Fruit and vegetable consumption and the risk of cardiovascular diseases

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Abstract

Background: Prospective cohort studies have shown that the consumption of total fruit and vegetables is associated with a lower risk of coronary heart disease (CHD) and stroke. It is not known which aspects of fruit and vegetable consumption contribute to these beneficial associations. The objective of this PhD research was to investigate different aspects of fruit and vegetable consumption, i.e. amount, processing, variety and color, in relation to 10-year incidence of CHD and stroke.

Methods: Data were used from the Monitoring Project on Risk Factors and Chronic Diseases in the Netherlands (MORGEN Study). This is a prospective population-based cohort study in over 22,000 men and women aged 20 to 65 years who were enrolled from 1993 to 1997. We selected 20,069 participants who were free of cardiovascular diseases (CVD) at baseline and were followed for an average of 10 years for non-fatal and fatal cases of CVD. All participants completed a validated 178-item food frequency questionnaire at baseline to measure habitual dietary intake in the previous year.

Results: During follow-up, 245 cases of CHD and 233 cases of stroke were documented. An inverse dose-response relationship was observed between total fruit and vegetable consumption and incident CHD, but not for incident stroke. Participants with a total fruit and vegetable consumption of more than 475 grams per day had a 34% lower risk of CHD (Q4: HR: 0.66; 95% CI:0.45-0.99) compared to those with a low intake (Q1: ≤241 g/d). High intake of raw fruit and vegetables (Q4: >262 vs Q1: ≤92 g/d) was associated with a 30% lower risk of either CHD (HR: 0.70; 95% CI: 0.47-1.04) or stroke (HR: 0.70; 95% CI: 0.47-1.03). Variety was strongly associated with total fruit and vegetable consumption, not with incident CHD or stroke. The intake of deep orange fruit and vegetables and especially carrots was inversely associated with CHD (per 25 g/d increase; HR: 0.74; 95% CI:0.55-1.00). High intake of white fruit and vegetables (Q4: >171 vs Q1: ≤78 g/d), such as apples and pears, was associated with a 52% lower risk of stroke (HR: 0.48; 95% CI:0.29-0.77).

Conclusion: The findings presented in this thesis suggest that total fruit and vegetable consumption was inversely related to incident CHD, but not to incident stroke. Raw fruit and vegetable consumption, however, may protect against CHD and stroke incidence. These results suggest that to prevent CVD at least 50% of the recommended daily amounts of fruit and vegetables should comprise raw fruit and vegetables. Before solid recommendations on different aspects of fruit and vegetable consumption can be made, results from additional prospective cohort and intervention studies are needed.

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Chapter 1

Introduction

Fruit and vegetables are rich sources of micronutrients and bioactive compounds. Evidence from prospective cohort studies showed that a high consumption of fruit and vegetables may prevent cardiovascular diseases (CVD). Based on these findings, it is recommended to consume sufficient amounts of fruit and vegetables to ensure an adequate intake of micronutrients and to prevent CVD[1,2]. Evidence from prospective cohort studies on which aspects of fruit and vegetable consumption may contribute to these beneficial associations, however, is lacking. This PhD research focuses on different aspects of fruit and vegetable consumption, i.e. amount, processing, variety and color, in relation to the incidence of coronary heart disease (CHD) and stroke.

Definition of fruit and vegetables

In plant taxonomy, the botanical term 'vegetables' refers to the edible part of a plant[3,4]. Thus, fruits are actually a subclass of vegetables. The botanical term 'fruit' refers to the ripened ovary of a flower that contains seeds. This implies that plant foods, such as cereals, legumes, nuts, cucumbers and tomatoes, are all fruits. All other parts of the plants, such as stems, roots, and leaves, can be biologically considered as vegetables.

From a nutritional perspective, it is more useful to define fruit and vegetables based on their nutritional value, taste and culinary use[3,4]. Though no universally agreed definition for fruit and vegetables exists, it is generally accepted for some plant foods. Cucumbers, tomatoes and sweet peppers, for example, are botanically considered as fruits. However, since they are used as vegetables and have a savory flavour they are considered as vegetables. Cereals are clearly different from fruit and vegetables since they contain approximately 70% starch and are a valuable source of protein and dietary fiber[4,5]. For other plant foods controversies exist whether they should be considered as fruit or vegetable. Potatoes and legumes are rich sources of starch and legumes also of protein[6]. Nuts are energy dense and high in mono-unsaturated fatty acids[6].

In this PhD research, fruits and vegetables are defined based on their nutritional value, taste and culinary use. Other plant foods such as cereals, legumes, potatoes and nuts are not considered as fruit or vegetables since their nutritional value is significantly different.

Evidence from prospective cohort studies

From the 1990's onwards, epidemiologic evidence started to emerge that consuming fruit and vegetables may protect against CVD. In a meta-analysis of 6 prospective cohort studies, each additional portion of 106 grams of fruit or vegetables was associated with a 4% lower risk of incident CHD[7]. In a later meta-analysis that combined the results of 12 prospective cohort studies, it was observed that participants with a daily fruit and vegetable consumption of more than 391 grams had a 17% lower risk of incident CHD compared to those consuming less than 235 grams[8].

With regard to stroke, Dauchet *et al.* observed in a meta-analysis including 7 prospective cohort studies that each additional portion of fruit and vegetables of 106 grams was associated with a 5% lower risk of stroke[9]. He *et al.* observed based on 9 prospective cohort studies a 26% lower risk of incident stroke for participants consuming more than 391 grams per day of fruit and vegetables compared to those who consumed less than 235 grams per day[10]. Based on these findings, it can be concluded that consumption of ample amounts of fruit and vegetables may contribute to the prevention of CHD and stroke.

Findings from prospective cohort studies showed that consumption of ample amounts of fruit and vegetables may prevent the occurrence of CHD and stroke.

Cardioprotective constituents of fruit and vegetables

Fruit and vegetables are rich sources of many different micronutrients and bioactive compounds, e.g. dietary fiber, vitamin C, potassium, carotenoids, flavonoids and other polyphenols[11]. These components may underlie the observed inverse associations of fruit and vegetable consumption with CVD. In the Netherlands, fruit and vegetables contribute substantially to the daily intake of vitamin C (~48%) and dietary fiber (~25%)[12].

Evidence is accumulating that constituents of fruit and vegetables may play a protective role in the development of CVD. Results of a pooled analysis of 8 prospective cohort studies showed that fiber from fruit is consistently associated with a lower risk of CHD[13]. Randomized placebo-controlled intervention studies found that increased intake of dietary fiber had a small blood pressure lowering effect[14,15] and resulted in lower serum total and LDL cholesterol levels[16]. Evidence from a meta-analysis of prospective cohort studies suggest an important role of dietary potassium in the prevention of stroke and CHD[17], possibly mediated by blood pressure lowering effects[18].

Prospective cohort studies suggested that dietary vitamin C and β-carotene may lower the risk of CHD[19,20]. However, beneficial effects of supplementation of β-carotene, vitamin C or its combination in relation to CVD could not be demonstrated in randomized placebo-controlled trials[21,22]. Recently, it was observed that serum α-carotene concentrations were inversely associated with CHD mortality among US adults[23]. Circulating carotenoids were also inversely associated with markers of inflammation, oxidative stress, and endothelial dysfunction[24] and may protect against early atherosclerosis[25,26]. For flavonols, meta-analyses of prospective cohort studies observed a 20% lower risk of fatal CHD[27] and incident stroke[28]. It is suggested that the protective effect of fruit and vegetables is most likely due to synergistic effects of their different bioactive compounds in their natural food matrix[29,30].

Fruit and vegetables are rich sources of many different micronutrients and bioactive compounds that may be cardioprotective through various pathways.

Different aspects of fruit and vegetable consumption

The Health Council of the Netherlands recommends to consume sufficient amounts of fruit, vegetables, legumes and whole grain foods to ensure an adequate intake of micronutrients and bioactive compounds and to prevent CVD[1]. The recommendation to consume fruit and vegetables to prevent CVD is based on the evidence provided by the meta-analyses showing beneficial associations of higher intakes of fruit and vegetables in relation to CHD[7,8] and stroke[9,10]. This recommendation is translated by the Netherlands Nutrition Center into a daily intake of 150 to 200 gram of vegetables and 200 gram of fruit for men and women from the age of 19 onwards[31]. It is unknown whether aspects of fruit and vegetable consumption, e.g. processing, variety and color groups, contribute to these beneficial associations. Different aspects of fruit and vegetable consumption are therefore not addressed in the current recommendations and need further investigation.

Processing
Processing of fruit and vegetables may alter their structure and induces changes in their chemical composition, nutritional value, digestibility and bioavailability of bioactive compounds. Though fruit and vegetable juices are lower in fiber content compared with their raw counterparts, they may be a good source of bioactive compounds[32,33]. During cooking, water-soluble and heat-sensitive bioactive compounds, such as carotenoids, can be lost but their bioavailability may improve[34,35]. The prospective cohort studies included in the meta-analyses of Dauchet *et al.* and He *et al.* made no distinction between raw or processed fruit and vegetables or it was not reported[7-10]. It is currently advised in the Dietary Guidelines to consume a diet rich of fruit and vegetables either raw, cooked, whole, cut up, mashed or as 100% fruit juice[1,2]. In addition, it is advised to consume whole fruits rather than fruit juice. To the best of our knowledge, no previous prospective cohort studies have yet investigated raw or processed fruit and vegetable consumption separately in relation to incident CHD and stroke.

Variety
A variety of fruit and vegetables provides many different micronutrients and bioactive compounds that may underlie the observed inverse associations with CVD[7-10]. Based on this knowledge, it is also advised to choose a variety of fruit and vegetables daily. To date, no prospective cohort studies evaluated the importance of variety in fruit and vegetable consumption in relation to incident CHD and stroke.

Color groups
Fruit and vegetable color groups provide a different array of micronutrients and bioactive compounds[36]. Possibly, several color groups may be responsible for the association with CHD and stroke. Several previous prospective cohort studies investigated a single fruit or vegetable in relation to CVD. Some evidence was obtained for carrots with both fatal CHD[37] and fatal CVD[38-40]. For stroke, inverse associations were found for the intake of citrus fruit[41-45] and apples and pears[41,43,46,47], although the latter were not statistically significant. Recently, Pennington and Fisher defined 10 coherent fruit and vegetable subgroups based on their unique nutritional value and characteristics, namely part of the plant, color, botanical family and total antioxidant capacity[4,36]. These subgroups can be classified into 4 color groups. Which color groups of fruit and vegetables contribute most to the inverse association with CHD an stroke remains unclear.

Rationale of the thesis

The main objective of this PhD research is to investigate the role of amount (Chapter 2 and 3), processing (Chapter 2 and 3), variety (Chapter 4) and color groups (chapter 5 and 6) of fruit and vegetable consumption in relation to 10-year incidence of CHD and stroke in a Dutch population free from CVD. For this purpose, we used data from the Monitoring Project on Risk Factors and Chronic Diseases in the Netherlands (MORGEN Study), a Dutch population-based prospective cohort study among men and women aged 20 to 65 years.

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Chapter 2

Raw and processed fruit and vegetable consumption and 10-year coronary heart disease incidence in a population-based cohort study in the Netherlands

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Abstract

Background: Prospective cohort studies have shown that high fruit and vegetable consumption is inversely associated with coronary heart disease (CHD). Whether food processing affects this association is unknown. Therefore, we quantified the association of fruit and vegetable consumption with 10-year CHD incidence in a population-based study in the Netherlands and the effect of processing on these associations.

Methods: Prospective population-based cohort study, including 20,069 men and women aged 20 to 65 years, enrolled between 1993 and 1997 and free of cardiovascular disease at baseline. Diet was assessed using a validated 178-item food frequency questionnaire. Hazard ratios (HR) were calculated for CHD incidence using multivariable Cox proportional hazards models.

Results: During a median follow-up time of 10.5 years, 245 incident cases of CHD were documented, which comprised 211 non-fatal acute myocardial infarctions and 34 fatal CHD events. The risk of CHD incidence was 34% lower for participants with a high intake of total fruit and vegetables (>475 g/d; HR: 0.66; 95% CI: 0.45-0.99) compared to participants with a low total fruit and vegetable consumption (≤241 g/d). Intake of raw fruit and vegetables (>262 g/d vs ≤92 g/d; HR: 0.70; 95% CI: 0.47-1.04) as well as processed fruit and vegetables (>234 g/d vs ≤113 g/d; HR: 0.79; 95% CI: 0.54-1.16) were inversely related with CHD incidence.

Conclusion: Higher consumption of fruit and vegetables, whether consumed raw or processed, may protect against CHD incidence.

Introduction

Prospective cohort studies have shown that a high consumption of fruit and vegetables is inversely associated with coronary heart disease (CHD)[1,2]. Fruit and vegetables are rich sources of fiber, vitamins, polyphenols and other bioactive phytochemicals that may contribute to a lower CHD risk[3]. In particular, antioxidants have received considerable attention. Although prospective cohort studies have shown inverse associations between dietary intake of antioxidant vitamins and CHD risk[4,5], randomized trials using vitamin supplements have failed to demonstrate a beneficial effect[6,7]. These results may be explained by methodological issues such as the relatively short follow-up period, the high doses of antioxidants compared to habitual diets and differences in bioavailability of natural and synthetic sources of antioxidants. However, the negative results of these trials could suggest that the protective effect of fruit and vegetables may be due to combined and synergistic effects of the different constituents in their natural food matrix and not to a particular antioxidant[8].

Processing of fruit and vegetables alters their structure and induces significant changes in chemical composition, nutritional value and bioavailability of bioactive compounds, which may induce different effects on CHD risk. Fruit juices, for example, have a lower content of fiber than raw fruit, but they may be a good source of phytochemicals[9]. Prospective studies that examined intake of citrus fruit juice in relation to risk of CHD[10] and cardiovascular diseases[11], respectively, found no association. Vegetables are often cooked before consumption, which induces loss of water-soluble and heat-sensitive bioactive compounds[12,13]. On the contrary, processing can enhance the availability of bioactive compounds[14]. It has been shown that heat treatment improves the bioavailability of lycopene from tomatoes[15,16] and carotenoids from carrots[17]. Furthermore, processing could convert folate polyglutamate in vegetables into monoglutamate, which has better bioavailability as well[18,19].

To the best of our knowledge, no prospective cohort study has focused specifically on raw and processed fruit and vegetable consumption in relation to CHD incidence. Therefore, we investigated the associations of total, raw and processed fruit and vegetable consumption with incident CHD in a population-based follow-up study in the Netherlands.

Methods

Population

The present study was conducted in a Dutch population-based cohort: the Monitoring Project on Risk Factors and Chronic Diseases in the Netherlands (MORGEN Study)[20]. Random samples of men and women aged 20 to 59 years were drawn from the municipal registers of two towns in the Netherlands (Amsterdam and Maastricht). In addition, men and women aged 26 to 65 years from the town of Doetinchem, who participated in a similar project between 1987 and 1991 were re-invited to participate in the MORGEN Study. Information on diet, lifestyle and cardiovascular risk factors was collected between 1993 and 1997. The Medical Ethics Committee of the Netherlands Organization for Applied Scientific Research (TNO) approved the study protocol and all participants signed informed consent form. Of the total 22,654 participants, we excluded respondents without informed consent for vital status follow-up (n=701), with incomplete dietary assessment (n=72), with reported total energy intake <500 or >4500 kcal per day for women or <800 or >5000 kcal per day for men (*n*=97), with a history of myocardial infarction or stroke at baseline (*n*=442) and with self-reported diabetes or using lipid-lowering or anti-hypertensive drugs (*n*=1,273). This resulted in a study population of 20,069 participants, including 8,988 men and 11,081 women.

Dietary assessment

Information on habitual food consumption of 178 food items, covering the previous year, was collected using a validated, self-administered, semi-quantitative food frequency questionnaire (FFQ) developed for the Dutch cohorts of the European Prospective Investigation Into Cancer (EPIC) Study[21]. Participants indicated their habitual consumption of food items as absolute frequencies in times per day, per week, per month, per year or as never, e.g. 'How often do you habitually eat raw vegetables during a meal in the winter?'. The questions used to assess the frequency of fruit and vegetable consumption were specified as to season and preparation methods. For several food items, additional questions were included about the consumption frequency of different sub-items or about preparation methods using the following categories: always/mostly, often, sometimes and seldom/never, in answer to questions such as: 'Which types of vegetables do you eat?'. For 21 food items, mainly vegetables, colored photographs were used to estimate portion sizes. Frequencies per day were multiplied with standard household measures, natural units or indicated portion sizes to obtain grams per day for each food item. The Dutch food composition database of 1996 was used to calculate values for energy and nutrient intakes[22].

The FFQ comprised 35 fruit and vegetable items in total, including 9 raw fruits, 7 raw vegetables, 13 cooked vegetables, 2 vegetable juices/sauces and 4 fruit juices/sauces. Processed fruits comprised fruit juices and apple sauce that were mainly consumed as industrially produce from concentrates. Processed vegetables comprised home-cooked vegetables including canned and frozen vegetables and tomato sauce. We did not consider potatoes and legumes, except French beans, as vegetables, because the nutritional value of these food items differs significantly from that of vegetables[22].

The reproducibility of the FFQ after 12 months and relative validity of the FFQ against 12 repeated 24-h recalls for food group and nutrient intake were tested in 63 males and 58 females with mean ages of 42.6 and 49.0 years, respectively[21,23]. In men, reproducibility of the FFQ after 12 months, expressed as Spearman's correlation coefficients, was 0.67 for raw vegetables and 0.69 for cooked vegetables. Similar correlation coefficients for raw and cooked vegetable intake were found in women. The reproducibility for total fruit intake was 0.61 in men and 0.77 in women. The validity against 12 repeated 24-h recalls varied between 0.32 and 0.49 for raw vegetables, 0.21 and 0.41 for cooked vegetables and 0.56 and 0.68 for total fruit intake. Jansen *et al.* validated fruit and vegetable intake by using plasma carotenoids and found that the plasma β-cryptoxanthin level was related to the sum of vegetables, fruit and juices (r=0.41 and 0.35 for men and women) and fruit (r=0.32 for both men and women), of which citrus fruit was the major dietary source. Lutein, mainly from green leafy vegetables, was the best indicator of vegetable intake with correlation coefficients of 0.27 and 0.19 for men and women, respectively[24].

Risk factors

The baseline measurements were previously described in detail by Verschuren *et al.*[25]. Body weight, height and blood pressure of the participants were measured by trained research assistants during a physical examination at a municipal health service site. Non-fasting venous blood samples were collected and total cholesterol concentrations were determined using an enzymatic method. Information on cigarette smoking, educational level, physical activity, use of anti-hypertensive and lipid lowering drugs, past or present use of hormone replacement therapy and the history of myocardial infarction of the participants' parents were obtained through a self-administered questionnaire. Dietary supplement use (yes/no) and alcohol intake were obtained from the FFQ. The most commonly reported dietary supplements included vitamin C (28%), multivitamins (26%) and vitamin B (11%). Alcohol intake was expressed as the number of glasses of beer, wine, port wines and strong liquor consumed per week. From 1994 onwards, the type, frequency and duration of physical activity was assessed using a validated questionnaire developed for the EPIC Study, including questions on leisure time and occupational physical activity[26]. The most frequently reported physical activities were walking, cycling, gardening and sports, of which cycling and sports were activities of at least 4 metabolic equivalents[27]. These 4 types of physical activities were related to 3-day activity patterns repeated 4 times[26].

Ascertainment of fatal and non-fatal events

After enrollment, information on the participants' vital status up to 1 January 2006 was monitored using the municipal population register. For participants who died, information on the primary cause of death was obtained from Statistics Netherlands. The hospital discharge register provided clinically diagnosed acute myocardial infarction (AMI) admissions by a cardiologist based on the definition of the European Society of Cardiology[28]. It has been shown on the national level that data from the Dutch hospital discharge register can be uniquely matched to a single person for at least 88% of the hospital admissions[29]. In a validation study with an overlap of 33% with our study population, 84% of the AMI cases in the cardiology information system of the University Hospital Maastricht corresponded with AMI cases identified in the hospital discharge register[30]. CHD incidence was defined as the first non-fatal AMI or fatal CHD event, not preceded by any other CHD event. Non-fatal AMI included code 410 of the 9th revision of the International Classification of Diseases[31]. Fatal CHD included ICD-10 codes I20-I25 as the primary cause of death[32]. Where the dates of hospital admission and death coincided the event was considered fatal. Until 1 January 2006, we documented in total 245 incident CHD events, including 211 non-fatal AMI cases and 34 fatal CHD cases.

Statistical analyses

For each participant, we calculated person time from date of enrollment until the first event (non-fatal AMI or fatal CHD), date of emigration (n=693), date of death or censoring date (1 January 2006), whichever occurred first. Quartiles of intake were computed for each fruit and vegetable group and the lowest quartile was used as the reference category. Hazard ratios (HR) for quartiles of fruit and vegetable consumption were estimated using Cox proportional hazards models. The Cox proportional hazards assumption was fulfilled in all models according to the graphical approach and Schoenfeld residuals. To test P for trend of the associations across increasing quartiles of intake, the median values of intake were assigned to each quartile and used as a continuous variable in the Cox model.

Besides an age (continuous) and gender adjusted model, we used a multivariable model that included total energy intake (kcal), smoking status (never, former, current smoker of <10, 10-20, ≥20 cigarettes/d), alcohol intake (never, moderate and high consumption of >1 glass per day in

Table 2.1. Demographic and lifestyle characteristics by quartiles of fruit and vegetable consumption of 20,069 Dutch participants[1]

	Quartiles of fruit and vegetable consumption			
	Q1:[2] **≤241 g/d**	**Q2:** **241-346 g/d**	**Q3:** **346-475 g/d**	**Q4:** **>475 g/d**
Age, y	41.4 (10.7)	41.4 (11.0)	41.6 (11.1)	41.6 (11.5)
Men, %	57.3	45.9	40.2	35.8
Low educational level[3], %	54.4	47.2	43.7	42.4
Current smokers, %	45.9	37.9	32.6	29.8
High alcohol consumers[4], %	35.1	31.9	29.6	27.1
Dietary supplement use, %	25.4	29.7	32.0	36.4
Physically active[5], %	57.8	65.5	70.0	71.8
Body Mass Index, kg/m^2	25.1 (4.0)	24.8 (3.8)	24.7 (3.7)	24.7 (3.8)
Serum total cholesterol, mmol/L	5.3 (1.1)	5.3 (1.0)	5.2 (1.0)	5.2 (1.1)
Systolic blood pressure, mmHg	121 (16)	120 (16)	119 (15)	119 (16)
Family history of AMI[6], %	9.7	8.8	9.2	8.7
Hormone replacement therapy use, %	3.2	4.9	5.5	6.0
Vegetarians, %	1.1	2.7	3.1	5.7
Fish consumers[7], %	18.4	23.0	27.2	31.2

[1] Data are presented as mean (SD) or percentages.

[2] 100 gram of fruit and vegetables equals 1 medium-sized piece of fruit or 1 cup cut-up raw fruit, fruit juice, cooked vegetables, or 2 cups raw leafy vegetables.

[3] Defined as primary school and lower, intermediate general education.

[4] Defined as >1 glass per day in women and >2 glasses per day in men.

[5] Defined as engagement in cycling or sports of ≥4 metabolic equivalents. In sub sample of participants enrolled from 1994 onwards (*n*=15,433).

[6] Defined as occurrence of acute myocardial infarction before age 55 of the father or before age 65 of the mother.

