in the elderly may represent a surrogate for comorbidity, frailty, or subclinical disease[10, 15] . In the present study, we excluded subjects with coronary heart disease, stroke, diabetes or cancer and subjects with a BMI < 18.5 kg/m^2 and subjects who died during the first 2 years of follow-up, so almost all participants

with a poor health status were excluded from our analysis. After adjusting our model for age, and other possible confounders, we did not found a significant association between total cholesterol, LDL-cholesterol and mortality.

In our study, VLDL-cholesterol was not associated with mortality. In the Honolulu Heart Study, the triglycerides level at ages below 60 was an independent predictor of CHD, but not at older ages[5]. Other studies involving elderly participants did also not found an association between VLDL or triglycerides and mortality[11, 24]. In the study of Austin et al., the relative risk for triglycerides was 1.70 (p=0.01) for a 1 natural log unit increase in triglycerides (mg/dl), and remained statistically significant after adjustment for covariates[7]. However, after adjustment for the other cholesterol fractions, LDL and HDL-cholesterol, only the relative risk for HDL remained statistically significant (HR: 0.70, p =0.01)[7], suggesting that a low HDL-cholesterol is a stronger predictor for mortality in elderly.

In conclusion, even in elderly people between 70 and 90 years at baseline, HDL-cholesterol is inversely associated with mortality from cardiovascular diseases and all-causes, especially in women. However, total, LDL and VLDL were not related to mortality in the elderly.

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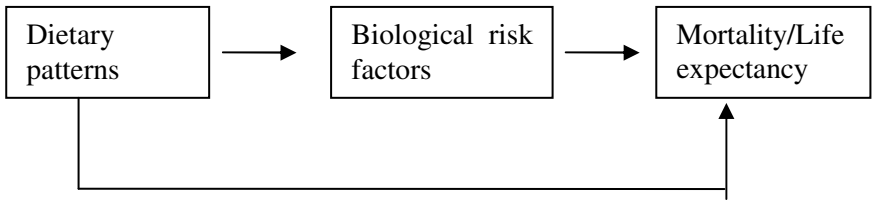
8
General Discussion

8.1 Main findings

Healthy aging comprises optimising both guantity and quality of life. Quantity of life is in this thesis operationalised as mortality. For preventive action, determinants of survival are of great interest. The conceptual model[1] on how determinants influence the risk of mortality is presented in figure 1. Dietary patterns exert an effect (positive or negative) on biological risk factors; biological risk factors influence the risk on mortality. However, dietary patterns can also affect mortality independent of their effects on biological risk factors. For example, n-3 fatty acids are inversely associated with coronary mortality. The most popular hypothesis to explain this association is that fatty acids prevent arrhythmias and therefore coronary mortality[2, 3]. This means that this association is independent of classical epidemiological risk factors for coronary mortality. The aim of this thesis was to investigate these associations in elderly subjects.

Figure 1: The conceptual model for diet and mortality in the population[1]



The main findings of the results presented in this thesis are summarized in table1.

We found that dietary patterns were associated with biological risk factors in elderly men in the Normative Aging Study. Homocysteine levels appeared to be lower and plasma B-vitamin concentrations higher

in a “prudent” dietary pattern compared to an “unhealthy” dietary patterns and a pattern characterized by “a relatively high alcohol and low fruit and vegetable intake”, although these differences were not statistically significant. After fortification with folic acid, folate intake and plasma folate levels increased significantly in all dietary patterns. Homocysteine tended to decrease in the cluster characterized by a high alcohol and low fruit and vegetable dietary pattern after fortification.

Dietary patterns defined by three European indexes were significantly associated with mortality in elderly men and women. Adherence to a Mediterranean type of diet and other healthy lifestyle factors (moderate alcohol consumption, being physical active and non-smoking) were associated with lower mortality rates in apparently healthy elderly and in post-myocardial infarction patients.

In the last part of this thesis, we found significant associations between diet-related biological risk factors and mortality. Weight loss was significantly associated with increased mortality in the study centres of Northern Europe while weight gain was weakly associated with mortality in these centres. HDL-cholesterol was inversely associated with mortality from cardiovascular diseases and all-causes, especially in women.

Table 1: Overview of the main results and conclusions of this thesis.

Investigated topic	Main results	Conclusion
Difference in intake and plasma B-vitamins, and in homocysteine levels before and after folic acid fortification, in relation to dietary patterns in the Normative Aging Study.	Dietary intake and plasma concentrations of folate increased significantly ($P<0.05$) among the three dietary patterns. Homocysteine tended to decrease in non-supplement users and in subjects in the high alcohol, low fruit and vegetable dietary pattern.	After fortification with folic acid, folate intake and plasma folate concentration increased significantly in all three dietary patterns.
Associations between dietary patterns and mortality using different European indexes of overall dietary quality in the HALE project.	The Mediterranean Diet Score (HR: 0.82 with 95% CI: 0.75–0.91), the Mediterranean Adequacy Index (HR: 0.83 with 95% CI: 0.75–0.92) and the Healthy Diet Indicator (HR: 0.89 with 95% CI: 0.81–0.98) were inversely associated with all-cause mortality.	The three different dietary scores were significantly inversely related with all-cause mortality.
A Mediterranean type of diet, being physically active, moderate alcohol use, and non-smoking in relation to mortality in the HALE project.	Adhering to a Mediterranean type of diet (HR: 0.77 with 95% CI: 0.68-0.88), moderate alcohol use, physical activity, and non-smoking were associated with a lower risk of all-cause mortality. The combination of 4 low risk factors lowered the all-cause mortality risk to 0.35 (95% CI, 0.28-0.44).	Adherence to a Mediterranean type of diet was associated with an approximately 20 % lower risk of all-cause mortality in apparently healthy elderly.
Healthy dietary and lifestyle behaviors and their impact on survival in post-myocardial infarction (MI) patients in the HALE project.	A Mediterranean type of diet lowered all-cause mortality by 20%. The combination with moderate alcohol consumption, physical activity, and non-smoking lowered the all-cause mortality risk to 0.58 (95% CI, 0.45-0.75).	Adherence to a Mediterranean type of diet was associated with approximately 20 % lower all-cause mortality in post-myocardial infarction patients.
The relationship between 5-year changes in body weight and mortality during 10 subsequent years of follow-up in the FINE Study.	Weight loss of more than 5 kg was strongly significantly related to mortality in Northern Europe (HR: 2.26; 95 % CI: 1.59-3.22) but not in Southern Europe. Weight gain of more than 5 kg was weakly associated with mortality in centers in Northern Europe (HR: 1.40; 95% CI: 0.90-2.17) but not in Southern Europe.	Weight changes are stronger associated with mortality in centres in Northern Europe compared to centres in Southern Europe.
Association between total, LDL, VLDL, HDL-cholesterol and 10-year cardiovascular and all-cause mortality in the HALE project.	Participants in the highest tertile versus the lowest tertile of HDL-cholesterol had a significant lower risk of mortality from cardiovascular diseases (HR: 0.72, 95% CI: 0.54-0.95) and all-causes (HR: 0.80, 95% CI: 0.66-0.96). The associations between HDL-cholesterol and mortality were strongest in women.	HDL-cholesterol is inversely associated with mortality from cardiovascular diseases and all-causes. These associations were strongest in women.

