PRO- AND ANTIOXIDANTS AND RISK OF ATHEROSCLEROSIS AND CORONARY HEART DISEASE IN THE ELDERLY

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PRO- AND ANTIOXIDANTS AND RISK OF ATHEROSCLEROSIS AND CORONARY HEART DISEASE IN THE ELDERLY

PRO- EN ANTIOXIDANTEN EN HET RISICO OP ATHEROSCLEROSE EN CORONAIRE HARTZIEKTEN BIJ OUDEREN

PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Erasmus Universiteit Rotterdam
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Prof. dr. P.W.C. Akkermans M.A.
en volgens het besluit van het College voor Promoties.

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Overige leden	Prof. Dr. U. Keil
	Prof. Dr. J.F. Koster
	Prof. Dr. M.L. Simoons

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K. Klipstein-Grobusch, J.C.M. Witteman, J.H. den Breeijen, R.A. Goldbohm, A. Hofman, P.T.V.M. de Jong, H.A. Pols and D.E. Grobbee. Dietary assessment in the elderly: application of a two-step semiquantitative food frequency questionnaire for epidemiologic studies. J Hum Nutr Diet. Accepted for publication.

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K. Klipstein-Grobusch, D.E. Grobbee, J.H. den Breeijen, H. Boeing, A. Hofman, J.C.M. Witteman. Dietary iron and risk of myocardial infarction in the Rotterdam Study. Am J Epidemiol 1999; 149:421-428.

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K. Klipstein-Grobusch, L. Launer, J.M. Geleijnse, H. Boeing, A. Hofman, J.C.M. Witteman: Serum carotenoids and atherosclerosis. The Rotterdam Study. Atherosclerosis. Accepted for publication.

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CHAPTER 1

Introduction

Coronary heart disease (CHD) is an increasing global problem carrying heavy social and economic costs. Coronary heart disease is responsible for about 50% of cardiovascular mortality, which itselfs accounts for 30-50% of all deaths in developed nations[1]. It is the major cause of premature death, in women as in men - women lagging behind men by some ten years in this age-related disease. Knowledge on risk factors for CHD has accumulated in recent years. Research evidence now lends strong support to the view that CHD is in part preventable by reduction of modifiable risk factors. The greater the number of risk factors known to be causally related to the disease, the greater the power to reduce the disease burden in the community by reducing the levels of such pathogenic risk factors, though depending on the modifiability of the risk factors and their strength related to disease. Diet is among the modifiable risk factors and provides a simple and non-pharmacologic method for prevention of atherosclerosis and coronary heart disease.

Steinberg and co-workers[2] hypothesized that low density lipoprotein (LDL) that has undergone oxidative damage is considerably more atherogenic than native LDL. Evidence that LDL oxidation occurs in vivo and contributes to the clinical manifestations of atherosclerosis supports the oxidative modification hypothesis. The link between oxidation of LDL and atherogenesis is hypothesized to be the basis for a beneficial effect of antioxidants on the incidence of subclinical and clinical coronary artery disease[3]. Observational studies have indicated that dietary antioxidants may exert a protective effect on incidence and mortality of coronary heart disease, though the evidence is not conclusive[4,5]. Free iron has been implicated in lipid peroxidation and ischaemic myocardial damage, and it has been suggested that iron is an independent risk factor for myocardial infarction[6]. Serum copper and ceruloplasmin as another complex of prooxidants operating through oxidative modification of LDL have been suggested to be independent risk factors for coronary heart disease[7].

The scope of this thesis is to describe and validate a new method of dietary assessment applied in the elderly population of the Rotterdam Study and subsequently focus on nutritional factors that may inhibit or promote the progression of atherosclerosis and coronary heart disease.

REFERENCES

1. Henderson AH: Coronary heart disease: overview. *Lancet* 1996; **348**:1-4.
2. Steinberg D, Parthasarathy S, Carew TE et al: Beyond cholesterol.: modifications of low-density lipoprotein that increase its atherogenicity. *N Engl J Med* 1989; **320**:915-924.
3. Diaz MN, Frei B, Vita JA et al.: Antioxidants and atherosclerotic heart disease. *N Engl J Med* 1997; **337**:408-416.
4. Jha P, Flather M, Lonn E et al.: The antioxidant vitamins and cardiovascular disease. A critical review of epidemiologic and clinical trial data. *Ann Intern Med* 1995; **123**:860-872.
5. Buring JE, Hennekens CH: Antioxidants and coronary heart disease: weighing the totality of the evidence. *J Cardiovasc Risk* 1996; **3**:343-345.
6. Sullivan JL: Iron and the sex difference in heart disease risk. *Lancet* 1981; **1**:1293-1294.
7. Salonen JT, Salonen R, Korpela H et al.: Serum copper and the risk of acute myocardial infarction: a prospective study in men in Eastern Finland. *Am J Epidemiol* 1989; **134**:268-276.

CHAPTER 2

Dietary assessment in the elderly

2.1

Dietary assessment in the elderly: validation of a semiquantitative food frequency questionnaire

ABSTRACT

Objective: The study was conducted to assess the relative validity of a 170-item semiquantitative food frequency questionnaire (SFFQ) adapted for use in the elderly.

Design & subjects: The study was carried out in a sample of 80 men and women aged 55 to 75 years participating in a community based prospective cohort study in Rotterdam, The Netherlands. The two-step dietary assessment comprised a simple self-administered questionnaire (20 min.) followed by a structured interview with trained dietitians (20 min.) based on the completed questionnaire. Multiple food records (FR) collected over a one year period served as reference method. 24-h urine urea was used as indirect marker for protein intake.

Results: Compared with FR, the SFFQ generally overestimated nutrient intake as reflected by difference in means and the ratio of SFFQ to FR. Energy adjustment reduced the observed overestimation. Pearson's correlation coefficients varied from close to 0.5 to about 0.9 for crude data, and after adjustment for age, sex, total energy intake, and for within-person variability in daily intake from 0.4 to 0.8. Cross-classification into quintiles resulted in correct classification into the same or adjacent quintile of 75.8% for crude and 76.8% for energy-adjusted data. Validation of protein intake estimated by SFFQ with protein excretion from 24-h urine urea indicated overestimation of protein intake by SFFQ. Spearman correlation coefficient between protein intake estimated from urea excretion and SFFQ was 0.67.

Conclusions: Adaptation of a SFFQ for use in the elderly resulted in a valid and time-efficient dietary assessment instrument. Its ability to adequately rank study subjects according to their dietary intake supports its application in epidemiological studies in the elderly.

INTRODUCTION

Dietary assessment has been difficult in epidemiological studies in elderly populations. Limited recall due to fading memory or disabilities in sight or attention may require a more complicated approach and may result in a higher respondent burden[1,2] and low participation rate. To simplify dietary assessment we adapted a previously validated semiquantitative food frequency questionnaire[3,4] for use in an elderly population. This paper describes the relative validity of the modified semiquantitative food frequency questionnaire (SFFQ) as compared to 15-d food records (FR) over a one year period in a sample of 80 men and women aged 55 to 75 years. As external marker for protein intake mean 24-h urine urea excretion of four non-consecutive days was used. The validity of the questionnaire is primarily defined as its ability to rank study subjects according to nutrient intake. Since the performance of any questionnaire also depends on the actual study population the present validation study was conducted within the population-based cohort of the Rotterdam Study.

METHODS

Subjects

Subjects recruited for the validation study comprised a sub-sample of the population-based Rotterdam Study[5] participating in a randomized double blind trial examining the effect of a reduced sodium and increased potassium and magnesium dietary intake on mild hypertension. Exclusion criteria were history of myocardial infarction, angina pectoris, diabetes mellitus, impaired renal function, a salt restricted diet on medical advice or antihypertensive treatment as previously described[6]. Of the 100 subjects (51 men, 49 women) enrolled in the study, complete follow-up was achieved in 97 persons. Two persons withdrew because of admission to hospital and one person because of other reasons. Dietary assessment by FR and SFFQ during the course of the validation study was successfully completed by 80 subjects (39 men, 41 women; participation rate 82.5%). Reasons for drop out during the one year period of dietary assessment were attributable to refusal and to unavailability or inability to manage keeping a food record diary.

Study design

Dietary assessment in the current validation study was undertaken as outlined in

figure 1. Multiple food records collected over a year to cover all seasons with respect to different food consumption patterns served as reference method. In total, fifteen days of food records distributed over six collection periods were obtained. Collection period one, five, and six consisted of each three consecutive days of dietary assessment. Collection period two, three, and four consisted of each two consecutive days of dietary assessment and fell in the intervention period of the trial[6]. The fifteen recording days were balanced across the days of the week. The modified semi-quantitative food frequency questionnaire (SFFQ) applied covered the habitual food intake during the preceeding year. It was administered during baseline recruitment of the Rotterdam Study and approximately two months after completion of the last three-day period of dietary assessment. Data for both SFFQs were obtained in 71 subjects and were used to estimate reproducibility of the SFFQ. The second SFFQ was used for assessment of relative validity of the SFFQ compared to FR.

Measurements

Semiquantitative food frequency questionnaire (SFFQ)

The SFFQ aims to assess habitual food intake during the past year. It contains 170 food items in 13 food groups and questions about dietary habits, supplementation, and prescribed diets. It is a version of a previously validated SFFQ[3,4] adapted for use in the elderly. The two-step dietary assessment comprised a simple self-administered questionnaire (20 minutes) followed by a structured interview with a dietitian (20 minutes allocated) based on the completed questionnaire. This method increases time efficiency and facilitates measuring diet in elderly subjects. The self-administered questionnaire consisted mainly of a meal-based checklist of foods which was filled in at home. Participants had to mark the foods they had consumed at least twice a month in the preceding year. The dietary interview was conducted at the study center by dietitians. The dietitians aimed at obtaining accurate information on amount and consumption frequency of food items noted by participants as consumed frequently in the questionnaire. For each item the frequency was recorded in times per day, week, or month. The number of servings per frequency was expressed in natural units (e.g. slice of bread, apple), household measures (e.g. cup, spoon) or grams (cooked vegetables, mixed dishes). Additional information about dietary habits, supplement use, (e.g. type, amount, frequency), and prescribed diets during the past year was also obtained.

Figure 1: Study design validation study

Time frame	1990-1992	Sept/Oct 1992	Dec/Jan 1992/3	Feb/Mar 1993	Apr/May 1993	Jun/Jul 1993	Jul/Aug 1993	Sept/Oct 1993
Status	Baseline Rotterdam Study	Baseline Validation Study						End of Validation Study
Measurements	SFFQ							SFFQ
		3-d FR 24-h urine	2-d FR 24-h urine	2-d FR 24-h urine	2-d FR 24-h urine	3-d FR	3-d FR	

SFFQ semiquantitative food frequency record
FR food record

Food records (FR)

The food record method was introduced and explained prior to the first recording period during a home visit by a dietitian. A food record diary including written instructions and examples was handed out. During the home visit the dietitian asked the participant to fill commonly used tableware and cutlery (glasses, spoons etc.) with water and recorded the obtained volume. Furthermore, the amount of butter, margarine, jam, spreads, cheese, and meat products used on bread were weighed and recorded, as was the amount of milk and sugar used in tea and coffee. Completed food record diaries were collected at the next visit to the study center. A dietitian checked for completeness of the food records while participants were present to answer any arising questions. Food records obtained during the dietary assessment collection period two to four fell into the intervention period of the trial (figure 1). Subjects assigned to the intervention group received a mineral salt for use in cooking and at the table (sodium:potassium:magnesium 8:6:1 mmol; SagaSalt© [Akzo Nobel, Netherlands]) and certain foods prepared with mineral salt. Controls received common salt and foods. Study foods included bread, cheese, meat products, and canned and instant soups. Salt and foods for both groups looked identical and were provided free-of-charge by means of a double blind coding system based on the randomization

numbers. Participants were asked to avoid changes in dietary habits and lifestyles during the intervention period. Whether they adhered to the protocol was checked by direct questioning. Provision of free-of-charge foods was monitored. To check if provision of free-of-charge foods influenced food and nutrient intake during this period, food group and estimated nutrient intakes were calculated separately for intervention (6 days of FR) and non-intervention period (9 days of FR). Differences in mean food intake due to intervention were evaluated by analysis of variance. Food intake during the intervention period was significantly higher for bread (146 vs. 139 g/d; $p = 0.047$), cheese (59 vs. 48 g/d; $p < 0.001$), and soups (300 vs. 269 g/d; $p = 0.045$) and significantly lower for vegetables (188 vs. 207 g/d; $p = 0.018$). Increased consumption of bread, cheese, and soups points towards elevated use of foods provided free-of-charge during the intervention period. However, decreased consumption of vegetables and increased consumption of soups may rather be attributable to seasonal influence, since the intervention period took place from December to May (figure 1). Food consumption during the intervention period translated into a statistically significant increased intake for energy, total protein, vegetable protein, total fat, monounsaturated fat, saturated fat, pyridoxine, niacin, sodium, calcium, and zinc. Adjustment for energy removed these effects for all nutrients except for iron, calcium, and sodium. To evaluate whether the intervention period affected the questionnaires ability to adequately rank study subjects according to their intake we compared Pearson's correlation coefficients between SFFQ and FR with (15-d FR) and without (9-d FR) the intervention period. Differences observed were in general minor. Deviations larger than 0.05 between the correlation coefficients of 9-d and 15-d of food records were only observed for saturated fat (0.50 versus 0.58), cholesterol (0.46 versus 0.53), mono/disaccharides (0.63 versus 0.70), and thiamine (0.50 versus 0.60) Given that an effect due to intervention was not clearly distinguishable from seasonal influence and because all seasons were to be balanced, we decided to consider 15 days of food recording for the present analysis.

Calculation of intake of nutrients and food groups

Mean individual nutrient intake per day was calculated from the FR as the average of the fifteen recording days. SFFQ data were converted to mean daily intake by multiplying consumption frequency, number of serving units and weight of unit (either standard or individual). The weight of a standard was derived from common Dutch

household measures. If the number of servings was omitted, the median number among all other questionnaires was taken instead. FR and SFFQ data were both converted to nutrient intake using the computerized Dutch Food Composition Table[7]. Nutrient intake through nutritional supplements was not considered since brand labels were not recorded with sufficient accuracy. For calculation of sodium intake only the sodium content of foods was considered.

Urine collections

Seventy-six participants completed the collection of four non-consecutive 24-h urine's as outlined in figure 1. They were instructed both in writing and orally about the method of urine collection and the necessity to obtain a complete 24-h urine collection. Completeness of the 24-h urines was checked by direct questioning when the urines were handed in. Data on the urine container were verified and, if necessary, missing information was obtained from participants. Urine samples were stored at -20°C before analysis. The urea concentration (mmol/l) was determined by spectrophotometry and multiplied by urine weight to obtain total urea (g/d). Assuming that urea N is a constant proportion (85%) of urinary nitrogen (U_n)[8], protein intake from 24-h urine collections was estimated as 6.25 x (U_n + 2) according to Isaksson[9]. A mean value of the four separate days was used to compare the estimated protein intake from urine excretion with the reported intake by FR and SFFQ.

Assessment of underreporting on group level

For evaluation of plausibility of dietary intake data, the ratio of energy intake (EI) to basal metabolic rate (BMR) was calculated. A cut-off depending on sample size and duration of dietary assessment identifying minimum plausible levels of energy expenditure expressed as a multiple of basal metabolic rate (BMR) for groups was defined according to Goldberg et al.[10]. Applying this cut-off enables detection of the extent of gross underreporting. In the current validation study BMR was predicted from standard equations based on the age-specific formulae published by Schofield et al.[11]. Subsequently, the ratio of EI to BMR for both SFFQ and FR was calculated and used for evaluation of underreporting that may have occurred and as a measure of external validation for intake of energy on group level. The cut-off for EI/BMR was predicted with 95% confidence interval to be 1.48 for both men and women on group level for FR and SFFQ.

Data analysis

Means and standard deviations for energy, nutrients, and energy-adjusted nutrients estimated by SFFQ and FR were calculated. Energy-adjusted nutrient intakes were derived by adding the median nutrient intake to the residuals from regression analysis of nutrient on energy intake[12]. In addition, the ratio of nutrient intake assessed by SFFQ to that assessed by FR and the difference between methods were calculated to enable a more detailed evaluation of agreement between methods. Mean differences in crude and energy-adjusted nutrient intake due to age and gender, period of dietary assessment, and day of the week were evaluated by analysis of variance.

Relative validity of the SFFQ compared to the food record method was assessed by calculating Pearson's correlation coefficients (unadjusted and adjusted for age, sex, and energy) between nutrient estimates of FR and SFFQ. To correct for variability in the day-to-day variation of food record measurements, correlation coefficients were deattenuated using the variance ratio (within-person variance divided by between-person variance) calculated from the repeated FR[13].

