Of Fats and Foods

Professor Daan Kromhout

Farewell address upon retiring as Professor of Public Health Research at Wageningen University on 16 April 2015

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Mr. Rector, colleagues, family and friends, ladies and gentlemen.

Nowadays it is customary to begin a presentation, even such a celebratory one as today´s, with a declaration of affiliations and support. My sponsors over the years were: the Dutch Heart Foundation, the European Union, the National Institutes of Health, USA, the National Institute of Public Health and the Environment (RIVM), the Ministry of Public Health, the Netherlands Organization of Scientific Research (NWO), the Netherlands Prevention Foundation (Praeventiefonds), the Royal Netherlands Academy of Arts and Sciences (KNAW), Unilever R&D, the University of Leiden and Wageningen University. I am very much indebted to these organizations. Without their financial support my research could not have been carried out.

Today in this symposium we have heard an excellent overview by esteemed colleagues on topics I have engaged over the past 40 years. "Looking back" helps us to see a logical order, not only in our science but also in our lives. The famous 19-th century philosopher Søren Kierkegaard from Denmark, summarized this in admonition: "Life can only be interpreted backwards but has to be lived forward."

I hope to show, in this farewell address, how the research on diet and cardiovascular diseases, including mine, has developed over time. First, from an emphasis on the effects on blood cholesterol levels, of fatty acids and other nutrients and bioactive compounds in the diet, to our current understanding that this reductive approach should be complemented by a food-based approach to best achieve our goals of cardiovascular diseases prevention and a healthy population.

Research on diet and cardiovascular diseases was not in my mind when I entered Wageningen University in 1968. Because my father was a florist, my higher education destiny became the Agricultural University in Wageningen and because of my limited laboratory skills, and an interest in determinants of health, I chose, in 1969, the first year of the newly developed BSc program on nutrition and health. Following the BSc in 1972 came seven months at the Karolinska Hospital in Stockholm where

my first research was analysis of data on body composition under professor Peter Reizenstein. Back in The Netherlands I joined Frits van der Haar and, with two other students and supervised by professor Jo Hautvast, conducted a pilot survey on serum cholesterol level and body fatness in 1,000 Wageningen schoolchildren. We found that 24% of the schoolchildren had elevated serum cholesterol levels.[1] This revealing finding triggered my interest in the role of diet and risk factors in cardiovascular diseases.

Wageningen school children project on serum cholesterol and body fatness 1973

From left to right Theo Koopman, Daan Kromhout, Frits van der Haar

Surveys on serum cholesterol and diet in school children

After using the pilot survey for the MSc thesis, Frits and I wrote two grant proposals for our doctoral projects and were funded respectively by Wageningen University and the Dutch Heart Foundation. We examined approximately 2,500 schoolchildren aged 4-12 in Heerenveen (1974), Roermond (1975), and in Harderwijk (1976), with the hypothesis that diets differed along a north-south gradient in the country and that diet differences would be reflected in serum cholesterol levels. The dietary surveys showed differences among food consumption patterns in the three towns while the energy and nutrient intake were similar. We concluded in our thesis that the population diet was not related to the serum cholesterol level due to lack of contrast in the diet. However, we observed that the average serum cholesterol levels of children aged 6-10 in 1974-1976 were 20% higher compared to that of children

surveyed 25 years earlier in Rotterdam and Leiden. This WAS in accord with CHANGED nutrient composition of the diet over the years. Our 1975 survey data showed that the intake of animal protein and total fat was higher and the intake of vegetable protein and carbohydrate lower than in the 1950 surveys.[2]

After defence of our PhD theses, Frits went into prevention of micronutrient-related deficiency diseases and I focused on cardiovascular disease epidemiology.

One of my heroes at that time was the American pathologist Russell Holman. He wrote just before his death in 1961 a provocative article entitled: Atherosclerosis – a pediatric nutrition problem?[3] As a geographic pathologist, he developed the concept that initial events in the development of atherosclerosis could be detected in the arterial wall already in childhood. In 1975, at the 10-day Teaching Seminar on Cardiovascular Epidemiology of the International Society of Cardiology (now World Heart Federation) in Mexico City, I heard for the first time about the Zutphen and Seven Countries Study in a lecture by Jeremiah Stamler from Chicago. Following the seminar I made a grand tour through the USA, thanks to the Dutch Heart Foundation, and visited amongst others, Holman´s colleague, Henry McGill, then in San Antonio, Texas. In his colony of baboons, changing from a traditional American diet to a prudent diet induced regression of atherosclerosis. That led me to a life-long interest in the role of diet in the causes and prevention of atherosclerotic complications such as heart attack.

My grand tour through the USA included also a visit to Minneapolis. On the day that I went to the Laboratory of Physiological Hygiene, formerly headed by Ancel Keys, I took a bus from my hotel to the Laboratory. I asked the man besides me where Stadium Gate 27 was. He said: " I know that place very well because I was one of the guinea pigs of Dr. Keys." Ancel Keys and Henry Blackburn were not present during my visit but I was entertained by Henry Taylor and David Jacobs. I found out that we shared a common interest in cardiovascular disease prevention through diet and physical activity.

The Zutphen Study and the Seven Countries Study

Back home, I visited professor Cees den Hartog, chairman of the advisory committee of the Zutphen Study and asked him about the status of that Study. He told me that the Principal Investigator, Professor Van Buchem had resigned in 1974 and that no one had been found with interest to head the study. Den Hartog said: "If you are interested, you can start tomorrow". I told him I was interested but first needed to finish my PhD thesis. Two and a half years later, with the degree, I had the opportunity to join the Institute of Social Medicine at the Medical Faculty of the

University of Leiden as assistant professor of nutrition and epidemiology. Den Hartog and I agreed that I took over the Zutphen Study and started in 1978.