8.2 Methodological considerations

In this thesis, the associations between dietary patterns, diet-related biological risk factors and mortality were described. We reflect first on the use of dietary patterns in investigating associations between diet and biological risk factors and between diet and mortality. These associations were examined in two longitudinal studies; the HALE project and the Normative Aging Study. Both studies were carried out in elderly subjects. Thereafter, we discuss the possible confounding effect of different characteristics of the study population on the results on dietary patterns in relation to diet-related biological risk factors and mortality.

8.2.1 Dietary patterns

Traditional analyses in nutritional epidemiology typically investigate the associations between a single or a few nutrients or foods and chronic diseases[4-6]. Although this type of analysis is valuable, it has several conceptual and methodological limitations that we presented in the general introduction[4-7]. Therefore, we used dietary patterns in this thesis. Two different approaches can be used to study dietary patterns; theoretically or "a priori" defined dietary patterns and empirically or "a posteriori" derived dietary patterns[4-7]. We used both methods in this thesis. In chapter 2, we used cluster analysis, a statistical exploratory method to derive dietary patterns in the Normative Aging Study. Cluster analysis seems to identify the major dietary patterns of a particular sample[5]. Dietary patterns, derived by cluster analysis did allow us to get insight into existing food consumption patterns in the Normative Aging Study. Although several studies find similar dietary patterns by cluster and factor analysis, the researcher has to make several choices in the

analysis that may significantly influence the results: the dietary patterns derived[5, 8]. How exactly these choices affect the outcome of the analysis is unclear. When greater consensus exists to guide decision-making in patterning methods, decisions can become more standardized, decreasing the subjectivity and increasing the reproducibility of clustering methods. Factor and cluster analysis may well be able to identify the major dietary patterns of a particular sample, but these patterns do not necessarily represent ideal diets. They are based on food consumption data and correlations of intakes between foods and ignore prior knowledge completely. These methods may therefore not be very useful to evaluate diet quality, but do allow us to gain insight into existing food consumption patterns within a population and patterns that may be associated with higher (or lower) health risk[8].

Therefore we used, in chapter 3, dietary indexes for dietary pattern evaluation in elderly. In literature, different indexes are described and used to study dietary patterns[4-8]. Besides indexes based on dietary guidelines or recommendations, indexes of diet quality can also be based on a diet that has proven to be healthy. The Mediterranean diet received increased attention in recent years because of a suggested association with a lower risk of coronary heart disease and several forms of cancer for people consuming such a type of diet[9]. Several indexes have been developed based on the traditional Mediterranean diet[7]. Dietary variables contained in the index are generally nutrients, foods or food groups that are assumed to be either healthful or detrimental. In addition to dietary components, dietary variety is also considered to contribute to a healthy diet and can be an individual index item[8]. In our studies, we did not

estimate variety. Therefore, we could not use scores based on variety to evaluate dietary patterns.

Table 1 gives an overview of existing theoretically defined indexes of overall diet quality and dietary variety scores[10-26]. Several of these indexes have been adapted and modified by others; however, these revised indexes have not been described in the table. The Healthy Eating Index, the Diet Quality index, the Healthy Diet Indicator, and the Mediterranean Diet Score are the indexes that have been referred to and/or validated most extensively[8].

In chapter 3, we compared differences in association between three different indexes and mortality; two dietary scores were based on a Mediterranean type of diet; the Mediterranean Diet Score and the Mediterranean Adequacy Index, while the third score was based on the WHO guidelines for prevention of chronic diseases. The reasons we chose these three diet scores and did not use the other scores are given in table 2. In chapter 3, the lowest hazard rate in relation to mortality was found for the Mediterranean Diet Score. Therefore, we used the Mediterranean Diet Score in chapter 4 and 5 to investigate the associations between a healthy dietary pattern and a healthy lifestyle in relation to mortality in apparently healthy elderly and post-myocardial infarction patients.

Although dietary patterns defined by dietary indexes are based on guidelines, recommendations or low-risk regional-based diets, there have still many decisions to be made to create an index. One of the decisions to be taken is which items should be included? Dietary variables

contained in the index are generally nutrients, foods or food groups that are assumed to be either healthy or detrimental. Some indexes consist solely of food groups or foods (Food-based quality index, Healthy Food Index), others only of nutrients (Nutrition Adequacy Ratio)[22-24,].

Most indexes however comprise both food groups and nutrients. The second decision to be made is which scoring method can be used and how to define the cut-off points. Most straightforward is to use a cut-off value for each component and to attribute a score of '0' if consumption is lower than this value (or higher if an unfavourable component is concerned) and '1' if consumption is higher (or lower) than the cut-off. The question remains however which cut-off value should be chosen[8]. Another decision to be made is how to adjust for energy. In the original Mediterranean Diet Score, intake of each component is adjusted to daily intakes of 2500 kcal for men en 2000 kcal for women[14]. In the Healthy Eating Index[11], the recommended number of servings depends on the recommended energy intake. For this index item scores reflect intake as a proportion of the number of servings recommended for the appropriate energy intake level, based on age and gender[11].