To evaluate the ability of both methods to classify individuals similarly in categories of nutrient intake, cross-classification of nutrient scores into quintile categories was carried out. Quintile categories were assigned for both assessment methods separately. The overall percentage of individuals classified into the same, the adjacent, or the extreme quintile category was determined. Furthermore, the study population was divided into quintile categories according to nutrient intake assessed by SFFQ. For each quintile category the corresponding mean nutrient intake as assessed from FR and SFFQ was calculated.

Skewed nutrient distributions were log-transformed (protein, total fat, saturated fat, monounsaturated fat, polyunsaturated fat, cholesterol, linolenic acid, mono/disaccharides, niacin, sodium, phosphorus, and magnesium) to reduce skewness and approximate normality. All statistical tests were performed on the transformed data. Analysis was performed by use of SAS® (Release 6.10; SAS Institute, Cary, NC, USA).

RESULTS

Table 1 shows selected characteristics for participants of the validation study and participants of the Rotterdam Study for whom dietary data were obtained during baseline recruitment. In general, there were no marked differences between both groups. Participants of the validation study were slightly younger (66.7 vs. 67.7 years) and heavier (75.9 vs. 73.6 kg). They were less often current cigarette smokers (18.8 vs. 23.3%), less often on a prescribed diet (7.0 vs. 13.4%) and used more often supplements (42.9 vs. 35.4%). The proportion of those living in a single household was considerably lower (17.7 vs. 26.2). During the one year period of dietary assessment in the validation study 7.5% of the subjects gained more than 3 kg of body weight and 18.8% lost more than 3 kg. A prescribed diet was followed by three women. None of them lost or gained more than 3 kg of body weight during the dietary assessment period.

For women, the mean ratio of energy intake to basal metabolic rate (EI/BMR) was below the cut-off (1.48) for both methods of dietary assessment (SFFQ: 1.46; FR: 1.41), whereas for men a value below the cut-off (1.48) was observed for FR (1.46) and above the cut-off for SFFQ (1.52). Comparison of EI/BMR for men and women combined with the predetermined cut-off indicates presence of underreporting of energy for FR (SFFQ: 1.49; FR: 1.44). Body mass index (BMI) was identified to be a determinant for underreporting of energy intake by SFFQ and FR. A significant inverse association (adjusted for age and sex) between BMI and EI/BMR (SFFQ: $r = -0.29$; $p = 0.010$; FR: $r = -0.36$; $p = 0.011$) was present. Other possible determinants in the occurrence of underreporting of energy such as weight change during the study or cognitive function showed no association with EI/BMR.

Crude and energy-adjusted nutrient intakes were significantly different for men and women and were age-dependent. Analysis of variance revealed that energy and nutrient intake for the collection period of FR were significantly different between the collection periods for most macronutrients (energy, total protein, vegetable protein, total fat, saturated fat, monounsaturated fat, polyunsaturated fat, total carbohydrates, mono/disaccharides, polysaccharides, water), and vitamin C. Adjustment for energy removed most of the difference between the collection periods; significant differences were only seen for total protein, water, magnesium, iron, sodium, potassium, and

Table 1: Comparison of baseline characteristics for participants of dietary assessment in the Rotterdam Study and participants of the current validation study (means [SD] if not otherwise stated)

	Study sample	
	Validation Study (n = 80)	Rotterdam Study (n = 5434)
Variables		
Women (%)	51.3	59.1
Age (years)	66.7 (4.6)	67.7 (7.8)
Body weight (kg)	75.9 (10.1)	73.6 (11.6)
Body mass index (kg/m^2)	27.0 (3.1)	26.3 (3.6)
Systolic blood pressure (mm Hg)	158 (14)	139(22)
Diastolic blood pressure (mm Hg)	90 (9)	74 (11)
Energy (kJ)[1]	8.89 (1.94)	8.26 (2.10)
Protein (% of energy)[1]	18.6 (3.0)	16.8 (3.1)
Fat (% of energy)[1]	42.3 (7.3)	43.2 (6.9)
Carbohydrates (% of energy)[1]	35.5 (6.9)	36.4 (6.2)
Obesity (%)[2]	16.3	14.3
Current cigarette smoking (%)	18.8	23.3
Consumption of alcohol (%)	85.9	79.2
Prescribed diet (%)	7.0	13.4
Use of supplements (%)	42.9	35.4
Single household (%)	17.7	26.2
Equivalent household income (%)		
< 1500 Hfl	18.8	20.8
> 3000 Hfl	5.0	10.0
College/university education (%)	3.8	8.5

[1] nutrient intake estimated from semiquantiative food frequency questionnaire

[2] defined as body mass index (BMI) ≥ 30

riboflavin. Linear trend analysis showed a decrease in nutrient intake with duration of dietary assessment.

In table 2 mean (SD) energy and nutrient intakes for SFFQ and FR are presented. For most macronutrients, minerals, and vitamins, mean intake as estimated by SFFQ was higher compared to intake estimated from (FR). Overestimation of SFFQ as compared to FR was also reflected by the mean difference of the nutrient estimates of both methods. The mean difference in energy intake of SFFQ to FR was 351 (SD1472) kJ/d. The difference in vegetable protein intake of 12.5 g/d (SD 8.3) and of mono/disaccharides of 8.2 g/d (SD 29.1) contributed most to the observed difference in energy intake. For further evaluation of agreement between methods, the ratio of nutrient intake assessed by SFFQ to that assessed by FR was calculated. For energy and all nutrients except for polyunsaturated fat, and sodium, values greater than 100% were obtained. Estimates of macronutrient intake by SFFQ were in general within 10% of the mean FR values. For most micronutrients the deviations from the FR results were higher than for the macronutrients.
Table 2 presents variance ratios used for deattenuation of the correlation coefficients. Also, correlation coefficients between SFFQ and FR obtained for crude data, data adjusted for age, sex, and energy, and data corrected for day-to-day variability of FR measurements are shown. Relative validity of SFFQ to FR as assessed by Pearson's correlation coefficients resulted in values for crude data ranging from 0.47 for water intake to 0.89 for alcohol intake (mean 0.65). Adjustment for age, sex, and energy resulted in slightly lower correlation coefficients for most nutrients (mean 0.57; range 0.39 (saturated fat) to 0.82 (alcohol). Correction of variability due to day-to-day variation of food record measurements yielded deattenuated and adjusted correlation coefficients in the range of 0.44 for iron to 0.85 for alcohol intake (mean 0.61). Due to their relatively large day-to-day variation, the effects of deattenuation were most pronounced for cholesterol, and vitamin C. In addition, crude data (intraclass correlation coefficient) on the reproducibility of the two SFFQ's administered on average two years apart are given in table 2. Intraclass correlation coefficients were between 0.49 for riboflavin and 0.88 for alcohol with a mean value of 0.70.

Cross-classification of nutrient scores into quintile categories was undertaken to evaluate the ability of both methods to classify individuals similarly in categories of nutrient

Table 2: Mean (SD) nutrient intake, SFFQ/FR ratio, mean difference (SD), and Pearson's correlation coefficient between SFFQ and FR

	SFFQ		FR 15 days		Variance ratio FR within/between subject variance	Difference SFFQ - FR			Ratio SFFQ/FR (%)	Intraclass corr. coeff.§ SFFQ0/SFFQ[2]	Pearson's correlation coefficient§		
	mean	SD	mean	SD		mean	SD	p[1]	mean	crude	crude	adj.[3]	adj.deatt.[3,4]
Energy (kJ)	8899	1937	8552	1828	1.09	351	1472	*	105	0.71	0.69	n.a.	n.a.
Total protein (g)	97.8	22.6	86.5	20.3	1.32	11.3	16.2	***	114	0.78	0.73	0.63	0.66
Vegetable protein (g)	42.7	10.4	30.2	7.7	1.10	12.5	8.3	***	145	0.56	0.62	0.57	0.59
Total fat (g)	84.6	25.7	83.3	24.0	1.55	1.2	21.6	n.s.	105	0.65	0.57	0.48	0.50
Monounsaturated fat (g)	29.5	9.8	30.0	9.0	1.74	0.3	8.8	n.s.	104	0.58	0.56	0.49	0.52
Polyunsaturated fat (g)	16.2	8.0	14.6	6.0	2.02	-0.5	8.6	n.s.	102	0.67	0.57	0.52	0.62
Saturated fat (g)	32.6	9.9	32.2	9.5	1.34	1.6	6.4	n.s.	116	0.64	0.49	0.39	0.52
Linolenic acid (mg)	13.4	7.5	10.5	5.5	1.01	2.9	6.2	**	140	0.56	0.54	0.52	0.54
Cholesterol (mg)	238	73	227	55	4.38	11.6	64.2	n.s.	107	0.77	0.53	0.48	0.59
Total carbohydrate (g)	224.1	59.1	217.6	50.5	0.81	6.5	41.6	n.s.	104	0.74	0.72	0.77	0.79
Mono/disaccharides (g)	106.9	40.7	98.7	30.2	1.01	8.2	29.1	*	110	0.68	0.70	0.74	0.76
Polysaccharides (g)	116.7	27.1	116.4	27.7	0.93	0.2	20.0	n.s.	101	0.63	0.73	0.64	0.66
Dietary fiber (g)	23.2	6.0	16.4	4.1	1.34	6.7	4.6	***	144	0.67	0.64	0.59	0.62
Alcohol[5] (g)	11.2	16.2	11.1	13.6	1.01	0.1	7.3	n.s.	109	0.81	0.89	0.82	0.85
Water (ml)	2634	605	2171	412	0.78	463	550	n.s.	123	0.88	0.47	0.52	0.53
Thiamine (mg)	1.33	0.34	1.06	0.24	2.26	0.27	0.27	***	128	0.72	0.60	0.42	0.45
Riboflavin (mg)	2.36	0.59	1.47	0.38	1.13	0.88	0.48	***	165	0.49	0.59	0.54	0.56
Pyrodixine (mg)	1.82	0.43	1.55	0.33	2.27	0.26	0.35	***	118	0.79	0.62	0.46	0.49
Vitamin C (mg)	105.2	45.8	91.5	39.6	2.70	13.5	38.4	**	121	0.75	0.68	0.64	0.70

table 2 continued

	SFFQ		FR 15 days		Variance ratio FR within/between subject variance	Difference SFFQ - FR			Ratio SFFQ/FR (%)	Intraclass corr. coeff.§ SFFQ0/SFFQ[2]	Pearson's correlation coefficient§		
	mean	SD	mean	SD		mean	SD	p[1]	mean	crude	crude	adj.[3]	adj.,deatt.[3,4]
Sodium[6](mg)	2360	756	2522	739	1.78	-161	577	*	95	0.71	0.73	0.55	0.58
Potassium (mg)	4725	1010	3550	701	1.42	1175	784	***	134	0.71	0.63	0.50	0.52
Iron (mg)	15.1	3.4	12.0	2.8	1.14	3.1	2.6	***	128	0.65	0.67	0.42	0.44
Calcium (mg)	1174	398	1022	364	0.96	152	305	***	119	0.79	0.73	0.70	0.72
Phosphorus (mg)	2108	810	1677	560	0.83	430	540	***	104	0.77	0.76	0.72	0.74
Magnesium (mg)	318.9	77.8	308.1	65.4	1.02	10.8	52.0	n.s.	114	0.80	0.75	0.69	0.71
Zinc (mg)	11.8	3.0	10.5	2.9	1.82	1.24	2.31	***	128	0.77	0.70	0.51	0.54

§ based on log-transformed values (protein, fat, saturated fat, monounsaturated fat, polyunsaturated fat, linolenic acid, cholesterol, mono/disaccharides, sodium, phosphorus, magnesium, niacin)

n.a. not applicable; n.s. not signifcant; * $p < 0.05$; ** $p < 0.001$; *** $p < 0.0001$

1 paired t-test (for polyunsaturated fat, linoleic acid, dietary fiber, alcohol, and pyridoxine Wilcoxon sign rank test)

2 intraclass correlation coefficient SFFQ0/SFFQ (SFFQ applied during baseline recruitment of the Rotterdam Study/SFFQ applied in the validation study); n = 71

3 correlation coefficient adjusted for age,sex, and energy

4 correlation coefficient deattenuated (corrected for within-person variation in food records)

5 alcohol intake for subjects consuming alcohol (FR: 84%; SFFQ: 88%); Spearman correlation coefficient

6 sodium content of foods only

Table 3: Comparison of SFFQ scores with mean daily nutrient intakes derived from FR, based on cross classification

	Overall proportion (%) of subjects classified into					
	same quintile category	adjacent quintile category	extreme quintile category	same quintile category	adjacent quintile category	extreme quintile category
				for energy - adjusted nutrients		
Energy	43.8	38.8	1.3	n.a.	n.a.	n.a.
Total protein	42.5	41.3	0.0	43.8	38.8	0.0
Vegetable protein	43.8	31.1	1.3	41.3	31.3	1.3
Total fat	36.3	36.3	0.0	32.5	36.3	0.0
Saturated fat	33.0	36.3	0.0	42.5	35.0	1.3
Monounsaturated fat	37.5	41.3	1.3	32.5	48.8	1.3
Polyunsaturated fat	27.5	53.8	1.3	35.0	43.8	1.3
Linolenic acid	31.3	43.8	1.3	30.0	42.3	1.3
Cholesterol	32.5	41.3	3.8	31.3	43.8	3.8
Total carbohydrates	43.8	37.5	0.0	48.8	40.0	0.0
Mono/disaccharides	50.0	36.3	0.0	51.3	30.0	0.0
Polysaccharides	50.0	36.3	0.0	36.3	50.0	1.3
Dietary fiber	42.5	37.5	0.0	35.0	42.5	2.5
Alcohol	70.0	22.5	0.0	75.0	26.3	1.3
Water	22.5	50.0	0.0	26.3	45.0	1.3
Thiamine	38.8	30.0	1.3	37.5	41.3	1.3
Riboflavin	37.5	33.8	0.0	33.8	35.0	1.3
Pyrodixine	37.5	40.0	0.0	38.8	40.0	1.3
Vitamin C	46.3	30.0	0.0	47.5	32.5	0.0
Sodium	48.8	35.0	1.3	47.5	37.5	0.0
Potassium	41.3	36.3	1.3	40.0	41.3	1.3
Iron	42.5	42.5	0.0	40.0	35.0	0.0
Calcium	36.3	42.5	0.0	42.5	33.8	0.0
Phosphorus	55.0	36.3	0.0	48.8	33.8	1.3
Magnesium	46.3	32.0	0.0	43.8	42.5	0.0
Zinc	34.0	46.3	0.0	33.8	46.3	0.0

n.a. not applicable

Table 4: Mean nutrient intake assessed by food records according to quintile categories of nutrient intake assessed by FR and SFFQ, respectively

	Quintile categories based on FR					Quintile categories based on SFFQ				
	Q1	Q2	Q3	Q4	Q5	Q1	Q2	Q3	Q4	Q5
Energy (kJ)	6393	7489	8251	9280	11339	6774	8050	8318	9063	10506
Percent energy from										
Protein	14.3	15.8	16.6	17.8	20.7	14.9	16.3	16.9	18.1	18.9
Fat	29.7	33.8	36.3	39.3	42.8	34.2	34.9	36.7	36.6	39.5
Carbohydrates	33.1	39.6	43.3	47.0	51.2	35.6	40.0	44.2	44.2	50.3
Total protein (g)	63.4	75.7	83.8	93.5	116.1	70.9	74.4	86.3	93.6	107.4
Vegetable protein (g)	20.6	25.7	30.1	32.9	41.7	23.1	26.8	32.5	32.8	35.8
Total fat (g)	55.9	68.1	79.9	93.0	119.8	65.6	76.4	78.5	87.2	109.0
Saturated fat (g)	21.3	27.4	30.7	36.1	45.7	25.5	30.6	31.1	32.8	41.2
Monounsaturated fat (g)	19.1	24.3	28.6	34.4	43.7	23.7	26.0	30.7	31.8	37.9
Polyunsaturated fat (g)	7.6	11.1	13.4	16.2	24.5	9.7	12.2	14.3	17.4	19.2
Linolenic acid (mg)	4.7	7.2	9.2	11.9	19.7	6.6	8.7	10.0	11.5	15.9
Cholesterol (mg)	157.0	193.8	219.2	258.1	306.1	209.2	190.4	220.3	242.7	271.8
Total carbohydrate (g)	151.2	190.0	216.2	238.1	292.1	166.1	200.9	214.0	236.5	270.3
Mono/disaccharides (g)	60.1	80.3	93.6	115.9	143.9	67.5	87.5	99.5	115.5	123.7
Polysaccharides (g)	81.1	99.5	116.1	127.4	157.8	87.7	103.6	113.9	135.3	141.4
Dietary fiber (g)	10.9	14.0	16.3	18.3	22.6	13.1	14.8	16.0	17.7	20.4
Alcohol (g)	0.6	3.1	8.8	16.9	35.4	1.9	4.2	8.6	15.6	33.9
Water (ml)	1620	1949	2171	2371	2744	1890	1977	2220	2252	2516
Thiamine (mg)	0.75	0.92	1.02	1.17	1.41	0.88	0.99	0.97	1.17	1.28
Riboflavin (mg)	0.98	1.27	1.44	1.66	2.02	1.17	1.30	1.53	1.60	1.77
Pyridoxine (mg)	1.11	1.37	1.56	1.73	2.01	1.22	1.43	1.57	1.73	1.82
Vitamin C (mg)	44.7	68.6	86.7	108.0	149.4	58.3	74.0	94.9	103.0	127.4
Sodium from food (mg)	1663	2097	2452	2744	3652	1846	2291	2483	2620	3368
Potassium (mg)	2608	3160	3532	3924	4529	2853	3371	3589	3853	4086
Iron (mg)	8.4	10.6	11.8	12.9	16.1	9.2	10.8	11.7	14.0	14.2
Calcium (mg)	575	833	1003	1175	1522	645	1014	976	1095	1379
Phosphorus (mg)	1062	1337	1577	1870	2541	1182	1352	1616	1917	2320
Magnesium (mg)	223.8	272.8	301.0	333.8	409.3	236.7	287.7	292.5	351.6	372.2
Zinc (mg)	7.3	9.0	10.2	11.3	14.8	8.3	9.3	10.4	11.4	13.3

intake. Table 3 presents the proportion of subjects classified into the same, the adjacent, and the extreme quintile category for crude and energy-adjusted nutrients. On average 39.0 % (crude) and 38.9 % (energy-adjusted) of the subjects were correctly classified into the same quintile category by both methods of dietary assessment. Severe misclassification i.e. classification of subjects into the extreme quintile category occurred only for 0.5 % (crude data) respectively 1.0 % (energy-adjusted data) subjects. Overall, classification into the same or adjacent quintile category was 75.8 %, respectively 76.8 % (energy-adjusted data). The SFFQ's ability to separate nutrient intake as estimated by FR by quintile derived from the SFFQ is demonstrated in table 4, where nutrient intakes of FR according to quintile categories of nutrient intake determined by both SFFQ and FR are presented. Mean nutrient intakes as determined by FR reflected the greater variance in estimates of nutrient intakes compared to SFFQ.