[7] Defined as the highest quartile of fish intake (median: 17 grams per day, i.e. ~1 portion of fish per week).

women and >2 glasses per day in men), educational level (4 categories), dietary supplement use (yes/no), past or present use of hormone replacement therapy (yes/no), family history of AMI before 55 years of the father or before 65 years of the mother (yes/no) and body mass index (BMI, kg/m^2). Additionally, we extended the model with dietary covariates including intake of whole grain foods (g/d), processed meat (g/d) and fish (quartiles). Depending on the exposure variable under investigation, we made mutual adjustments for raw or processed fruit and vegetables. Participants with missing data of one or more lifestyle factors were excluded from the analyses of the second and third model (1.2% of the total population). With regard to participants enrolled after 1994, we evaluated whether physical activity was a potential confounder by adding this as a dummy variable to the multivariable model ('active' being defined as physically active on at least 5 days a week for 30 minutes or more with an intensity of 4 or more metabolic equivalents). Therefore, we calculated HR with and without adding physical activity into the multivariable model. Stratified analyses were

Table 2.2. Dietary intake by quartiles of fruit and vegetable consumption of 20,069 Dutch participants[1]

	Quartiles of fruit and vegetable consumption			
	Q1: ≤241 g/d	**Q2: 241-346 g/d**	**Q3: 346-475 g/d**	**Q4: >475 g/d**
Fruit and vegetables, g/d	185 [144-215]	292 [266-319]	404 [374-436]	589 [521-699]
Total energy intake, Kcal	2198 (651)	2243 (653)	2283 (657)	2363 (697)
Total protein, en%	15	15	15	15
Total fat, en%	37	36	35	34
Saturated fatty acids, en%	15	15	15	14
Monounsaturated fatty acids, en%	14	14	14	13
Polyunsaturated fatty acids, en%	7	7	7	7
Trans fatty acids, g/d	4 (2)	4 (2)	4 (2)	4 (2)
Total carbohydrates, en%	43	45	46	48
Mono- disaccharides, g/d	106 (46)	115 (44)	126 (44)	149 (50)
Polysaccharides, g/d	133 (46)	135 (46)	134 (47)	133 (49)
Dietary fiber, g/d	21 (6)	24 (6)	26 (7)	29 (7)
Fruit fiber, g/d	1 (1)	2 (1)	4 (1)	6 (3)
Vegetable fiber, g/d	3 (1)	3 (1)	4 (1)	4 (2)
Dietary fiber from other sources than fruit and vegetables, g/d	18 (6)	18 (6)	19 (6)	18 (6)
Vitamin C, mg/d	62 (16)	88 (15)	113 (19)	164 (42)
Potassium, g/d	3.5 (0.9)	3.7 (0.9)	4.0 (0.9)	4.4 (1.0)

[1] Data are presented as mean (SD) or percentages.

performed for major cardiovascular risk factors. We examined potential effect modification by age (<50 vs ≥50 years), gender and smoking status (never vs current) visually as well as by entering cross-product terms into the multivariable model. The log likelihood ratio test was used to compare the models with and without the cross-product terms. *P* values <0.05 (two-tailed) were considered statistically significant. Analyses were performed using the Statistical Analysis System (version 9.1; SAS Institute, Inc. Cary, NC, USA).

Results

Participants with a high intake of fruit and vegetables were more often women, had a higher educational level, were less likely to smoke, used alcohol less often, were more likely to be physically active, used dietary supplements more often and were more often vegetarian compared to participants with a low intake of fruit and vegetables (**Table 2.1**). Age and BMI did not differ among quartiles. Participants with a high intake of fruit and vegetables had a higher intake of energy, fruit fiber, vitamin C and potassium compared to participants with a low intake fruit and vegetables (**Table 2.2**).

The average daily fruit and vegetable intake in our cohort was 378 g/d for the total study population, of which 188 g/d was consumed as raw and 190 g/d as processed fruit and vegetables. The total fruit and vegetable intake was more strongly correlated with raw (r=0.80) than with processed fruit and vegetables (r=0.69). Raw fruit and vegetables were only weakly related to processed fruit and vegetables (r=0.20). The raw fruit and vegetable intake mainly comprised raw fruit, with apples (22% of raw fruit intake) and oranges (18%) as major contributors. Raw vegetables were mainly cucumber (23% of raw vegetable intake) and tomatoes (18%). Processed fruit and vegetables mainly comprised processed fruit, of which citrus juice (24%) and apple juice (22%) were the most important contributors. Processed vegetables were mainly cabbages (24%) and French beans (14%).

The median follow-up time was 10.5 years (interquartile range: 9.2-11.8 years). After adjustment for potential confounders, high consumption of total fruit and vegetables was inversely associated with CHD incidence (>475 g/d; HR: 0.66; 95% CI: 0.45-0.99; **Table 2.3**) compared to participants with a low intake (≤241 g/d). Fruit intake (>328 vs ≤125 g/d; HR: 0.85; 95% CI: 0.58-1.27) as well as vegetable intake (>162 vs ≤96 g/d; HR: 0.88; 95% CI: 0.60-1.30) were inversely related with CHD incidence, although not statistically significant (data not shown). Compared to participants with a low intake, a borderline significant inverse association was observed for a high raw fruit and vegetable intake with CHD incidence (>262 vs ≤92 g/d; HR: 0.70; 95% CI: 0.47-1.04) as well as for a high processed fruit and vegetable intake (>233 vs ≤113 g/d; HR: 0.79; 95% CI: 0.54-1.16).

The associations between total fruit and vegetable intake and CHD did not differ significantly between strata of gender, age, smoking status, educational level, alcohol consumption and dietary supplement use (**Table 2.4**). Correspondingly, we found no evidence for effect modification relative to age, gender or smoking status between raw, processed or total fruit and vegetable intake with CHD incidence. Furthermore, we evaluated whether physical activity was a potential confounder for total fruit and vegetable intake with CHD incidence within participants enrolled from 1994 onwards (n=15,433). HRs for CHD incidence did not change, i.e. 0.51 (95% CI: 0.31-0.84) without physical activity in the model and 0.51 (95% CI: 0.31-0.85) with physical activity included in the model for top vs bottom quartiles of total fruit and vegetable intake.

Discussion

In the present study, an inverse association of total fruit and vegetable consumption with CHD incidence was observed. This inverse association was present for both raw and processed fruit and vegetables, although these findings did not attain statistical significance.

We had almost complete follow-up for cause-specific mortality as well as for non-fatal events obtained from the hospital discharge register. It has been shown in a validation study that the hospitalized AMI cases corresponded in 84% with AMI cases registered in the hospital discharge register[30]. We may have missed mild AMI cases that were not hospitalized. However, we expect this to be random and not related to fruit and vegetable intake. Therefore, it is unlikely that this has influenced the relation of fruit and vegetable consumption with CHD incidence.

Table 2.3. Hazard ratios and 95% confidence intervals of CHD incidence by quartiles of fruit and vegetable consumption of 20,069 Dutch participants[1]

	Quartiles of fruit and vegetable consumption				***P* for**
	Q1[2]	**Q2**	**Q3**	**Q4**	**trend**
Total fruit and vegetables					
Median intake, g/d	185	292	404	589	
Cases, *n*	88	62	51	44	
Model 1	1.00	0.75 (0.54-1.04)	0.64 (0.46-0.91)	0.58 (0.40-0.84)	0.003
Model 2	1.00	0.85 (0.61-1.19)	0.76 (0.53-1.09)	0.64 (0.43-0.95)	0.02
Model 3	1.00	0.87 (0.62-1.21)	0.79 (0.55-1.13)	0.66 (0.45-0.99)	0.04
Raw fruits and vegetables[3]					
Median intake, g/d	56	127	197	337	
Cases, *n*	81	67	54	43	
Model 1	1.00	0.81 (0.59-1.12)	0.67 (0.48-0.95)	0.52 (0.36-0.76)	<0.001
Model 2	1.00	0.87 (0.62-1.22)	0.81 (0.56-1.16)	0.66 (0.45-0.98)	0.04
Model 3	1.00	0.89 (0.64-1.25)	0.85 (0.59-1.22)	0.70 (0.47-1.04)	0.08
Processed fruits and vegetables[4]					
Median intake, g/d	87	137	196	301	
Cases, *n*	81	70	47	47	
Model 1	1.00	0.99 (0.72-1.36)	0.72 (0.50-1.03)	0.79 (0.55-1.14)	0.10
Model 2	1.00	1.01 (0.73-1.40)	0.77 (0.53-1.11)	0.76 (0.52-1.11)	0.08
Model 3	1.00	1.02 (0.74-1.42)	0.79 (0.55-1.14)	0.79 (0.54-1.16)	0.14

[1] Hazard ratios (95% CIs) were obtained from Cox proportional hazards models. Model 1 was adjusted for age and gender (*n*=20,069) Model 2 was the same as model 1 with additional adjustments for energy intake, alcohol intake, smoking status, educational level, dietary supplement use, use of hormone replacement therapy, family history of AMI before 60, BMI (*n*=19,819) Model 3 was adjusted as model 2 with additional adjustments for intake of fish, whole grain foods and processed meat (*n*=19,819).

[2] Reference group.

[3] Additionally adjusted for processed fruit and vegetable intake.

[4] Additionally adjusted for raw fruit and vegetable intake.

In the present study, participants with a high intake of fruit and vegetables were more often found to be women who had in general a healthier lifestyle and dietary pattern than men. We observed that confounding was mostly due to age and gender, since additional adjustment for lifestyle and dietary factors did not significantly change the observed associations. However, since fruit and vegetable intake is part of a healthy diet and lifestyle, we cannot rule out residual confounding completely. Furthermore, information was not available on incident diabetes, hypertension or hypercholesterolemia. These participants may have changed their diet intentionally, which may have attenuated the association between fruit and vegetable intake and risk of CHD.

Table 2.4. Hazard ratios and 95% confidence intervals of CHD incidence by high versus low fruit and vegetable intake among 20,069 participants, stratified by major risk factors[1]

	Total fruit and vegetable intake		
Risk factor	***n*/cases**	**Low**	**High**
Gender			
Men	8,988/171	1.00	0.82 (0.59-1.14)
Women	11,061/74	1.00	0.72 (0.44-1.18)
Age			
< 50 y	14,655/104	1.00	0.86 (0.56-1.31)
≥ 50 y	5,414/141	1.00	0.76 (0.53-1.09)
Cigarette smoking			
No	12,701/111	1.00	0.74 (0.50-1.11)
Yes	7,325/133	1.00	0.78 (0.53-1.12)
Educational level			
Low	9,371/148	1.00	0.81 (0.56-1.15)
High	10,061/93	1.00	0.79 (0.51-1.21)
Alcohol consumption			
Never or low	13,865/165	1.00	0.85 (0.61-1.18)
Higher	6,204/80	1.00	0.63 (0.38-1.03)
Dietary supplement use			
No	13,820/180	1.00	0.84 (0.61-1.15)
Yes	6,171/58	1.00	0.63 (0.36-1.11)

[1] Hazard ratios (95% CIs) were obtained from Cox proportional hazards models adjusted for age, gender, energy intake, alcohol intake, smoking status, educational level, dietary supplement use, use of hormone replacement therapy, family history of AMI before 60, BMI, and for intake of fish, whole grain foods and processed meat.

Another limitation of our study was the use of a self-reported FFQ that was assessed at baseline only. Measurement error in self-reported data and changes in dietary habits during follow-up are inevitable and may result in exposure misclassification and attenuation of the results. The weaker associations found for raw and processed fruit and vegetables as well as for subgroup analyses may be a result of the narrower range of intake and limited number of participants. Furthermore, salt is often added to cooked vegetables. Because the FFQ is not a reliable method to assess salt intake, we could not adjust for salt intake and this may have attenuated the results.

We observed an inverse association of total fruit and vegetable intake with CHD incidence, as well as for fruit and vegetables separately. These results are in agreement with meta-analysis data[1,2]. On the basis of 12 prospective cohort studies, He *et al.* observed that participants consuming more than 391 g/d of fruit and vegetables had a 17% lower risk of non-fatal and fatal CHD incidence than those who consumed less than 235 g/d[2]. This corresponds to an 11% lower risk per 100 g/d increase, whereas we observed a 6% lower CHD risk. An earlier meta-analysis of 6 cohort studies showed a weaker association, i.e. a 4% lower risk of CHD incidence for each additional portion fruit or vegetables of 106 grams[1].

In the present study, we found that raw fruit and vegetable consumption was inversely related with CHD incidence. To the best of our knowledge, this is the first prospective cohort study that examined raw fruit and vegetables using an integral approach. However, previous prospective cohort studies have provided partial information on raw fruit and vegetables. With regard to raw vegetables, two prospective cohort studies found inverse associations of salads with fatal CVD[33,34] and one found no association of higher frequencies of raw vegetable intake with CHD incidence[35]. As concerns raw fruit, prospective cohort studies observed inverse associations of citrus fruit intake and CHD[10,35] and CVD risk[11]. Raw fruit and vegetables are rich sources of nutrients and bioactive phytochemicals that may have beneficial effects on CHD incidence. It has been demonstrated that a diet rich in fruits and vegetables favorably affects blood pressure levels[36]. These effects may be explained by dietary fiber and potassium, which have been shown to reduce blood pressure levels[37,38]. Fiber from fruit has been consistently associated with a lower risk of CHD[39], probably through the reduced serum total and LDL cholesterol levels[40,41]. Flavonoids may also lower CHD risk. In a meta-analysis comprising 7 cohort studies dietary flavonoids lowered CHD mortality by 20%[42].

It is well-known that processing improves the bioavailability and bioaccessibility of bioactive compounds in fruits and vegetables. Heat treatment enhances the bioavailability and strengthens the beneficial health effects of lycopene[15,16] as well as carotenes[14,17] and folate[18,19]. Lycopene, for example, the main carotenoid in tomatoes, accounts for ~50% of the carotenoids in human blood and is suggested to protect against CVD. Participants with high lycopene concentrations in adipose tissue had a lower risk of AMI[43] and Paran *et al.* reported beneficial effects of tomato extracts on blood pressure in moderate hypertensive participants with uncontrolled hypertension[44].

Processed fruit and vegetables were also inversely related with CHD incidence in the present study. Processed fruits were mainly consumed as fruit juices, which have a lower dietary fiber content but may be good sources of phytochemicals[9]. Moreover, their liquid state affects volume and chewing and may result in decreased satiety and increased energy intake[45]. However, the positive effect of processed fruit was not confirmed in previous prospective cohort studies on citrus fruit juice and CHD[10] and CVD risk[11]. Processed vegetables comprised mainly cooked vegetables. Cooked vegetables also have a generally lower dietary fiber content than raw ones, vitamin C can be lost in cooking water[46] and salt is often added. One study found no association between higher frequencies of baked vegetable intake and CHD incidence[35]. Although fruit juices and cooked vegetables have lost intact cell walls and insoluble fiber[47], these findings suggest that improved bioavailability of bioactive compounds, e.g. flavonoids and carotenoids, may have contributed to the lower risk of CHD incidence.

In conclusion, the results of the present study suggest that a high consumption fruit and vegetables, whether consumed raw or processed, may protect against CHD incidence. Processing of fruit and vegetables affected the observed association with CHD to a small extent.

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Conflicts of interest

The authors declare that there is no conflict of interest related to any part of the study. The sponsors did not participate in the design or conduct of the study; in the collection, analyses, or interpretation of the data; or in the preparation, review, or approval of the manuscript.

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Chapter 3

Raw and processed fruit and vegetable consumption and 10-year stroke incidence in a population-based cohort study in the Netherlands

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Abstract

Background/objectives: Prospective cohort studies have shown that high fruit and vegetable consumption is related to a lower risk of stroke. Whether food processing affects this association is unknown. We evaluated the associations of raw and processed fruit and vegetable consumption independently from each other with 10-year stroke incidence and stroke subtypes in a prospective population-based cohort study in the Netherlands.

Subjects/methods: We used data of 20,069 men and women aged 20 to 65 years and free of cardiovascular diseases at baseline who were enrolled from 1993 to 1997. Diet was assessed using a validated 178-item food frequency questionnaire. Hazard ratios (HR) were calculated for total, ischemic and hemorrhagic stroke incidence using multivariable Cox proportional hazards models.

Results: During a mean follow-up time of 10.3 years, 233 incident stroke cases were documented. Total and processed fruit and vegetable intake were not related to incident stroke. Total stroke incidence was 30% lower for participants with a high intake of raw fruit and vegetables (Q4: >262 g/d; HR: 0.70; 95% CI: 0.47-1.03) compared with those with a low intake (Q1: ≤92 g/d) and the trend was borderline significant (*P* for trend = 0.07). Raw vegetable intake was significantly inversely associated with ischemic stroke (>27 vs ≤27 g/d; HR: 0.50; 95% CI: 0.34-0.73) and raw fruit borderline significantly with hemorrhagic stroke (>120 vs ≤120 g/d HR: 0.53; 95% CI: 0.28-1.01).

Conclusion: High intake of raw fruit and vegetables may protect against stroke. No association was found between processed fruit and vegetable consumption and incident stroke.

Introduction

Two meta-analyses of prospective cohort studies concluded that fruit and vegetable intake is associated with a lower risk of stroke[1,2]. These findings provided evidence for the 2005 Dietary Guidelines for Americans, which recommend a daily consumption of 2 cups of fruit and 2.5 cups of vegetables for a reference 2,000-calorie intake[3]. According to these guidelines, fruit and vegetables can be consumed as equivalent amounts of raw or cooked, whole, cut-up, mashed or as 100% fruit juice. In addition, whole fruits (fresh, frozen, canned or dried) rather than fruit juice is advised[3], but recommendations whether fruit or vegetables should be eaten as raw or processed were not specified.

Most prospective cohort studies included in both meta-analyses of Dauchet *et al.* or He *et al.* categorized fruit and vegetables together and no distinction was made between raw and processed[4-9] or this was not reported[10,11]. Four studies found an inverse relation between citrus fruit and stroke incidence[5,6,8,12], of which two were statistically significant[8,12]. Two studies observed that high leafy vegetable intake was inversely associated with ischemic stroke[6,8]. Joshipura *et al.* showed that fruit juice was inversely related to ischemic stroke[6], while Johnsen *et al.* observed no association between fruit and vegetable juice and ischemic stroke[8]. To the best of our knowledge, no prospective studies have yet investigated raw or processed fruit and vegetable consumption separately in relation to stroke incidence.

Raw fruit and vegetables are rich sources of fiber, potassium, folate, vitamins, minerals, flavonoids, lignans and other bioactive phytochemicals and are low in energy density[13]. Processing of fruit and vegetables alters their structure and changes their chemical composition, nutritional value, digestibility and bioavailability of bioactive compounds. Although processed fruit and vegetables are lower in fiber compared with their raw counterparts, they may be a good source of phytochemicals[14,15]. During processing water-soluble and heat-sensitive bioactive compounds can be lost, such as carotenoids, vitamins and minerals, but the bioavailability may improve[16,17]. Therefore, the objective of the present study was to evaluate the associations of the intake of raw and processed fruit and vegetables independently from each other with 10-year stroke incidence and stroke subtypes in a population-based follow-up study in the Netherlands.

Methods

Population

Our analyses were conducted in a Dutch population-based cohort study; the Monitoring Project on Risk Factors and Chronic Diseases in the Netherlands (MORGEN Study) carried out from 1993 to 1997[18]. The Medical Ethics Committee of the Netherlands Organization for Applied Scientific Research approved the study protocol.

Of 22,654 participants, we excluded respondents without informed consent for vital status follow-up (*n*=701), with incomplete dietary assessment (*n*=72), with reported total energy intake <500 or >4500 kcal per day for women or <800 or >5000 kcal per day for men (*n*=97), with prevalent myocardial infarction or stroke (*n*=442) and self-reported diabetes and those using lipid-lowering or anti-hypertensive drugs (*n*=1,273). This resulted in a study population of 20,069 participants, 8,988 men and 11,081 women.

Dietary assessment
Information on habitual food consumption of 178 food items, covering the previous year, was collected using a validated, self-administered semi-quantitative food frequency questionnaire (FFQ) developed for the Dutch cohorts of the European Prospective Investigation Into Cancer (EPIC) study[19]. Participants indicated their usual consumption of food items as absolute frequencies in times per day, per week, per month, per year or as never. For several food items, additional questions were included about the consumption frequency of different sub-items or about preparation method using the following categories; always/mostly, often, sometimes and seldom/never. For 21 food items, mainly vegetables, colored photographs were used to estimate portion sizes. Frequencies per day were multiplied with standard household measures, natural units or indicated portion sizes to obtain grams per day for each food item. The Dutch food composition database of 1996 was used to calculate values for energy and nutrient intakes[20]. Intake of carotenoids was calculated using the Dutch food composition database of 2001[21].

Specific information on preparation methods was collected to distinguish whether fruit or vegetables were consumed as raw or cooked, or as juice or sauce[22]. The FFQ comprised 35 fruit and vegetable items in total, including 9 raw fruit items, 7 raw vegetable items, 13 cooked vegetable items, 2 vegetable juices/sauces and 4 fruit juices/sauces. Processed fruits comprised fruit juices and apple sauce that were mainly consumed as industrially produce from concentrates. Processed vegetables comprised home-cooked vegetables including canned and frozen vegetables and tomato sauce. We did not consider potatoes and legumes, except French beans, as vegetables, because the nutritional value of these food items differs significantly from that of vegetables[20]. Fruit and vegetable consumption during winter and summer was assessed separately to take seasonal differences of intake into account.

In men, reproducibility of the FFQ after 12 months expressed as Spearman's correlation coefficients was 0.67 for raw vegetables and 0.69 for cooked vegetables[19] was tested against 12 repeated 24-h recalls to estimate the reliability of the dietary assessment. Spearman's rank correlation coefficients varied between 0.32 and 0.49 for raw vegetables, 0.21 and 0.41 for cooked vegetables and between 0.56 and 0.68 for total fruit intake.

Risk factors
The baseline measurements were previously described by Verschuren *et al.*[18]. Body weight, height and blood pressure were measured by trained research assistants during a physical examination at a municipal health service site. Non-fasting venous blood samples were collected and total cholesterol concentrations were determined using an enzymatic method. Information on cigarette smoking, educational level, physical activity, use of anti-hypertensive and lipid lowering drugs, ever use of hormone replacement therapy and both the participants' and their parents' history of previous myocardial infarction were obtained by a self-administered questionnaire. Dietary supplement use (yes or no) and alcohol intake were obtained from the FFQ. Alcohol intake was expressed as the number of glasses of beer, wine, port wines and strong liquor consumed per week. From

1994 onwards, type, frequency and duration of physical activity were assessed using a validated questionnaire, developed for the EPIC-Study including questions on leisure time and occupational physical activity[24].

Ascertainment of fatal and non-fatal events

After enrollment, information on the participants' vital status up to 1 January 2006 was monitored using the municipal population register. For participants who died, information on the primary cause of death was obtained from Statistics Netherlands. The hospital discharge register provided clinically diagnosed stroke admissions. Stroke incidence was defined as the first non-fatal or fatal stroke event, not preceded by any other non-fatal stroke event and included codes G45, I60-I67 and I69 of the tenth revision of the International Classification of Diseases (ICD-10)[25]. Ischemic stroke included cerebral infarction (ICD-10: I63) and Transient cerebral Ischemic Attack (ICD-10: G45). Hemorrhagic stroke included ICD-10 codes I60-I62. Other and unspecified strokes were defined as ICD-10 codes I64-I67 and I69. For hospital admission data, corresponding ICD-9 codes were used[26]. If the dates of hospital admission and death coincided, the event was considered fatal.

Statistical analyses

For each participant, we calculated follow-up time from date of enrollment until the first event (non-fatal or fatal stroke), date of emigration (n=693), date of death or censoring date (1 January 2006), whichever occurred first. Quartiles of intake were computed for each fruit and vegetable group. Hazard ratios (HR) and 95% confidence intervals (95% CI) for quartiles of fruit and vegetable consumption compared with the lowest quartile were estimated using Cox proportional hazards models. The Cox proportional hazards assumption was fulfilled in all models according to the graphical approach and Schoenfeld residuals. To test the P for trend of the associations across increasing categories of intake, the median values of intake were assigned to each quartile and used as a continuous variable in the Cox model. To analyze subtypes of stroke, HR and 95% CI for high vs low fruit and vegetable intake according to the median and for each 50 gram increase were calculated for power reasons. The correlation between the raw and processed fruit and vegetables was assessed with the Spearman's rank correlation test.