Also, these decisions are still yet not made in quantifying a Mediterranean type of diet. Although there are already different scores which measures a Mediterranean type of diet, according to experts, there is a need for a more precise and quantified definition of the Mediterranean dietary pattern[7].

Table 2: Dietary patterns derived by dietary indexes[10-27]

Dietary patterns	Index	Reason for not having used in chapter 3
Dietary guidelines	1. Diet Quality Index[10]	Based on the U.S. recommendations on Diet and Health, but we are studying European populations.
	2. Healthy Eating Index[11]	Including an item based on variety, which we don't have in our study.
	3. Healthy Diet Indicator[12]	Used in chapter 3
	4. Dietary Guidelines Index[13]	This Index included BMI and physical activity. We used these items as lifestyle factors in our analysis. Index contains a variety item for grains: ">6 different grain food items", which we don't have in our study.
Mediterranean Diet	5. Mediterranean Diet Score[14]	Used in chapter 3
	6. Mediterranean Diet Quality Index[15]	Score included number of cigarettes. To our opinion, smoking is not part of a dietary patterns but a separate lifestyle factor. Score included also olive oil, a component which we don't have separately
	7. Mediterranean Adequacy Index[16]	Used in chapter 3
	8. KIDMED[17]	Developed for children and adolescents. Contains information about skipping breakfast, a variable that we don't have in our study.
	9. Mediterranean Score[18]	Scoring based on the Mediterranean Pyramid. It contains items that we don't have separately in our study; olive oil and olives.
	10. Dietary Score[19]	Distinction made in grains; whole-grains, which we don't have.
	11. Mediterranean Diet Pattern Score[20]	Contains items we don't have separately: olive oil, butter, milk cream, margarine.
	12. Mediterranean Dietary Pattern Adherence Index[21]	Contains items we don't have in our study; such as trans fatty acids.
Food	13. Food Based Quality Index[22]	Based on recommended quantities of basic food groups formulated by the Netherlands Bureau for Nutrition Education. Food groups are often country-specific. Because we included different countries in our studies, we did not use country-specific guidelines.
	14. Healthy Food Index[23]	The score is based on only 4 items; butter/margarine/animal fat, vegetables, coarse bread, and fruit.
Nutrition	15. Nutrition Adequacy Ratio[24]	The score contains a ratio of intake of a nutrient relative to RDA. This score is often used to evaluate indexes of diet quality. It is not really an indicator of a dietary pattern.
Dietary variety	16. Recommended Food Score[25]	Number of different recommended foods consumed over a given period
	17. Dietary Variety Score[26]	Number of different food groups (dairy, meat, grain, fruit, and vegetable) consumed daily.
	18. Dietary Diversity Score[27]	Score included the number of different (nutrient-dense) foods consumed over a given period.

However, reaching consensus about the definition of a Mediterranean dietary pattern is complex since the pattern may be related to a specific Mediterranean region and to a selected period of time that are adopted as references. Problems with the definition of the pattern indirectly affect pattern evaluation methods and components used[7]. There are debates on which items need to be included. For example on the type of fat to be included in the definition (mono-unsaturated versus poly-unsaturated), on how to include dairy products, on the importance of different types of meat, on the classification of refined cereals as protective or 'non-protective' foods, definition of moderate alcohol intake and on the presence of nuts and fish as independent components[7,27]. The variability in choosing cut-off points in the score and distributing the population into different intake groups according to the grade of adherence may influence the interpretation of results. Also, the use of indexes and cut-off points by other authors is a limitation since the population in which the index is applied may differ from the population for which the index was originally designed. Another point is also how to adjust for energy intake. In the original Mediterranean Diet Score, intake of each component is adjusted to daily intakes of 2500 kcal for men en 2000 kcal for women. In the Mediterranean Adequacy Index, all items are expressed as percentage of energy.

In the Mediterranean Diet Score, we used in chapter 4 and 5, different improvements can be made. A distinction between low-fat dairy products and high-fat dairy products could be made[27]. For cereals, it is necessary to make a distinction in type of grains, for example whole-grains[27]. Also the question still remains which cut-off points should be used; population-based medians or the medians of the original Mediterranean

Diet Score. If we used the cut-off points of the original Mediterranean Diet Score, some countries, would score above the median while other countries would score under de median. This means that some countries will be defined as using a Mediterranean type of diet, while other countries score as not using a Mediterranean type of diet. In our study, we used the cut-off pints of our population, so that for all countries subjects scored under and above the median of the population. If the complete study population consumes an unhealthy diet, the medians for the healthy items will still be too low, resulting in subjects who score high on the Mediterranean Diet Score but who still have an unhealthy diet. Therefore, it may have been better to use a diet indicator which is based on dietary guidelines; the guidelines are not country-specific, the cut-off points are clear because they are according the guidelines, and third they take energy intake into account. However, some items of the Healthy Diet Indicator also discriminate not satisfactory; for example only a few people met the guideline of 50–70% energy intake of complex carbohydrates. So the same problem as for an index based on a Mediterranean type of diet could maintain and the cut-off point based on a median may not be a bad solution. However, besides the disadvantages of the Mediterranean Diet Score, an index based on a Mediterranean type of diet seems to be related to mortality in different study populations; in Greek studies, in Danish studies, in Australian studies, and in other European studies[7].

8.2.2 Study population characteristics

Elderly people

In studies involving elderly people, respondents represent a select group of survivors. Given a high response rate, the elderly cohorts are expected to be representative for the result of the aging process. Survival selection may have resulted in a weaker strength of observed associations between dietary patterns, biological risk factors and mortality, compared to middle-aged populations. This may be due to selective removal of those susceptible to these risk factors, leading to a weakening of associations especially with respect to former strong risk factors[28, 29].

In the HALE project, a prospective cohort study, distributions of dietary and lifestyle factors in the study population may have been affected by selective participation in the different cohorts. However, we had almost 100% mortality follow-up, so the relationships between dietary patterns, biological risk factors and mortality should not have been confounded[30].

In our study, diet was measured in 1990 and related to mortality during 10 years of follow-up. Although diet (and lifestyle) habits can change in time, generally they are a characteristic of a person's way of living and we assume that they reflect lifelong health habits[31, 32].