Protein intake as calculated from the mean urea excretion from four 24-h urine collections was 81.2 g/d (SD 16.0) and was on average 6.2 g/d (SD 14.1) lower than protein intake from FR (87.4 g/d (SD 19.9)), and 17.8 g/d (SD 15.4) from SFFQ (99.0 g/d (SD 21.8)). The mean difference in protein intake was somewhat higher in men than in women. Spearman correlation coefficients between protein intake estimated from urea excretion and FR and SFFQ were 0.69 ($p = 0.001$) and 0.67 ($p < 0.001$) respectively. The proportion of subjects classified into the same, adjacent, and extreme quintile category by protein intake estimated from urea excretion and methods of dietary assessment was 42.1%, 38.2% and 0% for SFFQ and 43.4%, 38.2% and 1.3% for FR, respectively. Discrepancy in protein intake by FR and SFFQ to protein intake calculated from urea excretion was not associated with age, BMI, body weight, or weight change during the study.

DISCUSSION

We evaluated the relative validity of nutrient intake estimated by a SFFQ adapted for dietary assessment in the elderly as compared to 15-day estimated food records. Measures of concordance used in the present validation study encompassed the correlation coefficient, cross-classification by quintile categories, the difference between means, and the ratio of SFFQ to FR. Correlation coefficient and cross-classification are usually considered as an index of accuracy by which the

questionnaire assessment can rank individuals by dietary intake level, while the difference between means and the ratio between the two methods of dietary assessment express the average tendency of individuals to over- or underestimate their dietary intake. Compared to FR, the SFFQ generally overestimated nutrient intake as reflected by difference in means and the ratio of SFFQ to FR greater than 100% for all nutrients except sodium. Overestimation of protein intake estimated from SFFQ was furthermore indicated by independent validation of protein intake with protein excretion calculated from 24-h urine urea. The ability of the SFFQ to rank subjects adequately according to their dietary intake was reflected by Pearson's correlation coefficients varying from close to 0.5 to about 0.9 for crude, and 0.4 to 0.8 for data adjusted for age, sex, total energy intake and within-person variability in daily intake, respectively, and a degree of correct classification into the same or adjacent quintile of 75.8% for crude and 76.8% for energy-adjusted data, respectively.

Given that neither method of dietary assessment is perfect, it is crucial that the errors of both methods be as independent as possible to avoid spuriously high estimates of validity. Among the available and feasible comparison methods for validating a (S)FFQ, diet records are likely to have the least correlated errors[14]. Probably the main source of error in the reference method is the possibility of a change in diet due to the measurement process itself, while the main sources of error in the questionnaire are the ability of the subject to describe the usual frequency of intake, restrictions imposed on a fixed list of foods, memory, interpretation of questions, and the assumption of average serving sizes for most foods. Diet records are open-ended and do not depend on memory. They allow a more direct assessment of portion sizes, and errors in interpretation relate to the dietitian coding the records rather than to the subjects[15]. The choice of recording pattern for estimated food records was based on the consideration that it is likely that several short recording periods are less demanding for the elderly participants than one long period. Thereby, we expected to reduce the chances of having changes and simplifications of the diet during the recording days.

Ideally, the subjects in a validation study should be a random sample of the study population in which the questionnaire is being used. In the present study, validation was carried out in a subsample of the population-based cohort of the Rotterdam Study, recruited for a study on blood pressure[6]. Since the main focus of the study was on

blood pressure reduction subjects may have been less focused on their diet than participants recruited specifically for a validation study. With respect to the cohort of the Rotterdam Study this may have resulted in a less selected and more representative study sample, a view that is supported by comparison of participants by selected baseline characteristics (table 1) with participants of the Rotterdam Study that underwent dietary assessment. However, embedding the validation study in a hypertension trial may have had a direct effect on dietary intake given that certain foods were provided free-of-charge during the intervention period. To check whether inclusion of these records affected the results of the current validation study we compared mean dietary energy-adjusted intake on group level for the intervention period with the non-intervention period and found only minor significant differences in energy-adjusted nutrient intake. These differences could possibly be explained by seasonal effects since the intervention period took place during the winter time. We also investigated whether exclusion of the six recording days that fell into the intervention period had an effect on Pearson correlation coefficients, e.g. on the questionnaire's ability to adequately rank study subjects. Again, differences observed were minor and not of the magnitude to suspect serious bias. Based on these results we decided to include the dietary records collected during the intervention period to have all seasons balanced and to obtain more stable estimates.

Conscious or unconscious underreporting of foods can have a profound effect on the quality of the reference data and thus on the apparent validity of the method to be evaluated. Presence of underreporting is now generally considered for all dietary assessment methods[16]. For evaluation of possible underreporting that may have occurred in estimates of nutrient intake and as a measure of indirect validation for energy intake on group level in the current study, the ratio of energy intake to basal metabolic rate was used (EI/BMR). EI/BMR is regarded to be a satisfactory independent validator of energy intake[17-19]. Comparing mean EI/BMR for FR and SFFQ with a pre-determined cut-off indicated the presence of underreporting of energy on group level for FR. Declining motivation of participants to record their food intake with duration of dietary assessment or possibly use of the recording period as an opportunity for weight loss by some participants may be likely explanations. The observed significantly inverse relation between BMI and energy intake observed in the

current study and several other studies[20-25] points towards marked underreporting by obese subjects.

Besides the use of EI/BMR for evaluation of possible underreporting, we used 24-h urine urea excretion collected over four non-consecutive days as an independent measure of validity of dietary protein intake. The reported protein intake tended to be higher than the protein output as calculated from urea excretion. This was more pronounced for SFFQ than FR. Overall, mean urea excretion per day in the current study corresponded well with reference values for analytes of 24-h urine collections known to be complete[8]. The Spearman correlation coefficient obtained was of similar magnitude than in other studies[26,27] indicating the questionnaire's ability to adequately rank subjects acccording to their protein intake.

The SFFQ validated in the present study is an adapted version for use in the elderly of a previously validated SFFQ compared to three 3-day FR conducted 4-5 months apart. On average, the original questionnaire covered 85% on food level and 91% on nutrient level of the food record intake. Food intake of the original SFFQ was lower for most food groups except for vegetables, citrus fruit, bread, and added fats compared to three 3-day FR. On nutrient level, intake estimated by the questionnaire resulted in a lower intake for most nutrients except for vegetable protein, polyunsaturated fat, dietary fiber, and vitamin C. Correlation coefficients between the original version of the SFFQ and 9-d FR ranged from 0.40 - 0.83 for crude and 0.33 - 0.79 adjusted for age and sex, respectively[3]. Questionnaire modification was mainly determined by use of a two-step dietary assessment approach and more detailed information on vegetable, fruit, and meat consumption, and inclusion of some additional items. In combination with the different way of administration - partly self-administered (a questionnaire mainly consisting of a meal-based checklist of foods to fill in at home) and partly by a dietary interview conducted by trained dietitians - these modifications may have contributed to the differences in the results of both methods.

In epidemiological studies nutrient intakes are usually classified in quintiles, quartiles or tertiles for calculation of measures of disease association. Thus information of the ability of the applied questionnaire to correctly assign subjects according to their dietary intake is important to obtain correct associations and risk estimates. The SFFQ

validated in the current study showed a relatively high degree of correct classification within one quintile category of dietary intake (75.8 % for crude, respectively 76.8 % for energy-adjusted data) and a low level of severe misclassification (0.5 % for crude, respectively 1.0 % for energy-adjusted data). In comparison to other studies conducted in the elderly[15,19,29,30] the questionnaire demonstrated similar ability to correctly assign individuals according to their dietary intake.

Published correlation coefficients between nutrient intakes recorded by a (S)FFQ and by a reference method have varied considerably depending e.g. on the reference method applied and the number of days recorded. The correlation coefficients observed in the current study ranged from close to 0.5 to about 0.9 for crude and from 0.4 to 0.8 for adjusted data indicated relatively good validity and were similar to results of studies where either a (S)FFQ or dietary history were validated in the elderly[15,18,19,28,30-34]. In comparison with results obtained in validation studies conducted in middle-aged populations, results obtained in the elderly were by no means less reliable and valid[3,4,29,35-41]. This supports the idea that age has little adverse effects on the validity of questionnaires if administered appropriate, e.g. by interview as pointed out by Block & Hartman[42] by use of memory strategies, prior notification of a dietary interview[43], or by combination of methods[2], and adaptation of existing dietary assessment methods as advocated in the present study. Our adapted SFFQ based on a two-step dietary assessment may be used with confidence in dietary assessment of older subjects in epidemiologic studies.

References

1. Kelsey JL, O'Brien LA, Grisso JA, et al. Issues in carrying out epidemiologic research in the elderly. *Am J Epidemiol* 1989; 130:857-866.
2. van Staveren WA, de Groot LCPGM et al. Assessing diets of elderly people: problems and approaches. *Am J Clin Nutr* 1994; 59:221S-223S.
3. Goldbohm RA, van den Brandt PA, Brants HAM et al. Validation of a dietary questionnaire used in a large-scale prospective cohort study on diet and cancer. *Eur J Clin Nutr* 1994; 48:253-265.
4. Goldbohm RA, van't Veer P, van den Brandt PA et al. Reproducibility of a food frequency questionnaire and stability of dietary habits determined from five annually repeated measurements. *Eur J Clin Nutr* 1995; 49:420-429.
5. Hofman A, Grobbee DE, De Jong PTVM et al. Determinants of disease and disability in the elderly: The Rotterdam Elderly Study. *Eur J Epidemiol* 1991; 7:403-422.

6. Geleijnse JM, Witteman JCM, Bak AAA et al. Reduction in blood pressure with a low sodium, high potassium, high magnesium salt in older subjects with mild to moderate hypertension. *BMJ* 1994; **309**:436-440.
7. Food and Nutrition Council. *(Dutch food composition table NEVO).* 1993. The Hague, Netherlands: Voorlichtingsbureau voor de Voeding (in Dutch).
8. Bingham SA, Williams R, Cole TJ et al. Reference values for analytes of 24-h urine collections known to be complete. *Ann Clin Biochem* 1988; **25**:610-619.
9. Isaksson B. Urinary nitrogen output as a validity test in dietary surveys. *Am J Clin Nutr* 1980; **33**:4-5.
10. Goldberg GR, Black AE, Jebb SA et al. Critical evaluation of energy intake data using fundamental principles of energy physiology: 1. Derivation of cut-off limits to identify under-recording. *Eur J Clin Nutr* 1991; **45**:569-581.
11. Schofield WN, Schofield C & James WTP. Basal metabolic rate. *Hum Nutr Clin Nutr* 1985; **39C**, Suppl 1:1-96.
12. Willett WC, Stampfer MJ. Total energy intake: implications for epidemiologic analysis. *Am J Epidemiol* 1986; **124**:17-27.
13. Beaton GH, Milner J, Corey P et al. Source of variance in 24-hour dietary recall data: implications for nutrition study design and interpretation. *Am J Clin Nutr* 1979; **32**:2546-2559.
14. Willett W. *Nutritional Epidemiology.* 1990. Oxford University Press, Oxford.
15. Horwarth CC. Validity of a short frequency questionnaire for estimating nutrient intake in elderly people. *Br J Nutr* 1993; **70**:3-14.
16. Black AE, Goldberg GR, Jebb SA et al. Critical evaluation of energy intake data using fundamental principles of energy physiology: 2. Evaluating the results of published surveys. *Eur J Clin Nutr* 1991; **45**:583-599.
17. Garrow JS. Validation of methods for estimating habiutal diet: proposed guidelines. *Eur J Clin Nutr* 1995; **49**:231-232.
18. Rothenberg E. Validation of the food frequency questionnaire with the 4-day record method and analysis of 24-h urinary nitrogen. *Eur J Clin Nutr* 1994; **48**:725-735.
19. Nes M, Frost Andersen L, Solvoll K et al. Accuracy of a quantitative food frequency questionnaire applied in elderly Norwegian women. *Eur J Clin Nutr* 1992; **46**:809-821.
20. Schoeller DA. How accurate is self-reported dietary energy intake? *Nut Rev* 1990; **48**:373-379.
21. Livingstone MBE, Prentice AM, Strain JJ et al. Accuracy of weighed dietary records in studies of diet and health. *BMJ* 1990; **300**:708-712.
22. Heitmann BL. The influence of fatness, weight change, slimming history and other lifestyle variables on diet reporting in Danish men and women aged 35-65 years. *Int J Obes Relat Metab Disord* 1993; **17**:329-336.
23. Lichtman SW, Pisarska K, Berman ER et al. Discrepancy between self-reported and actual caloric intake and excercise in obese subjects. *N Eng J Med* 1992; **327**:1893-1898.
24. Johnson RK, Goran MI, Poehlmann ET. Correlates of over- and underreporting of energy intake in healthy older men and women. *Am J Clin Nutr* 1994; **59**:1286-90.

25. Klesges RC, Eck LH, Ray JW. Who underreports dietary intake in a dietary recall? Evidence from the Second National Health and Nutrition Examination Survey. *J Consult Clin Psychol* 1995; 6:438-444.
26. Bingham SA, Cassidy A, Cole TJ et al. Validation of weighed records and other methods of dietary assessment using the 24-h urinary technique and other biological markers. *Br J Nutr* 1995; 73:531-550.
27. Porrini M, Gentile MG, Fidanza F. Biochemical validation of a self-administered semiquantitative food-frequency questionnaire. *Br J Nutr* 1995; 74:323-333.
28. Mares-Perlman JA, Klein BEK, Klein R et al. A diet history questionnaire ranks nutrient intakes in middle-aged and older men and women similarly to multiple food records. *J Nutr* 1993; 123:489-501.
29. Jain M, Howe GR, Rohan T. Dietary assessment in epidemiology: comparison of food frequency and a dietary history questionnaire with a 7-day food record. *Am J Epidemiol* 1996; 143:953-960.
30. Tjoenneland A, Overvad K, Haraldsdóttir J et al. Validation of a semiquantitative food frequency questionnaire developed in Denmark. *Int J Epidemiol* 1991; 20:906-912.
31. van Staveren WA, Burema J, Livingstone MBE et al. Evaluation of the dietary history method used in the SENECA study. *Eur J Clin Nutr* 1996; 50:S47-S55.
32. Grootenhuis PA, Westenbrink S, Sie CMTL et al. A semiquantitative food frequency questionnaire for use in epidemiologic research among the elderly: Validation by comparison with dietary history. *J Clin Epidemiol* 1995; 48:859-868.
33. Mahalko JR, Johnson LAK, Gallagher SK et al. Comparison of dietary histories and seven-day food records in a nutritional assessment of older adults. *Am J Clin Nutr* 1985; 42:542-533.
34. Munger RG, Folsom AR, Kushi LH et al. Dietary assessment of older Iowa women with a food frequency questionnaire: nutrient intake, reproducibility, and comparison with 24-hour dietary recall interviews. *Am J Epidemiol* 1992; 136:192-200.
35. Block G, Hartman AM, Naughton D. A reduced dietary questionnaire: development and validation. *Epidemiology* 1990; 1:58-64.
36. Rimm EB, Giovannucci EL, Stampfer MJ et al. Reproducibility and validity of an expanded self-administered semiquantitative food frequency questionnaire among male health professionals. *Am J Epidemiol* 1992; 135:1114-1126.
37. Willett WC, Sampson L, Stampfer MJ et al. Reproducibility and validity of a semiquantitative food frequency questionnaire. *Am J Epidemiol* 1985; 122:51-65.
38. Pietinen P, Hartman AM, Haapa E et al. Reproducibility and validity of dietary assessment instruments. I. A self-administered food use questionnaire with a portion size picture booklet. *Am J Epidemiol* 1988; 128:655-666.
39. Pietinen P, Hartman AM, Haapa E et al. Reproducibility and validity of dietary assessment instruments. II. A qualitative food frequency questionnaire. *Am J Epidemiol* 1988; 128:667-676.
40. Engle A, Lynn LL, Koury K et al. Reproducibility and comparability of a computerized, self-administered food frequency questionnaire. *Nutr Cancer* 1990; 13:281-292.