Principal Investigators of the Zutphen Study

Frans van Buchem
1897-1979

Cees den Hartog
1904-1993

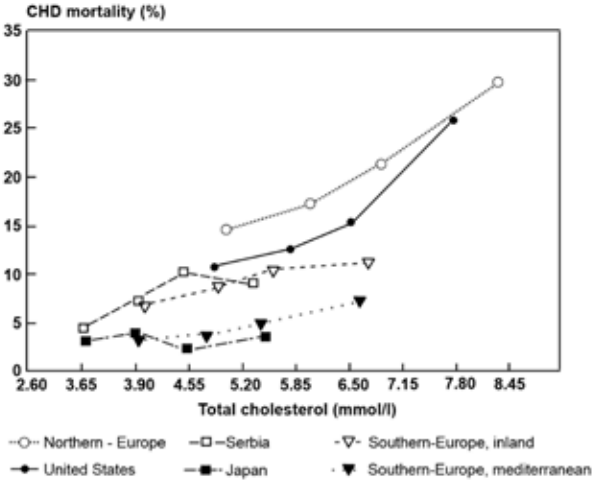
In 1979, in my new role with the Zutphen Study, I was invited to a planning conference of Seven Countries Study investigators on the island of Crete. Ancel Keys had finished his magnum opus, the monograph on the 10-year results of the Seven Countries Study.[4] The group met to discuss the future of the Study. Ancel Keys had retired, the supporting grants had expired, and Henry Blackburn, his successor, was expanding the Division in Minnesota with large demonstration projects such as the Minnesota Heart Survey and Heart Health Program. The question was whether and how to continue an effective Seven Countries Study. From that meeting, Alessandro Menotti from Rome took responsibility for continuing the mortality follow-up. I also told my future colleagues that in my research emphasis would thereafter be devoted to diet and cardiovascular diseases. From that moment on, Alessandro and I, with the moral support of Henry Blackburn, started an intensive collaboration to continue and coordinate the Seven Countries Study. Today it has achieved 50 years of follow-up.

Omega-6 fatty acids, serum cholesterol and coronary heart disease

Serum cholesterol and coronary heart disease (CHD) have been a main topic for researchers interested in diet and cardiovascular diseases at the individual and the population level. The unique opportunity in the Seven Countries Study (SCS) was that the relation between serum cholesterol and CHD could be studied at both levels simultaneously. Keys showed in the 1970 SCS monograph that average serum cholesterol was strongly related to 5-year incidence of CHD at the population level[5]. Cohorts with a low level of serum cholesterol had a low incidence of CHD and those

with a high level had a high CHD incidence and the correlations were linear and strong. It took 25 years of follow-up before a more detailed association could be explored. With PhD student Monique Verschuren as first author, we showed that the strength of the association (relative risk) of serum cholesterol with 25-year CHD mortality was similar in all cohorts but that the absolute risk differed.[6] Cohorts in Northern Europe had, at the same serum cholesterol level, a 5 times higher absolute risk of CHD compared to those in Mediterranean Southern Europe. This implies that at the same elevated level of serum cholesterol many more high-risk persons need to be treated in Northern Europe compared to Mediterranean Southern Europe.

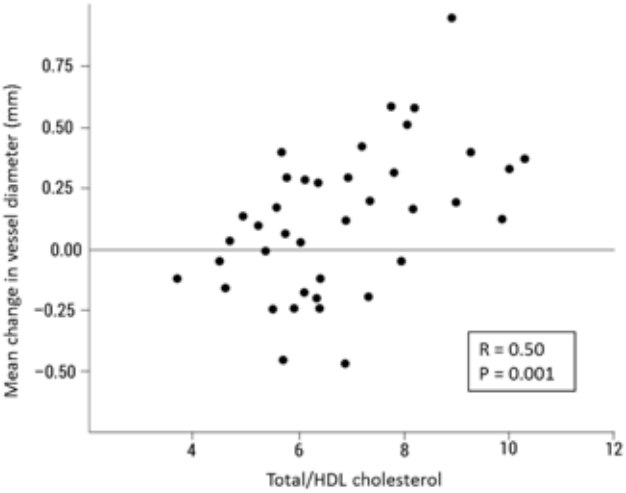
CHD mortality and serum total cholesterol in the Seven Countries Study[6]



Another important question to be addressed was: Can progression of atherosclerosis be reduced by diet? That question was posed by Alexander Arntzenius, professor of Cardiology at the University of Leiden. Arntzenius invited me to participate in his Leiden Intervention Trial. He had selected 39 patients with stable angina pectoris and severe atherosclerosis who were put on a diet low in saturated fat and high in the omega-6 fatty acid linoleic acid with a P/S ratio of 2. Coronary angiograms were taken at baseline and after 2 years of intervention. This study showed that the total/HDL-cholesterol ratio was strongly positively related to coronary lesion growth demonstrated on angiograms.[7] A high ratio was significantly associated with progression of atherosclerosis and a low ratio with regression.

The current controversy about whether saturated fat should be replaced by polyunsaturated fat or carbohydrate was already solved by the Leiden Intervention Trial but not fully realized at that time. The study showed that replacement of saturated fat by polyunsaturated fat reduced total cholesterol but not HDL-cholesterol.[8] Replacement by carbohydrate i.s.o. polyunsaturated fat is inferior because it reduces both the total and HDL-cholesterol level but does not reduce the total/HDL-cholesterol ratio, a strong predictor of CHD risk.

Change in coronary lesions and total/HDL cholesterol in the Leiden Intervention Study[7]



In conclusion, the Seven Countries Study showed that serum total cholesterol is a strong predictor of CHD mortality both at the population and at the individual level. In the Leiden Intervention Trial a high ratio of total/HDL cholesterol was strongly related to progression and a low ratio to regression of atherosclerosis. The Trial also showed that replacement of saturated fat by polyunsaturated fat reduced the total/HDL cholesterol ratio. These results provide evidence that an optimal fatty acids composition of the diet has positive effects not only on serum cholesterol but also on atherosclerosis and CHD risk.

Fish, omega-3 fatty acids and coronary heart disease

In 1982 I attended the International Congress of Atherosclerosis in Berlin and met with Alexander Arntzenius and Frans Kok, now head of the Division of Human Nutrition. Arntzenius found that research on diet and CHD was too much focused on saturated fat. He suggested a broader view and told us the story about white fish and

the low CHD mortality risk among the Inuit. Two years later he called me and said that he would retire soon and that I should give a talk on diet and CHD in his farewell symposium. I asked him which topic. He responded: "that is up to you." Then I remembered his Inuit story and decided to analyze the association between fish consumption and CHD mortality in the Zutphen Study.