Health status

Confounding by sub-clinical disease is a major point of concern in studying associations between dietary patterns, biological risk factors and mortality in population-based studies in elderly people. We excluded participants with major chronic diseases (coronary heart disease, stroke, diabetes, and cancer) at baseline to minimize the possibility that diet and lifestyle factors have changed in response to clinical disease as has be

done by others[33]. Besides the participants with major chronic diseases at baseline, we excluded also participants who died during the first 2 years of follow-up. In the second part of this thesis, in which biological risk factors are described, also participants with a BMI < 18.5 kg/m^2 were excluded. These participants were also excluded because the mortality risk increases steeply in persons with a BMI < 18.5 kg/m^2.

However, it is questionable whether we have to remove participants with chronic diseases, those who died during the first 2 years of follow-up and those with a BMI < 18.5 kg/m^2 in the analyses of dietary patterns and biological risk factors in relation to mortality. Aging is associated with an increase in chronic diseases, such as cardiovascular diseases, diabetes, and cancer. Evidence is accumulating that these diseases are preventable, and that the occurrence of these disease can be postponed by dietary and lifestyle factors[8, 16, 17]. Therefore, chronic diseases could be seen as a result of the effect of influencing dietary and lifestyle factors. If participants, who consume an unhealthy diet and have a less healthy lifestyle, will develop chronic diseases, removing these participants from the analyses will attenuate the outcome measurements. We always carried out our analyses with and without these participants with chronic diseases, those who died during the first 2 years of follow-up and those with a BMI < 18.5 kg/m^2. We indeed saw that excluding these participants attenuates our estimates. Where we study usual aging these high risk participants should not be removed from the population. However, in studying healthy aging, these participants should be removed.

Multi-centre study

The HALE project is a multi-centre study, which involved 18 study centres. Before pooling the different study centres and pooling the SENECA and the FINE Study, it is important to test whether the centres and studies could be pooled because factors across centers could exert powerful independent influence on study outcomes. Correlation or clustering, resulting from the similarity of outcomes among subjects within a centre, requires adjustment of confidence intervals and p-values, especially in observational studies and in randomized multi-centre studies in which treatment is allocated by centre rather than by individual patient. Therefore, we used a random effects model to test if the different study centers and the two studies could be pooled. For all-cause mortality, the random effect parameter was not significant in the model, indicating that there was no random effect for study centre or study (SENECA/FINE). To be sure that the relationships between diet, biological risk factors and mortality could not be explained by a study effect, this variable was included as potential confounder in the analyses.

Although national heritage could be a confounder in our analyses, as suggested by Craighead[34], we did not adjust for region (Northern or Southern Europe). The variable region has a strong cultural component, which could be responsible for (a part of) the variation in dietary patterns. Adjustment for region would result in overadjustment or underestimation of the true association between diet scores and mortality.

8.3 Public health implications

This thesis showed that a healthy dietary pattern is associated with a lower risk of mortality. Elderly who followed a Mediterranean type of diet had lower mortality rates of all-causes, coronary heart disease, cardiovascular diseases and cancer. A Mediterranean type of diet is characterised by a relatively high consumption of bread, cereals, legumes, vegetables, fruit, fish, vegetable oils, in combination with a relative low intake of milk, cheese, meat, potatoes, eggs, animal fats and hard margarines, sweet beverages, cakes, pies and cookies, sugar, beer and spirits and a moderate wine consumption[14,16]. Besides a Mediterranean diet, a dietary pattern which follows the recommendations of the WHO study group, translated in the Healthy Diet Indicator by Huijbregts et al[12]., is associated with a lower risk of mortality seen in the HALE project[10]. This is in line with other studies using these dietary scores[12, 14, 35-37]. Probably there is a considerable overlap between these two definitions of a healthy dietary pattern. Despite the different criteria that were used to define the dietary patterns, these findings confirm the beneficial effect of a healthy diet on mortality in elderly. In this thesis, the hazard rate for apparently healthy elderly consuming a Mediterranean type of diet was 0.77 for mortality from all-causes, 0.61 for mortality from coronary heart disease, 0.71 for mortality from cardiovascular diseases and 0.90 for mortality from cancer. This indicates that subjects who consume a Mediterranean type of diet, have an approximately 10-40 % lower risk of different causes of death, compared to elderly who do not eat a healthy diet. In intervention studies, the relative risks for modifying dietary patterns were even stronger. In the review of Iestra et al., combinations of dietary changes in coronary artery patients were

associated with a reduced mortality risk, (RR: 0.56; 95 % CI: 0.42-0.74)[38]. In the prospective, randomized single-blinded secondary prevention trial of de Lorgeril et al, an adjusted risk ratio of 0.30 (95% CI 0.11-0.82, p = 0.02) was found for post-MI patients who followed an alpha-linolenic acid enriched Mediterranean type of diet[39].

Besides a healthy dietary pattern, this thesis showed that also a healthy lifestyle is associated with a significantly lower mortality risk. Moderate alcohol consumption and at least 30 minutes of physical activity per day, in combination with non-smoking is associated with a low mortality rate in the HALE project. The more healthful dietary and lifestyle factors a participant had, the lower the risk for all-cause mortality and cause-specific mortality. In the current study, 60% to 64% of mortality was associated with lack of adherence to this low-risk pattern. This thesis indicates that these guidelines are not only important for apparently healthy elderly; also elderly who already had a myocardial infarction, have benefits by following these guidelines.

These guidelines are in line with the guidelines for a healthy diet 2006 of the Health Council of the Netherlands[40]. The report of the Health Council emphasises that, in the context of diet-related chronic diseases, the focus needs to be on dietary pattern, not on individual foods or food components. The best way to reduce the risk of chronic diseases is to have a diet which is rich in fruit, vegetables, whole-grain cereal products and the regular consumption of vegetable oil, fish, low-fat dairy and meat products, in combination with a physically active lifestyle, moderate alcohol consumption and abstinence from smoking. For reducing the risk of various chronic diseases, it is important that the low current average level of physical activity is increased by encouraging more people to

comply with the Dutch Standard for Healthy Physical Activity. For adults, the recommendation is at least 30 minutes of moderate to vigorous physical activity – brisk walking, cycling, gardening, etc… – at least five days a week, but preferably every day[40].