41. Gnardellis C, Trichopoulou A, Katsouyanni K et al. Reproducibility and validity of an extensive semiquantiative food frequency questionnaire among Greek school teachers. *Epidemiology* 1995; 6:74-77.
42. Block G, Hartman AM. Issues in reproducibility and validity of dietary studies. *Am J Clin Nutr* 1990; 50:1133-1138.
43. Chianetta MM, Head MK. Effect of prior notification on accuracy of dietary recall in the elderly. *J Am Diet Assoc* 1993; 93:572-579.

2.2

Dietary assessment in the elderly: application of a two-step semiquantitative food frequency questionnaire for epidemiologic studies

ABSTRACT

Objective: Description and application of an adapted semiquantitative food frequency questionnaire (SFFQ) for dietary assessment in the elderly population of the Rotterdam Study

Design: Dietary assessment consisting of a two-step approach was performed in 5434 participants (2225 men, 3029 women) of the Rotterdam Study from 1990 to 1993, a population-based prospective cohort of 7983 subjects aged 55 to 95 years (participation rate 78%).

Statistical analysis: Nutrient intake was calculated for men and women in four age groups (55-64 years, 65-74 years, 75-84 years, 85-95 years) and linear trend analysis for differences in mean nutrient intake across age groups (55-64 years, 65-74 years, and 75-95 years) by regression analysis was conducted. Influence of baseline characteristics on energy and nutrient intakes adjusted by age and sex was investigated by one-way-analysis of variance.

Results: The adapted SFFQ made it possible to measure nutrient intake in the elderly within a limited time frame (2 x 20 min) across a wide age range (55 to 95 years). For nutrient intake we observed a general decline in mean intake of energy and most nutrients with age in men. In women the relation with age was not consistent: for most nutrients mean intake showed a decrease by age (e.g. water, magnesium, potassium), for some an increase (e.g. total fat, saturated fat, mono/di-saccharides), and some nutrients showed no substantial change (e.g. calcium, retinol). Reported nutrient intake was influenced by body mass index, smoking status, socioeconomic status, and activites of daily living. A prescribed diet was reported by 12.9% of participants and 34.6% used supplements on a regular basis.

Conclusions: The described two-step approach for dietary assessment in the elderly facilitated collection of data on dietary habits across a wide age range and within a limited time frame making it a suitable instrument for application in large-scale epidemiological studies in the elderly .

INTRODUCTION

Diet is an important aspect of lifestyle and may affect life expectancy, morbidity and mortality of older adults. Data on the nutrient intake and the nutritional status of the elderly in large-scale studies are scarce, especially for those older than 75 years of age. So far, application of appropriate methods for dietary assessment in epidemiologic studies has been difficult in elderly populations. Limited recall due to fading memory or disabilities in sight or attention may require a more complicated dietary assessment and may result in a higher respondent burden[1,2] and low participation rate. To simplify dietary assessment without loss of validity and precision, a semiquantitative food frequency questionnaire was adapted for use in an elderly population. In this paper the method of dietary assessment will be introduced and nutrient intake in participants of the Rotterdam Study will be described.

METHODS

Study Design and Subjects

The Rotterdam Study is a community based prospective cohort study among 7983 subjects aged 55 years and over, living in Ommoord, an urban district in Rotterdam, the Netherlands. The rationale and design of the Rotterdam Study have been described previously[3]. In short, the Rotterdam Study is a large-scale prospective cohort study on prevalence, incidence, and determinants of cardiovascular, neurologic, locomotor, and ophthalmologic diseases. At baseline the study comprised an extensive general home interview (participation rate 78%) and two visits at a research center for a clinical examination (participation rate 69%). The study has been approved by the Medical Ethics Committee of Erasmus University, Rotterdam, and written consent was obtained from participants. Dietary assessment was undertaken to investigate the role of nutrition and nutrition-related factors in the development of chronic diseases. Dietary assessment was performed during the second visit of the participants (n = 7006) at the research center by trained dietitians. It was not performed in participants of the pilot study (n = 277), in those living in nursing homes (n = 479), and in persons with known reduced cognitive function assessed by a neuropsychological test prior to dietary assessment (n = 122). Due to logistic reasons (e.g. unavailability of a dietitian) no dietary assessment could be carried out in an additional 482 persons. A dietary interview was thus obtained in 5646 persons. 212 subjects were excluded from analysis because dietitians considered

their reported dietary intake unreliable, i.e because participants# answers during the dietary interview were either too inconsistent or too incomplete. This resulted in 5434 participants, 2225 men (41%) and 3209 women (59%), aged 55 to 95 years of age, for the present dietary analysis.

Dietary Assessment by Semiquantitative Food Frequency Questionnaire (SFFQ)

The instrument to assess nutrient intake in the Rotterdam Study is a modification of a self-administered mailed semiquantitative food frequency questionnaire (SFFQ) used in a large-scale prospective cohort study on diet and cancer in the Netherlands (NLCS)[4]. Data on the validity and reproducibility of the original questionnaire with 150 food items have been reported[5,6]. Modification consisted mainly of a different mode of administration, collection of more detailed information on vegetable, fruit, and meat consumption, and inclusion of some additional items. The modified SFFQ contained 170 food items in 13 food groups and general questions about dietary habits (e.g. type of coffee, use of milk/sugar in coffee, change in food habits) to assess habitual food intake during the past year. The dietary assessment was undertaken in two consecutive phases as outlined in figure 1. First, participants were asked to report foods consumed regularly (at least twice a month) by use of a self-administered questionnaire consisting mainly of a meal-based checklist of foods. The questionnaire was explained to each participant during a home visit by a trained research assistant. It was filled in at home and collected at the research center. Participants had to mark the foods they had consumed at least twice a month in the preceeding year. Questions on fruit and vegetables were asked with respect to season (summer and winter), and preparation (raw, cooked). In addition, type and brand of dietary fats and oils used on bread, for cooking or as salad dressing were asked. Finally, subjects were asked whether additional foods not included in the questionnaire were consumed on a regular basis (frequency and amount specified). Average time for completion of the questionnaire was 20 minutes. The availability of the completed checklist of foods allowed for a more time-efficient dietary interview at the research center. The dietary interview was conducted by dietitians trained in the use of this method. The dietitians aimed at obtaining accurate information on amount and consumption frequency of food items noted by participants as consumed frequently. For each item the frequency was recorded in times per day, week, or month. The number of servings per frequency was expressed in natural units (e.g. slice of bread, apple), household measures (e.g. cup, spoon) or grams (cooked vegetables, mixed dishes).

General questions to check the consistency of the completed dietary questionnaire were asked, such as for the total number of warm meals per week.
Additional information about dietary habits and supplement use (e.g. type, amount, and frequency) during the past year was obtained, as well as comprehensive data on prescribed diets that were currently followed. The time allocated to the dietary interview was 20 minutes, thus, time needed to complete both parts of the dietary assessment was approximately 40 minutes.

Figure 1: Dietary assessment in the Rotterdam Study

	Phase 1: **Self-administered questionnaire**	***Phase 2:*** **Dietary interview**
Place of application	at home	at the study center
Method of dietary assessment	meal-based checklist of foods	structured interview
Mode of application	self-administered	by dietitian
Type of information collected	foods consumed at least twice a month over the past year	quantification (amount, frequency) of reported food items ***based on the self-administered questionnaire*** over the past year
	in addition questions on • dietary fats/oils (type) • vegetables/fruits (season; preparation) • additional foods consumed regularly and not listed in the questionnaire	in addition questions on • reliability • dietary habits • prescribed diets • supplements
Time for completion	20 min (average)	20 min (allocated)

All data were entered into an interactive computer programme. Average time for data entry was 20 minutes. For calculation of frequency and amounts of foods consumed a computer application was developed. Questionnaires were checked for completeness by

an interactive data-entry programme and automatically coded for later conversion into nutrients. The program checked further for internal consistency, range, and other response errors. The conversion from foods to energy and nutrient intakes was established with a computerized version of the Dutch Food Composition Table[7]. Intake through dietetic products and nutritional supplements was not taken into account since brand labels were not recorded with sufficient accuracy.

Validity of the modified SFFQ was assessed in a subsample of 80 men and women aged 55 to 75 years of the Rotterdam Study as previously described[8]. Multiple food records (FR) collected over a one year period served as reference method. 24-h urine urea was used as indirect marker for protein intake. Compared with FR, the SFFQ generally overestimated nutrient intake as reflected by difference in means and the ratio of SFFQ to FR. Energy adjustment reduced the observed overestimation. Validation of protein intake from SFFQ with estimated protein excretion from 24-h urea indicated overestimation of protein intake by SFFQ. The ability of the SFFQ to rank subjects adequately according to their dietary intake was reflected by Pearson's correlation coefficients varying from 0.44 to 0.85 adjusted for age, sex, total energy intake[9] and within-person variability in daily intake[10] and by correct classification into the same or adjacent quintile (75.8 % of crude and 76.8% for energy-adjusted data).

Data Analysis

Nutrient intake was calculated for men and women in four age groups (55-64 years, 65-74 years, 75-84 years, 85-95 years). Linear trend analysis for differences in mean nutrient intake across three age groups (55-64 years, 65-74 years, and 75-95 years) was carried out by regression analysis. Influence of baseline characteristics such as smoking status, body mass index (BMI), disability, and reported difficulty with food intake on energy and nutrient intakes adjusted by age and sex was investigated by one-way-analysis of variance. When testing for statistical significance natural logarithm (energy, protein, vegetable protein, total fat, satured fat, monounsaturated fat, carbohydrates, mono/disaccharides, fiber, water, calcium, phosphor, potassium, iron, zinc, retinol, B vitamins, vitamin E, and ß-carotene), respectively square root transformation (polyunsaturated fat, linoleic acid, polyssaccharides, cholesterol, sodium, magnesium, and vitamin C) was used for skewed nutrients to improve their distribution towards normality.

Table 1: Characteristics of the study population

Characteristic	Men				Women			
	55-64 years	65-74 years	75-84 years	85-95 years	55-64 years	65-74 years	75-84 years	85-95 years
mean(SD)	n = 948	n = 902	n = 352	n = 24	n = 1331	n = 1193	n = 612	n = 73
Weight (kg)	80.7 (10.6)	78.6 (10.1)	75.6 (9.7)	72.1 (8.2)	70.4 (11.3)	70.5 (10.9)	68.1 (10.9)	67.8 (9.0)
Height (cm)	176.4 (6.6)	174.7 (6.3)	172.2 (6.3)	171.1 (7.2)	163.7 (6.0)	161.7 (6.2)	158.6 (6.5)	157.3 (5.8)
Body mass index (kg/m^2)	25.9 (2.9)	25.8 (2.8)	25.5 (3.0)	24.7 (2.6)	26.3 (4.0)	27.0 (4.0)	27.1 (4.4)	27.4 (3.2)
Energy intake/basal metabolic rate	1.36 (0.33)	1.36 (0.32)	1.47 (0.29)	1.52 (0.34)	1.32 (0.33)	1.34 (0.31)	1.38 (0.33)	1.47 (0.42)
Serum cholesterol (mmol/l)	6.5 (1.2)	6.3 (1.1)	6.1 (1.1)	6.0 (0.8)	6.9 (1.1)	7.0 (1.2)	6.8 (1.2)	6.5 (1.3)
Percentage								
Current cigarette smoking	33.7	25.8	25.6	33.3	25.7	18.2	9.5	2.7
Alcohol drinking	88.8	86.5	83.5	79.2	78.2	72.3	64.1	63.0
Diabetes	6.4	11.6	12.6	25.0	4.8	11.1	15.5	13.7
Hypertension*	16.8	19.5	24.1	29.2	14.0	22.0	31.1	47.2
Obesity (BMI ≥ 30 kg/m^2)	7.6	7.4	5.7	0.0	16.2	20.8	23.4	16.7
Poor performance‡	1.7	3.6	6.3	16.7	4.5	9.4	22.1	50.7
Single household	9.3	8.8	17.1	50.0	19.8	39.4	63.3	83.6
Difficulties with food intake	2.2	5.4	10.3	16.7	11.3	15.1	20.8	35.6
Use of prescribed diet	9.8	12.2	9.7	12.5	12.0	16.9	18.6	13.7
Use of supplements	25.8	23.9	25.5	25.0	39.7	37.2	37.8	34.2

* Hypertension Systolic blood pressure ≥ 160 mm Hg, diastolic blood pressure ≥ 90 mm Hg or use of blood pressure lowering drugs
‡ Poor performance High score on disability index: mean of ability to perform dressing, rising, eating, walking, hygiene, reaching, grip, activity

For evaluation of plausibility of dietary intake data, the ratio of energy intake (EI) to basal metabolic rate (BMR) was calculated. EI/BMR is regarded to be a satisfactory independent validator of energy intake[11,12]. BMR was predicted from standard equations based on age-specific formulae[13].

RESULTS

Descriptive Characteristics

Table 1 summarizes selected characteristics of the study group. Mean age was 67 years (range 55-93) for men and 68 years (range 55-94) for women. Significant trends towards lower measured body weight and lower measured height at older age were observed. These changes with age were more pronounced in men. Body Mass Index (BMI) decreased with age in men, whereas it increased slightly in women. Calculated basal metabolic rate averaged 6.9 MJ/d (SD 0.7) for men and 5.6 (SD 0.5) for women and declined significantly with age. Mean ratio of energy intake to basal metabolic rate (EI/BMR) was 1.38 (SD 0.32) for men and 1.34 (SD 0.33) for women. The ratio of energy intake to basal metabolic rate (EI/BMR) was significantly related to age.

Indicators of activities of daily living as well as the percentage of those living independently decreased steadily with age. More women were living in a single household than men. Percentage with a low monthly equivalent household income (<1500 Hfl [<680 EURO]) was higher for women (26.4%) than for men (12.6%). Education level was generally lower for women and decreased with age. A completed primary education as highest degree obtained was reported by 24.0% of men and 42.1% of women, whereas 14.7% of men and 4.3% of women reported a college or university education. Comparison of baseline characteristics as given in table 1 for the study population undergoing dietary assessment with the total study population revealed no differences for the majority of characteristics. The prevalence of obesity was somewhat lower for women in the total study population, whereas the percentage of those with poor physical performance (activities of daily living), living alone, difficulties with food intake and a low income was higher in the total population. Stratification by age revealed that these differences were mainly due to aberrant values in the oldest age group (85 - 95 years).