Besides an age (continuous) and gender adjusted model, we used a multivariable model that included total energy intake (kcal), smoking status (never, former, current smoker of <10, 10-20, ≥20 cigarettes per day), alcohol intake (never, moderate, high), educational level (4 categories), dietary supplement use (yes or no), ever use of hormone replacement therapy (yes or no), family history of myocardial infarction before age of 55 of the father or before age 65 of the mother (yes or no) and body mass index (BMI, continuous). Additionally, we extended the model with dietary covariates including intake of whole grain foods (g/d), processed meat (g/d) and fish (quartiles). Depending of the exposure variable under investigation, we further adjusted for raw or processed fruit and vegetable intake. Within participants enrolled after 1994, we evaluated whether physical activity was a potential confounder by comparing hazard ratios with and without adding physical activity to the multivariable model.

We examined potential effect modification by age (<50 vs ≥50 years), gender and smoking status (never vs current) by entering cross-product terms into the multivariable models. Log likelihood ratio

Table 3.1. Demographic and lifestyle characteristics by quartiles of raw or processed fruit and vegetable intake of 20,069 Dutch participants[1]

	Raw fruit and vegetable consumption				**Processed fruit and vegetable consumption**			
	Q1: ≤92 g/d	**Q2: 92-150 g/d**	**Q3: 150-262 g/d**	**Q4: >262 g/d**	**Q1: ≤113 g/d**	**Q2: 113-165 g/d**	**Q3: 165-233 g/d**	**Q4: >233 g/d**
Age, y	40.1 (11.0)	41.2 (11.1)	41.6 (11.0)	43.1 (11.0)	43.0 (10.6)	41.8 (10.7)	41.1 (11.2)	40.1 (11.6)
Men, %	57.6	46.0	39.7	35.8	51.4	47.0	41.8	38.9
Low educational level[2], %	54.3	48.1	42.9	42.4	52.1	47.5	43.9	44.2
Current smokers, %	47.9	38.0	32.4	28.0	39.7	36.7	35.5	34.5
High alcohol consumers[3], %	34.5	29.9	31.1	28.1	33.8	31.4	31.0	27.4
Dietary supplement use, %	25.9	29.5	32.3	35.8	26.7	29.7	32.4	34.7
Physically active[4], %	56.7	64.6	70.9	73.1	63.1	66.2	67.5	68.5
Body Mass Index, kg/m^2	25.0 (4.0)	24.8 (3.8)	24.7 (3.7)	24.9 (3.7)	25.2 (3.9)	24.8 (3.8)	24.7 (3.7)	24.7 (3.9)
Serum total cholesterol, mmol/L	5.3 (1.1)	5.2 (1.1)	5.2 (1.0)	5.3 (1.1)	5.4 (1.1)	5.3 (1.0)	5.2 (1.0)	5.2 (1.1)
Systolic blood pressure, mmHg	121 (16)	120 (15)	119 (16)	120 (15)	122 (16)	120 (115)	119 (15)	119 (15)
Family history of AMI[5], %	9.2	8.9	9.4	8.8	9.4	9.6	8.6	8.7
Hormone replacement therapy use, %	3.1	4.0	5.4	7.1	4.5	4.9	5.2	4.9
Vegetarians, %	1.2	2.2	3.4	5.8	2.3	3.0	3.4	3.9
Fish consumers[6], %	19	23	27	31	21	23	27	30

[1] Data are presented as mean (SD) or percentages.

[2] Defined as primary school and lower, intermediate general education.

[3] Defined as >1 glass per day in women and >2 glasses per day in men.

[4] Defined as engagement in cycling or sports of ≥4 metabolic equivalents. In sub sample of participants enrolled from 1994 onwards (n=15,433).

[5] Defined as occurrence of AMI before age 55 of the father or before age 65 of the mother.

[6] Defined as the highest quartile of fish intake (median: 17 grams per day, i.e. ~1 portion of fish per week).

testing showed no significant interactions between these factors and fruit and vegetable groups in relation to stroke. *P* values <0.05 (two-tailed) were considered statistically significant. Analyses were performed using Statistical Analysis System (version 9.1; SAS Institute, INc. Cary, NC, USA).

Results

Average daily fruit and vegetable consumption was 378 g/d for the total study population, of which 188 g/d was consumed as raw fruit and vegetables and 190 g/d as processed fruit and vegetables. Raw fruit and vegetable intake mainly comprised raw fruit, with apples (22% of raw fruit intake) and citrus fruit (25%) as major contributors. Raw vegetables were mainly cucumber (23% of raw vegetable intake) and tomatoes (18%). Processed fruit and vegetables mainly comprised processed fruit, of which citrus juice (49% of processed fruit intake) and apple juice (22%) were the most important contributors. Processed vegetables were mainly cabbages (24%) and French beans (14%). Raw fruit and vegetable intake was weakly related to processed fruit and vegetable intake (Spearman's *r*=0.20).

High raw fruit and vegetable consumers were more often women, tended to be older, had a higher educational level, were less likely to smoke, more likely to be physically active, used more often dietary supplements and were more often vegetarians compared with low raw fruit and vegetable consumers (**Table 3.1**). High processed fruit and vegetable consumers also showed a healthier lifestyle, but were younger than low processed fruit and vegetable consumers. High raw fruit and vegetable consumers had a higher intake of fruit fiber, vitamin C and potassium compared with low raw fruit and vegetable consumers, while energy intake and dietary fiber intake from other sources did not differ (**Table 3.2**). High processed fruit and vegetable consumers had higher intake of energy, dietary fiber from other sources, vitamin C and potassium compared with low processed fruit and vegetable consumers.

After an average follow-up period of 10.3 years, 19 fatal and 226 non-fatal stroke cases had occurred, of which 12 fatal cases had a non-fatal stroke previously. In all, 233 first-ever incident strokes remained for the present analysis (139 ischemic, 45 hemorrhagic and 49 other or unspecified strokes). After multivariable adjustment, total fruit and vegetable intake (>475 vs ≤241 g/d; HR: 0.97; 95% CI: 0.66-1.44, **Table 3.3**) and processed fruit and vegetable intake were not related to total stroke incidence (>234 vs ≤113 g/d; HR: 1.20; 95% CI: 0.81-1.76). High raw fruit and vegetable consumption (>262 g/d) was inversely related to stroke incidence (HR: 0.70; 95% CI: 0.47-1.03) compared with participants with a low intake (≤92 g/d) and was borderline significant (*P* for trend = 0.07). Raw vegetable intake was inversely associated with stroke incidence (>48 vs ≤14 g/d; HR: 0.53; 95% CI: 0.36-0.80), but raw fruit intake was not associated (>234 vs ≤65 g/d; HR: 1.01; 95% CI: 0.68-1.50).

To analyze stroke subtypes, we divided fruit and vegetable consumption according to the median intake. Compared with raw fruit and vegetable intake below the median, raw fruit and vegetable intake above the median was borderline significantly associated with ischemic stroke incidence (>150 vs ≤150 g/d; HR: 0.69; 95% CI: 0.48-1.00), but not with hemorrhagic stroke (**Table 3.4**). Raw vegetable intake above the median was inversely associated with ischemic stroke (>27 vs ≤27 g/d;

Table 3.2. Nutrient intake by quartiles of raw or processed fruit and vegetable intake of 20,069 Dutch participants[1]

	Raw fruit and vegetable consumption				Processed fruit and vegetable consumption			
	Q1: ≤92 g/d	**Q2: 92-150 g/d**	**Q3: 150-262 g/d**	**Q4: >262 g/d**	**Q1: ≤113 g/d**	**Q2: 113-165 g/d**	**Q3: 165-233 g/d**	**Q4: >233 g/d**
Total energy intake, Kcal	2,282 (673)	2,273 (660)	2,265 (662)	2,266 (674)	2,115 (636)	2,254 (634)	2,297 (662)	2,421 (700)
Total protein, en%	14	15	15	15	15	15	15	15
Total fat, en%	37	36	36	34	36	36	36	35
Saturated fatty acids, en%	15	15	15	14	15	15	15	14
Polyunsaturated fatty acids, en%	7	7	7	7	7	7	7	7
Trans fatty acids, g/d	4 (2)	4 (2)	4 (2)	3 (2)	4 (2)	4 (2)	4 (2)	4 (2)
Total carbohydrates, en%	44	45	45	47	44	45	46	47
Mono- disaccharides, g/d	114 (51)	119 (47)	124 (46)	139 (48)	106 (43)	117 (45)	126 (46)	147 (52)
Dietary fiber, g/d	22 (7)	24 (7)	26 (7)	29 (7)	22 (7)	25 (7)	26 (7)	27 (7)
Fruit fiber, g/d	1 (1)	2 (1)	4 (1)	7 (3)	3 (2)	3 (2)	4 (3)	5 (3)
Vegetable fiber, g/d	3 (1)	3 (1)	4 (1)	4 (2)	2 (1)	3(1)	4 (1)	4 (2)
Vitamin C, mg/d	67 (24)	90 (23)	112 (27)	158 (44)	79 (34)	95 (34)	111 (38)	142 (48)
Potassium, g/d	3.6 (1.0)	3.8 (0.9)	3.9 (0.9)	4.2 (1.0)	3.5 (0.9)	3.8 (0.9)	3.9 (0.9)	4.3 (1.0)
Carotenoids, mg/d	8.2 (3.6)	9.2 (3.6)	10.0 (4.2)	10.6 (4.2)	6.7 (2.4)	9.0 (2.7)	10.1 (3.5)	12.2 (4.8)
Flavonoids, mg/d	39.7 (39.3)	50.7 (39.6)	58.5 (42.3)	69.8 (45.5)	47.2 (42.8)	54.4 (44.3)	56.7 (41.8)	60.5 (42.7)

[1] Data are presented as mean (SD) or percentages.

HR: 0.50; 95% CI: 0.34-0.73), but not with hemorrhagic stroke. Raw fruit intake was not related to ischemic stroke, but was borderline significantly related to hemorrhagic stroke (>120 vs ≤120 g/d; HR: 0.53; 95% CI: 0.28-1.01). Processed fruit and vegetable intake was neither associated with ischemic nor with hemorrhagic stroke. We found similar results for cerebral infarction compared with ischemic stroke including Transient Ischemic Attack (data not shown). Furthermore, we also found similar results as we repeated the analysis in those with complete data compared with the presented analysis.

We evaluated whether physical activity was a potential confounder for raw fruit and vegetable intake with incident stroke within participants enrolled from 1994 onwards (*n*=15,433). HRs for incident stroke did not change, that is, 0.69 (95% CI: 0.43-1.11) without and 0.67 (95% CI: 0.42-1.10) including physical activity in the model for highest vs lowest quartiles of raw fruit and vegetable intake.

Discussion

The present study showed that raw fruit and vegetable consumption was inversely related to total stroke incidence. This inverse relationship was due to the inverse association between raw vegetables and ischemic stroke and borderline significant association between raw fruit and hemorrhagic stroke. Processed and total fruit and vegetable consumption were not related to incident stroke.

We had almost complete cause-specific mortality follow-up. With respect to non-fatal events, it has been shown on the national level that data from the Dutch hospital discharge register can be uniquely matched to an individual for at least 88% of the hospital admissions[27]. In the Netherlands, brain imaging (computed tomography or magnetic resonance imaging) is used to identify stroke and its subtypes in 98% of admitted patients[28]. This is in accord with the Dutch guideline for the diagnosis of stroke subtypes[29]. In the present study, 61% of all non-fatal strokes including Transient Ischemic Attack was ischemic, 18% was hemorrhagic and 21% was other or unspecified. Although misclassification is inevitable, we assume that based on the diagnostic procedures used in Dutch hospitals, misclassification of stroke and its subtypes was limited.

High raw and processed fruit and vegetable consumers were more often women who have as shown in the baseline characteristics a healthier lifestyle and dietary pattern than men. In the multivariable model, we were able to adjust for most relevant known potential confounders. However, as fruit and vegetable intake is part of a healthy diet and lifestyle, which involves many different factors, residual confounding can never be ruled out completely. Furthermore, information was not available on incident diabetes, hypertension or hypercholesterolemia. These participants may have changed their diet intentionally, which may have attenuated the association between fruit and vegetable intake and the risk of stroke. Additionally, we used a self-reported FFQ at baseline only. Measurement error in self-reported data and changes in dietary habits during follow-up are inevitable and may result in exposure misclassification and attenuation of the results.

In the present study, raw vegetables were inversely related to ischemic stroke and raw fruit with hemorrhagic stroke. The number of stroke cases, especially of hemorrhagic stroke, was rather small

Table 3.3. Hazard ratios and 95% confidence intervals of total stroke incidence by quartiles of fruit and vegetable intake of 20,069 Dutch participants[1]

	Quartiles of intake				*P* for trend
	Q1[2]	Q2	Q3	Q4	
Raw fruits and vegetables[3]					
Median intake, g/d	56	127	197	337	
Cases, *n*	74	61	49	49	
Model 1	1.00	0.75 (0.54-1.06)	0.59 (0.41-0.85)	0.55 (0.38-0.80)	0.001
Model 2	1.00	0.82 (0.58-1.16)	0.69 (0.47-1.00)	0.69 (0.47-1.00)	0.03
Model 3	1.00	0.83 (0.59-1.18)	0.72 (0.49-1.05)	0.70 (0.47-1.03)	0.07
Raw fruits[4]					
Median intake, g/d	34	94	154	293	
Cases, *n*	61	64	51	57	
Model 1	1.00	0.98 (0.69-1.39)	0.72 (0.50-1.05)	0.75 (0.52-1.08)	0.07
Model 2	1.00	1.13 (0.79-1.62)	0.85 (0.57-1.25)	0.93 (0.63-1.37)	0.47
Model 3	1.00	1.17 (0.82-1.69)	0.89 (0.60-1.32)	1.01 (0.68-1.50)	0.75
Raw vegetables[5]					
Median intake, g/d	8	20	36	66	
Cases, *n*	86	67	41	39	
Model 1	1.00	0.81 (0.59-1.11)	0.51 (0.35-0.74)	0.51 (0.35-0.75)	<0.001
Model 2	1.00	0.82 (0.59-1.14)	0.54 (0.37-0.79)	0.51 (0.34-0.76)	<0.001
Model 3	1.00	0.84 (0.61-1.16)	0.56 (0.38-0.82)	0.53 (0.36-0.80)	<0.001
Processed fruits and vegetables[6]					
Median intake, g/d	86	137	196	301	
Cases, *n*	64	52	63	54	
Model 1	1.00	0.89 (0.61-1.28)	1.12 (0.79-1.58)	1.03 (0.71-1.48)	0.65
Model 2	1.00	0.96 (0.66-1.40)	1.23 (0.86-1.76)	1.11 (0.76-1.63)	0.41
Model 3	1.00	0.97 (0.67-1.41)	1.30 (0.91-1.86)	1.20 (0.81-1.76)	0.22
Processed fruits[7]					
Median intake, g/d	8	39	95	176	
Cases, *n*	70	55	57	51	
Model 1	1.00	0.90 (0.63-1.28)	0.97 (0.68-1.37)	0.92 (0.64-1.33)	0.79
Model 2	1.00	0.97 (0.68-1.39)	1.07 (0.75-1.53)	1.03 (0.71-1.50)	0.77
Model 3	1.00	0.98 (0.69-1.41)	1.12 (0.78-1.60)	1.10 (0.75-1.60)	0.53

Table 3.3. Continued

	Quartiles of intake				*P* for trend
	Q1[2]	Q2	Q3	Q4	
Processed vegetables[8]					
Median intake, g/d	55	82	106	145	
Cases, *n*	60	52	59	62	
Model 1	1.00	0.89 (0.61-1.29)	1.03 (0.72-1.48)	1.05 (0.74-1.50)	0.61
Model 2	1.00	0.92 (0.63-1.34)	1.08 (0.75-1.56)	1.07 (0.75-1.55)	0.55
Model 3	1.00	0.92 (0.63-1.34)	1.10 (0.76-1.58)	1.14 (0.79-1.65)	0.35
Total fruit and vegetables					
Median intake, g/d	185	292	404	589	
Cases, *n*	67	61	53	52	
Model 1	1.00	0.90 (0.64-1.28)	0.78 (0.54-1.13)	0.77 (0.53-1.11)	0.13
Model 2	1.00	0.98 (0.69-1.40)	0.89 (0.61-1.30)	0.89 (0.60-1.31)	0.49
Model 3	1.00	1.02 (0.72-1.46)	0.95 (0.65-1.39)	0.97 (0.66-1.44)	0.81

[1] Hazard ratios (95% CIs) were obtained from Cox proportional hazards models. Model 1 was adjusted for age and gender (*n*=20,069). Model 2 was the same as model 1 with additional adjustments for energy intake, alcohol intake, smoking status, educational level, dietary supplement use, use of hormone replacement therapy, family history of AMI, BMI (*n*=19,819). Model 3 was adjusted as model 2 with additional adjustments for intake of fish, whole grain foods and processed meat (*n*=19,819).

[2] Reference group.

[3] Additionally adjusted for intake of processed fruit and vegetables.

[4] Additionally adjusted for intake of processed fruit and vegetables and raw vegetables.

[5] Additionally adjusted for intake of processed fruit and vegetables and raw fruits.

[6] Additionally adjusted for intake of raw fruit and vegetables.

[7] Additionally adjusted for intake of raw fruit and vegetables and processed vegetables.

[8] Additionally adjusted for intake of raw fruit and vegetables and processed fruits.

and thus more vulnerable to chance findings. Because of the limited power, we cannot conclude that raw vegetables are in particularly related to ischemic stroke and raw fruit to hemorrhagic stroke. However, our results suggest that raw fruit and vegetable consumption is related to total stroke incidence. Results of other prospective cohort studies with larger numbers of cases are needed to confirm these associations.

Raw fruit and vegetables contain many nutrients and bioactive phytochemicals that may have beneficial effects on stroke. High raw fruit and vegetables consumers had a higher intake of fiber, vitamin C and potassium, but similar energy intake compared with low raw fruit and vegetable consumers. Citrus fruit and apples contributed for ~47% to raw fruit intake. Citrus fruit is a rich source of vitamin C and apples are an important source of the flavonol quercetin[20,30]. Vegetables, raw or cooked, are high in flavonols and provide ~80% of dietary nitrate intake. Recently, it was

Table 3.4. Hazard ratios and 95% confidence intervals of stroke subtypes for high vs low and per 50 increase of fruit and vegetable intake of 20,069 Dutch participants[1]

	Ischemic stroke (*n*=139)			Hemorrhagic stroke (*n*=45)		
	Low[2]	**High**	**Per 50 g increase**	**Low[2]**	**High**	**Per 50 g increase**
Raw fruits and vegetables[3]						
Median intake, g/d	92	262	150	92	262	150
Cases, *n*	83	56	139	25	20	45
Model 1	1.00	0.63 (0.45-0.89)	0.95 (0.89-1.02)	1.00	0.67 (0.37-1.21)	0.93 (0.82-1.05)
Model 2	1.00	0.69 (0.48-0.98)	0.97 (0.91-1.04)	1.00	0.79 (0.43-1.47)	0.97 (0.86-1.10)
Model 3	1.00	0.69 (0.48-1.00)	0.97 (0.91-1.05)	1.00	0.88 (0.47-1.65)	0.99 (0.87-1.12)
Raw fruits[4]						
Median intake, g/d	65	234	120	65	234	120
Cases, *n*	70	69	139	29	16	45
Model 1	1.00	0.88 (0.63-1.23)	0.97 (0.90-1.04)	1.00	0.45 (0.24-0.83)	0.91 (0.79-1.04)
Model 2	1.00	0.95 (0.67-1.35)	0.99 (0.93-1.07)	1.00	0.52 (0.27-0.98)	0.95 (0.83-1.09)
Model 3	1.00	0.99 (0.70-1.41)	1.00 (0.93-1.08)	1.00	0.53 (0.28-1.01)	0.96 (0.83-1.10)
Raw vegetables[5]						
Median intake, g/d	14	48	27	14	48	27
Cases, *n*	95	44	139	25	20	45
Model 1	1.00	0.50 (0.35-0.72)	0.55 (0.37-0.80)	1.00	0.80 (0.44-1.44)	1.11 (0.70-1.76)
Model 2	1.00	0.50 (0.34-0.72)	0.55 (0.37-0.82)	1.00	0.86 (0.47-1.57)	1.17 (0.74-1.85)
Model 3	1.00	0.50 (0.34-0.73)	0.56 (0.37-0.83)	1.00	0.96 (0.52-1.78)	1.29 (0.82-2.04)
Processed fruits and vegetables[6]						
Median intake, g/d	113	234	165	113	234	165
Cases, *n*	66	73	139	22	23	45
Model 1	1.00	1.27 (0.91-1.77)	1.00 (0.93-1.08)	1.00	1.07 (0.60-1.93)	1.04 (0.92-1.18)
Model 2	1.00	1.38 (0.97-1.94)	1.01 (0.93-1.09)	1.00	1.15 (0.63-2.09)	1.05 (0.93-1.19)
Model 3	1.00	1.43 (1.01-2.03)	1.01 (0.94-1.10)	1.00	1.25 (0.68-2.30)	1.07 (0.95-1.21)
Processed fruits[7]						
Median intake, g/d	21	133	61	21	133	61
Cases, *n*	71	68	139	23	22	45
Model 1	1.00	1.11 (0.80-1.55)	1.01 (0.92-1.10)	1.00	1.02 (0.57-1.83)	1.04 (0.90-1.19)
Model 2	1.00	1.21 (0.85-1.70)	1.01 (0.93-1.11)	1.00	1.09 (0.60-1.99)	1.05 (0.91-1.21)
Model 3	1.00	1.26 (0.89-1.78)	1.03 (0.94-1.12)	1.00	1.13 (0.61-2.08)	1.05 (0.91-1.21)

Table 3.4. Continued

	Ischemic stroke (*n*=139)			Hemorrhagic stroke (*n*=45)		
	Low[2]	**High**	**Per 50 g increase**	**Low[2]**	**High**	**Per 50 g increase**
Processed vegetables[8]						
Median intake, g/d	70	121	94	70	121	94
Cases, *n*	70	69	139	20	25	45
Model 1	1.00	1.02 (0.73-1.42)	0.97 (0.79-1.19)	1.00	1.21 (0.67-2.18)	1.12 (0.80-1.55)
Model 2	1.00	1.06 (0.75-1.48)	0.98 (0.80-1.21)	1.00	1.25 (0.69-2.27)	1.14 (0.82-1.59)
Model 3	1.00	1.09 (0.77-1.53)	1.01 (0.82-1.24)	1.00	1.31 (0.72-2.38)	1.18 (0.84-1.64)
Total fruit and vegetables						
Median intake, g/d	240	475	346	240	475	346
Cases, *n*	75	64	139	23	22	45
Model 1	1.00	0.88 (0.63-1.23)	0.98 (0.93-1.02)	1.00	0.86 (0.48-1.56)	0.98 (0.91-1.06)
Model 2	1.00	0.96 (0.68-1.37)	0.99 (0.94-1.04)	1.00	1.01 (0.55-1.87)	1.01 (0.93-1.09)
Model 3	1.00	0.98 (0.69-1.40)	0.99 (0.94-1.04)	1.00	1.16 (0.62-2.17)	1.03 (0.95-1.11)

[1] Hazard ratios (95% CIs) were obtained from Cox proportional hazards models. Model 1 was adjusted for age and gender (*n*=20,069). Model 2 was the same as model 1 with additional adjustments for energy intake, alcohol intake, smoking status, educational level, dietary supplement use, use of hormone replacement therapy, family history of AMI, BMI (*n*=19,819). Model 3 was adjusted as model 2 with additional adjustments for intake of fish, whole grain foods and processed meat (*n*=19,819).
[2] Reference group.
[3] Additionally adjusted for intake of processed fruit and vegetables.
[4] Additionally adjusted for intake of processed fruit and vegetables and raw vegetables.
[5] Additionally adjusted for intake of processed fruit and vegetables and raw fruits.
[6] Additionally adjusted for intake of raw fruit and vegetables.
[7] Additionally adjusted for intake of raw fruit and vegetables and processed vegetables.
[8] Additionally adjusted for intake of raw fruit and vegetables and processed fruits.

suggested that nitrate could have potential cardiovascular benefits[31]. The protective effect of raw fruit and vegetables is most likely due to synergistic effects of these different compounds[32].