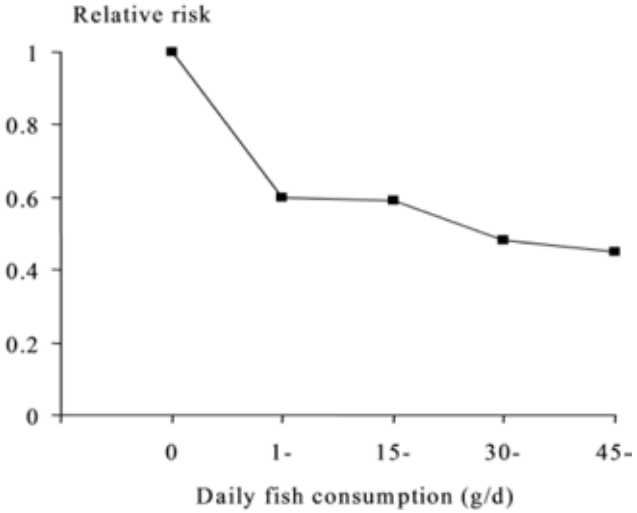
International Atherosclerosis Congress, Berlin 1982

From left to right Frans Kok, Alexander Arntzenius and Daan Kromhout

When I started the analysis I saw that the average fish consumption of the Zutphen men was only 20 g/day, which is 20 times lower than the amount of seafood consumed by the Inuit! That tempered my enthusiasm for the analysis; such a large difference in exposure may make the chance small to find an association. However, I observed a 50% lower risk of fatal CHD for men who consumed fish once or twice a week compared to non-users.

After the farewell symposium I prepared a manuscript about the results on fish consumption and CHD mortality and at the same time Arntzenius worked on a manuscript about the main findings of the Leiden Intervention Trial. We finished our manuscripts at the same time and I asked him to which journal we should submit our papers. His answer was: "the Lancet." Both papers were refused and then I suggested him to send the papers to the New England Journal of Medicine and to our surprise both were accepted.[7,9] This shows the capriciousness in publishing scientific papers.

Fish consumption and CHD mortality in the Zutphen Study[9]



I reproduced with several PhD students the results on fish consumption and fatal CHD in other Dutch cohorts and showed that especially fatty fish might be protective against fatal CHD.[10,11,12,13]. Similar results were found in cohort studies in other Western countries and formed the basis for randomized controlled trials. In Wales the DART trial was carried out in 2,000 cardiac patients in which the experimental group got the advice to eat oily fish at least twice a week while the control group got no such advice. After 2 years of follow-up the fish advice group had a reduced rate of fatal CHD by 33% and all-cause mortality by 29%.[14] These results provide evidence that eating fatty fish once a week or lean fish twice a week may prevent fatal CHD.

In 1999 the results were published of the GISSI-Prevenzione trial from Italy. In this trial with 11,000 cardiac patients fish oil capsules containing 900 mg/d of the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were used as active treatment but the control group did not receive a placebo capsule. These fatty acids were studied because EPA and DHA are the most likely candidates for explaining the seeming protective effects of fatty fish. This trial showed a strong protective effect of an additional amount of EPA-DHA on fatal CHD and sudden cardiac death.[15]

The results of prospective cohort studies suggested that an amount of 200 mg/d EPA-DHA may be enough to lower the risk of fatal CHD. The GISSI-Prevenzione trial showed that an additional amount approximately 600 mg/d EPA-DHA actually

consumed reduced fatal CHD an sudden cardiac death. I designed the Alpha Omega Trial, a randomized double-blind placebo-controlled trial in which the effect was evaluated of an additional amount of 400 mg/d EPA-DHA or 2 g/d of alpha-linolenic acid (ALA), the precursor fatty acid of EPA present in plant food, or the combination versus a placebo. (www.alphaomegatrial.com) The neutral fatty acid oleic acid was used as placebo. The omega-3 fatty acids or placebo were incorporated in margarines that did not differ in taste, smell and colour. In total 4,837 cardiac patients from 32 hospitals in The Netherlands were randomized and followed for 40 months.[16] No effect of omega-3 fatty acids was observed on fatal CHD and major cardiovascular events.[17] Similar results were obtained in other trials published at the same time.

What could be the explanation for these negative results? Colleagues from the UK suggested that the negative results could be due to effective drug treatment in the cardiac patients.[18] In the Alpha Omega Trial 98% of the patients used antithrombotic drugs, 90% antihypertensive drugs and 85% cholesterol-lowering statins. The consequence of the excellent cardiovascular risk factor treatment was a much lower absolute risk of fatal CHD and cardiovascular events in recent trials compared to the earlier DART and the GISSI-Prevenzione trial. This hypothesis was tested in the Alpha Omega Trial by comparing the effects of additional amounts of omega-3 fatty acids in patients who were state–of- the-art drug-treated, with those who did not use statins. In a paper with PhD student Simone Eussen as first author we showed compared to the patients who did not use statins, a 50% reduction in cardiovascular events among those who got extra omega-3 fatty acids.[19] This supports the hypothesis that effects of omega-3 fatty acids on cardiovascular events are only observed in high-risk cardiac patients.

In the Alpha Omega Trial 20% of the cardiac patients also had diabetes and they had a 30% higher risk of cardiovascular events, a 90% higher risk of fatal CHD and a 200% higher risk of severe arrhythmias compared to those without diabetes. Detailed analysis on the effects of additional amounts of omega-3 fatty acids on severe arrhythmias showed a 50% lower risk in cardiac patients with diabetes who got an additional amount of 400 mg/d EPA-DHA or 2 g/d ALA and an 80% lower risk in those who got both an additional amount of EPA-DHA plus ALA.[20] These results also suggest that a high absolute risk is a prerequisite for an effect of added amounts of omega-3 fatty acids on severe arrhythmias.