These guidelines are also in line with the recommendations given by the WHO about diet, nutrition and the prevention of chronic diseases (2003) and the dietary guidelines for Americans 2005[41, 42]. They both recommend a diet rich in fruit, vegetables, whole-grain products and fish and low in salt and saturated fat. Besides a healthy diet, the advice is to use alcohol in moderation and to be physically active, for at least 30 minutes a day.

Following these recommendations will also help to achieve a desirable body weight, blood cholesterol, blood pressure and glucose level.

The results of this thesis indicate that it is important for elderly people to maintain their body weight and cholesterol levels. Our data show that in apparently healthy elderly from Northern Europe between 70 and 90 years old, weight loss and possible weight gain are associated with a higher risk of mortality from all-causes, even after adjusting for chronic diseases at baseline. We also observed that a high HDL-cholesterol level, even in old age, might protect against mortality from cardiovascular diseases and all-causes.

Conclusion

From this thesis we conclude that a healthy dietary pattern, based on a Mediterranean diet or based on the WHO-guidelines for the prevention of chronic diseases, both rich in fruit, vegetables, whole-grains, legumes/nuts/seeds, in combination with regular consumption of vegetable oils, fish and low-fat dairy and meat products is associated with lower mortality rates in elderly men and women, aged 70-90 years. Besides a healthy diet, it is important for elderly people to be physically active, use alcohol in moderation and stop smoking.

It is also important to maintain constant body weight and to keep blood cholesterol at a desirable level, especially to maintain a high HDL-cholesterol level.

Healthy aging comprises optimising both life expectancy and optimising quality of life. In this thesis, we investigated only determinants for optimising survival and therefore life expectancy. For future research it is important also to investigate quality of life. Different measures are already proposed; QUALY (Quality Adjusted life year), DALY's (Disability Adjusted Life Years) and Healthy Life Expectancy. Healthy Life Expectancy combines information on life expectancy and health. To obtain tools for improving quality of life it is important to study determinants of these indexes.

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Summary

The world has experienced dramatic improvements in longevity. Life expectancy at birth has increased by 8 years in both sexes over the last 45 years. Before 1950 most of the gain in life expectancy was due to a reduction in premature deaths. In recent decades it has mostly been due to an improvement in the survival of people over 65. The percentage of the population aged 65 and over, which started to rise sharply from the latter part of the last century, is continuing to rise.

In industrialized countries, about 75% of deaths in persons over the age of 65 are now from cardiovascular diseases and cancer. Regardless of predisposing factors, diet, lifestyle and biological factors influence morbidity and mortality during the course of life. Because of the accumulation of adverse effects of risk factors throughout life, it is important to advise older persons to adopt diet and lifestyle practices that minimize their risk of morbidity and mortality and maximize their prospects for healthful aging. In this thesis we focused on the determinants of survival. The aim of this thesis was to investigate the associations between dietary patterns, biological risk factors and mortality.

In **chapter 2**, we described the association between dietary patterns and biological risk factors. Differences in B-vitamin intake, in plasma B-vitamin and homocysteine levels, before and after folic acid fortification, in relation to dietary patterns were investigated in the Normative Aging Study. The study included 2,280 male volunteers, ages 21 till 81 years (mean 42 years) which were recruited around Boston between 1961 and 1970. Three dietary patterns were identified by cluster analysis: a prudent dietary pattern, with relatively high intake of fruit, vegetables, low-fat

milk and breakfast cereals; an unhealthy pattern, with high intake of baked products, sweets and added fats; and a low fruit and vegetable but relatively high alcohol intake pattern. Dietary intake and plasma concentrations of folate increased significantly ($P<0.05$) among all dietary patterns. The average increase in folate intake was +100 (μg/day) and the average increase in plasma folate concentration was +15.5 nmol/L. Homocysteine decreased in non-supplement users (-0.38 μmol/L) and in subjects in the high alcohol, low fruit and vegetable dietary pattern (-0.46 μmol/L) but these differences were not statistically significant.

In **chapter 3**, the associations between three dietary patterns and mortality were investigated in the HALE project. This project included participants of the Survey in Europe on Nutrition and the Elderly: a Concerted Action (SENECA) and Finland, Italy, the Netherlands, Elderly (FINE) studies who were examined in 1988-1991 and were followed up for 10 years.

The SENECA study started in 1988 and consisted of a random age- and gender stratified sample of inhabitants, born between 1913 and 1918, in 19 European towns. In the HALE project, 13 centers, which carried out a 10-year mortality follow-up, were included. At baseline 1,162 men and 1,173 women participated in the SENECA Study. All men and women of the following towns were included: Hamme, Belgium; Roskilde, Denmark; Strasbourg, France; Valence, France; Iraklion, Greece; Monor, Hungary; Padua, Italy; Culemborg, the Netherlands; Vila Franca de Xira, Portugal; Betanzos, Spain; and Yverdon, Burgdorf, and Bellinzona, Switzerland.

The FINE Study consists of the survivors of 5 cohorts of the Seven Countries Study; Ilomantsi, East Finland; Poytya, and Mellila, West Finland; Crevalcore and Montegiorgio, Italy; and Zutphen, the Netherlands. The FINE Study which started in 1984-1985 and continued to 2000, recruited men who were born between 1900 and 1920. In the FINE Study, 2,284 men were enrolled in 1984-1985 at baseline. Surveys and measurements were repeated in 1989-1991, in 1994-1995 and in 1999-2000. For the HALE project, we used the measurements of 1989-1991 of FINE as baseline measures.

Three different European diet scores were used to measure dietary patterns. The Mediterranean Diet Score (MDS) (HR: 0.82 with 95% CI: 0.75–0.91), the Mediterranean Adequacy Index (MAI) (HR: 0.83 with 95% CI: 0.75–0.92) and the Healthy Diet Indicator (HDI) (HR: 0.89 with 95% CI: 0.81–0.98) were all inversely associated with all-cause mortality.