Citrus (49%) and apple juice (22%) were the largest contributors to processed fruit intake. Citrus juice and oranges have comparable levels of vitamin C and quercetin, but citrus juice is low in dietary fiber (0.3g/100g) compared with oranges (1.8g/100g)[20,30,33]. Apple juice contains no fiber or quercetin, while apples with or without peel have high levels of both dietary fiber (~2.3g/100g) and quercetin (3.6mg/100g)[20,30,33]. The lower dietary fiber and flavonoid content of fruit juices may explain the lack of association of processed fruit and vegetables with incident stroke. Additionally, the liquid state of juices and added sugars to juices and products such as applesauce may have contributed to the higher energy intake of those with a high processed fruit and vegetable intake[34]. Cauliflower is a major contributor to processed vegetable consumption. Cooked cauliflower is a poor source of dietary fiber (1.5/100g) and vitamin C (40 mg/100g) compared with 2.5 g fiber per 100 g and 80 mg vitamin C per 100 g in raw cauliflower[20,30]. This illustrates that cooked vegetables have a generally lower dietary fiber content and have lost vitamin C in the cooking water. Furthermore, salt is often added to cooked vegetables. The FFQ is not a reliable method to assess salt intake, therefore, we were not able to adjust for salt intake. The low content of fiber and vitamin C and the addition of salt to cooked vegetables could be an explanation that processed vegetables were in contrast to raw vegetables not related to stroke incidence. However, we cannot rule out that different types of vegetables comprising various micronutrients and phytochemicals may have influenced these results. Raw vegetables comprised mainly tomatoes and cucumber, while cabbages and French beans were the largest contributors of processed vegetables. Further research is needed to examine whether specific fruit and vegetable types influence the association with stroke independently from processing.

The results of the present study suggest that high raw fruit and vegetable consumption, in contrast to high processed fruit and vegetable intake, may protect against stroke incidence. The higher amount and possible synergy of fiber, vitamin C, potassium, flavonoids and other bioactive compounds of raw fruit and vegetables may explain these results. More research is needed to confirm the possible beneficial effects of raw vegetable consumption on ischemic stroke and of raw fruit consumption on hemorrhagic stroke risk.

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Conflicts of interest

The authors declare that there is no conflict of interest related to any part of the study. The sponsors did not participate in the design or conduct of the study; in the collection, analyses or interpretation of the data; or in the preparation, review or approval of the manuscript.

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Chapter 4

Variety in fruit and vegetable consumption and 10-year incidence of coronary heart disease and stroke

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Abstract

Background: Consuming a variety of fruit and vegetables provides many different micronutrients and bioactive compounds. Whether this contributes to the beneficial association between fruit and vegetables and incident coronary heart disease (CHD) and stroke is unknown.

Methods: Prospective population-based cohort study of 20,069 generally healthy men and women aged 20 to 65 years. Participants completed a validated 178-item food frequency questionnaire. Variety in fruit and vegetables was defined as the sum of different items consumed at least once per 2 weeks over the previous year. Hazard ratios (HR) for variety in relation to incident CHD and stroke were calculated using multivariable Cox proportional hazards models additionally adjusted for quantity of fruit and vegetables.

Results: Variety and quantity in fruit and vegetables were highly correlated (r=0.81). Variety was not associated with total energy intake (r=-0.01) and positively associated with nutrient intakes, particularly vitamin C (r=0.70). During 10 years of follow-up, 245 cases of CHD and 233 cases of stroke occurred. Variety in vegetables (HR per 2 items: 1.05; 95% CI: 0.94-1.17) and in fruit (HR per 2 items: 1.00; 95% CI: 0.87-1.15) were not related to incident CHD. Variety in vegetables (HR per 2 items: 0.93; 95% CI: 0.83-1.04) and in fruit (HR per 2 items: 1.03; 95% CI: 0.89-1.18) were also not related to incident stroke.

Conclusion: More variety in fruit and vegetable consumption was associated with higher intakes of fruit and vegetables and micronutrients. Independently of quantity, variety in fruit and vegetables was neither related to incident CHD nor to incident stroke.

Introduction

Consuming a variety of fruit and vegetables is accompanied with an intake of a wide spectrum of micronutrients and bioactive compounds that may underlie the observed inverse associations with cardiovascular diseases[1-4]. Randomized controlled trials have shown that increased fruit and vegetable intakes resulted in raised plasma concentrations of carotenoids and vitamin C[5-7]. Intervention studies focusing on single nutrients, however, failed to demonstrate beneficial effects on cardiovascular diseases[8,9]. The beneficial effects of eating more fruit and vegetables may, therefore, be explained by other mechanisms. Intervention studies showed that a diet rich in fruit and vegetables may also favorably affect blood pressure levels[7,10]. Possibly, the combined or even synergistic effects of different bioactive components in their natural food matrix could be more important in relation to cardiovascular diseases[11]. In this context of a fruit and vegetable rich diet, the 2010 Dietary Guidelines for Americans recommends to choose a variety of fruit and vegetables daily[12].

To the best of our knowledge, no previous prospective cohort studies to date have evaluated the association between variety of fruit and vegetable consumption and risk of incident CHD and stroke. Cross-sectionally, it was found that variety but not quantity of fruit and vegetable intake was associated with less inflammation and with a lower risk of CHD using the Framingham Risk Score[13]. In the field of cancer research, the importance of variety in fruit and vegetable consumption has been investigated in several prospective cohort studies. These studies found no associations between variety in fruit and vegetables and total cancer or subtypes[14-17]. For variety in vegetables only an inverse association with total cancer and non-lung epithelial cancer was found[15].

Different aspects of fruit and vegetable consumption may contribute to the inverse association with fruit and vegetables, e.g. amount, processing, color and variety. Previously, we found that both raw and processed fruit and vegetables and deep orange fruit and vegetable may protect against CHD[18,19]. With regard to stroke, inverse associations were observed for intake of raw and white fruit and vegetables[20,21]. In the present study, we examined the associations between variety in fruit and vegetable consumption with 10-year incident CHD and stroke in a population-based cohort study in the Netherlands.

Methods

Population

The present study was conducted in a Dutch population-based cohort of men and women aged 20 to 65 years; the Monitoring Project on Risk Factors and Chronic Diseases in the Netherlands (MORGEN Study). The baseline measurements including dietary assessment were carried out between 1993 and 1997[22]. The Medical Ethics Committee of the Netherlands Organization for Applied Scientific Research (TNO) approved the study protocol and all participants signed informed consent. Of 22,654 participants, we excluded respondents without informed consent for vital status follow-up (*n*=701), who did not fill out a food frequency questionnaire (FFQ; *n*=72), with reported total energy intake <500 or >4500 kcal per day for women or <800 or >5000 kcal per day for men (*n*=97), with prevalent myocardial infarction or stroke (*n*=442) and self-reported diabetes and those using lipid-lowering or anti-hypertensive drugs (*n*=1,273). This resulted in a study population of 20,069 participants, including 8,988 men and 11,081 women.

Dietary assessment

Information on habitual food consumption of 178 food items, covering the previous year, was collected using a validated, self-administered and semi-quantitative FFQ developed for the Dutch cohorts of the European Prospective Investigation into Cancer (EPIC) Study[23]. Participants indicated their consumption as absolute frequencies in times per day, per week, per month, per year or as never. For several food items, additional questions were included about consumption frequency of different sub-items or preparation method using the following categories: always/mostly, often, sometimes and seldom/never. Consumed amounts were calculated using standard household measures, natural units or indicated portion sizes by colored photographs. The photographs showed different portion sizes to assess consumed quantities of 21 food items, mainly vegetables. Frequencies per day and portion sizes were multiplied to obtain grams per day for each food item. The Dutch food composition database of 1996 was used to calculate values for energy and nutrient intakes[24].

The fruit and vegetables assessed were those commonly consumed in the Netherlands. Fruit and vegetable consumption during winter and summer was assessed separately to take seasonal variation into account. Fruit and vegetable juices and sauces were excluded and we did not consider potatoes and legumes as vegetables, because their nutritional value differs significantly from that of vegetables[24].

The reproducibility of the FFQ after 12 months was expressed as Spearman's correlation coefficients and were 0.76 in men and 0.65 in women for vegetable intake and 0.61 in men and 0.77 in women for fruit[23]. The validity against 12 repeated 24-h recalls varied between 0.31 and 0.38 for vegetables and between 0.56 and 0.68 for fruit consumption.

Variety

The FFQ comprised 9 fruit items, 7 raw vegetables and 13 cooked vegetables. Each different fruit or vegetable that was consumed at least once per 2 weeks over the previous year contributed 1 point to the variety score. Several vegetable items that were essentially the same food but appeared in different forms, e.g. raw and cooked carrots, contributed only 1 point if their combined intake was at least once per 2 weeks. Several items were combined in single questions and could, therefore, not be distinguished from one another, i.e. apples and pears, and cabbages, and leek and onions. Variety scores ranged from 0 to 22 for fruit and vegetables together, from 0 to 9 for fruit, and from 0 to 13 for vegetables.

Risk factors

Body weight, height and blood pressure of the participants were measured by trained research assistants during a physical examination at a municipal health service site. Non-fasting venous blood samples were collected and serum total and HDL cholesterol concentrations were determined using an enzymatic method. Information on cigarette smoking, educational level, physical activity, use of anti-hypertensive and lipid modifying drugs, ever use of hormone replacement therapy and both the participants' and their parents' history of acute myocardial infarction (AMI) were obtained by a self-administered questionnaire. Dietary supplement use (yes/no) and alcohol intake were obtained from the FFQ. Alcohol intake was expressed as the number of glasses of beer, wine, port wines and strong liquor consumed per week. From 1994 onwards, physical activity was assessed using a validated questionnaire that was developed for the EPIC-Study[25]. Physical activity was defined as engaging in activities with an intensity of ≥4 metabolic equivalents on at least 5 days per week for at least 30 minutes.

Ascertainment of fatal and non-fatal events

Information on the participants' vital status up to 1 January 2006 was monitored using the municipal population register. Information on the primary cause of death was obtained from Statistics Netherlands. The hospital discharge register provided clinically diagnosed AMI and stroke admissions. In a validation study, 84% of the AMI cases in the cardiology information system of the University Hospital Maastricht corresponded with AMI cases identified in the hospital discharge register[26]. CHD incidence was defined as non-fatal AMI event or fatal CHD event. Similarly, incident stroke was defined as non-fatal or fatal stroke event. Fatal CHD as the primary cause of death included codes I20-I25 of the tenth revision of the International Classification of Diseases (ICD-10) and non-fatal AMI comprised code 410 of the ninth revision of the International Classification of Diseases (ICD-9). Fatal stroke comprised ICD-10 codes I60-I67 and I69 and non-fatal stroke including Transient Ischemic Attack comprised ICD-9 codes 430-438. If the dates of hospital admission and death coincided, the event was considered fatal.

Statistical analyses

For each participant, we calculated person time from date of enrollment until the first event (incident CHD or stroke), date of emigration (n=693), date of death or censoring date (January 1 2006), whichever occurred first. Correlations between variety score in fruit and vegetable items and intake of selected foods and nutrients were calculated using the Spearman's rank correlation test.

Participants were divided into tertiles of variety scores. We used multivariable Cox proportional hazards models to estimate hazard ratios (HR) for the incidence of CHD and stroke for each tertile of variety compared to the lowest and continuously per increase of 2 or 4 items. The Cox proportional hazards assumption was fulfilled in all models according to the graphical approach and Schoenfeld residuals. To test P for trend across increasing tertiles of variety, median values of variety were assigned to each tertile and used as a continuous variable in Cox models.

Besides an age (continuous) and gender adjusted model, we used a multivariable model that included total energy intake (kcal), smoking status (never, former, current smoker of <10, 10-19, ≥20 cigarettes per day), alcohol intake (never, moderate or high consumption, i.e. >1 glass per day in women and >2 glasses per day in men), educational level (4 categories), dietary supplement use (yes/no), past or present use of hormone replacement therapy (yes/no), family history of premature AMI (before 55 years of the father or before 65 years of the mother, yes/no) and body mass index (BMI; kg/m^2). Additionally, we extended the model with dietary covariates including intake of whole grain foods (g/d), processed meat (g/d), fish (quartiles) and quantity of fruit and vegetable consumption (g/d). For participants enrolled from 1994 onwards, we evaluated whether

Table 4.1. Demographic and lifestyle characteristics by tertiles of variety in fruit and vegetable consumption of 20,069 Dutch participants[1]

	Tertiles of variety in fruit and vegetable consumption		
	T1	**T2**	**T3**
n	6,768	6,571	6,730
Variety score, mean	5.7	10.5	15.3
Age, y	41.0 (11.2)	41.5 (11.2)	42.0 (10.8)
Men, %	56.7	45.1	32.5
Low educational level[2], %	55.0	46.9	38.8
Current smokers, %	43.3	35.4	31.0
Moderate alcohol consumers[3], %	53.7	58.1	58.7
High alcohol consumers[4], %	31.6	30.0	31.1
Dietary supplement use, %	25.5	30.8	36.3
Physically active[5], %	26.5	31.8	37.4
Body Mass Index, kg/m^2	25.1 (4.0)	24.8 (3.8)	24.7 (3.7)
Serum total cholesterol, mmol/L	5.3 (1.1)	5.2 (1.1)	5.2 (1.1)
Serum HDL cholesterol, mmol/L	1.3 (0.4)	1.4 (0.4)	1.4 (0.4)
Systolic blood pressure, mmHg	121 (16)	120 (15)	119 (15)
Family history of AMI[6], %	9.2	9.0	9.0
Ever use of hormone replacement therapy in women, %	3.4	4.6	6.7

[1] Data are presented as mean (SD) or percentages.
[2] Defined as primary school and lower, intermediate general education.
[3] Defined as ≤1 glass per day in women and as ≤2 glass per day in men.
[4] Defined as >1 glass per day in women and >2 glasses per day in men.
[5] Defined as engagement in cycling or sports of ≥4 metabolic equivalents. In sub sample of participants enrolled from 1994 onwards (*n*=15,433).
[6] Defined as occurrence of aucte myocardial infarction before age 55 of the father or before age 65 of the mother.

physical activity was a potential confounder ('active' being defined as engagement in cycling or sports of ≥4 metabolic equivalents) by comparing HRs with and without adding physical activity to the multivariable model.

Stratified analyses and the log-likelihood test using cross-product terms in the multivariable models showed no evidence for potential effect modification by age (<50 vs ≥50 years), gender or smoking status (never including former vs current). *P* values <0.05 (two-tailed) were considered statistically significant. Analyses were performed using the Statistical Analysis System (version 9.2; SAS Institute, Inc. Cary, NC, USA).

Results

Participants with a greater variety in fruit and vegetable consumption were more often women, had a higher educational level, were less likely to smoke, more likely to be physically active and used more often dietary supplements (**Table 4.1**). The mean scores for each tertile of variety in fruit and vegetables were 5.7, 10.5 and 15.3, respectively. Fruit and vegetable intake was 2.5 fold higher among participants in the highest compared to the lowest tertiles of variety. Variety was strongly correlated with total fruit and vegetable intake (Spearman's *r*= 0.81) and with fruit intake (Spearman's *r*= 0.72) and less strongly with vegetable intake (Spearman's *r*= 0.53). Greater variety in fruit and vegetable consumption was not associated with total energy intake and positively with the intake of vitamin C, carotenoids, flavonoids and dietary fiber (**Table 4.2**). Eighty percent of the population consumed apples and pears, citrus fruit, cabbages and allium vegetables at least once per 2 weeks over the previous year.

Table 4.2. Daily mean (SD) intake of selected foods and nutrients by tertiles of variety in fruit and vegetable consumption of 20,069 Dutch participants

	Tertiles of variety in fruit and vegetable consumption			
	T1	**T2**	**T3**	***R***[1]
n	6,768	6.571	6,730	
Variety score, mean	5.7	10.5	15.3	
Fruit and vegetables, g/d	155 (69)	259 (98)	393 (141)	0.81
Fruit, g/d	67 (60)	144 (101)	248 (131)	0.72
Vegetables, g/d	88 (38)	115 (43)	145 (52)	0.53
Whole grain foods, g/d	51 (71)	64 (73)	73 (70)	0.18
Processed meat, g/d	48 (36)	44 (33)	39 (31)	-0.14
Fish, g/d	8 (9)	10 (10)	12 (12)	0.18
Total energy intake, kcal/d	2,262 (667)	2,296 (677)	2,258 (658)	-0.01
Total protein, en%	15 (2)	15 (2)	15 (2)	0.10
Total fat, en%	36 (5)	36 (5)	35 (5)	-0.15
Saturated fatty acids, en%	15 (3)	15 (2)	14 (2)	-0.16
Polyunsaturated fatty acids, en%	7 (2)	7 (2)	7 (2)	0.04
Total carbohydrates, en%	45 (6)	45 (6)	46 (6)	0.13
Dietary fiber, g/d	22 (7)	25 (7)	27 (7)	0.31
Vitamin C, mg/d	75 (27)	104 (34)	141 (45)	0.70
Potassium, g/d	3.6 (1.0)	3.9 (1.0)	4.1 (1.0)	0.21
Carotenoids, mg/d	7.9 (3.3)	9.4 (3.6)	11.1 (4.3)	0.39
Flavonoids, mg/d	43.1 (39.3)	54.3 (41.9)	66.7 (44.8)	0.32

[1] Spearman's correlation coefficients were calculated between variety score in fruit and vegetable consumption and intake of selected foods and nutrients. Adjustment for total energy intake showed similar Spearman's correlation coefficients except for dietary fiber (*r*=0.42) and potassium (*r*=0.35).

Table 4.3. Hazard ratios and 95% confidence intervals of incident CHD by tertiles of variety in fruit and vegetable consumption of 20,069 Dutch participants[1]

	Tertiles of variety in fruit and vegetable consumption			*P* for trend[2]	Continuously
	T1	T2	T3		
Fruit and vegetables					
Variety score	<9	9-12	≥13		Per 4 items
n	6,768	6,571	6,730		
Cases, *n*	104	84	57		245
Model 1	1.00	0.87 (0.65-1.17)	0.65 (0.47-0.90)	0.01	0.86 (0.77-0.98)
Model 2	1.00	0.99 (0.73-1.33)	0.77 (0.55-1.09)	0.15	0.93 (0.82-1.06)
Model 3	1.00	1.10 (0.80-1.53)	0.99 (0.63-1.57)	1.00	1.07 (0.89-1.29)
Fruit					
Variety score	<4	4-6	≥7		Per 2 items
n	7,520	6,156	6,393		
Cases, *n*	123	67	55		245
Model 1	1.00	0.66 (0.49-0.89)	0.56 (0.41-0.78)	<0.001	0.87 (0.80-0.95)
Model 2	1.00	0.76 (0.56-1.03)	0.70 (0.50-0.98)	0.04	0.93 (0.85-1.02)
Model 3	1.00	0.81 (0.58-1.13)	0.80 (0.50-1.29)	0.40	1.00 (0.87-1.15)
Vegetables					
Variety score	<5	5-7	≥8		Per 2 items
n	6,493	7,699	5,877		
Cases, *n*	90	89	66		245
Model 1	1.00	0.94 (0.70-1.26)	1.01 (0.73-1.39)	0.99	0.98 (0.89-1.08)
Model 2	1.00	0.98 (0.73-1.32)	1.09 (0.78-1.51)	0.65	1.00 (0.90-1.11)
Model 3	1.00	1.03 (0.76-1.40)	1.26 (0.89-1.79)	0.21	1.05 (0.94-1.17)

[1] Hazard ratios (95% CIs) obtained from Cox proportional hazards models. Model 1 was adjusted for age and gender (*n*=20,069). Model 2 was the same as model 1 with additional adjustments for energy intake, alcohol intake, smoking status, educational level, dietary supplement use, use of hormone replacement therapy, family history of AMI, BMI, (*n*=19,819). Model 3 was the same as model 2 with additional adjustment for intake of whole grain foods, processed meat, fish and quantity of fruit and vegetable consumption (*n*=19,819).

[2] *P* for trend was tested across increasing tertiles of variety.

During 10 years of follow-up, we documented 245 first cases of CHD, of which 34 were fatal. Furthermore, 233 first cases of stroke occurred (7 fatal cases), of which 139 were ischemic, 45 hemorrhagic and 49 other or unspecified strokes. After adjustment for lifestyle and dietary factors including quantity of fruit and vegetables, we found that fruit variety (HR per 2 items: 1.00; 95% CI: 0.87-1.15, **Table 4.3**), vegetable variety (HR per 2 items: 1.05; 95% CI: 0.94-1.17) and its combination (HR per 4 items: 1.07; 95% CI: 0.89-1.29) were not related to incident CHD. With regard to incident stroke, we observed that fruit variety (HR per 2 items: 1.03; 95% CI: 0.89-1.18, **Table 4.4**) and the

Table 4.4. Hazard ratios and 95% confidence intervals of incident stroke by tertiles of variety in fruit and vegetable consumption of 20,069 Dutch participants[1]

	Tertiles of variety in fruit and vegetable consumption			*P* for trend[2]	Continuously
	T1	T2	T3		
Fruit and vegetables					
Variety score	<9	9-12	≥13		Per 4 items
n	6,768	6,571	6,730		
Cases, *n*	96	69	68		233
Model 1	1.00	0.72 (0.53-0.98)	0.70 (0.51-0.96)	0.03	0.85 (0.75-0.96)
Model 2	1.00	0.80 (0.58-1.10)	0.82 (0.82-1.14)	0.24	0.91 (0.80-1.03)
Model 3	1.00	0.83 (0.59-1.18)	0.90 (0.58-1.41)	0.65	0.92 (0.77-1.11)
Fruit					
Variety score	<4	4-6	≥7		Per 2 items
n	7,520	6,156	6,393		
Cases, *n*	99	67	67		233
Model 1	1.00	0.77 (0.56-1.05)	0.74 (0.54-1.01)	0.08	0.92 (0.84-1.00)
Model 2	1.00	0.89 (0.64-1.22)	0.88 (0.63-1.22)	0.48	0.97 (0.89-1.07)
Model 3	1.00	0.94 (0.67-1.33)	0.99 (0.62-1.58)	0.98	1.03 (0.89-1.18)
Vegetables					
Variety score	<5	5-7	≥8		Per 2 items
n	6,493	7,699	5,877		
Cases, *n*	91	92	50		233
Model 1	1.00	0.90 (0.67-1.20)	0.66 (0.46-0.93)	0.02	0.89 (0.80-0.99)
Model 2	1.00	0.93 (0.69-1.24)	0.70 (0.49-1.00)	0.06	0.91 (0.82-1.01)
Model 3	1.00	0.96 (0.71-1.29)	0.76 (0.52-1.10)	0.17	0.93 (0.83-1.04)

[1] Hazard ratios (95% CIs) obtained from Cox proportional hazards models. Model 1 was adjusted for age and gender (*n*=20,069). Model 2 was the same as model 1 with additional adjustments for energy intake, alcohol intake, smoking status, educational level, dietary supplement use, use of hormone replacement therapy, family history of AMI, BMI, (*n*=19,819). Model 3 was the same as model 2 with additional adjustment for intake of whole grain foods, processed meat, fish and quantity of fruit and vegetable consumption (*n*=19,819).