Our conclusion from 30 years of research on fish, omega-3 fatty acids and fatal CHD is that eating fatty fish once a week or lean fish twice a week may prevent fatal CHD in primary prevention. However, added amounts of omega-3 fatty acids do not reduce CHD risk in state-of-the-art drug-treated cardiac patients.

Flavonoids and coronary heart disease

In 1986 I was invited to present a paper on micronutrients and cancer for a conference in Portugal organized by the International Life Sciences Institute (ILSI). Besides a literature review I decided to analyze as well the relation of diet with lung cancer in the Zutphen Study.[21] In Zutphen vitamin C was inversely related to lung cancer incidence, even after taking smoking into account. I also analyzed the association of foods with lung cancer incidence. Because of the association of vitamin C with lung cancer I decided to focus on fruits and divided them as citrus fruit, mainly oranges, and hard fruits, mainly apples. In contrast to my expectation the association was strongest for apple consumption and lower rates of lung cancer. I wondered which bioactive compounds in apples could be responsible for this inverse association.

To make a long story short, on one of my frequent visits to Minneapolis, I visited Lee Wattenberg, an internationally renowned expert on anticarcinogens. He suggested that flavonoids, compounds with strong anti-oxidant properties, could be the reason for the inverse association of apples with lung cancer. I discussed this hypothesis with the food chemist Peter Hollman in Wageningen and we initiated a study on bioactive compounds and cancer risk. In 1990 we organized a meeting in Wageningen with international experts on anticarcinogens who concurred that flavonoids were the most likely candidate for explaining the inverse association of apples with lung cancer, if the association was actually causal.

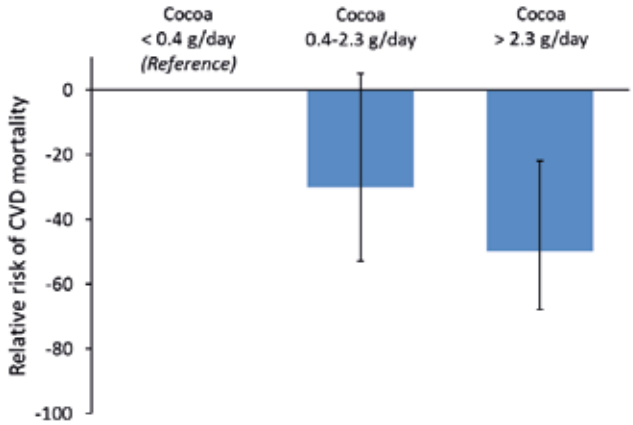
International Workshop on Anticarcinogens, Wageningen 1990

Standing: Lee Wattenberg, Minneapolis (first left), Daan Kromhout (third left). Sitting: Michaël Hertog (second right), Peter Hollman (third right)

The next question to be answered was which flavonoids should be measured? There are 4,000 individual flavonoids present in plant foods divided in six main types. We decided to study two types: the flavonols and flavones that were present in fruits and vegetables. Peter Hollman and PhD student Michaël Hertog developed and validated a method for determining the two types of flavonoids in vegetables, fruits and beverages. The most common dietary sources of these flavonoids in the Dutch diet were tea, onions and apples.

The intake of these flavonoids was not associated with either lung cancer or total cancer in the Zutphen Elderly Study.[22] Because of accumulating evidence on the potential role of antioxidants in inhibition of LDL oxidation we decided also to study the intake of these flavonoids in relation to CHD risk, where we observed a 68% lower risk of CHD mortality among the Zutphen men with a high intake of these flavonoids compared to those with a low intake.[23]

Cocoa intake and cardiovascular mortality in the Zutphen Elderly Study[25]



The next type of flavonoids we studied was flavanols (catechins), using the same procedure for these flavonoids as in the earlier study on flavonols and flavones. Peter Hollman and PhD student Ilja Arts found that the richest sources of flavanols were tea, apples and chocolate. The Zutphen Elderly Study showed that men with a high intake of flavanols had a 51% lower risk of fatal CHD compared to those with a low intake.[24] However, in the Dutch diet the intake of flavonols and flavanols was strongly correlated because tea is the major source of both flavonol (61%) and flavanol (87%) intake. Men who drank more than 2 cups of tea per day had a 60% lower risk of fatal CHD.[23] Besides tea, the intake of cocoa was inversely associated with cardiovascular

risk as shown in a paper with PhD student Brian Buijsse as first author. Men with an intake of 4.2 g/day of cocoa (comparable to 2 small chocolate Easter eggs) had a 50% lower cardiovascular mortality risk compared to those who did not use cocoa.[25] Tea is a rich source of the flavonol quercetin and contains also the flavanol epicatechin for which cocoa is a very rich source but does not contain the flavonol quercetin.

The next question to be addressed, which of the two flavonoids is more important, requires a causal trial. Therefore we designed a cross-over randomized placebo-controlled trial in which the effects of pure quercetin and epicatechin on endothelial function and insulin resistance were tested. These markers of atherosclerosis were selected because meta-analyses of small intervention studies showed that 3 cups of tea and 19-54 g/day cocoa improved endothelial dysfunction and insulin resistance and in cohort studies 3 cups of tea were associated with a 16% lower risk of diabetes.[26,27,28] In a paper with PhD student James Dower as first author, we recently showed in a cross-over trial that an additional amount of 100 mg/day epicatechin, but not quercetin, improved insulin resistance significantly and endothelial dysfunction borderline significantly.[29] Added quercetin did not affect insulin resistance and endothelial dysfunction.

After 25 years of research on flavonoids and CHD the results of prospective cohort studies and a small trial suggest that in the domain of 4,000 flavonoids, epicatechin is a promising one in relation to CHD risk.