Table 2a: Mean daily intake of energy and selected nutrients, energy distribution and nutrient density for selected nutrients of men in the Rotterdam Study

	Men				Test for trend†
	55-64 years	65-74 years	75-84 years	85-95 years	
	n = 948 mean (SD)	n = 902 mean (SD)	n = 352 mean (SD)	n = 24 mean (SD)	
Nutrient					
Energy (MJ)	9.7 (2.3)	9.3 (2.1)	8.9 (1.7)	9.1 (1.9)	***
Protein					
total (g)	93.3 (22.1)	86.2 (18.4)	82.0 (19.1)	82.4 (17.4)	***
vegetable protein (g)	32.6 (9.3)	30.8 (8.4)	29.2 (7.2)	30.2 (7.0)	***
Fat					
total (g)	94.6 (29.8)	90.9 (28.4)	87.6 (24.3)	90.6 (27.5)	***
saturated fat (g)	36.8 (13.0)	35.4 (12.4)	34.7 (10.6)	36.8 (12.2)	*
monounsaturated fat (g)	32.6 (11.7)	31.0 (10.8)	29.6 (9.7)	31.3 (13.2)	***
polyunsaturated fat (g)	18.3 (8.3)	17.9 (8.5)	16.9 (7.9)	16.2 (8.2)	**
linoleic acid (g)	15.2 (8.1)	14.8 (8.2)	13.9 (7.8)	13.4 (8.1)	**
Carbohydrates					
total (g)	243.3 (70.8)	233.1 (64.4)	228.8 (53.7)	229.5 (54.8)	**
mono/disaccharides (g)	118.1 (50.4)	114.7 (46.1)	115.9 (39.8)	112.1 (43.6)	ns
polysaccharides (g)	124.1 (35.3)	117.5 (30.9)	112.0 (26.8)	115.9 (30.4)	***
Cholesterol (mg)	268 (94)	254 (85)	247 (83)	254 (94)	***
Dietary fiber (g)	18.6 (5.6)	17.6 (5.3)	16.9 (4.3)	16.7 (4.4)	***
Water (ml)	2543 (655)	2344 (538)	2279 (504)	2166 (441)	***
Alcohol (g)‡	19.9 (19.7)	18.8 (19.2)	16.0 (16.3)	18.5 (13.7)	*
Energy density					
Cholesterol (mg/MJ)	27.7 (7.2)	27.5 (7.0)	27.8 (7.8)	28.2 (7.0)	ns
Dietary fiber (g/MJ)	2.0 (0.5)	1.9 (0.5)	1.9 (0.5)	1.9 (0.5)	ns

table 2 a continued

	Men				Test for trend†
	55-64 years	65-74 years	75-84 years	85-95 years	
	n = 948 mean (SD)	n = 902 mean (SD)	n = 352 mean (SD)	n = 24 mean (SD)	
Energy distribution					
Energy from:					
protein %	16.3 (2.8)	15.8 (2.8)	15.5 (2.8)	15.5 (2.9)	***
fat %	36.3 (6.0)	36.7 (6.0)	36.8 (6.3)	37.3 (5.8)	ns
saturated fat %	14.1 (3.0)	14.3 (3.0)	14.6 (3.2)	15.1 (3.1)	*
carbohydrates %	41.9 (6.9)	42.2 (7.1)	43.2 (7.1)	42.6 (7.2)	**
mono/disaccharides %	20.2 (6.3)	20.6 (6.2)	21.8 (6.3)	20.5 (6.0)	***

† test for trend across age groups (55-64 years, 65-74 years, 74-95 years)
ns = not significant, * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$
‡mean alcohol consumption of men drinking alcohol (87%)

Table 2b: Mean daily intake of energy and selected nutrients, energy distribution and nutrient density for selected nutrients of women in the Rotterdam Study

	Women				Test for trend†
	55-64 years	65-74 years	75-84 years	85-95 years	
	n = 1331 mean (SD)	n = 1193 mean (SD)	n = 612 mean (SD)	n = 73 mean (SD)	
Nutrients					
Energy (MJ)	7.5 (1.7)	7.4 (1.6)	7.4 (1.7)	7.9 (2.2)	ns
Protein					
total (g)	78.6 (16.8)	75.1 (16.3)	74.1 (17.4)	75.1 (22.4)	***
vegetable protein (g)	26.7 (7.2)	25.7 (7.0)	24.4 (6.5)	25.2 (7.1)	***
Fat					
total (g)	72.1 (23.4)	72.3 (22.8)	74.0 (23.2)	82.1 (29.3)	*
saturated fat (g)	28.3 (10.3)	29.0 (10.2)	30.5 (10.6)	33.5 (13.2)	***
monounsaturated fat (g)	24.6 (8.5)	24.3 (8.6)	24.8 (8.6)	27.6 (11.1)	ns
polyunsaturated fat (g)	13.6 (6.7)	13.4 (6.7)	13.0 (7.0)	14.7 (7.3)	*
linoleic acid (g)	11.4 (6.5)	11.1 (6.4)	10.6 (6.6)	11.6 (6.7)	**
Carbohydrates					
total (g)	195.4 (53.9)	195.5 (49.6)	195.6 (52.3)	207.8 (68.5)	ns
mono/disaccharides (g)	97.8 (36.6)	99.7 (35.0)	103.5 (38.6)	111.5 (47.4)	***
polysaccharides (g)	96.9 (26.9)	95.2 (24.5)	91.5 (23.8)	94.7 (29.6)	***
Cholesterol (mg)	217 (70)	214 (71)	219 (74.5)	243 (93)	ns
Dietary fiber (g)	16.5 (4.8)	15.8 (4.5)	15.3 (5.0)	16.0 (8.8)	***
Water (ml)	2483 (614)	2391 (596)	2334 (554)	2216 (632)	***
Alcohol (g) ‡	9.4 (11.7)	8.4 (10.9)	5.8 (8.3)	5.4 (8.8)	***
Energy density					
Cholesterol (mg/MJ)	29.1 (7.6)	29.0 (7.7)	29.6 (7.6)	30.6 (7.0)	ns
Dietary fiber (g/MJ)	2.3 (0.6)	2.2 (0.6)	2.1 (0.7)	2.0 (0.7)	***

table 2b continued

	Women				Test for trend†
	55-64 years	65-74 years	75-84 years	85-95 years	
	n = 1331 mean (SD)	n = 1193 mean (SD)	n = 612 mean (SD)	n = 73 mean (SD)	
Energy distribution					
Energy from:					
protein %	17.8 (3.2)	17.2 (3.0)	17.0 (3.1)	16.2 (3.2)	***
fat %	35.7 (6.3)	36.2 (6.19	37.2 (6.4)	38.6 (6.3)	***
saturated fat %	14.0 (3.2)	14.5 (3.3)	15.4 (3.6)	15.7 (3.2)	***
carbohydrates %	43.6 (6.9)	44.2 (6.6)	44.2 (6.7)	43.8 (5.8)	*
mono/disaccharides %	21.7 (6.2)	22.4 (5.8)	23.3 (6.3)	23.3 (5.3)	***

† test for trend across age groups (55-64 years, 65-74 years, 75-95 years)
ns = not siginificant; * $p < 0.05$; ** $p < 0.001$; *** $p < 0.0001$

‡mean alcohol consumption of women drinking alcohol (73%)

Energy and Nutrient Intake

Men reported significantly higher mean intakes of energy and all nutrients from food intake compared compared to women except for water and calcium, which were similar, and vitamin C which was lower in men. Mean alcohol consumption among drinkers of any alcohol (men 87%; women 73%) was higher in men than in women with 18.9 g/d compared to 8.3 g/d. Linear trend analysis across age groups showed a general decline in energy and nutrient intake with advancing age in men which was significant for most nutrients (table 2a, 3a). However, percentage of energy from saturated fat, mono/disaccharides and carbohydrates increased whereas percentage of energy from protein decreased. In women, mean intake of most nutrients decreased (e.g. water, protein, magnesium, potassium, zinc, pyridoxine, ß-carotene), but some increased (e.g total fat, saturated fat, mono/disaccharides, or the percentage of energy of these nutrients, respectively), and some showed no substantial changes (e.g. calcium, retinol) (table 2b, 3b).

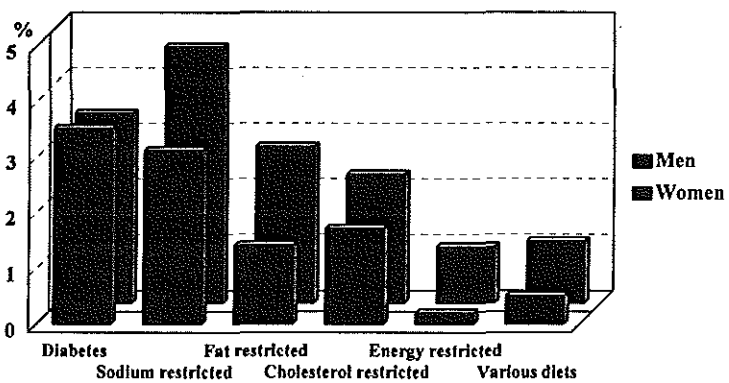
Some baseline characteristics (age- and sex-adjusted) were related to energy and nutrient intake. A significant inverse relationship between BMI and reported energy intake was present for both men and women which was consistent over age groups. Current cigarette smokers had significantly higher intakes of macronutrients (e.g. carbohydrates, mono/disaccharides, saturated fat) and significantly lower intakes of micronutrients (e.g. calcium, vitamin C) compared to nonsmokers and former smokers. Lower educational attainment was associated with a higher intake of macronutrients in general and a less favourable fat composition (van Rossum, unpublished observations). Disability and reported difficulty with food intake were associated with lower intakes of energy and most nutrients. Living independently compared to living in a service flat only marginally influenced energy and nutrient intakes.

Dietary regimen

A prescribed diet was reported by 231 men (10.4%) and 469 women (15.3%). Figure 2 shows the percentage of men and women currently following different types of a diet. Only for women a significant increase with age in the percentage of prescribed diets was seen (ANOVA; $p < 0.0001$) (table 1). Of the 700 men and women reporting a prescribed diet, 153 followed a combined dietary regime. The most commonly used combinations were a sodium/fat-restricted diet, a fat/cholesterol-restricted diet, or a diabetes diet with

sodium or fat restriction. Subjects currently following a prescribed diet generally reported lower energy and nutrient intakes compared to subjects not on a dietary regimen. The observed decrease in energy and nutrient intake was more pronounced for macronutients than for micronutrients with the exception of sodium. The reported energy and nutrient intake between different dietary regimes differed according to the aim of the prescribed diet.

Figure 2: Men and women following different types of prescribed diet in the Rotterdam Study



Supplementation

Information on supplement use during the past year was obtained in 5334 persons (2183 men, 3151 women). Of those, 1267 women (40.2%) and 580 men (26.6%) reported regular use of supplements. Consumption of more than two different supplements was reported by 4.8% of men and 9.3% of women. The type of supplements consumed included vitamins, minerals and a variety of preparations such as garlic, brewer's yeast, ginseng, and fish oil. Use of supplementation showed no clear trend with age (table 1). The most popular supplement was garlic (n = 572), followed by tonicum supplement (n = 107) and brewer's yeast (n = 86). Use of vitamin/mineral supplementation was reported by 307 men (14.1%) and 793 women (25.2%). The most frequently consumed mineral/vitamin supplements were multivitamins (n = 329), vitamin C (n = 309), calcium (n = 229), vitamin B preparations (n = 135), multiminerals (n = 123) and vitamin D (n = 83).

Table 3a: Mean daily intake of selected vitamins and minerals of men in the Rotterdam Study

	Men				Test for trend†
	55-64 years	65-74 years	75-84 years	85-95 years	
	n = 948 mean (SD)	n = 902 mean (SD)	n = 352 mean (SD)	n = 24 mean (SD)	
Minerals					
Calcium (mg)	1205 (471)	1117 (414)	1081 (386)	1035 (297)	***
Phosphor (mg)	1843 (820)	1673 (802)	1604 (646)	1514 (432)	***
Iron (mg)	13.8 (3.2)	12.8 (2.9)	12.3 (2.8)	11.8 (2.5)	***
Sodium from food (mg)	2584 (761)	2385 (681)	2317 (628)	2408 (893)	***
Potassium (mg)	4083 (910)	3823 (817)	3653 (739)	3630 (743)	***
Magnesium (mg)	350.9 (86.3)	320.0 (74.5)	306.9 (68.1)	307.4 (68.4)	***
Zinc (mg)	12.1 (3.0)	11.2 (2.5)	10.7 (2.6)	10.2 (2.3)	***
Vitamins					
Vitamin A (mg RE)	0.93 (0.47)	0.87 (0.38)	0.88 (0.36)	0.78 (0.23)	*
ß-carotene (mg)	1.66 (0.70)	1.49 (0.59)	1.42 (0.56)	1.19 (0.47)	***
Thiamin (mg)	1.27 (0.34)	1.17 (0.31)	1.13 (0.36)	1.09 (0.24)	***
Riboflavin (mg)	1.72 (0.62)	1.58 (0.53)	1.57 (0.50)	1.57 (0.42)	***
Pyridoxine (mg)	1.84 (0.45)	1.71 (0.40)	1.63 (0.38)	1.63 (0.34)	***
Niacin (mg)	12.9 (4.7)	11.0 (3.8)	9.9 (3.0)	10.1 (3.1)	***
Tocopherol (mg eqv)	15.6 (6.6)	15.3 (6.9)	14.7 (6.3)	13.3 (6.8)	**
Vitamin C (g)	116.6 (52.4)	114.8 (49.5)	112.8 (46.7)	92.5 (33.5)	ns

† test for trend across age groups (55-64 years, 65-74 years, 74-95 years)
ns = not significant
* $p < 0.05$
** $p < 0.001$
*** $p < 0.0001$

Table 3b: Mean daily intake of selected vitamins and minerals of women in the Rotterdam Study

	Women				Test for trend†
	55-64 years	65-74 years	75-84 years	85-95 years	
	n = 1331 mean (SD)	n = 1193 mean (SD)	n = 612 mean (SD)	n = 73 mean (SD)	
Minerals					
Calcium (mg)	1125 (386)	1090 (349)	1115 (381)	1134 (551)	ns
Phosphor (mg)	1697 (754)	1612 (727)	1610 (724)	1619 (820)	***
Iron (mg)	11.5 (2.5)	11.0 (2.4)	10.4 (2.5)	10. 8 (3.5)	***
Sodium from food (mg)	2092 (582)	1993 (532)	1954 (564)	2061 (699)	***
Potassium (mg)	3590 (765)	3469 (729)	3381 (798)	3450 (998)	***
Magnesium (mg)	298 (66)	285 (62)	277 (65)	285 (80)	***
Zinc (mg)	10.2 (2.3)	9.7 (2.3)	9.6 (2.5)	9.6 (2.9)	***
Vitamins					
Vitamin A (mg RE)	0.76 (0.37)	0.76 (0.37)	0.77 (0.34)	0.89 (0.47)	ns
ß-carotene (mg)	1.61 (0.79)	1.46 (0.79)	1.41 (0.94)	1.37 (0.53)	***
Thiamin (mg)	1.07 (0.26)	1.02 (0.25)	0.99 (0.27)	1.00 (0.28)	***
Riboflavin (mg)	1.51 (0.48)	1.47 (0.46)	1.50 (0.50)	1.60 (0.74)	ns
Pyridoxine (mg)	1.50 (0.34)	1.46 (0.32)	1.42 (0.34)	1.40 (0.38)	***
Niacin (mg)	9.7 (2.7)	8.9 (2.7)	8.1 (2.4)	8.0 (2.6)	***
Tocopherol (mg eqv)	12.8 (5.5)	12.8 (5.5)	12.5 (5.7)	13.9 (5.9)	ns
Vitamin C (g)	125.8 (55.0)	123.5 (2.7)	123.3 (64.2)	121.6 (47.7)	ns

† test for trend across age groups (55-64 years, 65-74 years, 75-95 years)
ns = not siginificant;
* $p < 0.05$
** $p < 0.001$
*** $p < 0.0001$

DISCUSSION

In this paper we described a new approach to improve feasability of dietary assessment by SFFQ in the elderly. The assessment comprised a simple self-adminstered dietary questionnaire which formed the basis for an interview with a trained dietitian. Average time for completion of the self-administered questionnaire was 20 minutes, and a further 20 minutes were allocated to conduct the dietary interview. Information concerning dietary intake was obtained in 5434 participants of the Rotterdam Study, and intake of a variety of nutrients was calculated. In men, a general decline in intake of energy and most nutrients was observed with age. In women, the relation with age was not consistent: mean intake for most nutrients showed a significant decline, some showed an increase, and others showed no substantial change with age. Body mass index, smoking status, socioeconomic status, and activities of daily living were observed to influence reported nutrient intake.

Before interpreting the results, some methodological aspects of the study need to be addressed. The Rotterdam Study is a community based prospective cohort study in Rotterdam. Baseline data were collected by means of an extensive general home interview and two visits at the research center. In general, relatively healthy community dwelling elderly are more likely to participate in a study such as the Rotterdam Study[1,14]. Application of exclusion criteria for dietary assessment analysis such as exclusion of nursing home residents and persons with a known reduced cognitive function may have further contributed to the formation of a relatively healthy cohort. Thus, we cannot exclude the possibility of an overrepresentation of persons with a dietary pattern similar to a healthier population, particularly in the older age groups. If this is true, the observed decreased intake of most nutrients with age, especially in men as compared to the general population of this age is probably underestimated.