[2] *P* for trend was tested across increasing tertiles of variety.

combination of fruit and vegetable variety was not associated (HR per 4 items: 0.92; 95% CI: 0.77-1.11) and greater variety in vegetables were not significantly related (HR per 2 items: 0.93; 95% CI: 0.83-1.04). Within participants enrolled from 1994 onwards (*n*=15,433), the HRs for incident CHD and stroke did not change after adjustment for physical activity. For example, the HRs for incident CHD changed from 1.06 (95% CI: 0.61-1.36) to 1.05 (95% CI: 0.58-1.92) after adjustment for physical activity for high vs low variety in fruit and vegetable consumption.

Discussion

In this population-based cohort study of generally healthy men and women, we found that greater variety in fruit and vegetables was associated with higher intakes of particularly vitamin C as well as carotenoids, flavonoids and dietary fiber but not with energy intake. Variety and quantity of fruit and vegetable consumption were strongly correlated. After adjustment for quantity, we found no relation between variety in fruit and vegetable consumption and the risk of either incident CHD or stroke.

Major strengths of this study are its prospective and population-based study design and large sample size. With respect to non-fatal events, it was shown on the national level that data from the Dutch hospital discharge register can be uniquely matched to an individual for at least 88% of the hospital admissions[27]. Possible misclassification is expected to be random and not related to fruit and vegetable consumption. Therefore, the strengths of the associations may have been underestimated.

Although we used a detailed FFQ that was validated for the intake of food groups as well as nutrients, it was difficult to operationalize variety. First, the FFQ assessed only the intake of fruit and vegetable items that were most commonly consumed in the Netherlands. Second, several fruit and vegetable items were combined in single questions and could not be distinguished from one another. Third, some fruit and vegetables are typically consumed during summer or winter and their consumed frequencies were calculated as frequencies per day during the previous year. Due to these limitations, variety scores could be underestimated, which may have led to little variation in variety in fruit and vegetables. Greater variety can possibly be achieved by combining different dietary assessment methods, e.g. a dietary history methods and a FFQ or by extending a FFQ with questions on less commonly consumed fruit and vegetables during the season that they were on the market[15].

After adjustment for quantity of fruit and vegetables, we found no clear associations between variety and incident CHD or stroke. Consistent with results of previous cohort studies, we found that a more varied fruit and vegetable consumption is accompanied by higher amounts of fruit and vegetables[13-17]. Our reported correlation coefficients between variety and quantity were comparable to those found among American adults of the Nurses' Health Study and the Health Professionals' Follow-up Study (r = 0.77 for fruits; r = 0.73 for vegetables)[14]. It is possible that after adjustment for quantity the potential benefits of a varied fruit and vegetable consumption were overadjusted.

Fruit and vegetables are rich sources of micronutrients and bioactive phytochemicals that may play an important role in the prevention of CHD and stroke and are relatively low in energy density[28]. We found that more variety in fruit and vegetables was associated with higher intakes of vitamin C, carotenoids, flavonoids and dietary fiber. This is in line with a previous study that showed that variety within fruit and vegetables was correlated with nutrient adequacy[29]. Variety in fruit and vegetable consumption may promote higher intakes of total fruit and vegetables and thus micronutrients and bioactive compounds. These compounds may act synergistically to prevent CHD or stroke[11] and this supports the current recommendation to eat a diet rich of fruit and vegetables daily[12].

Particularly, eating a variety of nutrient-dense foods between and within basic food groups may ensure that adequate amounts of micronutrients and bioactive compounds are consumed. This has been translated in the 2010 USDA Dietary Guidelines for Americans to “Choose a variety of fruit and vegetables daily”[12]. The implication of this recommendation, however, is unclear because variety was not defined. In different studies, in which variety scores were calculated based on FFQ data, different time periods were used, e.g. per month, per 2 weeks or per week, and fruit juices and sauces or herbs were included in the definition[13-17]. A clear definition of variety is urgently needed to examine the importance of variety in relation to CHD or stroke.

In conclusion, we found that greater variety in fruit and vegetable consumption was accompanied by higher intakes of fruit and vegetables and of micronutrients and bioactive compounds. Evidence from population-based cohort studies including the present one does not support the recommendation to consume a variety of fruit and vegetables to reduce CHD or stroke risk. Results from prospective cohort studies with more detailed data on variety are needed to underpin this recommendation.

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Conflicts of interest

Dr Geleijnse obtained an unrestricted grant from the Product Board for Horticulture, Zoetermeer, the Netherlands. The other authors did not report financial disclosures. The sponsors did not participate in the design or conduct of the study; in the collection, analyses or interpretation of the data; or in the preparation, review or approval of the manuscript.

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Chapter 5

Colors of fruit and vegetables and 10-year incidence of coronary heart disease

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Abstract

The colors of the edible part of fruit and vegetables indicate the presence of specific micronutrients and phytochemicals. The extent to which fruit and vegetable color groups contribute to coronary heart disease (CHD) protection is unknown. We therefore examined the associations between fruit and vegetables of different colors and their subgroups and 10-year CHD incidence. We used data from a prospective population-based cohort including 20,069 men and women aged 20 to 65 years who were enrolled between 1993 and 1997. Participants were free of cardiovascular diseases at baseline and completed a validated 178-item food frequency questionnaire. Hazard ratios (HR) for the association between green, orange/yellow, red/purple, white fruit and vegetables and their subgroups with CHD were calculated using multivariable Cox proportional hazards models. During 10 years of follow-up, 245 incident cases of CHD were documented. For each 25 g/d increase in the intake of the sum of all four colors of fruit and vegetables, a borderline significant association with incident CHD was found (HR: 0.98; 95% CI: 0.97-1.01). No clear associations were found for the color groups separately. However, each 25 g/d increase in intake of deep orange fruit and vegetables was inversely associated with CHD (HR: 0.74; 95% CI: 0.55-1.00). Carrots, their largest contributor (60%), were associated with a 32% lower risk of CHD (HR: 0.68; 95% CI: 0.48-0.98). In conclusion, though no clear associations were found for the four color groups with CHD, a higher intake of deep orange fruit and vegetables and especially carrots may protect against CHD.

Introduction

Prospective cohort studies have shown that a high consumption of fruit and vegetables lowers the risk of coronary heart disease (CHD)[1,2]. Various subgroups of fruit and vegetables provide a different array of micronutrients and phytochemicals[3], which may underlie the observed association with CHD. Consistent evidence for subgroups of fruit and vegetables in relation to CHD is lacking since prospective cohort studies have focused on only a limited number of fruit and vegetables that were selected on the basis of their botanical family or content of one specific micronutrient or bioactive compound.

Previous cohort studies have shown inconsistent results for specific fruit and vegetables. Thus, two prospective cohort studies have observed inverse associations between intake of citrus fruit and incident CHD[4,5], while two other studies have not found an association with fatal CHD[6,7]. Intake of berries was found to lower the risk of fatal cardiovascular diseases (CVD)[7-9], but not the risk of incident CHD in male smokers[10]. Also, two prospective cohort studies have found that apples were not significantly inversely related to fatal CHD[11-13]. Vegetables rich in carotenoids[14], tomatoes and tomato-based products, however, were inversely related to fatal CVD[15] as well as to incident CVD[16]. Carrots were inversely associated with both fatal CHD[17] and fatal CVD[6,15,18]. Cruciferous vegetables were inversely related to incident CHD[4] and broccoli to fatal CHD[13]. With regard to incident CHD only, inverse relationships were observed for intake of green leafy and vitamin C-rich vegetables[4].

Randomized trials focusing on antioxidant supplements have failed to demonstrate a beneficial effect on CVD[19,20]. Although this could be explained by methodological issues, such as a relatively brief follow-up period or the use of high doses of antioxidants, this could also indicate that the protective effect of fruit and vegetables may be due to the combined or even synergistic effects of the various components in their natural food matrix and not to one particular antioxidant[21]. Fruit and vegetable subgroups, therefore, need to be classified according to similarities in micronutrient and phytochemical content. Pennington and Fisher[3,22] defined ten fruit and vegetable subgroups based on their unique nutritional value and characteristics, e.g. edible part of the plant, color, botanical family or total antioxidant capacity.

The color of the edible part of fruit and vegetables reflects the presence of pigmented phytochemicals, e.g. carotenoids and flavonoids, and therefore indicates their nutritional value[23]. Drewnowski[24] found that consumers perceive the most colorful vegetables as the most nutritious and suggested that fruit and vegetable colors may be an important factor in food selection. Heber and Bowerman[25] has suggested using fruit and vegetable colors as a tool to translate the science of phytochemical nutrition into dietary guidelines for the public. The 2010 Dietary Guidelines for Americans recommend selecting vegetables from five subgroups, i.e. dark green, red-orange, legumes, starchy and other vegetables to reach the recommendation[26]. However, there have been no prospective cohort studies to date that focus on fruit and vegetable color groups in relation to incident CHD.

Our investigation, therefore, focuses on the associations of fruit and vegetable color groups and their subgroups with 10-year CHD incidence in a population-based follow-up study in the Netherlands.

Methods

Population

We used data from the Monitoring Project on Risk Factors and Chronic Diseases in the Netherlands (MORGEN Study), a Dutch population-based cohort[27,28]. The baseline measurements were carried out between 1993 and 1997. The present study was conducted in accordance with the guidelines laid down in the declaration of Helsinki and all procedures involving human subjects were approved by the Medical Ethics Committee of the Netherlands Organization for Applied Scientific Research. Written informed consent was obtained from all participants. Of the total 22,654 participants we excluded respondents without informed consent for vital status follow-up (*n*=701), with incomplete dietary assessment (*n*=72), with reported extreme total energy intakes of <2,094 or >18,844 kJ per day for women or <3,350 or >20,938 kJ per day for men (*n*=97), with a history of myocardial infarction or stroke at baseline (*n*=442) and with self-reported diabetes or use of lipid-lowering or anti-hypertensive drugs (*n*=1,273). This resulted in a study population of 20,069 participants, including 8,988 men and 11,081 women.

Dietary assessment

Information on habitual food consumption of 178 food items, covering the previous year, was collected using a validated, self-administered, semi-quantitative food frequency questionnaire (FFQ) developed for the Dutch cohorts of the European Prospective Investigation into Cancer (EPIC) Study[29]. Participants indicated their consumption as absolute frequencies in times per day, per week, per month, per year, or as never. For several food items, additional questions were included about consumption frequency of different sub-items or preparation methods using the following categories: always/mostly, often, sometimes and seldom/never. Consumed amounts were calculated using standard household measures, natural units or portion sizes indicated by colored photographs. Frequencies per day and portion sizes were multiplied to obtain grams per day for each food item. The Dutch food composition database of 1996 was used to calculate values for energy and nutrient intakes[30]. To calculate the intake of carotenoids and flavonoids from fruit and vegetables, the Dutch food composition database of 2001 was used[31].

The FFQ was designed to assess habitual intake during summer and winter of 35 commonly used fruit and vegetables in the Netherlands, including juices and sauces. Potatoes and legumes were not included, because their nutritional value differs significantly from that of vegetables[30]. The reproducibility of the FFQ after 12 months and relative validity against 12 repeated 24-h recalls for food group and nutrient intake were tested in 63 males and 58 females[29,32]. Reproducibility of the FFQ after 12 months expressed as Spearman's correlation coefficients for vegetables was 0.76 in men and 0.65 in women; for fruit intake it was 0.61 in men and 0.77 in women. The validity against 12 repeated 24-h recalls over a period of one year, varied between 0.31 and 0.38 for vegetables and between 0.56 and 0.68 for fruit.

In 284 men and 287 women of the MORGEN Study, Jansen *et al.*[33] validated fruit and vegetable intake using plasma carotenoids and found that intake of several fruit and vegetable subgroups was positively associated with plasma levels of specific carotenoids. Participants in the highest quartile of carrot intake showed a 31% higher α-carotene level compared to participants in the lowest

Table 5.1. Classification of fruit and vegetables according to color group[1]

Color group	Fruit and vegetable subgroup	Fruit and vegetable items
Green	Cabbages (18%)	Broccoli, Brussels sprouts, and green cabbages (Chinese, green, oxheart, sauerkraut, savoy, white)
	Dark green leafy vegetables (15%)	Kale and spinach
	Lettuces (13%)	Endive and lettuce
	Other green fruit and vegetables (54%)	French beans, green sweet pepper, honeydew melon, and kiwi fruit
Orange/yellow	Citrus fruits (78%)	Citrus fruit juices, grapefruit, orange, and tangerine
	Deep orange fruit and vegetables (22%)	Cantaloupe, carrot, carrot juice, and peach
Red/purple	Berries (41%)	Cherries, grapes, grape and berry juices, and strawberries
	Red vegetables (59%)	Red beet, red beet juice, red cabbage, red sweet pepper, tomato, tomato juice, and tomato sauce
White	Hard fruits (55%)	Apple, apple juice, apple sauce and pear
	Allium family bulbs (10%)	Garlic, leek, and onion
	Other white fruit and vegetables (35%)	Banana, cauliflower, chicory, cucumber, and mushroom

[1] Fruit and vegetables were classified into subgroups as proposed by Pennington and Fisher.

quartile. For tomatoes, 26% higher β-carotene and 21% higher lycopene levels were observed. For cabbages, β-carotene levels were 17% higher and lutein levels were 13% higher.

Classification of fruit and vegetables

Fruit and vegetables were classified into color groups and subgroups (**Table 5.1**). First, we categorized fruit and vegetables into 4 fruit and vegetable color groups according to the color of the primarily edible part; green, orange/yellow, red/purple and white. Second, we subdivided fruit and vegetables within these color groups, resulting in 9 fruit and vegetable subgroups and 2 groups with 'other' fruit and vegetables, as recently proposed by Pennington and Fisher[3,22]. We made small adjustments in the classification of subgroups to make it more compatible with our FFQ and the Dutch situation. Cabbages were classified according to their color as green, red/purple and white cabbages. As apples and pears are commonly consumed in the Netherlands and are an important source of flavonoids[34], we created the specific subgroup of hard fruits. Several green and white fruit and vegetables that could not be classified because of their unique micronutrient composition were allocated to an 'other' group.

Risk factors

The baseline measurements were previously described in detail by Verschuren *et al.*[27]. Body weight, height and blood pressure of the participants were measured by trained research assistants during a physical examination at a municipal health service site. Non-fasting venous blood samples were collected and serum total and HDL cholesterol concentrations were determined using an enzymatic method. Information on cigarette smoking, educational level, physical activity, use of anti-hypertensive and lipid-lowering drugs, past or present use of hormone replacement therapy and the history of myocardial infarction of the participants' parents were obtained through a self-administered questionnaire. Dietary supplement use (yes/no) and alcohol intake were obtained from the FFQ. Alcohol intake was expressed as the number of glasses of beer, wine, port wines and strong liquor consumed per week. From 1994 onwards, physical activity was assessed using a validated questionnaire that was developed for the EPIC-Study[35]. Physical activity was defined as engaging in cycling and/or sports on at least 5 days per week during ≥30 minutes with an intensity of ≥4 metabolic equivalents. In this subsample both cycling and sports were related to cardiovascular diseases[36].

Ascertainment of fatal and non-fatal events

After enrollment, the participants' vital status up to 1 January 2006 was monitored using the municipal population register. For participants who died, information on cause of death was obtained from Statistics Netherlands. The hospital discharge register provided information on clinically diagnosed acute myocardial infarction (AMI) discharges. CHD incidence was defined as the first non-fatal AMI or fatal CHD event that was not preceded by any other CHD event. Non-fatal AMI comprised code 410 of the 9th revision of the International Classification of Diseases[37]. Fatal CHD included ICD-10 codes I20-I25 as the primary cause of death[38]. Where the dates of hospital admission and death coincided, the event was considered fatal.

Statistical analyses

For each participant, we calculated person time from date of enrollment until the first event (non-fatal AMI or fatal CHD), date of emigration (*n*=693), date of death or censoring date (1 January 2006), whichever occurred first. The intake of the total of fruit and vegetable color groups was calculated by summing the intake of fruit and vegetable color groups. Quartiles of intake were computed for each fruit and vegetable color group. Tertiles of intake were calculated for each fruit and vegetable subgroup. Hazard ratios (HR) for each category of fruit and vegetables compared to the lowest category and per 25 g/d increase in intake were estimated using Cox proportional hazards models. The Cox proportional hazards assumption was fulfilled in all models according to the graphical approach and Schoenfeld residuals. To test *P* for trend across increasing categories of intake, median values of intake were assigned to each category and used as a continuous variable in the Cox model.

Besides an age- (continuous) and gender-adjusted model we used a multivariable model that included total energy intake (continuous), smoking status (never, former, current smoker of <10, 10-20, ≥20 cigarettes per day), alcohol intake (never, moderate and high consumption of >1 glass per day in women and >2 glasses per day in men), educational level (4 categories), dietary supplement use (yes/no), past or present hormone replacement therapy (yes/no), family history of AMI before 55 years of the father or before 65 years of the mother (yes/no) and body mass index (BMI; kg/m^2). In addition, we extended the model with dietary covariates, including intake of whole grain foods and processed meat (g/d), fish (quartiles) and mutually for the sum of intake of the other fruit and vegetable color groups or subgroups. With regard to the participants enrolled from 1994

Table 5.2. Baseline characteristics of 20,069 Dutch men and women for high and low fruit and vegetable intake[1]

	Men		Women	
	Low	**High**	**Low**	**High**
n	5,177	3,811	4,857	6,224
Age, y	42.0 (10.8)	41.9 (11.1)	40.7 (10.9)	41.4 (11.4)
Low educational level[2], %	44.6	38.5	57.4	45.8
Current smoking, %	40.1	36.4	44.0	31.0
High alcohol consumption[3], %	39.6	31.4	27.0	26.5
Physically active[4], %	28.3	36.7	27.3	35.3
Dietary supplement use, %	21.6	26.4	33.8	39.0
Fish consumers[5], %	21.5	29.1	19.8	29.3
Body Mass Index, kg/m^2	25.3 (3.5)	25.2 (3.3)	24.5 (4.3)	24.5 (4.0)
Serum total cholesterol, mmol/L	5.3 (1.1)	5.2 (1.1)	5.2 (1.0)	5.2 (1.0)
Serum HDL cholesterol, mmol/L	1.2 (0.3)	1.2 (0.3)	1.5 (0.4)	1.5 (0.4)
Systolic blood pressure, mmHg	124 (15)	124 (15)	117 (16)	117 (15)
Family history of AMI[6], %	9.2	9.0	9.3	9.1
Nutrient intake				
Total energy intake, Kcal	2,522 (644)	2,740 (692)	1,899 (486)	2,067 (526)
Saturated fatty acids, %	15 (3)	14 (2)	15 (3)	14 (2)
Dietary fiber, g/d	25 (7)	31 (8)	20 (5)	25 (6)
Vitamin C, mg/d	75 (21)	138 (42)	75 (20)	139 (41)
Carotenoids, mg/d	8.1 (3.2)	11.0 (4.4)	8.0 (3.0)	10.9 (4.2)
Flavonoids, mg/d	41.9 (37.5)	57.7 (41.3)	50.2 (43.5)	67.0 (44.8)
Fruit and vegetable intake, g/d				
Total	225 (72)	516 (176)	244 (67)	525 (162)
Green	50 (23)	78 (33)	54 (23)	86 (34)
Orange/yellow	51 (31)	154 (86)	57 (32)	160 (79)
Red	40 (18)	80 (36)	44 (18)	87 (36)
White	81 (37)	191 (84)	84 (34)	180 (71)

[1] Data are presented as mean (SD) or percentages.

[2] Defined as primary school and lower, intermediate general education.

[3] Defined as >1 glass per day in women and >2 glasses per day in men.

[4] Defined as engagement in cycling or sports of ≥4 metabolic equivalents. In subsample of subjects enrolled from 1994 onwards (*n*=15,433).

[5] Defined as the highest quartile of fish intake (median: 17 g/d, i.e. ~1 portion of fish/week).

[6] Defined as occurrence of acute myocardial infarction before age of 55 of the father or before age of 65 of the mother.

onwards, we evaluated whether physical activity was a potential confounder ('active' being defined as engagement in cycling or sports of ≥4 metabolic equivalents). We calculated the HR with and without physical activity in the multivariable model.

According to stratified analyses and the log-likelihood test using cross-product terms in the multivariable model, no evidence was observed for potential effect modification by age (<50 vs ≥50 y), gender, or smoking status (never vs current). *P* values <0.05 (two-tailed) were considered statistically significant. Analyses were performed using the Statistical Analysis System (version 9.1; SAS Institute, Inc. Cary, NC, USA).

Results

Participants were 42±11 years old at baseline and 45% were male. Women had a higher fruit and vegetable consumption, had a lower educational level, used alcohol less often and used die tary supplements more often than men (**Table 5.2**). Women had a lower intake of energy and dietary fiber, but a higher intake of vitamin C and flavonoids than men.

Participants had an average daily fruit and vegetable intake of 378±193 g/d. The largest contributors to total fruit and vegetable consumption were white (36%) and orange/yellow (29%) fruit and vegetables (**Table 5.1**). The most commonly consumed items in the white fruit and vegetable range were hard fruits (55%). Orange/yellow fruit and vegetables comprised citrus fruits (78%) and deep orange fruit and vegetables (22%). Green fruit and vegetables consisted of several vegetable subgroups, e.g. cabbages (18%), dark leafy vegetables (15%) and lettuces (13%) and other green fruit and vegetables (54%). Red/purple fruit and vegetables comprised red vegetables (59%) and berries (41%). Spearman's correlation coefficients between fruit and vegetable color groups ranged from 0.38 for green vs orange/yellow fruit and vegetables to 0.60 for orange/yellow vs white fruit and vegetables.

After a median follow-up of 10.5 (interquartile range: 9.2-11.8) years, we documented 245 incident CHD events, which comprised 211 non-fatal cases of AMI and 34 fatal cases of CHD. After adjustment for lifestyle and dietary factors, we observed for each 25 g/d increase in intake of the sum of green, orange/yellow, red/purple and white fruit and vegetables a borderline significant association with incident CHD (HR: 0.98; 95% CI: 0.97-1.01, **Table 5.3**). No clear associations were found between intake of the 4 fruit and vegetable color groups separately and incident CHD.

In addition, we analyzed the subgroups of fruit and vegetables as proposed by Pennington and Fisher[22]. After adjustment for lifestyle and dietary factors, continuous analysis per 25 g/d increase in intake of deep orange fruit and vegetables was inversely associated with CHD (HR: 0.74; 95% CI: 0.55-1.00, Table 5.4). Carrots were the largest contributor to deep orange fruit and vegetables (60%). Each 25 g/d increase in intake of carrots was associated with a 32% lower risk of CHD (HR: 0.68; 95% CI: 0.48-0.98), whereas each 25 g/d increase in intake of the sum of the other fruit and vegetable subgroups was weakly associated (HR: 0.99; 95% CI: 0.97-1.01). The consumption of the other fruit and vegetable subgroups was not associated with CHD (**Table 5.4**).