Food patterns, coronary and all-cause mortality

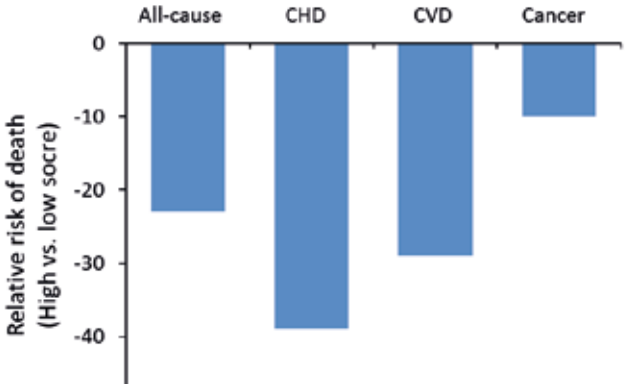
During the first 25 years of the Seven Countries Study the emphasis was on studying risk factors in relation to CHD both at the population and the individual level and on differences in the frequency of CHD, serum cholesterol levels and fatty acids in the diet among populations. In the subsequent 25 years risk factors were not only studied in relation to long-term CHD mortality but also in regard to healthy ageing (www.sevencountriesstudy.com).

The baseline dietary surveys of the Seven Countries Study were carried out in samples of 20-50 men in each of the 16 cohorts. The seven-day food record in combination with precise weighing was used as the preferred dietary survey method. Aliquots of foods consumed by the participants during a week were prepared for chemical analysis. The food samples were analyzed for water, ash, protein, total fat, and fatty acids in central laboratories in Minneapolis and Naples. The baseline average population intake of saturated fatty acids was related to the baseline population average serum cholesterol level and the 5-year frequency of CHD incidence in the 16 cohorts.[5]

Because of the emphasis on chemical analysis of nutrients, especially fatty acids, the food records of the 16 cohorts were coded only in 1986. Annemarie Jansen, the dietician in my group, visited her colleagues in the different countries and coded the records collected in the baseline surveys. (The exception were the records from the two cohorts in Crete and Corfu. They were no longer accessible and the foods consumed were taken from a publication by Keys and colleagues. For fruit and vegetable consumption the data were taken from the food balance sheets in Greece.[30,31])

The Northern European (Finland and The Netherlands) diet was characterized by a high consumption of milk, potatoes, butter or hard margarines, and sugar products. The Mediterranean cohorts from Greece, Croatia and Italy had olive oil as the principal source of fat in combination with either a high intake of fruit (Greece) or fish (Croatia) or pasta (Italy).

Mediterranean Diet Score and mortality in the HALE project[32]



The traditional Cretan Mediterranean diet is characterized by a high intake of olive oil, dark bread, fruits, vegetables and legumes, moderate intake of fish and a low intake of meat and dairy products.[31] From these foods a Mediterranean Diet score is derived in which an intake of five plant foods and fish above the median and an intake of meat and dairy products below the median were the reference. In the HALE (Healthy Ageing Longitudinal study in Europe) project in which amongst others elderly from the Seven Countries Study cohorts from Finland, The Netherlands and Italy participated, showed that the Mediterranean Diet Score was inversely related to CHD and all-cause mortality. In a paper with PhD student Kim Knoops as first

author, we showed that a high score was associated with a 39% lower CHD mortality risk and a 23% lower risk of all-cause mortality.[32] These results were confirmed in meta-analyses of many prospective cohort studies.

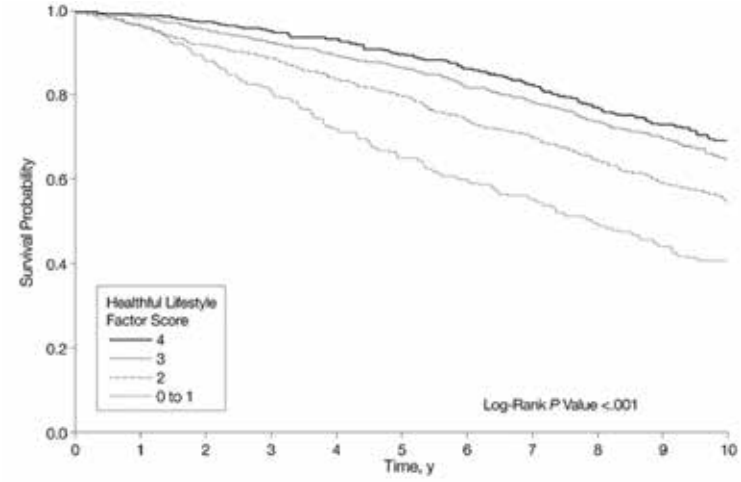
The association of a traditional Mediterranean diet with low CHD and all-cause mortality obtained in prospective cohort studies does not prove causality. Therefore, results are needed from randomized controlled trials. Few trials with hard endpoints such as CHD and all-cause mortality have been carried out. The eight small trials on replacement of saturated fat by polyunsaturated fat carried out up to 1992 showed that replacement of 10% of energy from saturated fat by polyunsaturated fat reduced CHD incidence by 20%.[33] However, the replacement of saturated fat by polyunsaturated fat did not decrease all-cause mortality.

Besides the trials on fatty acids only a small number of food-based trials have been carried out. The DART trial in which the intervention was advice to cardiac patients to eat at least two portions of oily fish per week, reduced CHD mortality by 33% and all-cause mortality by 29% compared to no advice.[14] The largest food-based trial, is the PREDIMED study in which added amounts of extra virgin olive oil or nuts were compared with a low-fat diet. This trial was carried out in high-risk patients and showed that adding 50 ml of extra-virgin olive oil per day to the diet reduced cardiovascular events by 30% but did not decrease all-cause mortality. A 28% reduction in cardiovascular risk was observed for 30 g nuts added per day.[34]

These results suggest that improving the quality of fat in the diet and added amounts of oily fish, extra–virgin olive oil and nuts positively influence the risk of CHD death or cardiovascular events. All-cause mortality was not significantly reduced in the trials with the exception of oily fish consumed by high-risk cardiac patients. More and larger trials are needed to determine any effect of recommended diets on all-cause mortality.

Diet is not the only lifestyle-related factor of importance in relation to cardiovascular disease prevention. We showed in the HALE project that that adherence to a healthful diet in combination with recommended levels of physical activity, non-smoking and moderate alcohol consumption, even in elderly men and women is associated with a more than 50% lower risk of CHD, cardiovascular diseases and all-cause mortality.[32] Taking the evidence from prospective cohort studies and clinical trials together, I conclude that a healthful diet and lifestyle reduce the rate of CHD and cardiovascular diseases mortality and possibly also of all-cause mortality in high-risk populations.