Efficiency in dietary assessment was aimed by conduct of two consecutive steps (figure 1). A checklist of foods completed by the participants at home made the subjects aware of their food consumption and the items marked by the participants informed the dietitian about the meal pattern and the foods consumed regularly. This, subsequently, allowed the dietitian to focus during the interview only on specification of amounts and frequencies of reported items during the interview. Thus, respondent burden, which is seen as a specific problem of dietary assessment in the elderly[1,2], was kept low. Time for

completion of the self-administered questionnaire averaged 20 minutes and another 20 minutes were allocated for the dietary interview. Thus, total time for dietary assessment was approximately 40 minutes. Combination of methods, use of memory strategies, or prior notification of a dietary interview have been shown previously to be useful in obtaining accurate and complete recalls in elderly people[15].

Visual check of formal completeness of the self-administered questionnaire and by the dietitians, their professional judgement concerning unreliable dietary assessment (3.8% unreliable dietary assessments), and logical checks build in the interactive data entry programme further ensured to obtain complete recalls in our study.

The presence of over- and underreporting in dietary assessment has been extensively documented for various methods and for diverse populations and age groups[12]. Studies using the doubly labeled water method to assess total energy expenditure confirmed that self-reports of energy and food intake tend to be underestimated[16-18]. Underestimation of food intake may be due to the tendency of subjects to conceal their true dietary intake consiously or unconciously. Some authors suggested underreporting to be dependent on the degree of obesity[19,20] and for elderly women adiposity was shown to be a independent predictor of underreporting[20]. In our study population of 5434 elderly subjects the ratio of energy intake to basal metabolic rate (EI/BMR) was significantly associated with age (table 1) partially to be explained by the observed significant decrease of estimated BMR with age. EI/BMR was also found to be significantly inverse associated with BMI (Pearson's $r = 0.36$, $p > 0.0001$). Stratification by age strata (<65, 65-74, >74 years) revealed that EI/BMR significantly decreased across categories of BMI (<25, 25-30, >30) suggesting underreporting of obese subjects independent of age.

The results of this study are generally in accordance with other surveys of smaller size conducted in this age group in the Netherlands, despite the use of a different FFQ or a different methodology for dietary assessment[22-25]. However, some differences were observed. Intakes of protein and vitamin C intake were consistently lower, whereas intakes of fat, saturated fat, cholesterol, and dietary fiber were consistently higher in the present study. Differences may be partly attributable to the use of an updated food composition table in the present study. Changes in the composition of foods and improvements in the quality of data in the current food composition table were shown to

be the major cause for changes in dietary fiber and fat intake estimates in the Dutch National Food Consumption Survey in 1987/88 compared to 1992[26].
Comparison with large-scale studies in the elderly conducted in the US revealed some differences[27-29]. Nutrient estimates derived in the Rotterdam Study showed good agreement on macronutrient level with nutrient estimates obtained by FFQ method in the Iowa's Women Study[29]. For calcium, however, the Dutch estimates were considerably higher (circa 300 mg/d), whereas for vitamin A (retinol and ß-carotene), vitamin C, and the B vitamins the estimates were lower. In the Beaver Dam Study[28] nutrient estimates obtained by diet history questionnaire (age group 65-84 years) were considerably lower compared to the Rotterdam Study except for vitamin B complex and vitamin C. Similar results were found for the Ross Laboratories Elderly Dietary Survey (RLEDS)[30], in which diet was assessed by 24-h recall and in the 1987 National Health Interview Survey (NHIS)[27] using a 60-item FFQ. In summary, dietary intake of most nutrients in the elderly of the Rotterdam Study compared to the US studies cited has been found to be in general higher, except for vitamin C and B-vitamins (thiamin, riboflavin, pyridoxine, niacin). The widespread use of products fortified with vitamin B-complex and other vitamins/minerals in the USA may explain this observation.
A substantial part of the subjects in our study followed a prescribed dietary regimen as is in accord with other studies in the elderly[24]. Prescribed diets were more prevalent among women than among men. The number of persons reporting a prescribed diet increased significantly with age (table 1). Because we assume that prescribed diets are consumed over a relatively long period and because we are interested in average dietary intake over a longer period (preceeding year) we did not exclude subjects following a prescribed diet from the analysis.

Dietary supplements, especially vitamin and mineral preparations, are generally regarded as important sources of micronutrients. Supplement use in the Rotterdam Study was not recorded with sufficient accuracy to incorporate vitamin and mineral intake through supplements in the computation of nutrient intakes. However, data on supplement use as collected in our study provide enough precision to classify individuals correctly as users or non-users of those supplements in the past year[31]. In a validation study using the same method a sensitivity of 65.9% and a specificity of 98.5% were reported with a kappa statistic of 0.69[31] for classification of users or non-users of the past year. The use of supplements respectively vitamin/mineral supplements in the Rotterdam Study was

somewhat higher than reported in the Dutch National Food Consumption Survey[24,32] and and the National Diet and Nutrition Survey in the elderly in the UK[33], but considerably lower than that observed in American elderly, where figures range from 28% in the NHIS[26] to 60% in the Iowa's Women Study[28].

CONCLUSION

We described a novel two-step dietary assessment approach for use in epidemiological studies in the elderly, applied it across a wide age range in a large elderly population, and reported estimates of nutrient intake. We conclude that the adapted SFFQ facilitated collection of data on dietary habits in the elderly within a limited time frame and consider it to be a suitable instrument for dietary assessment in large scale epidemiologic studies.

REFERENCES

1. Kelsey JL, O'Brien LA, Grisso JA et al. Issues in carrying out epidemiologic research in the elderly. *Am J Epidemiol* 1989; 130:857-866
2. Van Staveren WA, de Groot LCPGM et al. Assessing diets of elderly people: problems and approaches. *Am J Clin Nutr* 1994; 59:221S-223.
3. Hofman A, Grobbee DE, De Jong PTVM et al. Determinants of disease and disability in the elderly: The Rotterdam Elderly Study. *Eur J Epidemiol* 1991; 7:403-422.
4. Van den Brandt PA, Goldbohm RA, van't Veer P et al. A large-scale prospective cohort study on diet and cancer in the Netherlands. *J Clin Epidemiol* 1990; 121:783-790
5. Goldbohm RA, van den Brandt PA, Brants HAM et al. Validation of a dietary questionnaire used in a large-scale prospective cohort study on diet and cancer. *Eur J Clin Nutr* 1994; 48:253-265.
6. Goldbohm RA, van't Veer P, van den Brandt PA et al. Reproducibility of a food frequency questionnaire and stability of dietary habits determined from five annually repeated measurements. *Eur J Clin Nutr* 1995; 49:420-429.
7. Food and Nutrition Council. Dutch food composition table (NEVO). 1993. The Hague, Netherlands: Voorlichtingsbureau voor de Voeding (in Dutch).
8. Klipstein-Grobusch K, den Breeijen JH, Goldbohm RA et al. Dietary assessment in the elderly: validation of a semiquantitative food frequency questionnaire. *Eur J Clin Nutr* 1998; 52:588-596.
9. Willett WC, Stampfer MJ. Total energy intake: implications for epidemiologic analysis. *Am J Epidemiol* 1986; 124:17-27.

10. Beaton GH, Milner J, Corey P et al. Source of variance in 24-hour dietary recall data: implications for nutrition study design and interpretation. *Am J Clin Nutr* 1979; **32**:2546-2559.
11. Garrow JS. Validation of methods for estimating habitual diet: proposed guidelines. *Eur J Clin Nutr* 1995; **49**:231-232.
12. Black AE, Goldberg GR, Jebb, SA et al. Critical evaluation of energy intake data using principles of energy physiology: 2. Evaluating the results of published surveys. *Eur J Clin Nutr* 1991; **45**:583-599.
13. Department of Health: Dietary reference values for food energy and nutrients for the United Kingdom. Annex 2. London, U.K. HMSO, 1994.
14. Herzog AR, Rodgers WL. Age and response rates to interview sample surveys. *J Gerontol* 1988; **43**:S2000-2005.
15. Chiannetta MM, Head MK. Effect of prior notification on accuracy of dietary recall in the elderly. *J Am Diet Assoc* 1992; **92**:741-743.
16. Black AE, Prentice AM, Goldberg GR et al. Measurements of total energy expenditure provide insight into the validity of dietary measurements of energy intake. *J Am Diet Assoc* 1993, **93**:572-579.
17. Goran MI, Poehlman ET. Total energy expenditure and energy requirements in healthy elderly persons. *Metabolism* 1992; **41**:744-753.
18. Livingstone MBE, Prentice AM, Strain JJ et al. Accuracy of weighed dietary records in studies of diet and health. *BMJ* 1990; **300**:708-712.
19. De Vries JH, Zock PL, Mensink RP et al. Underestimation of energy intake by 3-d records compared with energy intake to maintain body weight in 269 nonobese adults. *Am J Clin Nutr* 1994; **60**:855-860.
20. Lichtman SW, Pisarska K, Berman ER et al. Discrepancy between self-reported and actual caloric intake and exercise in obese subjects. *N Engl J Med* 1992; **327**:1893-1898.
21. Johnson RK, Goran MI, Poehlman ET. Correlates of over-and underreporting of energy intake in healthy older men and women. *Am J Clin Nutr* 1994; **59**:1286-1290.
22. Grootenhuis PA, Westerbrink S, Sie CM et al. A semiquantitative food frequency questionnaire for use in epidemiologic research among the elderly: validation by comparison with dietary history. *J Clin Epidemiol* 1995; **48**:859-868.
23. Löwik MRH, Westenbrink S, Hulshof KFAM et al. Nutrition and aging: Dietary intake of "apparently healthy" elderly - The Dutch Nutrition Surveillance System. *J Am Coll Nutr* 1990; **8**:347-356.
24. Löwik MRH, Brussaard JH, Hulshof KFAM et al. Adequacy of the diet in the Netherlands in 1987-1988. Dutch Nutrition Surveillance System. *Int J Food Sciences and Nutr* 1994; **45**:S1-S62.
25. Moreiras-Varela O, van Staveren WA, Amorim Cruz JA et al. Intake of energy and nutrients. Euronut SENECA Investigators. *Eur J Clin Nutr* 1991: **45**:S105-120.
26. Hulshof KFAM, Löwik MRH, Kistemaker L. Changes in the composition of the Dutch diet. Abstract; Second International Conference on Dietary Assessment Methods. Boston 1995

27. Block G, Subar AF. Estimates of nutrient intake from a food frequency questionnaire: The 1987 National Health Interview Survey. *J Am Diet Assoc* 1992; **92**:969-977.
28. Mares-Perlman JA, Klein BEK, Klein R et al. Nutrient supplements contribute to the dietary intake of middle- and older-aged adult residents of Beaver Dam, Wisconsin. *J Nutr* 1993; **123**:176-188.
29. Munger RG, Folsom AR, Kushi LH et al. Dietary assessment of older Iowa women with a food frequency questionnaire: Nutrient intake, reproducibility, and comparison with 24-hour dietary recall interviews. *Am J Epidemiol* 1992; **136**:192-200.
30. Ryan AS, Craig LD, Finn SC: Nutrient intakes and dietary patterns of older Americans: A national study. *J Gerontology* 1992; **47**:M145-150.
31. Dorant E, van den Brandt PA, Goldbohm RA et al. Agreement between interview data and a self-administered questionnaire on dietary supplement use. *Eur J Clin Nutr* 1994; **48**:180-188.
32. Dorant E, van den Brandt PA, Hamstra AM et al. The use of vitamins, minerals, and other dietary supplements in the Netherlands. *Internat J Vit Nutr Research* 1993; **63**:4-10.
33. Bates CJ, Prentice A, van der Pols JC et al. Estimation of the use of dietary supplements in the National Diet and Nutrition Survey: People aged 65 years and over. An observed paradox and a recommendation. *Eur J Clin Nutr* 1998; **52**:917-923.

CHAPTER 3

Prooxidants in coronary heart disease

3.1

Serum ceruloplasmin as coronary risk factor in the elderly

ABSTRACT

Serum copper and ceruloplasmin have been suggested to be independent risk factors for coronary heart disease operating through oxidative modification of low-density lipoprotein. However, given its function as an acute-phase protein, the question has been raised whether elevated ceruloplasmin is not merely an indicator of inflammation. In the current study, we investigated whether serum ceruloplasmin was associated with subsequent myocardial infarction taking into account indices of inflammation. The study population consisted of 210 cases of first myocardial infarction and controls, frequency-matched on age (5-year categories) and sex selected from the population-based cohort of the Rotterdam Study. Serum ceruloplasmin levels were significantly elevated in cases of myocardial infarction compared to controls (510 (SD 110) v. 470 (SD 100) mg/l; p=0.007). Risk of myocardial infarction for the highest compared to the lowest quartile of ceruloplasmin was 2.46 (95% CI 1.04-6.00; p_{trend}=0.043) after adjustment for age, sex, body mass index, packyears smoked, serum cholesterol, systolic blood pressure, and income. The relative risk was most evident in current smokers. Adjustment for C-reactive protein and white blood cell count reduced the excess risk by 33%. This suggests that a substantial part of the observed association between serum ceruloplasmin and coronary heart disease may be attributed to inflammation processes rather than to the pro-oxidant activity of ceruloplasmin.

INTRODUCTION

Elevated serum ceruloplasmin levels have been found in patients with cardiovascular disorders including arteriosclerosis, abdominal aneurysms, unstable angina, and vasculitis and peripheral artery disease[1]. Several prospective studies have indicated that serum copper or ceruloplasmin may be an independent risk factor for cardiovascular disease[2-7]. The increased risk has been attributed to pro-oxidant activity of ceruloplasmin and recent experimental studies demonstrating the ability of ceruloplasmin to oxidatively modify low-density lipoprotein[8,9] seem to underline this notion. However, the question has been raised whether elevated ceruloplasmin is not merely an indicator of inflammation given its acute-phase protein property. So far, most studies investigating the association between serum ceruloplasmin or copper lacked information on indicators of inflammation. In the current study we obtained information on C-reactive protein and white blood cell count as indicators of inflammation. We examined whether serum ceruloplasmin is associated with an increased risk of myocardial infarction taking into account possible markers of inflammation.

SUBJECTS AND METHODS

Study population and case ascertainment

The Rotterdam Study is a community based prospective cohort study of 7983 persons (response rate 78%) aged 55 years and over, living in Ommoord, an urban district in Rotterdam, the Netherlands. The aim of the study is to investigate the incidence of and the risk factors for chronic and disabling diseases, as described elsewhere[10]. The study was approved by the Medical Ethics Committee of the Erasmus University, and written informed consent was obtained from all participants.

Follow-up for coronary heart disease started after the baseline survey in 1990, and until April 1996 (mean 4 years) follow-up information was available for 94% of the cohort. With respect to the vital status of participants, information was obtained at regular intervals from the municipal health service in Rotterdam. Information on fatal and non-fatal endpoints was obtained from the general practitioners (GPs) working in the study district of Ommoord. All possible events reported by the GPs were verified by research physicians from the Rotterdam Study through records of the participating GPs and medical specialists. Cause and circumstances of death were obtained by questionnaire

from the GP and by scrutinising information from hospital discharge records in case of admittance or referral, shortly after reporting of death by the municipal health service or the GP. Classification of fatal and non-fatal events was based on the International Classification of Diseases, 10th edition[11]. For the present analysis only cases of first myocardial infarction (ICD-10 codes I21-I24) were selected. All events were classified independently by two research physicians. If there was disagreement, a consensus was reached in a special session. Finally, all these events were verified by a cardiovascular disease expert. In case of discrepancies, the judgement by this expert was considered definite.

The association between serum ceruloplasmin and risk of myocardial infarction was examined by use of a nested case-control design. Cases and controls were drawn from the population-based Rotterdam Study. For every subject with a myocardial infarction during follow-up (n= 202) a control without a myocardial infarction was selected. Age strata (5-year interval) and sex were used as matching variables. For determination of serum ceruloplasmin and C-reactive protein frozen sera were available for 255 subjects, 111 myocardial infarction cases and 144 controls. Availability of blood samples of cases and matched controls in this project depended on the date of the event and were not related to serum ceruloplasmin levels. Exclusion of subjects with history of myocardial infarction at baseline resulted in a study population of 210 subjects (83 cases and 127 controls) for the present analysis.

Measurements

Baseline information on current health status, medical history, drug use, education, income, and smoking behaviour was obtained with a computerised questionnaire during a home interview. Height and weight were measured, and body mass index (weight in kg/height in m^2) was calculated as a measure of obesity. Sitting blood pressure was measured on the right upper arm with a random-zero sphygmomanometer. The average of two measurements was used in the analysis. A venepuncture was performed and haematological parameters were obtained by standard clinical laboratory procedures. Serum total and high density lipoprotein (HDL) cholesterol concentrations were determined by an automated enzymatic procedure. Alcohol intake was estimated by use of a semiquantiative food frequency questionnaire assessing habitual food intake during the past year[12]. Frozen sera, preserved at -20°C, and collected from the cases and the controls simultaneously at study baseline were used to determine serum concentrations

of ceruloplasmin and C-reactive protein. Sera from cases and controls were analysed in the same run. Serum ceruloplasmin concentrations and C-reactive protein were determined by kinetic nephelometry by use of a Beckman-Array system (Munich, Germany). For five subjects (cases/controls) C-reactive protein could not be determined due to insufficient serum for analysis. Interassay coefficients of variation were 4.6% for ceruloplasmin and 3.8% for C-reactive protein.