Table 5.3. Hazard ratios and 95% confidence intervals of incident CHD by quartiles and per 25 g/d increase of fruit and vegetable color group intake of 20,069 Dutch participants[1]

	Quartiles of fruit and vegetable color group intake				*P* for trend	Per 25 g increase
	Q1[2]	Q2	Q3	Q4		
Green						
Median, g/d	34	54	72	105		
Cases, *n*	65	60	71	49		245
Model 1	1.00	0.89 (0.63-1.27)	1.02 (0.73-1.44)	0.69 (0.47-1.01)	0.08	0.91 (0.82-1.01)
Model 2	1.00	0.93 (0.65-1.33)	1.08 (0.76-1.52)	0.74 (0.51-1.09)	0.18	0.93 (0.84-1.03)
Model 3	1.00	0.95 (0.66-1.37)	1.14 (0.80-1.62)	0.83 (0.55-1.24)	0.47	0.95 (0.85-1.07)
Orange/yellow						
Median, g/d	30	66	110	193		
Cases, *n*	91	55	58	41		245
Model 1	1.00	0.66 (0.48-0.93)	0.75 (0.54-1.04)	0.54 (0.37-0.78)	0.003	0.93 (0.89-0.98)
Model 2	1.00	0.80 (0.56-1.12)	0.88 (0.62-1.24)	0.65 (0.44-0.96)	0.05	0.95 (0.91 -1.00)
Model 3	1.00	0.82 (0.58-1.17)	0.93 (0.63-1.36)	0.70 (0.44-1.12)	0.19	0.96 (0.91 -1.02)
Red/purple						
Median, g/d	29	48	67	100		
Cases, *n*	90	62	58	35		245
Model 1	1.00	0.80 (0.58-1.11)	0.85 (0.61-1.18)	0.58 (0.39-0.86)	0.01	0.86 (0.77-0.95)
Model 2	1.00	0.83 (0.59-1.15)	0.93 (0.66-1.32)	0.63 (0.41-0.96)	0.05	0.88 (0.78-0.98)
Model 3	1.00	0.86 (0.61-1.21)	1.00 (0.68-1.47)	0.70 (0.41-1.19)	0.29	0.89 (0.76-1.03)
White						
Median, g/d	57	98	142	216		
Cases, *n*	81	60	48	56		245
Model 1	1.00	0.77 (0.55-1.08)	0.64 (0.45-0.91)	0.74 (0.52-1.04)	0.07	0.98 (0.94-1.02)
Model 2	1.00	0.84 (0.59-1.18)	0.73 (0.50-1.06)	0.82 (0.57-1.18)	0.27	0.99 (0.95-1.03)
Model 3	1.00	0.92 (0.65-1.31)	0.88 (0.59-1.31)	1.11 (0.71-1.74)	0.67	1.04 (0.99-1.09)
Total of fruit and vegetable color groups						
Median, g/d	182	286	395	572		
Cases, *n*	88	62	51	44		245
Model 1	1.00	0.79 (0.57-1.09)	0.65 (0.46-0.92)	0.59 (0.41-0.86)	0.003	0.98 (0.96-1.00)
Model 2	1.00	0.89 (0.64-1.24)	0.77 (0.54-1.11)	0.66 (0.44-0.98)	0.03	0.98 (0.96-1.00)
Model 3	1.00	0.92 (0.66-1.28)	0.81 (0.56-1.16)	0.70 (0.47-1.04)	0.06	0.98 (0.97-1.01)

[1] Hazard ratios (95% CIs) obtained from Cox proportional hazards models. Model 1 was adjusted for age and gender (*n*=20,069). Model 2 was the same as model 1 with additional adjustments for energy intake, alcohol intake, smoking status, educational level, dietary supplement use, use of hormone replacement therapy, family history of AMI, BMI, (*n*=19,819). Model 3 was the same as model 2 with additional adjustment for intake of whole grain foods, processed meat, fish, and mutually for the sum of the other fruit and vegetable color groups.

[2] Reference group.

Table 5.4. Hazard ratios and 95% confidence intervals of incident CHD by tertiles and per 25 g/d increase in intake of fruit and vegetable subgroups[1] of 20,069 Dutch participants[2]

	Tertiles of intake			*P* for	Per 25 g
	T1[3]	T2	T3	trend	increase
Green cabbage family vegetables					
Median, g/d	5	10	19		
Cases, *n*	76	84	85		245
Model 1	1.00	1.08 (0.79-1.48)	1.14 (0.84-1.55)	0.43	0.96 (0.67-1.39)
Model 2	1.00	1.16 (0.85-1.60)	1.23 (0.90-1.69)	0.22	1.04 (0.72-1.50)
Model 3	1.00	1.18 (0.86-1.63)	1.26 (0.91-1.73)	0.19	1.13 (0.78-1.64)
Dark green leafy vegetables					
Median, g/d	2	8	18		
Cases, *n*	84	80	81		245
Model 1	1.00	0.96 (0.71-1.30)	1.01 (0.75-1.38)	0.89	1.00 (0.70-1.42)
Model 2	1.00	0.97 (0.71-1.32)	0.93 (0.68-1.27)	0.64	0.88 (0.62-1.26)
Model 3	1.00	0.97 (0.71-1.33)	0.94 (0.68-1.28)	0.68	0.89 (0.62-1.27)
Lettuce					
Median, g/d	2	6	16		
Cases, *n*	77	69	99		245
Model 1	1.00	0.80 (0.58-1.11)	0.96 (0.71-1.30)	0.88	0.94 (0.68-1.31)
Model 2	1.00	0.84 (0.60-1.17)	0.93 (0.68-1.27)	0.88	0.87 (0.62-1.21)
Model 3	1.00	0.84 (0.60-1.17)	0.93 (0.68-1.27)	0.89	0.87 (0.63-1.22)
Other green fruit and vegetables					
Median, g/d	17	31	55		
Cases, *n*	103	78	64		245
Model 1	1.00	0.71 (0.53-0.96)	0.59 (0.43-0.82)	0.002	0.82 (0.70-0.97)
Model 2	1.00	0.78 (0.58-1.05)	0.67 (0.48-0.93)	0.02	0.88 (0.75-1.03)
Model 3	1.00	0.80 (0.59-1.09)	0.73 (0.50-1.06)	0.1	0.94 (0.78-1.13)
Citrus fruits					
Median, g/d	21	64	142		
Cases, *n*	108	74	63		245
Model 1	1.00	0.79 (0.59-1.07)	0.69 (0.50-0.94)	0.02	0.94 (0.89-0.99)
Model 2	1.00	0.94 (0.69-1.27)	0.82 (0.59-1.14)	0.24	0.96 (0.91-1.01)
Model 3	1.00	1.01 (0.73-1.39)	0.94 (0.65-1.37)	0.73	0.98 (0.92-1.03)
Deep orange fruit and vegetables					
Median, g/d	9	20	36		
Cases, *n*	105	77	63		245
Model 1	1.00	0.73 (0.55-0.99)	0.61 (0.45-0.84)	0.003	0.65 (0.51-0.83)
Model 2	1.00	0.80 (0.59-1.08)	0.69 (0.50-0.96)	0.03	0.72 (0.56-0.92)
Model 3	1.00	0.82 (0.60-1.12)	0.75 (0.51-1.09)	0.13	0.74 (0.55-1.00)

Table 5.4. Continued

	Tertiles of intake			*P* for trend	Per 25 g increase
	T1[3]	T2	T3		
Berries					
Median, g/d	7	20	44		
Cases, *n*	105	80	60		245
Model 1	1.00	0.77 (0.58-1.04)	0.61 (0.44-0.84)	0.003	0.77 (0.65-0.92)
Model 2	1.00	0.84 (0.62-1.13)	0.72 (0.52-1.01)	0.06	0.84 (0.70-1.00)
Model 3	1.00	0.88 (0.64-1.21)	0.80 (0.53-1.22)	0.32	0.87 (0.69-1.09)
Red vegetables					
Median, g/d	19	33	54		
Cases, *n*	112	73	60		245
Model 1	1.00	0.86 (0.64-1.16)	0.89 (0.64-1.22)	0.44	0.88 (0.74-1.05)
Model 2	1.00	0.89 (0.65-1.20)	0.91 (0.65-1.27)	0.56	0.87 (0.73-1.05)
Model 3	1.00	0.95 (0.70-1.30)	1.03 (0.72-1.47)	0.89	0.93 (0.77-1.12)
Allium family bulbs					
Median, g/d	2	9	21		
Cases, *n* n	94	73	78		245
Model 1	1.00	0.84 (0.62-1.14)	0.93 (0.69-1.26)	0.76	0.99 (0.78-1.25)
Model 2	1.00	0.90 (0.66-1.23)	0.94 (0.69-1.28)	0.75	0.89 (0.68-1.16)
Model 3	1.00	0.91 (0.67-1.24)	0.94 (0.69-1.29)	0.77	0.91 (0.70-1.19)
Hard fruits					
Median, g/d	24	60	120		
Cases, *n*	93	74	78		245
Model 1	1.00	0.84 (0.62-1.15)	0.85 (0.63-1.15)	0.34	0.99 (0.94-1.05)
Model 2	1.00	0.92 (0.67-1.26)	0.96 (0.70-1.33)	0.86	1.00 (0.95-1.06)
Model 3	1.00	1.03 (0.74-1.42)	1.24 (0.86-1.79)	0.24	1.05 (0.99-1.11)
Other white fruit and vegetables					
Median, g/d	22	40	70		
Cases, *n*	101	79	65		245
Model 1	1.00	0.86 (0.64-1.16)	0.73 (0.54-1.00)	0.05	0.89 (0.78-1.00)
Model 2	1.00	0.94 (0.70-1.28)	0.85 (0.61-1.17)	0.31	0.93 (0.82-1.05)
Model 3	1.00	1.02 (0.75-1.39)	0.99 (0.68-1.44)	0.95	0.99 (0.86-1.14)

[1] Fruit and vegetables were classified into subgroups as proposed by Pennington and Fisher.

[2] Hazard ratios (95% CIs) obtained from Cox proportional hazards models. Model 1 was adjusted for age and gender (*n*=20,069). Model 2 was the same as model 1 with additional adjustments for energy intake, alcohol intake, smoking status, educational level, dietary supplement use, use of hormone replacement therapy, family history of AMI, BMI, (*n*=19,819). Model 3 was the same as model 2 with additional adjustment for intake of whole grain foods, processed meat, fish and mutually for the sum of the other fruit and vegetable subgroups.

[3] Reference group.

We evaluated whether physical activity was a potential confounder for the sum of green, orange/yellow, red/purple and white fruit and vegetables with incident CHD for participants enrolled from 1994 onwards (*n*=15,433). HRs for each 25 g/d increase of all fruit and vegetable color groups was 0.97 (95% CI: 0.95-0.99) and remained similar when physical activity was added to the model (HR: 0.97; 95% CI: 0.95-1.00).

Discussion

In the present study, we observed that consumption of the four fruit and vegetable color groups together was weakly related to a lower risk of CHD. A more detailed analysis of fruit and vegetable subgroups, as defined by Pennington and Fisher[3,22], showed that deep orange fruit and vegetables and their largest contributor, carrots, were strongly associated with a lower risk of incident CHD. The inverse relationship of consumption of fruit and vegetable color groups with incident CHD was attenuated after adjustment for potential confounders.

A major strength of the present study is the almost complete follow-up for CHD mortality. With respect to non-fatal events, it was shown on the national level that data from the Dutch hospital discharge register can be uniquely matched to an individual for at least 88% of the hospital admissions[39]. In a validation study, 84% of the AMI cases in the cardiology information system of the University Hospital Maastricht corresponded with AMI cases identified in the hospital discharge register[40]. Mild AMI cases where hospitalization was not necessary may have been missed, but we expect this to be random and not to be related to fruit and vegetable intake. It is unlikely, therefore, that this has influenced the relationship of fruit and vegetable color groups with CHD incidence.

A potential limitation of our study was that some vegetables, such as onions and cabbages are commonly used in mixed dishes, which complicates the estimation of intake using a FFQ. Furthermore, fruit and vegetable intake is part of a healthy lifestyle and diet. Although we adjusted for potential risk factors as well as important food groups in relation to CHD, we cannot rule out residual confounding. In addition, comparing studies on subgroups of fruit and vegetables is challenging, since the availability and range of intake of commonly consumed fruit and vegetables differ between countries[41].

In the present study, we found that consumption of the four fruit and vegetable color groups combined was weakly inversely related to incident CHD. Mixed fruit juices that could not be classified in color groups were not included in the present analysis. However, we reported previously that the intake of total fruit and vegetables, including mixed fruit juices, was associated with a 6% lower risk of incident CHD in the same population[42]. This finding confirms the results of previous meta-analyses that showed a 4-11% lower risk of CHD for each ~100 g/d increase of fruit and vegetable intake[1,2].

After adjustment for lifestyle and dietary factors, we did not observe significant associations of the sum of fruit and vegetable color groups as well as with the four color groups separately, with incident CHD. In this respect our study may have had insufficient power to detect statistically significant associations. Results of further prospective cohort studies with larger numbers of cases are therefore needed to investigate these associations.

A more detailed analysis of fruit and vegetable color groups defined by Pennington and Fisher[3,22] showed that intake of deep orange fruit and vegetables was associated with a lower risk of incident CHD. Carrots, the primary source of deep orange fruit and vegetables (60%), were inversely associated with incident CHD, while the intake of the remaining fruit and vegetables was not related. This suggests that the lower CHD risk of total fruit and vegetable intake could be driven by the strong inverse association of carrots, which, is consistent with findings of previous studies with fatal CHD[17] and fatal CVD[6,15,18] as endpoints. Carrots are a rich source of carotenoids[3,30]. Recently, it has been found that serum α-carotene concentrations were inversely associated with ischemic heart disease mortality among US adults[43]. Circulating carotenoids were also inversely associated with markers of inflammation, oxidative stress and endothelial dysfunction[44] and may protect against early atherosclerosis[45,46]. This suggests that carotenoids may lower CHD risk through different pathways.

In conclusion, we found that consumption of the sum of all four fruit and vegetable color groups was weakly inversely related to CHD. A more detailed analysis of different color groups showed that a higher intake of deep orange fruit and vegetables, especially carrots, may protect against incident CHD. Prospective cohort studies with larger numbers of cases are needed to replicate these findings.

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Conflicts of interest

The authors declare that there is no conflict of interest related to any part of the study. The sponsors did not participate in the design or conduct of the study; in the collection, analyses, or interpretation of the data; or in the preparation, review, or approval of the manuscript.

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Chapter 6

Colors of fruit and vegetables and 10-year incidence of stroke

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Abstract

Background: The color of the edible portion of fruit and vegetables reflects the presence of pigmented bioactive compounds, e.g. carotenoids, anthocyanidins and flavonoids. Which fruit and vegetable color groups contribute most to the beneficial association of fruit and vegetables with stroke incidence is unknown. Therefore, we examined the associations between consumption of fruit and vegetable color groups with 10-year stroke incidence.

Methods: This was a prospective, population-based cohort study, including 20,069 men and women age 20 to 65 years and free of cardiovascular diseases at baseline. Participants completed a validated, 178-item food frequency questionnaire. Hazard ratios (HR) were calculated for stroke incidence using multivariable Cox proportional hazards models adjusting for age, sex, lifestyle and dietary factors.

Results: During 10 years of follow-up, 233 incident cases of stroke were documented. Fruit and vegetables were classified in 4 color groups. Medians of green, orange/yellow, red/purple and white fruit and vegetable consumption were 62, 87, 57 and 118 g/d, respectively. Green, orange/yellow and red/purple fruit and vegetables were not related to incident stroke. Higher intake of white fruit and vegetables was inversely associated with incident stroke (Q4: >171 g/d vs Q1: ≤78 g/d; HR: 0.48; 95% CI: 0.29-0.77). Each 25 g/d increase in white fruit and vegetable consumption was associated with a 9% lower risk of stroke (HR: 0.91; 95% CI: 0.85-0.97). Apples and pears were the most commonly consumed white fruit and vegetables (55%).

Conclusion: High intake of white fruit and vegetables may protect against stroke.

Introduction

Prospective cohort studies have consistently shown that a high consumption of fruit and vegetables is associated with a lower risk of stroke[1,2]. Various subgroups of fruit and vegetables contain different micronutrients and phytochemicals[3]. However, which subgroups of fruit and vegetables contribute most to this inverse association remains unclear. Inconsistent results were found for citrus fruit juice[4,5], berries[6-8], cruciferous vegetables[4,8,9], leafy vegetables[4,9] and root vegetables[8,9]. However, five prospective cohort studies found that the intake of citrus fruit was inversely associated with incident stroke[4,8-11]. Apples and pears were inversely, but not significantly, related to incident stroke[5,10-12] and onions were not associated with stroke incidence[9,12].

Previous prospective cohort studies used different characteristics to categorize fruit and vegetables, e.g. botanical family or part of the plant. However, the beneficial effect of fruit and vegetables may also be caused by combined, or even synergistic, effects of these different components in their natural food matrix[13]. Recently, Pennington and Fisher defined 10 fruit and vegetable subgroups in a novel way based on a combination of their unique nutritional value and characteristics, e.g. edible part of the plant, color, botanical family and total antioxidant capacity[3,14].

The color of the edible portion of fruit and vegetables reflects the presence of pigmented phytochemicals, e.g. carotenoids and flavonoids. Their color could, therefore, be an indicator of their nutrient profile and could be used to group various fruit and vegetables[3,15]. Heber has suggested using fruit and vegetable colors as a tool to translate the science of phytochemical nutrition into dietary guidelines for the public[16]. This has also been acknowledged in the 2010 Dietary Guidelines for Americans, which advises selecting vegetables from 5 subgroups, i.e. dark green, red-orange, legumes, starchy and other vegetables to reach the recommendation[17]. However, to the best of our knowledge, no prospective studies have yet investigated fruit and vegetable color groups in relation to stroke incidence. In the present study, we investigated the associations of fruit and vegetable color groups with 10-year stroke incidence in a population-based, follow-up study in the Netherlands.

Methods

Population

The present study was conducted in a Dutch population-based cohort; the Monitoring Project on Risk Factors and Chronic Diseases in the Netherlands (MORGEN Study). The baseline measurements, including dietary assessment were carried out between 1993 and 1997[18]. The Medical Ethics Committee of the Netherlands Organization for Applied Scientific Research approved the study protocol and all participants signed informed consent. Of 22,654 participants, we excluded respondents without informed consent for vital status follow-up (*n*=701), who did not fill out a food frequency questionnaire (FFQ; *n*=72), with reported total energy intake <500 or >4500 kcal per day for women or <800 or >5000 kcal per day for men (*n*=97), with prevalent myocardial infarction or stroke (*n*=442) and self-reported diabetes and those using lipid-lowering or antihypertensive drugs (*n*=1,273). This resulted in a study population of 20,069 participants, comprising 8,988 men and 11,081 women.

Dietary assessment

Information on habitual food consumption of 178 food items, covering the previous year, was collected using a validated, self-administered and semi-quantitative FFQ developed for the Dutch cohorts of the European Prospective Investigation into Cancer (EPIC) Study[19]. Participants indicated their consumption as absolute frequencies in times per day, per week, per month or per year or as never. For several food items, additional questions were included about consumption frequency of different subitems or preparation method using the following categories: always/mostly, often, sometimes and seldom/never. Consumed amounts were calculated using standard household measures, natural units or indicated portion sizes by colored photographs. The photographs showed different portion sizes to assess consumed quantities of 21 food items, mainly vegetables. Frequencies per day and portion sizes were multiplied to obtain grams per day for each food item. The Dutch food composition database of 1996 was used to calculate values for energy[20].

The FFQ assessed habitual intake of commonly consumed fruit and vegetables, including juices and sauces. Fruit and vegetable consumption during winter and summer was assessed separately to take seasonal variation into account. We did not consider potatoes and legumes to be vegetables, because their nutritional value differs significantly from that of true vegetables[20].

Reproducibility of the FFQ after 12 months and relative validity of the FFQ against 12 repeated 24-h recalls for food group and nutrient intake were tested in 63 males and 58 females[19,21]. The reproducibility of the FFQ after 12 months expressed as Spearman's correlation coefficients for vegetables was 0.76 in men and 0.65 in women. The reproducibility for fruit intake was 0.61 in men and 0.77 in women. The validity against 12 repeated 24-h recalls, was 0.31 in women and 0.38 in men for vegetables and 0.56 in women and 0.68 in men for fruit consumption.

In 284 men and 287 women of the MORGEN Study, Jansen *et al.* validated fruit and vegetable intake using plasma carotenoids; they found that the intake of several specific fruit and vegetables was associated with plasma levels of specific carotenoids[22]. Participants in the highest quartile of carrot intake showed a 31% higher plasma α-carotene level compared with participants in the lowest quartile. For tomatoes, 26% higher plasma β-carotene and 21% higher plasma lycopene levels were observed. For cabbages, plasma β-carotene levels were 17% higher and plasma lutein levels were 13% higher.

Classification of fruit and vegetables

Fruit and vegetables were classified into four color groups (**Table 6.1**). These were made according to the color of the primarily edible portion; green, orange/yellow, red/purple and white. The color groups comprised 9 fruit and vegetable subgroups and 2 rest groups as recently proposed by Pennington and Fisher[3,14]. We made small adjustments in the classification of subgroups to make it more compatible with our FFQ. Cabbages were classified according to their color as green, red/purple and white cabbages. Apples and pears are commonly consumed in the Netherlands and are an important source of flavonoids[23]. Therefore, we created the specific subgroup of hard fruits. Several green and white fruit and vegetables that are unique in their micronutrient composition could not be classified into neat groups and were classified in two heterogeneous rest groups.

Table 6.1. Classification of fruit and vegetables according to color groups[1]

Colour group	Fruit and vegetable subgroup[2]	Fruit and vegetable items
Green	Cabbages (18%)	Broccoli, Brussels sprouts, and green cabbages (Chinese, green, oxheart, sauerkraut, savoy, white)
	Dark green leafy vegetables (15%)	Kale and spinach
	Lettuces (13%)	Endive and lettuce
	Other green fruit and vegetables (54%)	French beans, green sweet pepper, honeydew melon, and kiwi fruit
Orange/yellow	Citrus fruits (78%)	Citrus fruit juices, grapefruit, orange, and tangerine
	Deep orange fruit and vegetables (22%)	Cantaloupe, carrot, carrot juice, and peach
Red/purple	Berries (41%)	Cherries, grapes, grape and berry juices, and strawberries
	Red vegetables (59%)	Red beet, red beet juice, red cabbage, red sweet pepper, tomato, tomato juice, and tomato sauce
White	Hard fruits (55%)	Apple, apple juice, apple sauce and pear
	Allium family bulbs (10%)	Garlic, leek, and onion
	Other white fruit and vegetables (35%)	Banana, cauliflower, chicory, cucumber, and mushroom

[1] Fruit and vegetables were classified into subgroups as proposed by Pennington and Fisher.

[2] Proportion of subgroups to the color group in brackets.

Risk factors

The baseline measurements were previously described by Verschuren *et al.*[18]. Body weight, height and blood pressure of the participants were measured by trained research assistants during a physical examination at a municipal health service site. Non-fasting venous blood samples were collected and serum total and HDL cholesterol concentrations were determined using an enzymatic method. Data on cigarette smoking, educational level, physical activity, use of antihypertensive and lipid lowering drugs, ever use of hormone replacement therapy and both the participants' and their parents' history of acute myocardial infarction were obtained by a self-administered questionnaire. Dietary supplement use (yes/no) and alcohol intake were obtained from the FFQ. Alcohol intake was expressed as the number of glasses of beer, wine, port wines and strong liquor consumed per week. From 1994 onwards, physical activity was assessed using a validated questionnaire that was developed for the EPIC-Study[24]. Physical activity was defined as engaging in at least 5 days per week and ≥30 minutes in activities with an intensity of ≥4 metabolic equivalents.

Ascertainment of fatal and non-fatal events

Data on participants' vital status up to 1 January 2006 was monitored using the municipal population register. Information on the primary cause of death was obtained from Statistics Netherlands. The hospital discharge register provided clinically diagnosed stroke admissions. According to the Dutch guideline for the diagnosis of stroke subtypes[25], brain imaging (computed tomography or magnetic resonance imaging) is used in Dutch hospitals to identify stroke and its subtypes in 98% of admitted patients[26]. Stroke incidence was defined as the first nonfatal or fatal stroke event, not preceded by any other nonfatal stroke event. Stroke included codes I60-I67, I69, as well as G45 (Transient Ischemic Attack) of the 10th revision of the International Classification of Diseases (ICD-10). For hospital admission data corresponding ICD-9 codes were used. If the dates of hospital admission and death coincided, the event was considered fatal.