Healthful lifestyle factors and mortality from all-causes in the HALE project[32]



We recently finished the 50-year mortality follow-up of the Northern European cohort in Zutphen and the Mediterranean cohort of Crete. The survival curve of Crete is different from that of Zutphen. The median age of death was 81.6 years in Crete and 75.9 years in Zutphen. The dramatic 6-year difference in survival was associated with low mortality rates from CHD and lung cancer. These two causes of death are strongly related to smoking and diet. This suggests that that the difference in survival between Crete and Zutphen is in large part due to environmental causes.

Epilogue

When I became responsible for the Zutphen Study in 1978 my predecessors Van Buchem and Den Hartog had collected unique data of repeated measures of cardiovascular risk factors and diet. However, at that time the risk factor data were only partly digitized and the dietary data had still to be coded. With grants from the Nutrition Council and the Food Organization TNO the job was done in two years. Thereafter data analysis started and the yield of results began. My first grant application for a 20-year follow-up of the Zutphen cohort was turned down with the argument that an old study could not produce new results. This argument was used repeatedly in the past 40 years when I submitted grants for data analysis. A greater discrepancy between the results of the Zutphen Study and their importance for my career and the rejection of my grants for data analysis, is hardly imaginable!

In 1985 the title of my inaugural address at the University of Leiden was: "Nutritional epidemiology between dream and reality". My hope was to discover nutritional causes of cardiovascular diseases. Thirty years later I conclude that my mission has not been fulfilled. The evidence is still in sufficient that omega-3 fatty acids in fatty fish are a cause of CVD. The search to which flavonoids are protective for cardiovascular diseases is still in its infancy and the same holds for food patterns. Our science and our lives are unfinished business.

Acknowledgements

The late professor Jan Tinbergen, a Dutch Nobel Prize winner, described econometrists as well trained athletes who never competed in a match. The same can be said of epidemiologists who are so much dependent on results from other disciplines to complement their findings. I am very much indebted to many such collaborators and colleagues of which I only can mention a few.

I thank the other speakers in this symposium for their excellent contributions. I am grateful to Marianne Geleijnse for a decade of fine collaboration in the Alpha Omega Trial. I am thankful to my American colleagues David Jacobs, David Siscovick and Dariush Mozaffarian for long-term collaboration on research about on fatty acids, foods and food patterns, and to Andreas Flammer from Switserland for his contribution on cocoa flavanols and cardiovascular health.

Seven Countries Study Investigators Japan 1993

Standing from left to right: Hironori Toshima (Japan), Henry Blackburn (USA), Daan Kromhout (The Netherlands), Alessandro Menotti (Italy), Aulikki Nissinen (Finland), Srecko Nedeljkovic (Serbia), Fred Epstein (Switzerland). Sitting from left to right: Ratko Buzina (Croatia), Christ Aravanis (Greece), Ancel Keys (USA), Flaminio Fidanza (Italy), Martti Karvonen (Finland), Anastasios Dontas (Greece).

I am very much indebted to my colleagues in the Seven Countries Study. From the first meeting with the Seven Countries Study Investigators on Crete in 1979 I felt at home. I owe a lot to Ancel Keys, the leader of the Study and to long-term colleagues and members of the Coordinating Team: Henry Blackburn, Alessandro Menotti and David Jacobs and the fine colleagues in the different countries. I also like to thank Henry Blackburn for editing this lecture.

Now I like to change to my mother tongue to thank my Dutch colleagues and family.

In mijn loopbaan hebben alle drie de hoogleraren van de afdeling Humane voeding een rol gespeeld. Professor Cees den Hartog heb ik als student tot mijn kandidaats (BSc) meegemaakt. Hij had zoveel vertrouwen in mij dat hij de Zutphen Studie aan mij toevertrouwde op jeugdige leeftijd. Ik was zo jong dat tijdens de eerste bijeenkomst van de Zeven Landen Studie op Kreta in 1979 een van de senior onderzoekers vroeg of ik de kleinzoon was van een van de hoogleraren. Professor Jo Hautvast was de grondlegger van de schoolkinderen onderzoeken naar serum cholesterol en overgewicht en mijn promotor. Hij raakte het meest onder de indruk toen ik in mijn RIVM periode hem een keer met auto en chauffeur kwam ophalen. De meest recente hoogleraar Humane voeding en vriend Frans Kok en de voormalige Rector Bert Speelman ben ik zeer veel dank verschuldigd. Zij hebben mogelijk gemaakt dat ik vanaf 2005 naast het vicevoorzitterschap van de Gezondheidsraad ook tot deeltijd hoogleraar werd benoemd aan mijn Alma Mater.

Wageningen Professors of Nutrition and Health and heads of the Division

Cees dan Hartog
1954-1972

Jo Hautvast
1972-1997

Frans Kok
1997-2015

In 1985 kreeg ik van het Praeventiefonds (nu ZonMW) subsidie voor een onderzoek naar ouderen in Zutphen. De subsidie was net genoeg om 900 mannen van 65-84 jaar te onderzoeken. Het medisch onderzoek bestond uit een lichamelijk onderzoek, bloedafname, het opnemen van een ECG en het invullen van vragenlijsten en werd locaal gecoördineerd door de internist-geriater Edward Bosschieter, die vele jaren bij het onderzoek betrokken is geweest. Er was echter geen geld beschikbaar voor voedingsonderzoek. Ik heb toen contact opgenomen met de leiding van de afdeling Diëtetiek van de Haagse hogeschool die bereid was een groep van 25 vierde jaars diëtetiek studenten naar Zutphen te laten afreizen om bij de deelnemers thuis een cross-check dietary history af te nemen. Zij werden ondergebracht in een klooster in Eefde en moesten op britsen slapen. Ondanks deze Spartaanse omstandigheden hebben zij uitstekend werk verricht. Zonder hen was er geen modern voedingsonderzoek in de Zutphen Studie geweest! Ik ben hun zeer veel dank verschuldigd en ontzettend blij dat Annemarie Jansen er in is geslaagd om met een deel van haar jaargenoten vandaag aanwezig te zijn. Bennie Boemberg was jarenlang mijn steun en toeverlaat bij het opbouwen van de databases voor de Zutphen en Zeven Landen Studie.