Data Analysis

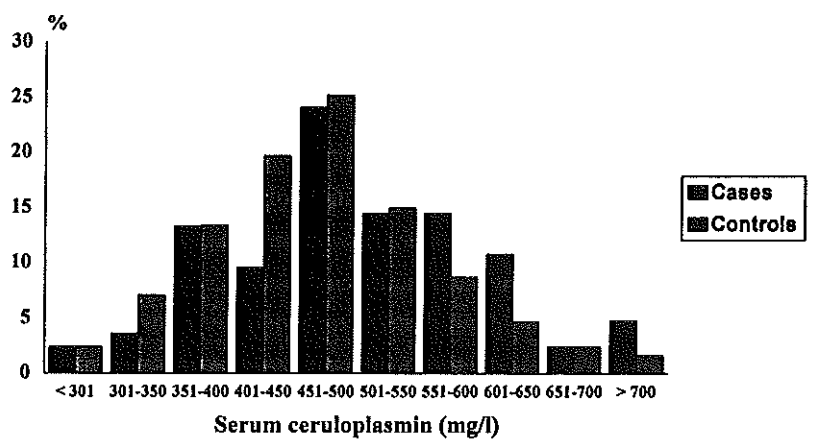
Associations between serum ceruloplasmin concentrations and risk factors for ischemic heart disease were estimated by use of Pearson's correlation coefficient (continous variables) and analysis of variance (categorical variables) adjusted for age and sex. The association between quartiles of serum ceruloplasmin and risk of myocardial infarction was investigated by multiple logistic regression comparing risk of myocardial infarction in the upper quartiles to the lowest quartile of serum ceruloplasmin. Analyses were initially adjusted for age and sex and subsequently for body mass index, packyears smoked, equivalent household income (five categories), serum cholesterol, and systolic blood pressure. Stratification procedures were used to evaluate whether smoking status, hypertension (systolic blood pressure $\geq$140 mm Hg and/or diastolic blood pressure $\geq$90 mm Hg and/or use of antihypertensive medication), hypercholesterolemia (serum cholesterol levels $\geq$6.5 mmol/l), or high-density lipoprotein cholesterol levels below the median modified the association between serum ceruloplasmin and myocardial infarction. We next examined to which extent ceruloplasmin reflects increased inflammation. First we included measures of inflammation in our multivariate logistic regression model - white blood cell count as continous, and C-reactive protein as a categorised variable using a cut-off point of 6 g/l. Second, we assessed whether exclusion of subjects with C-reactive protein levels above 6 g/l modified the serum ceruloplasmin - myocardial infarction association. Associations are expressed as odds ratios with 95% confidence interval (CI). Results were considered statistically significant at the two-sided 0.-05 alpha level. Statistical analysis was performed using SAS® statistical software package version 6.11 (SAS Institute, Cary, NC, USA).

RESULTS

Baseline characteristics of cases and controls of myocardial infarction are shown in table 1. Significant differences were seen for white blood cell count, percentage of subjects with C-reactive protein levels above 6 g/l, and income of at least 3000 Hfl (about £900)/month. Compared to controls cases of myocardial infarction had non-significantly higher serum levels of cholesterol, have more packyears smoked, and were more often current smokers.

Serum ceruloplasmin levels in the case-control population ranged from 220 to 790 mg/l with averages of 370, 460, 520, and 640 mg/l in subsequent quartiles. Ceruloplasmin was significantly higher in cases than controls of myocardial infarction (510 (SD 110) v. 470 (SD 100) mg/l; p=0.007). The distribution of serum ceruloplasmin for cases and controls is shown in figure 1 indicating a shift towards higher serum ceruloplasmin levels for incident cases of myocardial infarction compared to controls.

Figure 1: Serum ceruloplasmin levels among myocardial infarction cases and controls



Serum ceruloplasmin adjusted for age and sex correlated significantly with serum cholesterol (r=0.25; p=0.0002), white blood cell count (r=0.24, p=0.0004), and packyears smoked (r=0.24; p=0.0004), and was significantly inverse associated with

serum HDL-cholesterol (r=0.17, p=0.0170). No correlations were observed between ceruloplasmin and age, body mass index, waist-to-hip ratio, diastolic and systolic blood pressure, and alcohol intake. Mean ceruloplasmin was observed to be significantly higher (572 (SD 85) v. 470 (SD 94) mg/l; $p < 0.0001$) for subjects with C-reactive protein of at least 6 g/l. These subjects also reported to have more packyears smoked and they were more likely to be current smokers. No correlation between white blood cell count and packyears smoked was seen.

Table 1: Baseline characteristics of cases of myocardial infarction and controls (Mean values and standard deviations or percentage distribution)

	Cases of myocardial infarction		Controls		p*
Variables	*n = 83*		*n = 127*		
Men (%)	45.8		44.1		n.s.
Age (years)	76.4	(8.7)	76.8	(9.0)	n.s.
Body mass index (kg/m^2)	26.2	(2.8)	25.9	(3.5)	n.s.
Waist-to-hip ratio	0.94	(0.09)	0.92	(0.08)	n.s.
Ceruloplasmin (mg/l)	510	(110)	470	(100)	0.007
White blood cell count (10^9/l)	7.34	(2.20)	6.70	(1.73)	0.020
Serum cholesterol (mmol/l)	6.67	(1.37)	6.46	(1.37)	n.s.
HDL-serum cholesterol (mmol/l)	1.22	(0.26)	1.28	(0.32)	n.s.
Systolic blood pressure (mm Hg)	148	(22)	143	(23)	n.s.
Diastolic blood pressure (mmHg)	73	(14)	74	(12.2)	n.s.
Packyears smoked (%)	17.9	(26.6)	12.9	(27.0)	n.s.
Current smokers(%)	24.1		17.3		n.s.
C-reactive protein above 6 g/l (%)	21.3		9.9		0.019
High income§ (%)	1.2		9.5		0.013

* for continous variables: analysis of variance adjusted for age and sex; for categorical variables: Mantel-Haenszel Chi-square test adjusted for age and sex

§ equivalent household income > 3000 Hfl (around £900)

When adjusted for age and sex, serum ceruloplasmin levels were significantly associated with increased risk of myocardial infarction (table 2). The odds ratio for the highest compared to the lowest quartile of ceruloplasmin was 2.96 (95% CI, 1.29-6.84; p_{trend} =0.010). The association remained statistically significant after adjustment for BMI, packyears smoked, income (five categories), serum cholesterol, and systolicblood pressure (table 2). Figure 2 depicts graphically a trend of increasing myocardial infarction risk across the quartiles of serum ceruloplasmin. We next stratified by smoking status, hypertension, hypercholesterolemia, and low high-density lipoprotein cholesterol levels. Results showed a significantly increased risk of myocardial infarction with elevated ceruloplasmin in current smokers compared to

Table 2: Risk of myocardial infarction according to quartiles of serum ceruloplasmin concentration in the Rotterdam Study
(Values are odds ratios (OR) and 95% confidence intervals)

Variable	Quartiles				P value for trend
	1*f* (Lowest)	2	3	4 (Highest)	
No. of MI	20	16	20	27	
serum level (mg/l)	< 0.420	0.420 - 0.489	0.490 - 0.560	> 0.560	
Odds ratio (95% CI)					
age and sex adjusted	1	1.07 (0.47 - 2.42)	1.45 (0.65 - 3.25)	2.96 (1.29 - 6.84)	0.010
multivariate adjusted*	1	0.97 (0.42 - 2.22)	1.31 (0.58 - 2.98)	2.59 (1.10 - 6.24)	0.029
multivariate adjusted**	1	0.99 (0.43 - 2.27)	1.28 (0.57 - 2.93)	2.46 (1.04 - 6.00)	0.043
adjusted for CRP**	1	0.96 (0.41 - 2.22)	1.15 (0.49 - 2.68)	1.97 (0.76 - 5.18)	0.189
adjusted for white blood cell count**	1	0.91 (0.39 - 2.11)	1.15 (0.50 - 2.66)	2.02 (0.82 - 5.06)	0.127

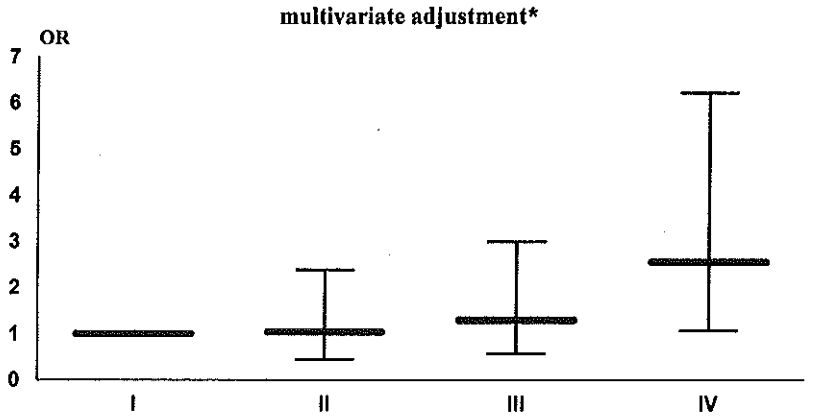
f reference category

* adjusted for age, sex, body mass index, packyears smoked, and equivalent houshold income (five categories)

** additonally adjusted for serum cholesterol and systolic blood pressure.

former and non-smokers. Risk estimates for quartiles of serum ceruloplasmin in smokers were 1, 2.08 (0.29-19.81), 3.54 (0.43-38.81), and 9.12 (1.41-88.09), respectively with 95% CI in parentheses and a p-value for trend of 0.021. For hypertension, hypercholesterolemia, and low high-density lipoprotein cholesterol levels no risk modifications were observed (data not shown).

Figure 2: Odds ratio and 95% confidence interval for myocardial infarction according to quartiles of serum ceruloplasmin



* adjusted for age, sex, body mass index, packyears smoked, equivalent household income (5 categories), alcohol intake (4 categories), serum cholesterol, and hypertension

The excess risk of myocardial infarction for the highest compared to the lowest quartile of serum ceruloplasmin was reduced by 33% when white blood cell count was taken into account or when C-reactive protein was included in the model (table 2). For both, white blood cell count and C-reactive protein, a non-significant increased risk of myocardial infarction was observed in the multivariate models. Exclusion of subjects with C-reactive protein above 6 g/l (28 subjects) resulted in an age- and sex-adjusted odds ratio of 2.18 (95% CI 0.88-5.49; p_{trend}=0.107) for the highest compared to the lowest quartile of serum ceruloplasmin. In the multivariate adjusted model, an odds ratio of 1.73 (95% CI 0.65-4.73, p_{trend}=0.300) was observed.

DISCUSSION

In the prospective cohort of the Rotterdam Study we observed a significant association between high baseline levels of serum ceruloplasmin and the subsequent risk for a first myocardial infarction. The association was most evident in current smokers. Serum ceruloplasmin was observed to be highly correlated with indices of inflammation. Exclusion of subjects with elevated C-reactive protein levels reduced the excess risk of myocardial infarction associated with high serum ceruloplasmin levels by 33%. For each 100 mg/l increase in serum ceruloplasmin we observed an increment of 41% in myocardial infarction risk. Comparable findings of an elevated risk of myocardial infarction[5] and incidence of coronary heart disease[6] among persons with high levels of serum ceruloplasmin have been previously reported. Several other studies reported associations of high levels of serum copper to elevated risk of increased carotid intima-media thickness[3], myocardial infarction[7], and mortality from coronary heart disease or cardiovascular disease[2,4].

Ceruloplasmin in which 90-95% of serum copper is bound, is considered a physiologic inhibitor of lipid peroxidation. However, besides its antioxidant properties such as its ability to scavenge superoxide and other reactive species and the inhibition of the Fenton reaction by conversion of Fe^{2+} to Fe^{3+} through ferroxidase activity, pro-oxidant activities of ceruloplasmin have been proposed[8]. To explain the pro-oxidant activity of ceruloplasmin a pathway involving lipid and lipoprotein oxidation has been suggested. Recent data indicate that ceruloplasmin by itself can oxidise LDL in vitro and possibly in vivo[8,13]. However, accessory factors derived from vascular cells may be modulatory or requisite during lipoprotein oxidation within the vessel wall[1].

An alternative explanation for the association between elevated serum ceruloplasmin levels and increased incidence of CHD is its property as acute-phase protein. Since an increase of ceruloplasmin can be mediated by many unspecific factors causing tissue injury, a high ceruloplasmin level may reflect response to injury or inflammation. Endothelial injury and inflammatory processes are thought to be involved in the pathogenesis of atherosclerosis and markers of inflammation and infection, such as white blood cell count, fibrinogen, and C-reactive protein have been shown to be independent risk factors for coronary heart disease[14-18]. Most previous studies have not taken into account markers of inflammation. We investigated whether the observed

associations in our study may have been due to the presence of inflammation. Including white blood cell count or C-reactive protein in the multivariate logistic regression model substantially reduced the risk estimate. We next excluded subjects with clinically elevated C-reactive protein from the analysis. Risk of myocardial infarction with serum ceruloplasmin levels was considerably decreased but still present. This suggests that a substantial part of the increased risk associated with high levels of serum ceruloplasmin may be attributed to inflammation processes. The remaining elevated risk may be due to other properties of ceruloplasmin, like its pro-oxidant activity or it may reflect low-level inflammation being involved in the cardiovascular disease process.

In summary, we observed an elevated risk of myocardial infarction with high serum ceruloplasmin levels in the elderly population of the Rotterdam Study. Adjustment of markers of inflammation substantially reduced the association between serum ceruloplasmin and myocardial infarction. This results suggest that at least part of the observed risk association with high levels of ceruloplasmin can be attributed to it's property as acute phase protein.

REFERENCES

1. Fox PL, Mukhopadhyay C, Ehrenwald E. Structure, oxidant activity, and cardiovascular mechanisms of human ceruloplasmin. *Life Sciences* 1995; **56**:1749-1758.
2. Kok FJ, van Duijn CM, Hofman A et al. Serum copper and zinc and the risk of death from cancer and cardiovascular disease. *Am J Epidemiol* 1988; **128**:352-359.
3. Salonen JT, Salonen R, Seppänen K et al. Interactions of serum copper, selenium, and low density lipoprotein cholesterol in atherogenesis. *BMJ* 1991; **302**:756-760.
4. Salonen JT, Salonen R, Korpela H et al. Serum copper and the risk of acute myocardial infarction: a prospective study in men in Eastern Finland. *Am J Epidemiol* 1991; **134**:268-276.
5. Reunanen A, Knekt P, Aaran R-K. Serum ceruloplasmin level and the risk of myocardial infarction and stroke. *Am J Epidemiol* 1992; **136**:1082-1090.
6. Mänttäri M, Manninen V, Huttunen JK et al. Serum ferritin and ceruloplasmin as coronary risk factors. *Eur Heart J* 1994; **15**:1599-1603.
7. Reunanen A, Knekt P, Marniemi J et al. Serum calcium, magnesium, copper, and zinc and risk of cardiovascular death. *Eur J Clin Nutr* 1996; **50**:431-437.
8. Ehrenwald E, Chisolm GM, Fox, PL. Intact human ceruloplasmin oxidatively modifies low density lipoprotein. *J Clin Invest* 1994; **93**:1493-1501.

9. Lamb DJ, Leake DS. Acidic pH enables caeruloplasmin to catalyse the modification of low-density lipoprotein. *FEBS Letters* 1994; **338**:122-126.
10. Hofman A, Grobbee DE, De Jong PTVM et al. Determinants of disease and disability in the elderly: The Rotterdam Elderly Study. *Eur J Epidemiol* 1991; **7**:403-422.
11. WHO (1992). *International Statistical Classification of Diseases and Related Health Problems*, 10th revision. Geneva, Vol 1.
12. Klipstein-Grobusch K, den Breeijen JH, Goldbohm RA et al. Dietary assessment in the elderly: validation of a semiquantitative food frequency questionnaire. *Eur J Clin Nutr* 1998; **52**:588-596.
13. Craig WY, Poulin SE, Palomaki GE et al. Oxidation-related analytes and lipid and lipoprotein concentrations in healthy subjects. *Arterioscl Thromb Vasc Biol* 1995; **15**:733-739.
14. Yarnell JW, Baker IA, Sweetnam PM et al. Fibrinogen, viscosity, and white blood cell count are major risk factors for ischemic heart disease. The Caerphilly and Speedwell collaborative heart disease studies. *Circulation* 1991; **83**:836-844.
15. Ernst E, Resch KL. Fibrinogen as a cardiovascular risk factor: a meta-analysis and review of the literature. *Ann Int Med* 1993; **118**:956-963.
16. Rosengren A, Wilhelmsen L. Fibrinogen, coronary heart disease and mortality from all causes in smokers and nonsmokers. The study of men born in 1933. *J Int Med* 1996; **239**:499-507.
17. Kuller LH, Tracy RP, Shaten J et al. Relation of C-reactive protein and coronary heart disease in the MRFIT nested case-control study. *Am J Epidemiol* 1996; **144**:537-547.
18. Haverkate F, Thompson SG, Pyke SDM et al. Production of C-reactive protein and risk of coronary events in stable and unstable angina. *Lancet* 1997; **349**:462-466.