Statistical analyses
For each participant, we calculated person time from date of enrollment until the first event (non-fatal or fatal stroke), date of emigration (*n*=693), date of death or censoring date (1 January 2006), whichever occurred first. We calculated the average consumption of fruit and vegetables in grams per day that were consumed during summer and winter. Quartiles of intake were computed for each fruit and vegetable color group. Hazard ratios (HR) for the incidence of stroke for each category of fruit and vegetables using the lowest category as reference and per 25 g/d increase in intake were estimated using Cox proportional hazards models. We repeated the analyses for ischemic stroke, but not for hemorrhagic stroke because of the small number of cases. In addition, we analyzed the most commonly consumed white fruit and vegetables, hard fruit, separately. The Cox proportional hazards assumption was fulfilled in all models according to the graphical approach and Schoenfeld residuals. To test *P* for trend across increasing categories of intake, median values of intake were assigned to each quartile and used as a continuous variable in the Cox models.

Besides an age- (continuous) and sex-adjusted model, we used a multivariable model that included total energy intake (kcal), smoking status (never, former, current smoker of <10, 10-20, ≥20 cigarettes per day), alcohol intake (never, moderate and high consumption of >1 glass per day in women and >2 glasses per day in men), educational level (4 categories), dietary supplement use (yes/no), past or present use of hormone replacement therapy (yes/no), family history of acute myocardial infarction before 55 years of the father or before 65 years of the mother (yes/no) and body mass index (BMI, kg/m^2). In addition, we extended the model with dietary covariates including intake of whole grain foods (g/d), processed meat (g/d) and fish (quartiles) and mutually for the sum of intake of the other fruit and vegetable color groups or subgroups. Within participants enrolled from 1994 onwards, we evaluated whether physical activity was a potential confounder ('active' being defined as engagement in cycling or sports) by comparing the HR with and without adding physical activity to the multivariable model.

Stratified analyses and the log-likelihood test using cross-product terms into the multivariable model showed no evidence for potential effect modification by age (<50 vs ≥50 years), sex or smoking status (never vs current). *P* values <0.05 (two-tailed) were considered statistically significant. Analyses were performed using Statistical Analysis System (version 9.2; SAS Institute, Inc. Cary, NC, USA).

Results

Participants were on average 42 ± 11 years old and 45% of the population were men. Women had lower educational level, consumed less alcohol and used dietary supplements more often than did men (**Table 6.2**). Women had higher total fruit and vegetable consumption and lower intake of

Table 6.2. Demographic and lifestyle characteristics for men and women separately[1]

	Men	Women
n	8,988	11,081
Age, y	42.0 (11.0)	41.1 (11.2)
Low educational level[2], %	42.0	50.9
Current smokers, %	36.4	36.7
Moderate alcohol consumption[3], %	55.6	57.8
High alcohol consumption[4], %	36.1	26.7
Any dietary supplement use, %	23.6	36.7
Physically active[5], %	31.9	31.8
Body Mass Index, kg/m^2	25.3 (3.4)	24.5 (4.1)
Serum total cholesterol, mmol/L	5.3 (1.1)	5.2 (1.0)
Serum HDL cholesterol, mmol/L	1.2 (0.3)	1.5 (0.4)
Systolic blood pressure, mmHg	124 (15)	117 (15)
Family history of AMI[6], %	9.0	9.1
Hormone replacement therapy, %	-	8.9
Total energy intake, Kcal/d	2,614 (673)	1,993 (516)
Whole grain foods, g/d	71 (84)	56 (59)
Processed meat, g/d	56 (38)	34 (25)
Fish consumers[7], %	24.7	25.1
Total fruit and vegetables, g/d	341 (185)	393 (184)
Green, g/d	62 (31)	72 (34)
Orange/yellow, g/d	94 (80)	115 (81)
Red/purple, g/d	57 (34)	68 (36)
White, g/d	128 (82)	138 (75)

[1] Data are presented as mean (SD) or percentages.

[2] Defined as primary school and lower, intermediate general education.

[3] Defined as ≤1 glass per day in women and as ≤2 glass per day in men.

[4] Defined as >1 glass per day in women and >2 glasses per day in men.

[5] Defined as engagement in cycling or sports of ≥4 metabolic equivalents. In sub sample of participants enrolled from 1994 onwards (*n*=15,433).

[6] Defined as occurrence of aucte myocardial infarction before age 55 of the father or before age 65 of the mother.

[7] Defined as the highest quartile of fish intake (median: 17 g/d, i.e. ~1 portion of fish/week).

energy, whole grain foods and processed meat than men.

Participants had an average daily fruit and vegetable intake of 378±193 g/d. The largest contributors to total fruit and vegetable consumption were white (36%) and orange/yellow (29%) fruit and vegetables (**Table 6.1**). The most commonly consumed white fruit and vegetables were hard fruits (55%). Orange/yellow fruit and vegetables comprised mainly citrus fruits (78%). Many different fruit and vegetables contributed to green fruit and vegetables and included cabbages (18%), dark leafy vegetables (15%) and lettuces (13%) as defined subgroups. Red/purple fruit and vegetables comprised mostly red vegetables (59%). Spearman's correlation coefficients ranged from 0.42 for white with green fruit and vegetables to 0.60 for white with orange/yellow fruit and vegetables.

During an average follow-up period of 10.3 years, 19 fatal and 226 non-fatal stroke cases occurred; of the 19 fatal cases 12 patients had a non-fatal stroke previously. 233 first-ever incident strokes remained for the present analysis (139 ischemic, 45 hemorrhagic and 49 other or unspecified strokes). Green, orange/yellow and red/purple fruit and vegetables were not related to incident stroke **(Table 6.3)**. After adjustment for lifestyle and dietary factors, higher consumption of white fruit and vegetables was inversely associated with incident stroke (Q4: >171 g/d; HR: 0.48; 95% CI: 0.29-0.77) compared to participants with a low consumption (Q1: ≤78 g/d). We found for each 25 g/d increase of white fruit and vegetable consumption a 9% lower risk of stroke (HR: 0.91; 95% CI: 0.85-0.97). Similar results were found when we repeated the analysis for ischemic stroke as well as when we stratified by age, sex or smoking status. In addition, we analyzed apples and pears (55%), the largest contributors of white fruit and vegetables separately. Each 25 g/d increase in intake of apples and pears was inversely associated with stroke (HR: 0.93; 95% CI: 0.86-1.00).

We evaluated whether physical activity was a potential confounder for white fruit and vegetables with incident stroke within participants enrolled from 1994 onwards (*n*=15,433). HRs for each 25 g/d increase of white fruit and vegetable consumption was 0.90 (95% CI: 0.84-0.98) and remained similar when physical activity was added to the model (HR: 0.91; 95% CI: 0.84-0.98).

Discussion

In this prospective cohort of healthy Dutch men and women, we found that a higher consumption of white fruit and vegetables was inversely associated with total stroke incidence. Green, orange/yellow and red/purple fruit and vegetables were not related to incident stroke.

Major strengths of this study include its prospective and population-based study design and large sample size. With respect to nonfatal events, it was shown on the national level that data from the Dutch hospital discharge register can be uniquely matched to an individual for at least 88% of hospital admissions[27]. We expect possible misclassification to be random and not to be related to fruit and vegetable consumption. Therefore, the strengths of the associations may have been underestimated. Our findings are based on the combined endpoint of total stroke. We were unable to perform separate analyses for subtypes of stroke, because of the rather small number of stroke cases. Results of other prospective cohort studies with larger numbers of cases are needed to distinguish between different types of stroke.

We used a detailed FFQ that was primarily designed to measure the consumption of different types of fruit and vegetables. This enabled us to classify fruit and vegetables according to their color. The relative validity of the FFQ for vegetable intake, however, remains of concern[19]. Possible reasons may be the narrower range of vegetable intake or measurement errors in the portion size estimation of vegetables. However, the correlation coefficients for vegetable intake were in the same range

Table 6.3. Hazard ratios and 95% confidence intervals of incident stroke by quartiles and per 25 g/d increase of fruit and vegetable color group intake of 20,069 Dutch participants[1]

	Quartiles of fruit and vegetable color group intake				*P* for trend	Per 25 g/d increase
	Q1[2]	Q2	Q3	Q4		
Green						
Median, g/d	34	54	72	105		
Cases, *n*	48	61	62	62		233
Model 1	1.00	1.19 (0.81-1.73)	1.12 (0.77-1.63)	1.04 (0.71-1.52)	0.93	1.01 (0.92-1.11)
Model 2	1.00	1.26 (0.86-1.85)	1.20 (0.81-1.76)	1.12 (0.76-1.66)	0.79	1.02 (0.93-1.12)
Model 3	1.00	1.30 (0.89-1.91)	1.28 (0.86-1.90)	1.25 (0.83-1.90)	0.41	1.06 (0.95-1.18)
Orange/yellow						
Median, g/d	30	66	110	193		
Cases, *n*	69	49	58	57		233
Model 1	1.00	0.73 (0.51-1.06)	0.88 (0.62-1.25)	0.84 (0.58-1.19)	0.58	0.99 (0.95-1.03)
Model 2	1.00	0.84 (0.58-1.22)	1.01 (0.70-1.45)	0.99 (0.68-1.44)	0.77	1.01 (0.96 -1.05)
Model 3	1.00	0.94 (0.64-1.38)	1.25 (0.84-1.85)	1.37 (0.87-2.14)	0.10	1.04 (0.99 -1.10)
Red/purple						
Median, g/d	29	48	67	100		
Cases, *n*	92	43	45	53		233
Model 1	1.00	0.51 (0.35-0.73)	0.57 (0.40-0.82)	0.70 (0.50-0.99)	0.09	0.93 (0.84-1.03)
Model 2	1.00	0.53 (0.37-0.77)	0.64 (0.44-0.93)	0.80 (0.55-1.15)	0.37	0.97 (0.88-1.08)
Model 3	1.00	0.56 (0.38-0.82)	0.69 (0.46-1.04)	0.90 (0.56-1.45)	0.88	1.02 (0.89-1.17)
White						
Median, g/d	57	98	142	216		
Cases, *n*	75	62	54	42		233
Model 1	1.00	0.81 (0.58-1.13)	0.71 (0.50-1.00)	0.54 (0.37-0.79)	0.001	0.93 (0.89-0.98)
Model 2	1.00	0.88 (0.62-1.23)	0.78 (0.54-1.13)	0.60 (0.40-0.91)	0.01	0.95 (0.90-0.99)
Model 3	1.00	0.83 (0.59-1.18)	0.70 (0.48-1.04)	0.48 (0.29-0.77)	0.002	0.91 (0.85-0.97)

[1] Hazard ratios (95% CIs) obtained from Cox proportional hazards models. Model 1 was adjusted for age and gender (*n*=20,069). Model 2 was the same as model 1 with additional adjustments for energy intake, alcohol intake, smoking status, educational level, dietary supplement use, use of hormone replacement therapy, family history of AMI, and BMI (*n*=19,819). Model 3 was the same as model 2 with additional adjustment for intake of whole grain foods, processed meat, fish and mutually for intake of the sum of the other fruit and vegetable color groups (*n*=19,819).

[2] Reference group.

as those of other studies[19,28]. In addition, Jansen *et al.* found positive assocations between plasma concentrations of carotenoids and individual vegetables, e.g. α-carotene with carrot intake that added further strength to the relative vality of our FFQ[22]. Furthermore, some vegetables, e.g. onions and cabbages, are commonly used in mixed dishes. This complicates the estimation of intake using a FFQ and may have led to underestimation of the intake of these vegetables.

We adjusted for potential risk factors as well as for important food groups, nevertheless, we cannot rule out the possibility of residual confounding. However, after adjustment for these confounders we found similar results in both men and women. This argues against residual confounding, because in men fruit and vegetable intake is less strongly related to healthy behavior.

Apples and pears were the most commonly consumed white fruit and vegetables, and were inversely associated with incident stroke. This is in line with four previous prospective cohort studies that found that apples and pears were inversely related to incident stroke, although not statistically significant[5,10-12]. Apples are a rich source of dietary fiber (~2.3 g/100g) and the flavonol quercetin (3.6 mg/100g)[20,23]. Two meta-analyses of randomized placebo-controlled intervention studies showed that dietary fiber had a small blood pressure lowering effect[29,30]. With regard to flavonols, Hollman *et al.* found in a meta-analysis of 6 prospective cohort studies that high intake of flavonols was associated with a 20% lower risk of incident stroke[31]. Another important contributor to white fruit and vegetable consumption was bananas. To our knowledge, no previous prospective cohort studies have investigated the association between bananas and incident stroke.

The results of the present study suggest that high consumption of white fruit and vegetables, comprising mainly apples and pears, may protect against incident stroke. This is the first prospective cohort study to our knowledge that examined consumption of fruit and vegetable color groups in relation to stroke incidence. However, our findings need to be confirmed in other prospective cohorts studies before recommendations for the consumption of white fruit and vegetables can be made.

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Conflicts of interest

None of the authors had a conflicts of interest related to any part of this study. The sponsors did not participate in the design or conduct of the study; in the collection, analyses or interpretation of the data; or in the preparation, review or approval of the manuscript.

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Chapter 7

General discussion

Evidence from meta-analyses of prospective cohort studies suggested that consumption of total fruit and vegetables may prevent coronary heart disease (CHD)[1,2] and stroke[3,4]. Based on these findings, it is recommended in the Dietary Guidelines to consume sufficient amounts of fruit and vegetables to ensure an adequate intake of micronutrients and to prevent cardiovascular diseases (CVD)[5,6]. However, it is not known which aspects of fruit and vegetable consumption may contribute to these beneficial associations. In most of the individual studies included in the meta-analyses, the definition of fruit and vegetables was ambiguous. The group of total fruit and vegetables comprised all fruit and vegetables regardless of whether they were consumed raw or processed[7-16] or included potatoes or legumes[11,16]. Furthermore, in most studies it was unclear or not reported which types of fruit and vegetables were included[14,17-24].

The research described in this thesis aimed to investigate different aspects of fruit and vegetable consumption, i.e. amount, processing, variety and color groups, in relation to 10-year incidence of CHD and stroke in a population-based cohort study of Dutch men and women (MORGEN Study). In this final chapter, the main findings are interpreted and discussed in the context of other studies. In addition, the potential causality of fruit and vegetables in the etiology of CVD and public health relevance are discussed.

Main findings

The findings described in this thesis are presented in **Table 7.1.** Within the MORGEN Study, total fruit and vegetable consumption was significantly inversely associated with incident CHD (Chapter 2), but not with incident stroke (Chapter 3). High intake of raw fruit and vegetables was borderline significantly inversely associated with either CHD (Chapter 2) or stroke (Chapter 3). Variety in fruit and vegetables was strongly associated with higher intakes of total fruit and vegetables. Independent of total fruit and vegetables, variety in fruit and vegetables was not related to CHD or stroke (Chapter 4). Deep orange fruit and vegetables and especially carrots were inversely associated with CHD (Chapter 5). White fruit and vegetables, such as apples and pears, were also inversely associated with stroke (Chapter 6).

Different aspects of fruit and vegetable consumption

In the MORGEN study, a validated food frequency questionnaire (FFQ) was used to assess long-term habitual dietary intake. FFQs are frequently used to rank participants based on their usual food intake in large-scale epidemiological studies[25]. FFQs are associated with measurement error due to several sources of uncertainties, e.g. in reported frequencies, portion sizes used to calculate amounts and grouping of food items together in one question.

Previously, Ocké *et al.* investigated the reproducibility of the FFQ after 6 and 12 months and the relative validity against 12 repeated 24-hour recalls used in the MORGEN Study[26,27]. The results of this validation study for the intake of total fruit and total vegetables were summarized in Chapter 4. Ocké *et al.* concluded that the reproducibility and relative validity for the ranking of participants according to total fruit intake was considered good[26]. Though the reproducibility of total vegetable intake was also good, the relative validity of total vegetable intake remained of concern. Estimating

Table 7.1. Main findings on the relation between different aspects of fruit and vegetable consumption and incident coronary heart disease and stroke in the MORGEN Study

	CHD[1]	Stroke[1]	Unit	CHD (HR: 95% CI)	Stroke (HR: 95% CI)
Amount					
Total fruit and vegetables	↓↓	0	Q4: >475 vs Q1: ≤241 g/d	0.66 (0.45-0.99), P=0.04	0.97 (0.66-1.44)
Processing					
Raw fruit and vegetables	↓	↓	Q4: >262 vs Q1: ≤92 g/d	0.70 (0.47-1.04), P=0.08	0.70 (0.47-1.03), P=0.07
Processed fruit and vegetables	0	0	Q4: >234 vs Q1: ≤113 g/d	0.79 (0.54-1.16)	1.20 (0.75-1.76)
Variety					
Fruit and vegetable variety	0	0	T3: >12 vs T1: ≤8	0.99 (0.63-1.57)	0.90 (0.58-1.41)
Color groups					
Green	0	0	Q4: >85 vs Q1: ≤44 g/d	0.83 (0.55-1.24)	1.25 (0.83-1.90)
Orange/yellow	0	0	Q4: >141 vs Q1: ≤48 g/d	0.70 (0.44-1.12)	1.37 (0.87-2.14)
- Deep orange	↓↓	0	Per 25 g/d increase	0.74 (0.55-1.00)	0.83 (0.63-1.10)
Red/purple	0	0	Q4: >80 vs Q1: ≤39 g/d	0.70 (0.41-1.19)	0.90 (0.56-1.45)
White	0	↓↓	Q4: >171 vs Q1: ≤78 g/d	1.11 (0.71-1.74)	0.48 (0.29-0.77), P<0.01
- Hard fruit	0	↓↓	Per 25 g/d increase	1.05 (0.94-1.17)	0.93 (0.86-1.00)

[1] ↓↓: P for trend < 0.05; ↓: borderline significant association; 0: no association.

vegetable intake is difficult due to the narrow range of intake, large variety, seasonal variation and problems in quantifying portion sizes. Overall, participants were ranked adequately according to their total fruit intake and reasonably according to total vegetable consumption.

Total fruit and vegetable consumption of more than 475 grams per day compared to less than 241 grams per day was associated with a 34% lower risk of CHD (HR: 0.66; 95% CI:0.45-0.99). Also, an inverse dose-response relationship was found for total fruit and vegetables and CHD. Each additional portion of 100 grams fruit and vegetables per day corresponded with a 6% lower CHD risk. This finding is in line with earlier prospective cohort studies that reported a 4-11% lower risk of CHD per 100 grams per day increase[1,2] and supports a role of total fruit and vegetable intake in the development of CHD. In contrast, no association was observed between total fruit and vegetables and stroke.

Meta-analyses of prospective cohort studies showed that total fruit and vegetables were inversely related to the incidence of CHD[1,2] and stroke[3,4]. Fruit and vegetable consumption is a characteristic of a healthy diet and lifestyle. The beneficial associations of fruit and vegetables with CVD incidence may be due to residual confounding. In cohort studies, correlations of fruit and vegetable intake with other dietary and lifestyle factors were taken into account by adjusting for these factors. In addition, fruit and vegetable intake is less strongly related to healthy behavior in men. In the studies presented in this thesis, comparable results were observed in both men and women, which argues against residual confounding.

Processing

In Chapters 2 and 3, it was hypothesized that the nutritional value of fruit and vegetables is dependent on processing. Processing alters their structure and induces changes in their chemical composition, nutritional value, digestibility and bioavailability of bioactive compounds. Fruit and vegetable juices are lower in fiber compared with their raw counterparts, but they may be a good source of phytochemicals[28,29]. Fruit juices and products, such as apple sauce, also have a lower viscosity and often sugars are added. Possibly, this affects volume and chewing and may result in decreased satiety and increased energy intake[30]. During cooking, water-soluble and heat-sensitive bioactive compounds, such as carotenoids, vitamins and minerals, can be lost but their bioavailability may improve[31,32]. Furthermore, salt is often added during cooking. Therefore, processing of fruit may influence the association of fruit and vegetables with CHD and stroke in both a positive and negative way.

Raw and processed fruit and vegetables contributed equally to total fruit and vegetable consumption and were only weakly correlated to each other (r=0.20). Consequently, raw and processed fruit and vegetables could be investigated independent of each other. Raw fruit and vegetables mainly consisted of raw fruit (80%) and only 20% of raw vegetables. Raw fruit and vegetable consumption was borderline significantly inversely associated with the risk of CHD or stroke. Processed fruit and processed vegetables contributed both 50% to the total group of processed fruit and vegetables. Processed fruit and vegetables were not related to incident CHD or stroke. These findings suggest that consumption of raw fruit and vegetables is important in the prevention of CHD and stroke.

Participants consumed both raw and processed fruit and vegetables and did not commonly replace one by the other. Stratified analysis for raw or processed fruit and vegetable consumers was therefore not possible. By means of substitution modeling[33], the association of substituting processed for raw fruit and vegetables was estimated. Raw and processed fruit and vegetable intake were included as continuous variables (per 50 g/d increase) in the same multivariable model and differences in regression coefficients were used to compute hazard ratios for replacement. Substituting processed for raw fruit and vegetables was not associated with CHD (**Table 7.2**). Substituting processed for raw fruit and vegetables was borderline significantly inversely associated with stroke. These results were comparable with those presented in Chapters 2 and 3. Substitution modeling can be considered, therefore, as another way of presenting the same results.

Table 7.2. Hazard ratios and 95% confidence intervals of incident CHD and stroke for substituting 50 grams of processed for raw fruit and vegetables

	HR	95% CI
CHD		
Raw instead of processed fruit and vegetables	0.99	(0.90-1.08)
Raw instead of processed vegetables	1.05	(0.83-1.32)
Raw instead of processed fruit	0.97	(0.91-1.05)
Stroke		
Raw instead of processed fruit and vegetables	0.95	(0.88-1.04)
Raw instead of processed vegetables	0.64	(0.46-0.89)
Raw instead of processed fruit	0.98	(0.89-1.07)

Different types of fruit and vegetables contribute to raw or processed fruit and vegetables. Comparing the group of raw with the group of processed fruit and vegetables is therefore not a proper comparison. It is preferable to replace comparable raw and processed fruit and vegetables, e.g. citrus fruit with citrus fruit juice. The effect of substitution can be tested in randomized controlled trials. To initiate such intervention studies, evidence from prospective cohort studies is needed. In Chapter 3, different relationships were observed for raw or processed fruit and vegetables with stroke.

Variety

Consuming a variety of fruit and vegetables provides many different micronutrients and bioactive compounds. In agreement with findings of a previous study[34], we found that more variety in fruit and vegetables was associated with higher intakes of vitamin C, carotenoids, flavonoids, and dietary fiber (Chapter 4). These findings support the recommendation to 'Choose a variety of fruit and vegetables daily' to ensure adequate intakes of micronutrients and bioactive compounds[5,35]. To date, no prospective cohort studies investigated the importance of variety in fruit and vegetable consumption in the prevention of CHD or stroke. In Chapter 4, we found that variety and the total intake of fruit and vegetables were strongly correlated (r=0.81). Independent of total intake, variety in fruit and vegetables was not related to incident CHD or stroke. Participants frequently consumed fruit and vegetables from each color group. Therefore, it was not possible to examine variety in fruit and vegetable color groups. These findings do not support the current recommendation to consume a variety of fruit and vegetables to prevent CVD.