The single and combined effects of a Mediterranean type of diet, physical activity, moderate alcohol consumption and non-smoking on mortality were investigated in apparently healthy elderly (**chapter 4**) and in post-myocardial infarction patients (**chapter 5**) of the HALE project. In apparently healthy elderly, adhering to a Mediterranean diet (hazard ratio [HR], 0.77; 95% confidence interval [CI], 0.68-0.88), moderate alcohol use (HR, 0.78; 95% CI, 0.67-0.91), physical activity (HR, 0.63; 95% CI, 0.55-0.72) and non-smoking (HR, 0.65; 95% CI, 0.57-0.75) were associated with a lower risk of all-cause mortality. Similar results were observed for mortality from coronary heart disease, cardiovascular diseases and cancer. The combination of 4 low risk factors lowered the

all-cause mortality rate to 0.35(95% CI, 0.28-0.44). Lack of adherence to this low-risk pattern was associated with a population attributable risk of 60% of all deaths, 64% of deaths from coronary heart disease, 61% from cardiovascular diseases and 60% from cancer.
In post-myocardial infarction patients, similar associations were found compared to apparently healthy elderly. A Mediterranean-type diet (HR 0.75; 95% CI 0.57–0.97), physical activity (HR 0.69; 95% CI 0.53–0.90), moderate alcohol consumption (HR 0.77; 95% CI 0.58–1.02) and non-smoking (HR: 0.62; 95% CI: 0.44–0.88) were associated with lower all-cause mortality rates. Presence of at least three healthy behaviours was associated with a 40% lower mortality rate in post-myocardial infarction patients.

Associations between biological risk factors and mortality were also investigated. **Chapter 6** described the associations between changes in body weight during 5 years and 10-year mortality in the FINE Study. After excluding men with chronic diseases in 1985 and 1990, men with a BMI < 18.5 kg/m ^{2} and men who died during the first 2 years of follow-up, men who had lost 2-5 kg of body weight (adjusted HR: 1.37; 95% CI: 1.03-1.83) and men who had lost more than 5 kg (adjusted HR: 1.94; 95% CI: 1.43-2.63) had a higher mortality risk compared to men with stable weight. However, analyses stratified for region showed that weight loss was strongly significantly related to mortality in Northern but not in Southern Europe. Weight gain of more than 5 kg was associated with mortality in centers in Northern Europe, although this association was not statistically significant (HR: 1.40; 95% CI: 0.90-2.17). Weight gain was not associated with mortality in Southern Europe.

In **chapter 7,** the associations were described between different serum cholesterol fractions and cardiovascular and all-cause mortality in European elderly men and women of the HALE project. In these men and women, a significant inverse association was found between HDL-cholesterol and 10-year cardiovascular and all-cause mortality. Men and women in the highest tertile versus the lowest tertile had a significantly lower risk of mortality from cardiovascular diseases (HR: 0.72, 95% CI: 0.54-0.95) and all-causes (HR: 0.80, 95% CI: 0.66-0.96). These associations remained significant after adjustments for gender, age, education, BMI, smoking, study centre and LDL and VLDL-cholesterol. The associations between HDL-cholesterol and mortality were strongest in women. Total cholesterol, LDL and VLDL-cholesterol were not significantly related to mortality.

In **chapter 8**, we summarized the main findings of this thesis and reflected on some methodological considerations. First, we discussed the different approaches to derive dietary patterns and the advantages and disadvantages of these methods. Second, we reflected on studying elderly people, their health status and multi-centre studies. At the end of this chapter, public health implications of our findings were discussed. Finally, general conclusions were presented.

From the results presented in thesis we conclude that a healthy dietary pattern, such as a Mediterranean diet or a dietary pattern based on the WHO-guidelines for the prevention of chronic diseases, both rich in fruit, vegetables, whole-grains, legumes/nuts/seeds and characterised by

regular consumption of fish, vegetable oils and low-fat dairy and meat products is associated with lower mortality rates in elderly men and women, aged 70-90 years. Besides a healthy diet, it is important for elderly people to be physically active, use alcohol in moderation and non-smoking.

It is also important to maintain constant body weight and to keep serum cholesterol at a desirable level, especially to keep HDL-cholesterol high.

Samenvatting
Voedingspatronen, biologische risicofactoren en survival bij oudere Europese mannen en vrouwen

In de loop van de voorbije eeuw is de levensverwachting van de mens sterk gestegen. Gedurende de afgelopen 45 jaar is de levensverwachting voor zowel mannen als vrouwen met 8 jaar toegenomen. Vóór 1950 is de toename in levensverwachting voornamelijk toe te schrijven aan de sterftedaling bij kinderen. Na 1950 wordt de toename in levensverwachting veroorzaakt door betere overlevingskansen bij ouderen. Door deze betere overlevingskansen bij ouderen is er een sterke toename in het percentage mensen ouder dan 65 jaar. Het percentage ouderen in de bevolking blijft nog steeds toenemen.
In geïndustrialiseerde landen sterft ongeveer 75 % van de ouderen boven de 65 jaar aan hart- en vaatziekten en kanker. Onafhankelijk van aangeboren factoren, worden morbiditeit en mortaliteit gedurende het hele leven beïnvloed door voeding en biologische factoren. Daarom is het belangrijk om oudere personen te adviseren hun voedingsgewoonten en leefstijl aan te passen zodat hun levensverwachting toeneemt en ze gezond ouder worden. In dit proefschrift zal de nadruk gelegd worden op de determinanten van survival. Het doel van dit proefschrift is het onderzoeken van de associaties tussen voedingspatronen, biologische risicofactoren en mortaliteit bij ouderen.

In **hoofdstuk 2** van dit proefschrift worden de associaties beschreven tussen voedingspatronen en biologische risicofactoren. De veranderingen in de vitamine B inname, plasma vitamine B concentraties en homocysteine concentratie in het bloed door het verrijken van voeding met foliumzuur werden onderzocht in relatie tot voedingspatronen in de "Normative Aging Study". Tussen 1961 en 1970 werden voor deze studie mannelijke vrijwilligers tussen de 21 en 81 jaar oud geselecteerd in de

omgeving van Boston. De totale populatie bestond uit 2280 mensen, met een gemiddelde leeftijd van 42 jaar. In deze studie werden drie voedingspatronen onderscheiden door middel van clusteranalyse; een "gezond" voedingspatroon gekenmerkt door een relatieve grote inname van fruit, groenten, magere melkproducten en ontbijtgranen; een "ongezond" voedingspatroon gekenmerkt door hoge inname van gebakken producten, suiker en toegevoegde vetten en een "laag fruit en groenten en relatief hoge alcohol consumptie" patroon. De foliumzuurinname en plasma foliumzuur stegen significant ($p < 0.05$) in alle voedingspatronen. De gemiddelde inname van foliumzuur bedroeg 100 µg/dag en de stijging in de ge middelde plasmaconcentratie was 15.5 nml/L. Het homocysteine gehalte daalde) bij de mensen die geen vitaminesupplementen gebruikten (-0.38 µmol/L) en bij de mensen met het "laag fruit en groenten en relatief hoge alcohol consumptie" voedingspatroon (-0.46 µmol/L) maar de ze verschillen waren statistisch niet significant.