Een van de grote onderzoeken in de Zutphen Studie was het flavonoïden project. De fascinerende zoektocht naar welke flavonoïden bescherming bieden tegen hart- en vaatziekten behoort tot de hoogtepunten van mijn loopbaan. Ik heb daarbij zeer veel te danken aan de grote klasse van het chemisch analytische werk van Peter Hollman. Peter, het was een feest om met jou samen te werken. Je enorme inzicht en heldere wijze van denken zijn een voorbeeld voor elke wetenschapper. Je behoort tot de top-10 van de Wageningse onderzoekers en werd onlangs uitgeroepen tot een van de wereldwijde onderzoekers die er echt toe doen!

Een ander groot project was de Alpha Omega Trial. De beroemde Engelse epidemiologen Archie Cochrane en Richard Peto wezen mij al vroeg in mijn loopbaan op het grote belang van een goed uitgevoerde grote interventiestudie als je wil bewijzen dat voeding een oorzaak is voor chronische ziekten. Dat is voor mij de Alpha Omega Trial geworden waarin bijna 5000 patiënten zijn geïncludeerd die een hartinfarct hadden doorgemaakt. Aan dit onderzoek hebben 32 ziekenhuizen deelgenomen, een derde van alle ziekenhuizen in Nederland. Ik ben de cardiologen en medewerkers van deze ziekenhuizen en de verpleegkundigen die het onderzoek bij de patiënten thuis uitvoerden, zeer veel dank verschuldigd voor hun bijdragen. Deze trial, waar meer dan 200 personen aan mee werkten, werd gecoördineerd door een team bestaande uit Marianne Geleijnse, Erik Giltay, Janette de Goede, Linda Oude Griep, Annemarie Teitsma en Eveline Waterham. Ik heb genoten van de samenwerking met dit voortreffelijke team!

Executive Committee Alpha Omega Trial, Stockholm 2010

From left to right: Eveline Waterham, Linda Oude Griep, Marianne Geleijnse, Annemarie Teitsma-Jansen, Daan Kromhout, Janette de Goede, Erik Giltay

De onderzoeken die ik heb opgezet zijn grotendeels uitgevoerd door promovendi. Deze jonge onderzoekers hopen in 4 jaar te promoveren, wat helaas niet altijd lukt. Ik ben aan hen zeer veel dank verschuldigd voor het vele werk dat zij hebben verzet. Op dit moment zijn er nog drie promovendi bezig met het afronden van hun proefschrift en hebben 48 promovendi hun proefschrift succesvol verdedigd, waarvan er 10 hoogleraar zijn geworden. Zij geven mijn passie voor onderzoek door aan de volgende generatie.

Voor het uitvoeren van de Zutphen en Zeven Landen Studie, en de Alpha Omega Trial was administratieve ondersteuning onontbeerlijk. De internationale oriëntatie van zowel Anke Roccuzzo bij het RIVM en van Lous Duym bij de Afdeling Humane Voeding in Wageningen maakte het mogelijk om deze projecten efficiënt te runnen. Het feit dat Anke het Italiaans perfect beheerst maakte veeleisende personen zoals professor Fidanza zo mak als een lammetje! Lous is zo goed ingevoerd in de Wageningse en internationale voedingswereld dat ze elke probleem moeiteloos oploste. Het elkaar begrijpen met weinig woorden maakte de samenwerking met jullie tot een feest!

Ik had al het onderzoek niet kunnen uitvoeren zonder de steun van mijn gezin. Dat is de plek waar ik volledig mezelf kan zijn. Waar enerzijds wordt gerelaxt maar waar

ook stevig wordt gediscussieerd en ik regelmatig scherper wordt aangevallen dan in menig wetenschappelijk debat. Het is een groot voorrecht om met Gerda, kinderen, hun partners en kleinkinderen oud te worden.

Tot slot. In 1960 kwam meester Van Horssen van de vierde klas van de koningin Emma school in Rijnsburg naar mijn ouders met de vraag welke vervolgopleiding ik zou gaan doen. Mijn ouders bleven hem het antwoord op die vraag schuldig. Hij adviseerde hen mij over te laten stappen naar een andere lagere school omdat de koningin Emma school alleen opleidde voor lager beroepsonderwijs en mulo. Mijn ouders willigden zijn verzoek in en lieten mij op weg gaan naar een stip aan de horizon die zij niet kenden. Als zij dit niet hadden gedaan, had ik hier vandaag niet gestaan.

Ik heb gezegd.

References

1. Kromhout D, van der Haar F, Hautvast JG. Coronary heart disease risk factors in Dutch schoolchildren--results of a pilot-study. Prev Med 1977;6:500-513.
2. Van der Haar F, Kromhout D. Food intake, nutritional anthropometry and blood chemical parameters in 3 selected Dutch schoolchildren populations. PhD thesis. H. Veenman & Zonen, Wageningen 1978: 1-239.
3. Holman RL. Atherosclerosis – a pediatric nutrition problem? Am J Clin Nutr 1961;9:565-569.
4. Keys A. Aravanis C, Blackburn H, et al. Seven countries. A multivariate analysis of death and coronary heart disease. Cambridge, MA; Harvard University Press, ISBN: 0-674-80237-3, 1980: 1-381.
5. Keys A. (Ed). Coronary heart disease in seven countries. Circulation 1970;41, Suppl 1: 1-211.
6. Verschuren WM, Jacobs DR, Bloemberg BP, et al. Serum total cholesterol and long-term coronary heart disease mortality in different cultures. Twenty-five-year follow-up of the Seven Countries Study. JAMA 1995;274:131-136.
7. Arntzenius AC, Kromhout D, Barth JD, et al. Diet, lipoproteins and the progression of atherosclerosis. The Leiden Intervention Trial. N Engl J Med 1985;312:805-811.
8. Kromhout D, Arntzenius AC, Kempen-Voogd N, et al. Longterm effects of a linoleic