Operationalizing variety by means of a FFQ is difficult. For example, several fruit and vegetables items were combined in single questions and could not be distinguished from each other. Also, the FFQ assessed the intake of the most commonly consumed fruit and vegetables in the Netherlands. In addition, since reported frequencies were calculated as frequencies per day during the previous year, fruit and vegetables that are typically consumed during summer or winter had only a small contribution to variety. This may have resulted in underestimation and small contrasts of variety scores. For future research, it is therefore suggested to extend the FFQ with questions concerning seasonal consumption of less commonly consumed fruit and vegetables[36].

The recommendation to choose a variety of fruit and vegetables daily does not give a clear definition of variety[5,6]. In Chapter 4, variety in fruit and vegetable consumption was defined as the sum of different fruit or vegetable items consumed at least once per 2 weeks over the previous year. Other cohort studies that calculated variety scores by means of a FFQ used different time periods, e.g. per month, per 2 weeks, or per week[36-40]. Furthermore, in several studies also processed fruit and vegetables contributed to variety[37,40] or this was not reported[38,39]. For future research, a clear definition of variety is essential to investigate the importance of variety in fruit and vegetables in the prevention of CVD.

Are fruit and vegetables causally related to cardiovascular diseases?

Color groups

In Chapters 5 and 6, it was hypothesized that the color of the edible portion of fruit and vegetables indicates their nutritional profile. To date, no previous prospective cohort studies examined which fruit and vegetable color groups contribute most to the beneficial association of fruit and vegetables with CHD and stroke. For this purpose, 10 fruit and vegetable subgroups previously defined by Pennington and Fisher[41,42] were combined into four color groups. Each fruit and vegetable color group is rich in specific micronutrients or bioactive compounds (**Table 7.3**).

Deep orange fruit and vegetables were strongly inversely associated with CHD (Chapter 5). Carrots were the largest contributor to deep orange fruit and vegetables (60%). Each increase of 25 grams per day of carrot intake was associated with a 32% lower risk of CHD. This result is consistent with findings of previous prospective cohort studies with fatal CHD[9] or fatal CVD[19,43,44]. Orange fruit and vegetables, and especially carrots, are rich sources of α- and β-carotene (**Table 7.3**). Serum α-carotene concentrations were inversely associated with CHD mortality among ~15,000 US adults[45]. Raw fruit and vegetables contributed 48% to orange fruit and vegetables (**Table 7.4**). Carrots, however, were mainly home-cooked (69%). It is well-known that heat-treatment enhances the bioavailability of carotenoids[32,46]. It is suggested that circulating carotenoids may lower CHD risk through inhibition of inflammation and endothelial dysfunction[47] and may protect against early atherosclerosis[48,49].

Table 7.3. Weighted averaged nutrient composition per 100 gram of fruit and vegetable color groups[1]

	Green	Orange/yellow	Red/purple	White
Vitamin C, mg	27	28	28	9
β-carotene, ug	904	903	392	50
α-carotene, ug	28	398	3	5
Lutein, ug	1650	70	98	103
Zeaxanthin, ug	27	27	9	3
β-cryptoxanthin, ug	4	69	42	1
Lycopene, ug	39	6	11717	0
Total carotenoids, ug	4428	2439	13048	468
Total catechines, mg	0	0	4	3
Quercetin, mg	1	0	1	3
Total flavonoids, mg	2	1	5	5
Potassium, mg	281	168	376	218

[1] Based on data of the 1996 and 2001 Dutch Food Composition Tables.

Each additional 25 grams per day of white fruit and vegetables was associated with a 9% lower risk of stroke. Apples and pears were the primary source of white fruit and vegetables (55%). In line with previous prospective cohort studies[8,50-52], apples and pears were inversely associated with stroke. White fruit and vegetables and apples are a source of quercetin as well as other flavonoids (**Table. 7.3**). More than 50% of white fruit and vegetables (**Table 7.4**) and apples and pears were consumed raw. A cardioprotective effect of raw fruit and vegetables implies that raw apples and pears are responsible for the inverse associations. Raw apples including skin are high in dietary fiber (~2.3 g/100g) and the flavonol quercetin (3.6 mg/100g)[53,54]. Contrary, apple juice contains no dietary fiber or flavonoids[53,54]. Two meta-analyses of randomized placebo-controlled intervention studies showed that dietary fiber had small blood pressure lowering effects[55,56]. In a meta-analysis of 6 prospective cohort studies, high intake of flavonols was associated with a 20% lower risk of stroke[57].

In spite of this evidence, we cannot conclude that single nutrients were responsible for the beneficial associations between total and raw fruit and vegetables and CVD. Studying independent associations of single nutrients is difficult. Nutrients are highly correlated to each other and to their dietary source and other nutrients. In addition, randomized placebo-controlled intervention studies failed to demonstrate beneficial effects of supplementation of β-carotene, vitamin C or its combination in relation to CVD[58,59]. This suggests that the beneficial association of fruit and vegetable may be due to combined or synergistic effects of various components in their natural food matrix[60,61].

The findings presented in Chapters 5 and 6, strengthens the evidence for an important role of orange and white fruit and vegetables, and especially for carrots and apples and pears, in the etiology of CVD. Before recommendations on fruit and vegetable color groups in the prevention of CVD can be made, these findings need to be confirmed in other prospective cohort studies and intervention studies. So far, there is insufficient evidence regarding the potential underlying mechanisms by

Table 7.4. Contribution of raw and processed fruit and vegetables to color groups

	Total	Raw		Processed	
	g/d	g/d	%	g/d	%
Green	68	26	38	42	62
Orange	106	51	48	55	52
Red	63	34	54	29	46
White	133	77	58	56	42

which carrots and apples and pears may lower CVD risk. Future research is needed to elucidate these mechanisms.

Plasma micronutrient levels

The results presented in this thesis showed that fruit and vegetable consumption is associated with higher intakes of vitamin C, carotenoids, flavonoids, and dietary fiber. This is in line with findings of relatively small and short-term intervention studies that effectively raised plasma levels of carotenoids and vitamin C by increasing total fruit and vegetables intakes[62-66]. These findings were confirmed in a large-scale intervention study of 690 healthy participants[67]. During 6 months of follow-up, encouragement of fruit and vegetable intake resulted in an increased self-reported intake by on average 1.4 portions per day in the intervention group. This led to a significant increase in plasma concentrations of α-carotene, β-carotene, lutein, β-cryptoxantin and vitamin C compared to the control group. In addition, two of these intervention studies reported substantially increased dietary fiber intakes in the intervention group[63,68]. The fruit and vegetables given in these trials included both raw and processed fruit and vegetables[62-64] or this was not reported[67]. For several specific processed fruit and vegetables, for instance orange juice, short-term intervention studies showed significantly higher plasma concentrations of vitamin C compared to the control group[69,70]. However, long-term effects of increased consumption of specific raw or processed fruit and vegetables on plasma concentrations of carotenoids and vitamins were not reported in these studies and need to be further examined.

The impact of fruit and vegetables on CVD risk factors

Until now, no intervention studies investigated the effect of increased fruit and vegetable consumption on incident CVD. However, there is some evidence from intervention studies that tested the effects of increased fruit and vegetable consumption on surrogate end points for CVD, including blood pressure, plasma or serum cholesterol[63,67,68,71], and markers of inflammation and endothelial dysfunction. Such trials are difficult to perform since long-term compliance to an increased fruit and vegetable intake is required. The longest intervention trial had a follow-up of 6 months[67]. Results of this kind of trials are important in determining the biological plausibility of a potential causal relationship.

In the Dietary Approaches to Stop Hypertension (DASH) trial, 154 untreated mildly hypertensive US individuals increased their fruit and vegetable consumption during a period of 8 weeks to a total of 8 servings of fruit and vegetables per day[72,73]. The fruit and vegetable diet reduced average systolic blood pressure by 2.8 mmHg and diastolic blood pressure by 1.1 mmHg compared to the control diet[73]. This beneficial effect was most pronounced in hypertensive individuals and was found among all demographic subgroups[74]. Blood pressure lowering effects of fruit and vegetables were confirmed in an intervention study of 6-months among 344 healthy community-dwelling individuals[67]. Fruit and vegetable intake increased from on average 3.4 to 4.9 portions per day and lowered average systolic blood pressure with 3.4 mmHg and diastolic blood pressure with 1.4 mmHg compared to the control group. A short-term intervention study among 48 apparently healthy participants, 400 grams of fruit and vegetables and 200 ml fruit juice per day had no significant effect on blood pressure levels[68]. This was probably due to limited statistical power, since the study was not specifically designed to test changes in blood pressure levels.

So far, there is insufficient evidence for an effect of fruit and vegetables on serum or plasma cholesterol levels[63,67,68,71], and markers of endothelial dysfunction[65,66] and inflammation[75]. Increased fruit and vegetable intakes did not change plasma or serum cholesterol levels[63,67,68,71]. Plasma lipoprotein oxidation lag time, a marker of oxidative damage, increased significantly after 25 days in those receiving 600 grams fruit and vegetables per day compared to the control group[65]. An intervention study of 8 weeks among 117 hypertensive participants showed that an increased fruit and vegetable intake of 6 portions significantly improved endothelium-dependent vasodilatation[66]. In the same study population, no significant changes were found in markers of inflammation, endothelial activation and insulin resistance[75]. To date, no intervention studies investigated the importance of fruit and vegetables in relation to type 2 diabetes.

Evidence from randomized controlled intervention studies and prospective cohort studies suggest that fruit and vegetables are important in the etiology of CVD. Based on findings described in this thesis, future cohort and intervention studies should differentiate between processed and color groups of fruit and vegetables. The blood pressure lowering effect of fruit and vegetables is a plausible biological mechanism and could partly explain the lower risk of CVD. High quality prospective studies on different aspects of fruit and vegetables and the development of atherosclerotic complications are needed to improve our understanding of their cardioprotective effects.

Public health implications

The results presented in this thesis showed a dose-response relationship between total fruit and vegetable consumption and incident CHD. This finding is in agreement with the two meta-analyses of previous prospective cohort studies[1,2]. Daily total fruit and vegetable consumption of more than 475 grams compared to less than 241 grams was associated with a 34% lower risk of CHD (Chapter 2). This supports the current recommendation of a daily consumption of 150-200 grams of vegetables and 200 grams of fruit[5,35].

Previous prospective studies did not make a distinction between raw and processed fruit and vegetables[1-4]. High intake of raw fruit and vegetables (>262 vs ≤92 g/d) was associated with a 30% lower risk of either CHD (Chapter 2) or stroke (Chapter 3). These findings suggest that raw fruit and vegetables are responsible for the inverse associations observed for total fruit and vegetables in previous prospective cohort studies.

It is recommended in the Dietary Guidelines to consume a diet rich in fruit and vegetables that can be raw or cooked, whole, cut up, mashed or as 100% juice[5,35,76]. Although it is advised to consume whole fruits rather than fruit juice, specific recommendations whether fruit or vegetables should be eaten as raw or processed are not given. The results presented in this thesis taken together with demonstrated blood pressure lowering effects in interventions studies suggest that to prevent CVD raw fruit and vegetables need to contribute at least 50% of the daily total fruit and vegetable intake. Before solid recommendations on different aspects of fruit and vegetable consumption can be made, results from additional prospective cohort and intervention studies are needed.

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Summary in Dutch (Samenvatting)

Groente- en fruitconsumptie en het risico op hart- en vaatziekten

Groente en fruit zijn rijk aan vitamines, mineralen en bioactieve stoffen, zoals voedingsvezels, vitamine C en kalium. Eerder uitgevoerde prospectieve cohortonderzoeken laten zien dat een hoge inname van groente en fruit het risico op hart- en vaatziekten verlaagt. Op basis van deze resultaten wordt aanbevolen om dagelijks 150-200 gram groente en 200 gram fruit te consumeren voor een adequate inname van voedingsstoffen en ter preventie van hart- en vaatziekten. Er is nog weinig bekend over het belang van verschillende aspecten van groente- en fruitinname, zoals bewerkingsgraad, variatie en kleur, in relatie tot preventie van hart- en vaatziekten. Het doel van het in dit proefschrift beschreven onderzoek was het bestuderen van verschillende aspecten van groente- en fruitconsumptie in relatie tot het risico op een hartinfarct of beroerte in een Nederlandse populatie.

Voor de onderzoeken beschreven in dit proefschrift wordt gebruik gemaakt van gegevens van het project ‘Monitoring van Risicofactoren en Gezondheid in Nederland’ (MORGEN-project) van het Rijksinstituut voor Volksgezondheid en Milieu (RIVM). In dit prospectieve cohortonderzoek zijn tussen 1993 en 1997 gegevens verzameld van meer dan 22.000 Nederlandse mannen en vrouwen van 20 tot 65 jaar uit Amsterdam, Doetinchem en Maastricht. Voor de analyses in dit proefschrift zijn 20.069 deelnemers van het MORGEN-project geselecteerd. Deze deelnemers hadden aan het begin van het onderzoek geen hart- en vaatziekten en een voedselfrequentievragenlijst ingevuld. Met behulp van deze vragenlijst werd de gebruikelijke voedselconsumptie gedurende dat afgelopen jaar gemeten. In de daarop volgende 10 jaar werd bij 245 deelnemers een hartinfarct en 233 deelnemers een beroerte geregistreerd.

In **Hoofdstuk 2** en **3** van dit proefschrift wordt beschreven of de totale groente- en fruitconsumptie is gerelateerd aan het optreden van hart- en vaatziekten. Een hogere inname van totale groente en fruit was gerelateerd aan een hogere inname van verschillende voedingsstoffen, zoals vitamine C, voedingsvezel en verschillende bioactieve stoffen. Deelnemers met een dagelijkse totale groente- en fruitconsumptie van meer dan 475 gram hadden een 34% lager risico op een hartinfarct (Hazard Ratios, HR: 0.66; 95% betrouwbaarheidsinterval: 0.45-0.99) vergeleken met deelnemers met een lage consumptie (≤241 g/d). Deze bevinding komt overeen met de resultaten van eerder uitgevoerde prospectieve cohortonderzoeken en ondersteunen het belang van een hoge groente- en fruitinname in de preventie van hartinfarcten. Er werd echter geen verband gevonden tussen totale groente- en fruitconsumptie en het optreden van beroertes.

Bewerking van groente en fruit beïnvloedt de structuur en de voedingswaarde. Groente- en vruchtensappen bevatten minder voedingsvezel dan het oorspronkelijke product, maar zijn een goede bron van bioactieve stoffen. Door het koken van groente gaan wateroplosbare en hitte-gevoelige vitamines verloren. Echter, de opname van andere bioactieve stoffen door de darm kan hierdoor ook juist verbeteren. In eerdere prospectieve cohortonderzoeken werd geen onderscheid gemaakt tussen rauwe en bewerkte groente en fruit of dit werd niet gerapporteerd. In **Hoofdstuk 2** en **3** werd gevonden dat deelnemers met een hoge inname van rauwe groente en fruit (>262 g/d) een 30% lager risico hadden op een hartinfarct (HR: 0.70; 0.47-1.04) en op een beroerte (HR: 0.70; 0.47-1.03) ten opzichte van deelnemers met een lage inname (≤92 g/d). Er werd geen verband gevonden tussen de inname van bewerkte groente en fruit en hart- en vaatziekten. Deze bevindingen suggereren dat met name consumptie van rauwe groente en fruit belangrijk is voor de preventie van hart- en vaatziekten.

Naast het consumeren van voldoende groente en fruit wordt geadviseerd om hierin te variëren. Variatie in groente- en fruitconsumptie levert veel verschillende voedingsstoffen en bioactieve stoffen. In **Hoofdstuk 4** is bestudeerd of variatie in groente en fruit belangrijk is voor preventie van hart- en vaatziekten. Dit was nog niet eerder onderzocht in prospectieve cohortonderzoeken. Variatie was sterk gerelateerd aan de totale inname van groente en fruit. Ongeacht de totale inname was variatie in groente en fruit niet gerelateerd aan het optreden van een hartinfarct of beroerte. Variatie is echter moeilijk te operationaliseren met behulp van een voedselfrequentievragenlijst. De inname van bepaalde soorten groente en fruit is seizoensgebonden, waardoor de gemeten variatie in dit onderzoek mogelijk lager was dan in werkelijkheid. Daarnaast is de aanbeveling om te variëren in groente en fruit niet duidelijk gedefinieerd. Om deze redenen is meer prospectief cohortonderzoek nodig naar het belang van variatie in groente en fruit voor de preventie van hart- en vaatziekten. Een duidelijke definitie van de aanbeveling om gevarieerd groente en fruit te eten is hiervoor noodzakelijk.

In **Hoofdstuk 5** en **6** werden op basis van de kleur van het eetbare deel van groente en fruit vier kleurgroepen samengesteld: groen, oranje/geel, rood/paars en wit. Elke kleurgroep van groente en fruit is rijk aan een specifieke voedingstoffen of bioactieve stoffen. Niet eerder werden kleurengroepen van groente en fruit in relatie tot hart- en vaatziekten onderzocht. Elke extra dagelijkse inname van 25 gram dieporanje groente en fruit verlaagt het risico op een hartinfarct met 26% (HR: 0.74; 0.55-1.00). Dieporanje groente en fruit bestond voornamelijk uit wortelen (60%), die rijk zijn aan α- en β-caroteen. Deelnemers met een hoge inname van witte groente- en fruitsoorten (>171 g/d) hadden een 52% lager risico op beroertes (HR: 0.48; 0.29-0.77) vergeleken met deelnemers met een lage inname (≤78 g/d). Appels en peren waren de belangrijkste bron van deze witte groep (55%) en zijn rijk aan voedingsvezel en de flavonoïde quercetine. Deze bevindingen suggereren dat met name de witte en oranje kleurgroepen van groente en fruit mogelijk van belang zijn bij het ontstaan van hart- en vaatziekten. Deze resultaten dienen te worden bevestigd in prospectieve en interventieonderzoeken voordat aanbevelingen kunnen worden gedefinieerd voor de consumptie van kleurgroepen van groente en fruit in relatie tot hart- en vaatziekten.

In **Hoofdstuk 7** zijn de belangrijkste bevindingen geïnterpreteerd en bediscussieerd in de context van ander onderzoek. Daarnaast zijn de potentiële causaliteit van groente en fruit bij het ontstaan van hart- en vaatziekten en de mogelijke betekenis voor de volksgezondheid besproken. Het in dit proefschrift beschreven gunstige verband tussen de consumptie van groente en fruit en de inname van verschillende voedingsstoffen wordt ondersteund door kortdurende interventieonderzoeken die verhoogde bloedwaarden van vitamine C en carotenoïden lieten zien. Tot op heden zijn geen interventieonderzoeken verricht naar het effect van groente- en fruitconsumptie op de preventie van hart- en vaatziekten, maar wel naar intermediaire eindpunten. Uit deze interventieonderzoeken blijkt dat een hogere groente- en fruitconsumptie de bloeddruk verlaagt.

Uit meta-analyse van prospectieve cohortonderzoeken is gebleken dat hoge groente- en fruitconsumptie beschermt tegen hart- en vaatziekten. De resultaten die beschreven zijn in dit proefschrift laten zien dat totale groente- en fruitconsumptie het risico op hartinfarcten verlaagde, maar niet het risico op beroertes. Echter, consumptie van rauwe groente en fruit beschermde tegen zowel hartinfarcten als beroertes. Naast het advies van het Voedingscentrum om maximaal 1 stuk fruit per dag te vervangen door vruchtensap, zijn tot op heden geen specifieke aanbevelingen gedefinieerd voor rauwe of bewerkte groente en fruit. De resultaten gepresenteerd in dit proefschrift tezamen met resultaten van andere prospectieve cohortonderzoeken en de bloeddrukverlagende effecten gevonden in interventieonderzoeken suggereren dat tenminste 50% van de dagelijkse groente- en fruitconsumptie zou moeten bestaan uit rauwe producten ter preventie van hart- en vaatziekten. Resultaten van toekomstige prospectieve cohort- en interventieonderzoeken zijn noodzakelijk om betrouwbare aanbevelingen te definiëren.

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About the author

Curriculum Vitae

Linda Oude Griep was born in Utrecht, May 21st 1979. After completing secondary school at 'Willem de Zwijger' college in Schoonhoven (1996), she started her BSc studies in Human Nutrition and Dietetics at the 'Hogeschool van Amsterdam'. For her BSc thesis, Linda was introduced into the field of nutritional research at Maastricht University. She obtained her BSc degree in 2000 and decided to continue her education in Human Nutrition and Health at Wageningen University. Linda completed her MSc thesis at TNO Quality of Life entitled 'Subjective and physiological parameters related to satiety' and obtained her MSc degree with a major in Physiology. After her graduation in June 2003, Linda started working at the Division of Human Nutrition of Wageningen University. She became member of the research staff of the Alpha Omega Trial. This is a large multi-centre intervention study investigating the effects of low doses omega-3 fatty acids on cardiovascular diseases in 4,837 post-myocardial infarction patients. She became experienced in epidemiological fieldwork that motivated her to start her PhD training in nutritional epidemiology in July 2006. She first prepared a grant proposal entitled: 'Fruit and vegetable consumption and the risk of cardiovascular diseases' that was funded by the Productboard of Horticulture. For her PhD research, she was guest researcher at the National Institute of Public Health and the Environment (RIVM), Bilthoven to perform epidemiological analyses using data of the MORGEN Study. In 2007, Linda participated in the Ten-day International Teaching Seminar on Cardiovascular Disease Epidemiology and Prevention of the World Heart Federation. Linda joined the educational program of the graduate school VLAG, was involved in teaching at the BSc and MSc level and chaired the PhD committee of the Division of Human Nutrition. She attended several (international) conferences and courses in the field of nutrition, epidemiology and cardiovascular diseases, and received a poster prize from the Dutch Epidemiology Society (WEON, Amsterdam, 2009). Currently, Linda is appointed as postdoctoral fellow at the Division of Human Nutrition, Wageningen University, and is preparing research proposals.

Publications in scientific journals

Oude Griep LM, Verschuren WMM, Kromhout D, Ocké MC, Geleijnse JM. Colors of fruit and vegetables and 10-year incidence of stroke. Stroke, In press, DOI: 10.1161/STROKEAHA.110.611152

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Training and educational activities

Courses

- Multilevel analysis, National Institute of Public Health and the Environment, Bilthoven (NL), 2010
- Survival Analysis by Dr. Kleinbaum, Erasmus Summer Program, Rotterdam (NL), 2008
- Regression Analysis by Dr. S. Lemeshow, Erasmus Summer Program, Rotterdam (NL), 2008
- 40th Ten day seminar on Epidemiology and Cardiovascular health, World Heart Federation, Sommarøy (NO), 2007
- Socrates Intensive Program 'Food and Health III' on Functional foods, Cluj-Napoca (RO), 2007

Meetings

- 11th FENS Nutrition European Conference, Madrid (SP), 2011
- 51st Conference on Cardiovascular Disease Epidemiology and Prevention -and- Nutrition, Physical Activity and Metabolism– American Heart Association, Atlanta, Georgia (USA), 2011
- European Society of Cardiology Congress, Stockholm (SW), 2010
- EGEA Congress on 'Social and health benefits of balanced diet, The role of fruit and vegetables', Brussels (BE), 2010
- 50st Conference on Cardiovascular Disease Epidemiology and Prevention -and- Nutrition, Physical Activity and Metabolism– American Heart Association, San Francisco, California (USA), 2010
- 49th Cardiovascular Disease Epidemiology and Prevention -and- Nutrition, Physical Activity and Metabolism – American Heart Association, Palm Harbor, Florida (USA), 2009
- Nutritional Science Forum 'Too Much-too little', Arnhem (NL), 2009
- 3rd International congress MeDiet, 'Traditional Mediterranean diet: past, present and future', Athens (Gr), 2007
- Annual meetings of NWO-nutrition, WEON, EPIC-NL, NHS and NVVL-FNLI

General

- Advanced presentation- and mediatraining, Wageningen (NL), 2011
- Scientific writing, Wageningen (NL), 2009
- Carreer coaching, Wageningen (NL), 2009
- PhD study tour USA, 2007

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