In **hoofdstuk 3** werden de associaties tussen voedingspatronen en mortaliteit onderzocht in het HALE project. Dit project omvat deelnemers van de SENECA (Survey in Europe on Nutrition and the Elderly: a Concerted Action) en de FINE (Finland, Italy, the Netherlands, Elderly) studie. De SENECA studie startte in 1988 en bestaat uit een random steekproef gestratificeerd voor leeftijd en geslacht van de inwoners, geboren tussen 1913 en 1918, van 19 Europese steden. In het HALE project worden de deelnemers van de 13 steden waar een mortaliteitsfollow-up van 10 jaar heeft plaatsgevonden opgenomen. In de SENECA studie namen 1162 mannen en 1172 vrouwen uit de volgende plaatsen deel aan de baseline metingen; Hamme, België ; Roskilde,

Denemarken; Straatsburg en Valence, Frankrijk; Iraklion, Griekenland; Monor, Hongarije; Padue, Italië; Culemborg, Nederland; Vila Franca de Xira, Portugal; Betanzos, Spanje; Yverdon, Burgdorf en Bellinzona, Zwitserland. De FINE studie bestaat uit de overlevenden van de "Seven Countries Study"; Ilomantsi, Poyota en Mellila, Finland; Crevalcore en Montegiorgio; Italië; Zutphen, Nederland. De studie startte in 1984-1985 met het opnemen van 2284 mensen geboren tussen 1900 en 1920. Metingen werden herhaald in 1989-1990 en in 1994-1995. Voor het HALE project werden de metingen in 1989-1990 gebruikt als baseline metingen.

Drie verschillende Europese voedingsscores werden gebruikt om voedingspatronen te definiëren. Twee voedingsscores waren gebaseerd op het Mediterrane voedingspatroon: de "Mediterranean Diet Score" en de "Mediterranean Adequacy Index. De derde voedingsscore was gebaseerd op de voedingsrichtlijnen van de Wereld Gezondheidsorganisatie. De "Mediterranean Diet Score" (HR: 0.82 met 95 % BI: 0.75-0.91), de "Mediterranean Adequacy Index" (HR: 0.83 met 95 % BI: 0.75-0.92) en de "Healthy Diet Indicator" (HR: 0.89 met 95 % BI: 0.81-0.98) waren alle drie omgekeerd significant geassocieerd met mortaliteit.

De associaties tussen een Mediterraan voedingspatroon, leefstijlfactoren en mortaliteit werden onderzocht bij gezonde ouderen (**hoofdstuk 4**) en bij patiënten met een myocardinfarct (**hoofdstuk 5**) in het HALE project. Bij gezonde ouderen is een Mediterraan voedingspatroon (HR: 0.77 met 95 % BI: 0.68-0.88), een matige alcoholconsumptie (HR: 0.78 met 95 % BI: 0.67-0.91), fysieke activiteit (HR: 0.63 met 95 % BI: 0.55-0.72) en

niet-roken (HR: 0.65 met 95 % BI: 0.57-0.75) geassocieerd met een lager risico op 10-jaar sterfte. Gelijkaardige resultaten werden gevonden voor mortaliteit van coronaire hartziekten, cardiovasculaire ziekten en kanker. De combinatie van deze 4 gezonde factoren (Mediterrane voeding, matig alcoholgebruik, matige tot intensieve lichamelijke activiteit en niet roken) verlaagde de hazard rate 0.35 (95 % BI: 0.28-0.44)**.** Het attributieve risico bedroeg 60 voor totale mortaliteit, 64 voor mortaliteit van coronaire hartziekten, 61 voor mortaliteit van cardiovasculaire ziekten en 61 voor kanker.
Bij patiënten met een myocardinfarct werden gelijkaardige associaties gevonden. Een Mediterraan voedingspatroon (HR: 0.75 met 95 % BI: 0.57-0.97), matig alcohol gebruik (HR: 0.77 met 95 % BI: 0.58-1.02), fysieke activiteit (HR: 0.69 met 95 % BI: 0.53-0.90) en niet-roken (HR: 0.62 met 95 % BI: 0.44-0.88) waren geassocieerd met een lagere kans op 10-jaars mortaliteit. De aanwezigheid van tenminste 3 gezonde leefstijlfactoren was geassocieerd met een 40 % lagere kans op overlijden.

In het tweede deel van deze thesis werd ingegaan op de associaties tussen biologische risicofactoren en mortaliteit. In **hoofdstuk 6** worden de associaties tussen veranderingen in lichaamsgewicht gedurende 5 jaar en 10-jaars kans op overlijden beschreven in de FINE studie. In dit hoofdstuk werden mensen met chronische ziekten, mensen met een BMI kleiner dan 18,5 kg/m^2 en mensen die gedurende de eerste twee jaar van de follow-up periode zijn overleden uitgesloten. Mannen met 2-5 kg gewichtsverlies (HR: 1.37; 95 % BI: 1.03 -1.83) en mannen met meer dan 5 kg gewichtsverlies (HR: 1.94; 95 % BI: 1.43 -2.63) hadden een

significant hogere kans op overlijden dan mensen die een stabiel gewicht hadden. Wanneer deze associaties echter apart werden bekeken voor Noord en Zuid-Europa, dan was het verband tussen gewichtsverlies en mortaliteit sterker in Noord-Europa. Een gewichtstoename van meer dan 5 kg was geassocieerd met mortaliteit in Noord-Europa, maar deze associatie was niet significant (HR: 1.40; 95 % BI: 0.90 -2.17). Gewichtstoename was niet geassocieerd met mortaliteit in Zuid-Europa.