acid enriched diet, changes in body weight and alcohol consumption on serum total and HDL cholesterol. Atherosclerosis 1987;66:99-105.
9. Kromhout D, Bosschieter EB, de Lezenne Coulander C. The inverse relation between fish consumption and 20-year mortality from coronary heart disease. N Engl J Med 1985;312:1205-1209.
10. Kromhout D, Feskens EJM, Bowles CH. The protective effect of a small amount of fish on coronary heart disease mortality in an elderly population. Int J Epidemiol 1995;24:340-345.
11. Oomen C, Feskens EJM, Räsänen L, et al. Fish consumption and coronary heart disease mortality in Finland, Italy and the Netherlands. Am J Epidemiol 2000;151:999-1006.
12. Streppel M, Ocke MC, Boshuizen HC, Kok FJ, Kromhout D. Long-term fish consumption and n-3 fatty acid intake in relation to (sudden) coronary heart disease death: the Zutphen Study. Eur Heart J 2008;29:2024-2030.
13. De Goede J, Geleijnse JM, Boer JMA, Kromhout D, Verschuren WMM. Marine (n-3) fatty acids, fish consumption, and the 10-year risk of fatal and nonfatal coronary heart disease in a large population of Dutch adults with a low fish intake. J Nutr 2010;140:1023-1028.
14. Burr ML, Fehily AM, Gilbert JF, et al. Effects of changes in fat, fish, and fibre on death and myocardial infarction: diet and reinfarction trial (DART). Lancet 1989;2:757-761.
15. GISSI-Prevenzione Investigators. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Lancet 1999;354:447-455.
16. Geleijnse JM, Giltay EJ, Schouten EG, et al. Effect of a low dose of n-3 fatty acids on cardiovascular diseases in 4,837 post-myocardial infarction patients: Design and baseline characteristics. Am Heart J 2010;140:539-556.
17. Kromhout D, Giltay EJ, Geleijnse JM; Alpha Omega Trial Group. n-3 fatty acids and cardiovascular events after myocardial infarction. N Engl J Med 2010;363:2015-2026.
18. Saravanan P, Davidson NC, Schmidt EB, Calder PC. Cardiovascular effects of marine omega-3 fatty acids. Lancet 2010;376:540-550.
19. Eussen SRBM, Geleijnse JM, Giltay EJ, Rompelberg CJM, Klungel OH, Kromhout D. Effects of n-3 fatty acids on major cardiovascular events in statin users and non-users with a history of myocardial infarction. Eur Heart J 2012;33:1582-1588.
20. Kromhout D, Geleijnse JM, de Goede J, et al. n-3 fatty acids, ventricular arrhythmia-related events, and fatal myocardial infarction in postmyocardial infarction patients with diabetes. Diabetes Care 2011;34:2515-2520.
21. Kromhout D. Essential micronutrients in relation to carcinogenesis. Am J Clin Nutr 1987;45:1361-1367.

22. Hertog MGL, Feskens EJM, Hollman PCH, Katan MB, Kromhout D. Dietary flavonoids and cancer risk in the Zutphen Elderly Study. Nutr Cancer 1994;22:175-184.
23. Hertog MG, Feskens EJ, Hollman PC, Katan MB, Kromhout D. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. Lancet 1993;342:1007-1011.
24. Arts IC, Hollman PC, Feskens EJ, Bueno de Mesquita HB, Kromhout D. Catechin intake might explain the inverse relation between tea consumption and ischemic heart disease: the Zutphen Elderly Study. Am J Clin Nutr 2001;74:227-232.
25. Buijsse B, Feskens EJ, Kok FJ, Kromhout D. Cocoa intake, blood pressure, and cardiovascular mortality: the Zutphen Elderly Study. Arch Intern Med 2006;166:411-417.
26. Ras RT, Zock PL, Draijer R. Tea consumption enhances endothelial-dependent vasodilatation; a meta-analysis. PLoS ONE 2011;6:e16974.
27. Hooper L, Kay C, Abdelhamid A, et al. Effects of chocolate, cocoa, and flavan-3-ols on cardiovascular health: a systematic review and meta-analysis of randomized trials. Am J Clin Nutr 2012;95:740-751.
28. Huxley R, Lee CMY, Barzi F, et al. Coffee, decaffeinated coffee, and tea consumption in relation to incident type 2 diabetes mellitus. A systematic review with meta-analysis. Arch Intern Med 2009;169:2053-2063.
29. Dower JI, Geleijnse JM, Gijsbers L, Zock PL, Kromhout D, Hollman PCH. Effects of the pure epicatechin and quercetin on vascular function and cardiometabolic health: a randomized double-blind, placebo-controlled, crossover trial. Am J Clin Nutr 2015;101:914-921.
30. Keys A, Aravanis C, Sdrin H. The diets of middle-aged men in two rural areas in Greece. Voeding 1966;27:575-586.
31. Kromhout D, Keys A, Aravanis C, et al. Food consumption patterns in the nineteen sixties in Seven Countries. Am J Clin Nutr 1989;49:889-894.
32. Knoops KT, de Groot LC, Kromhout D, et al. Mediterranean diet, lifestyle factors, and 10-year mortality in elderly European men and women: the HALE project. JAMA 2004;292:1433-1439.
33. Mozaffarian D, Micha R, Wallace S. Effects of coronary heart disease of increasing polyunsaturated fat in place of saturated fat: A systematic review and meta-analysis of randomized controlled trials. PLoS Med 2010;7:e1000252
34. Estruch R, Ros E, Salas-Salvado J, et al. Primary prevention of cardiovascular disease with a Mediterranean diet. N Engl J Med 2013;368:1279-1290.

Professor Daan Kromhout

'The traditional paradigm in nutrition research is a focus on nutrients and a translation from nutrients to foods for consumer education. Foods contain many nutrients and bioactive compounds. My epidemiologic research showed that nutrients (fatty acids) and bioactive compounds (flavonoids) were related to cardiovascular diseases. Even stronger associations were obtained for foods (fish and cocoa) and dietary patterns (traditional Mediterranean diet). Experimental studies are needed to verify these associations to underpin evidence-based dietary guidelines.'

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