In **hoofdstuk 7** worden de associaties beschreven tussen bloedlipiden-fracties en mortaliteit in het HALE project. Bij mannen en vrouwen werd een omgekeerd verband gevonden tussen HDL-cholesterol en mortaliteit ten gevolge van hart-en vaatziekten en totale mortaliteit. Mannen en vrouwen in het hoogste tertiel hebben een significant lager risico op mortaliteit van hart-en vaatziekten (HR: 0.72; 95 % BI: 0.54-0.95) en totale mortaliteit (HR: 0.80; 95 % BI: 0.66-0.96) ten opzichte van mannen en vrouwen in het laagste tertiel van HDL-cholesterol. Deze associaties bleven significant na correctie van geslacht, leeftijd, opleiding, BMI, roken, studie centrum, en LDL- en VLDL-cholesterol. De associaties waren sterker voor vrouwen dan voor mannen. Totaal cholesterol and VLDL-cholesterol waren niet significant geassocieerd met mortaliteit.

In **hoofdstuk 8** worden de belangrijkste resultaten van dit proefschrift samengevat en worden de methodologische aspecten van het proefschrift besproken. Eerst wordt ingegaan op de verschillende methoden om voedingspatronen af te leiden en op de voor-en nadelen van deze methoden. Vervolgens worden verschillende aspecten besproken van ouderenonderzoek, confounding van gezondheidstoestand en multicenter studies. Ten slotte worden enkele implicaties gegeven voor

volksgezondheid. Het hoofdstuk wordt afgesloten met een algemene conclusie van het proefschrift.

Uit dit proefschrift kan worden geconcludeerd dat een gezond voedingspatroon, gebaseerd op een Mediterraan voedingspatroon of op een voedingspatroon gebaseerd op de richtlijnen van de WHO, beiden rijk aan fruit, groenten, peulvruchten/noten/zaden, volgraanproducten, en een regelmatige consumptie van vis, plantaardige oliën, magere melkproducten en vleesproducten is geassocieerd met lagere 10-jaars overlijdenskansen bij oudere mannen en vrouwen tussen de 70 en 90 jaar. Naast gezonde voeding is fysieke activiteit, een matig alcohol gebruik en niet-roken belangrijk.
Tenslotte is het ook belangrijk om het lichaamsgewicht constant te houden en het cholesterolgehalte binnen de richtlijnen te houden, vooral om het HDL cholesterolgehalte hoog te houden.

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Kim

About The Author

Curriculum Vitae

Kim Tonia Benny Knoops was born on April 4, 1977, in Maaseik, Belgium. After completing her secondary school at the "Vrije Humaniora Sint Ursula" in Maaseik, she started the MSc program "Health Sciences" at the University Maastricht. As part of this program, she conducted a research project on "Heart failure, Antioxidants and Homocysteine" at the department of Epidemiology, in co-operation with the department Pharmacology and Toxicology at the University of Maastricht. In 2000, she obtained her MSc-degree in "Health Sciences" at the University of Maastricht. That same year, she started the MSc program "Applied Statistics" at the Limburgs Universitair Centrum (now called: University Hasselt) where she obtained her MSc-degree in 2001. In 2001, she started a PhD-fellowship at the Wageningen University to work on the project "Dietary determinants of healthy aging". Simultaneously she started the MSc program "Biostatistics" at the University Hasselt. In 2006, she obtained her MSc-degree in "Biostatistics". During her PhD appointment, she obtained a grant from "Stichting De Drie Lichten" to work for a 3-month period at the Jean Mayer USDA Human Nutrition Research Centre on Aging at the Tufts University in Boston, USA. She attended several conferences and took courses on nutrition, epidemiology, geriatrics and statistics.

Since July 2006, she is working as a statistical researcher at Statistics Netherlands in Heerlen, the Netherlands.

Educational program

Discipline Specific Activities

- "Third Annual IANA International Conference: Nutrition and Aging", Saint Louis (USA), 2005.
- "Associate Parliamentary Food and Health Forum: Expanding Europe: Eat, drink and be healthy?", Londen (United Kingdom), 2005.
- "Unilever Food and Health Research Institute Symposium: Healthy for Longer", Vlaardingen (The Netherlands), 2005.
- "7^{e} Nationaal Gerontologie Congres: Perspectief op een vergrijzende maatschappij", Ede (The Netherlands), 2004.
- "European Congress on Nutrition and Health in the Elderly", Toulouse (France), 2004.
- "3rd CELSE meeting: Conference on Epidemiological & Longitudinal Studies in Europe", Bristol (United Kingdom), 2004.
- WEON "Epidemiologisch Congres", 2003, 2004, 2005.
- "9th European Nutrition Conference", Rome (Italy), 2003.
- "Vth European Congress of Gerontology", Barcelona (Spain), 2003.
- "7th International Congress of Behavioural Medicine", Helsinki (Finland), 2002.
- Meeting of working group NWO "Nutrition", Arnhem (The Netherlands), 2002, 2003.
- International Conference "The coronary heart disease epidemic can be tamed by diet and lifestyle. 40-year results of the Seven Countries Study", Eefde (The Netherlands), 2002.
- Symposium "Homocysteine, folate and vitamin B12 in cardiovascular and neurological diseases", Ravenstein (The Netherlands), 2001.

General Courses

- "Mixed Models and Missing Data", Brussels (Belgium), 2005.
- "Good Clinical Practice", Maastricht (The Netherlands), 2005.
- Masterclass "Geriatric Nutrition: Diet, Functionality, and Disease", Wageningen (The Netherlands), 2004.
- Master class "Nutrition and Lifestyle Epidemiology", Wageningen (The Netherlands), 2003.
- "Scientific Writing", Wageningen (The Netherlands), 2003.
- "Written English", Wageningen (The Netherlands), 2003.
- "Epidemiological Data Analysis by K. Rothman", Bilthoven (The Netherlands), 2002.

Optional Courses and Activities

- Oldsmobiles, Wageningen University, 2001-2006.
- PhD Study Tour to Switzerland, Italy, and Germany, 2001.
- PhD Study Tour to Australia, 2003.
- Invitation Jean Mayer USDA Human Nutrition Research Centre on Aging at Tufts University in Boston (USA), 2006 (3 months).

Publications

Full papers

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