3.2

Serum ferritin and risk of myocardial infarction in the elderly

ABSTRACT

Background: Elevated body iron stores have been suggested to be an ischemic heart disease risk factor.

Objective: To examine whether elevated serum ferritin levels, other parameters of iron status, and dietary iron affect the incidence of myocardial infarction (MI) in an elderly population.

Design: A nested case-control study of 60 cases of first MI and 112 controls frequency-matched on age (5-year categories) and sex embedded in the population-based cohort of the Rotterdam Study.

Results: Age- and sex-adjusted risk of MI for subjects with serum ferritin levels above 200 μg/l was 1.82 (95% CI, 0.90-3.69; p=0.096). The odds ratio (OR) was 1.26 (95% CI 0.98-1.64; p=0.078) for the highest compared to the lowest tertile of serum ferritin and was slightly altered in a multivariate model. Risk of MI associated with the highest compared to the lowest tertile of ferritin was found to be most evident in current or former smokers (OR=1.68; 95% CI 1.17-2.47; p_{trend}=0.008) and in subjects with hypercholesterolemia (OR=1.43; 95% CI 0.99-2.11; p_{trend}=0.056) or diabetes (OR=2.50; 95% CI 1.15-8.05; p_{trend}=0.020). No association with risk of MI was observed for tertiles of serum iron, serum transferrin, or total dietary iron. For dietary heme iron risk of MI was significantly increased in a multivariate model, additionally adjusted for categories of dietary energy, fat, saturated fat, and cholesterol (OR= 4.01; 95% CI 1.17-15.87; p_{trend}=0.031).

Conclusion: The observed association of elevated serum ferritin levels with myocardial infarction in high risk strata in an elderly population suggests that ferritin may adversely affect coronary heart disease risk in the presence of other risk factors.

INTRODUCTION

Free iron - a catalyst of the production of free radicals - has been implicated in ischemic myocardial damage and lipid peroxidation. Plausible hypotheses as to how free iron may accelerate the progression of atherosclerosis or contribute to myocardial injury after an ischemic event have been generated from basic research. Direct evidence that high stored iron levels or high iron intake increase the incidence of coronary heart disease in humans, however, is limited. So far the strongest supporting evidence for iron as a coronary heart disease risk factor stems from a cohort study of Eastern Finnish men, where high levels of serum ferritin and dietary iron were positively associated with incidence of myocardial infarction[1]. Furthermore, serum ferritin was observed to be one of the strongest indicators of presence and progression of carotid artery disease[2,3], and blood donation, depleting iron stores in blood donors, was associated with reduced risk of myocardial infarction[4] and cardiovascular disease[5]. However, most subsequent studies investigating whether iron status or dietary iron intake are associated with increased risk of myocardial infarction or coronary heart disease have not provided consistent results[6-14]. Using a nested case-control approach, we studied whether serum ferritin and other parameters of iron status are associated with the incidence of myocardial infarction in the elderly population of the Rotterdam Study.

SUBJECTS AND METHODS

Study population and case ascertainment

The Rotterdam Study is a community based prospective cohort study of 7983 persons (response rate 78%) aged 55 years and over, living in Ommoord, an urban district in Rotterdam, the Netherlands. The aim of the study is to investigate the incidence of and the risk factors for chronic and disabling cardiovascular, neurodegenerative, locomotor, and ophthalmic diseases as described elsewhere[15]. The study was approved by the Medical Ethics Committee of the Erasmus University, and written informed consent was obtained from all participants.

Follow-up for coronary heart disease started after the baseline survey in 1990. Until April 1996 (mean 4 years) follow-up information was available for 94% of the cohort. With respect to the vital status of participants, information was obtained at regular intervals from the municipal health service in Rotterdam. Information on fatal and non-

fatal endpoints was obtained from the general practitioners (GPs) working in the study district of Ommoord. All possible events reported by the GPs were verified by research physicians from the Rotterdam Study through records of the participating GPs and medical specialists. Cause and circumstances of death were obtained from the GP and from hospital discharge records in case of admittance or referral, shortly after reporting of death by the municipal health service or the GP. Classification of fatal and non-fatal events was based on the International Classification of Diseases, 10th edition[16]. For the present analysis cases of first myocardial infarction (ICD-10:I21-I24) were used. All events were classified independently by two research physicians. If there was disagreement about case status, a consensus was reached in a special session. Finally, all these events were verified by a cardiovascular disease expert. In case of discrepancies, the judgment by this expert was considered definite.

The association between serum ferritin and risk of fatal and non-fatal myocardial infarction was examined by use of a nested case-control approach. For every subject with a first myocardial infarction during follow-up (n = 202) a control without a cardiac end-point was selected. Age strata (5-year intervals) and sex were used as matching variables. Frozen sera, stored at -20°C for determination of serum ferritin were available for 255 subjects (111 myocardial infarction cases and 144 controls). Blood samples were not available for all cases and controls allocated to this study due to multiple use of blood samples in the Rotterdam Study. Subjects with C-reactive protein values above 6 g/l or in case data for C-reactive protein were missing, erythrocyte sedimentation rate above 20 mm/h (cases n = 25, controls n = 15) indicating presence of inflammation or infection that could potentially lead to elevated ferritin values were excluded from analysis. Further exclusion of subjects with a verified history of myocardial infarction resulted in 172 subjects, 60 cases (35 nonfatal and 25 fatal) and 112 controls for analysis of serum ferritin and risk of myocardial infarction.

Baseline measurements

Information on current health status, medical history, drug use, education, income, and smoking behaviour was obtained with a computerized questionnaire during a home interview. Height and weight were measured, and body mass index (weight in kg/height in m^2) was calculated as a measure of obesity. Sitting blood pressure was measured on the right upper arm with a random-zero sphygmomanometer. The average of two

measurements was used in the analysis. A venepuncture was performed and hematological parameters were obtained by standard clinical laboratory procedures. Serum total and high density lipoprotein (HDL) cholesterol concentrations were determined at baseline by an automated enzymatic procedure. Serum samples were collected from case and control subjects simultaneously at study baseline and frozen at -20 °C until used to determine serum concentrations of ferritin, iron, transferrin, ceruloplasmin, and C-reactive protein. Sera from cases and controls were analyzed in the same run. Serum ferritin concentrations were determined by ELISA (Boehringer Mannheim, Germany). The coefficient of variation was 2.8%, 4.0%, and 10.4% for ferritin levels of 389, 139 and 27 µg/l, respectively. Serum transferrin and C-reactive protein (CRP) were measured by kinetic nephelometry by use of a Beckman-Array system (Munich, Germany) and serum iron was determined by photometry on EPOS (Boehringer Mannheim, Germany). Coefficients of variation were 4%, 2%, 4.6%, and 3.8% for transferrin, iron, ceruloplasmin, and C-reactive protein, respectively.

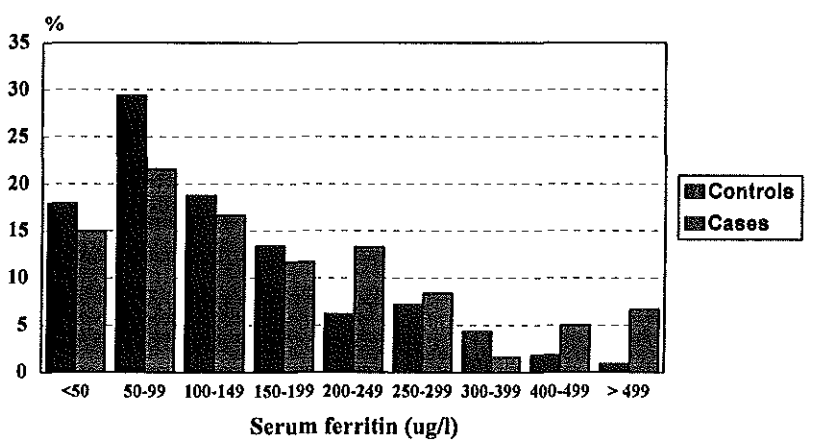
Dietary assessment

The semiquantitative food frequency questionnaire (SFFQ) completed during baseline aimed at assessing habitual food intake during the past year and included 170 food items in 13 food groups and questions about dietary habits, supplementation, and prescribed diets. SFFQ data were converted to nutrient intake using the computerized Dutch Food Composition Table[17]. Heme iron intake was estimated to account for 40% of the total iron found in meat, poultry, and fish[18]. Data for ß-carotene, retinol, and tocopherol were updated by use of an additional database by the Netherlands Institute of Public Health and Environmental Protection (YCJ Vollebregt, EJM Feskens, unpublished observations, 1993). Nutrient intake through nutritional supplements was not considered since brand labels, dose, and duration were not recorded with sufficient accuracy. The validity of the SFFQ was assessed in a subsample of 80 men and women aged 55 to 75 years. Nutrient intake estimated from the SFFQ was compared with estimated nutrient intake of in total 15 days of food records collected over a one-year period[19]. The ability of the SFFQ to adequately rank subjects according to their dietary intake was demonstrated by Pearson's correlation coefficients (0.5 to 0.9 for crude data) and a high degree of classification into the same or adjacent quintile (75.8 percent for crude data). Pearson's correlation coefficient between food records and SFFQ for iron was 0.67.

Data Analysis

Associations between serum ferritin and risk factors for ischemic heart disease were investigated by use of Pearson's correlation coefficient and for categorical variables by chi-square statistics. Analysis of variance was used to test for differences in baseline characteristics between cases and controls of myocardial infarction. All analyses were adjusted for age and sex. Serum ferritin was categorized equal or below/above 200 µg/l ferritin and risk of nonfatal and fatal myocardial infarction was investigated by multivariate logistic regression. To evaluate whether there was a graded association between serum ferritin levels and risk of myocardial infarction, analysis was performed for tertiles of serum ferritin. Analyses were initially adjusted for age and sex and subsequently for body mass index (BMI), packyears smoked, equivalent household income (five categories), and alcohol intake (five categories). To evaluate whether smoking status, hypertension, hypercholesterolemia, or diabetes modified the association between serum ferritin and myocardial infarction, stratified analyses were conducted. In addition, the association of other measures of iron status (serum iron and transferrin) and dietary iron intake (total iron and heme iron) to risk of myocardial infarction was evaluated.

Figure 1: Serum ferritin levels among cases and controls of myocardial infarction



For dietary intake of total and heme iron the multivariate model was furthermore adjusted for categories of energy, fat, saturated fat, and cholesterol. Associations are

Table 1: Means (SD) and distribution (%) of baseline characteristics for cases and controls of myocardial infarction

Variables	Cases of myocardial infarction *n = 60*		Controls *n = 112*		p†
Men (%)	44.6		45.0		n.s.
Age (years)	75.9	(8.5)	76.4	(8.9)	n.s.
Body mass index (kg/m^2)	26.2	(2.8)	26.0	(3.5)	n.s.
Waist-to-hip ratio	0.93	(0.09)	0.92	(0.08)	n.s.
Diastolic blood pressure (mmHg)	75	(12)	74	(12)	n.s.
Systolic blood pressure (mm Hg)	148	(21)	143	(23)	n.s.
Ferritin (µg/l)	183	(168)	144	(142)	n.s.
Transferrin (g/l)	2.52	(0.45)	2.59	(0.42)	n.s.
Serum iron (µmol/l)	16.4	(5.0)	16.9	(5.2)	n.s.
Serum cholesterol (mmol/l)	6.80	(1.22)	6.53	(1.33)	n.s.
HDL-serum cholesterol (mmol/l)	1.26	(0.28)	1.27	(0.32)	n.s.
Obesity (%)	10.0		13.4		n.s.
Hypertension (%)‡	36.7		32.1		n.s.
Hypercholesterolemia (%)*f*	55.0		44.6		n.s.
Diabetes (%)	20.3		20.6		n.s.
Current smokers (%)	18.3		16.1		n.s.
High alcohol intake¥	3.3		5.4		n.s.
Dietary iron (g/d)§	12.1	(2.7)	12.5	(3.5)	n.s.
Dietary heme iron (g/d)§	1.14	(0.46)	0.95	(0.36)	0.016

† for continous variables: ANOVA adjusted for age and sex
for categorical variables: Mantel-Haenszel Chi-square test adjusted for age and sex

‡ systolic blood pressure ≥ 160 mm Hg and/or diastolic blood pressure ≥ 95 mm Hg and/or use of medication

f serum cholesterol > 6.5 mmol/l

¥ alcohol intake > 20 g/d for females and > 30 g/d for males

§ dietary data available for 37 cases of myocardial infarction and 66 controls

Table 2: Odds ratio and 95% confidence intervals (CI) for myocardial infarction for tertiles of iron parameters

Variable	Tertiles			P value for trend
	1 (Lowest)*f*	2	3 (Highest)	
serum ferritin				
No. of MI /controls	17/41	17/40	26/31	
serum level (µg/l)	< 77	77 - 171	> 171	
Odds ratio (95% CI)				
age and sex adjusted	1.00	1.01 (0.67 - 1.51)	1.26 (0.98 - 1.64)	0.070
multivariate adjusted†	1.00	1.08 (0.71 - 1.64)	1.28 (0.98 - 1.67)	0.066
serum iron				
No. of MI/controls	23/35	16/42	21/35	
serum level (µmol/l)	< 14.1	14.1 - 18.9	> 18.9	
Odds ratio (95% CI)				
age and sex adjusted	1.00	0.75 (0.50 - 1.11)	0.95 (0.72 - 1.24)	0.699
multivariate adjusted†	1.00	0.77 (0.51 - 1.15)	0.97 (0.73 - 1.28)	0.788
serum transferrin				
No. of MI	25/49	20/31	15/32	
serum level (g/l)	< 2.5	2.5 - 2.7	> 2.7	
Odds ratio (95% CI)				
age and sex adjusted	1.00	1.12 (0.77 - 1.63)	0.97 (0.75 - 1.26)	0.909
multivariate adjusted†	1.00	1.15 (0.78 - 1.68)	0.96 (0.73 - 1.25)	0.624
dietary iron§				
No. of MI	12/23	14/20	11/23	
dietary intake (g/d)	< 11.1	11.1 - 13.1	> 13.1	
Odds ratio (95% CI)				
age and sex adjusted	1.00	1.73 (0.61 - 5.09)	1.33 (0.43 - 4.26)	0.641
multivariate adjusted†	1.00	1.49 (0.50 - 4.62)	1.35 (0.41 - 4.62)	0.606
multivariate adjusted‡	1.00	2.12 (0.57 - 8.45)	3.02 (0.50 - 20.52)	0.274
dietary heme iron§				
No. of MI	9/26	12/22	16/18	
dietary heme iron intake (g/d)	< 2.16	2.16 - 2.76	> 2.76	
Odds ratio (95% CI)				
age and sex adjusted	1.00	1.68 (0.59 - 4.96)	2.79 (1.01 - 8.13)	0.047
multivariate adjusted†	1.00	1.66 (0.56 - 5.08)	2.75 (0.92 - 8.64)	0.069
multivariate adjusted‡	1.00	1.85 (0.61 - 5.91)	4.01 (1.17 - 15.87)	0.031

f reference category

§ dietary data available for 37 cases of myocardial infarction and 66 controls

† adjusted for age, sex, BMI, packyears smoked, equivalent household income (five categories), alcohol intake (five categories.)

‡ additonally adjusted for categories of dietary energy, total fat, saturated fat, and cholesterol

expressed as an odds ratio with 95% confidence interval (CI). Two-sided p-values were calculated. Statistical analysis was performed using SAS® statistical software package version 6.11 (SAS Institute, Cary, NC, USA).

RESULTS

Serum ferritin levels in the case-control population ranged from 10 to 1221 µg/l and averaged 47, 119, and 309 µg/l per tertile. Median levels of serum ferritin were 129 µg/l for men and 101 µg/l for women. Serum ferritin was significantly inversely associated with serum transferrin ($r = -0.28$, $p = 0.002$) and was directly associated with serum iron, hemoglobin, and alcohol intake. For serum ferritin and heme iron intake a weak association was noted ($r = 0.14$; $p = 0.17$).

Figure 1 shows the distribution of serum ferritin for cases and controls indicating a shift towards higher serum ferritin levels for cases of myocardial infarction. Correspondingly, more cases of myocardial infarction had levels above the cut-off of 200 µg/l (33.3%) than controls (21.4%). Cases and controls of myocardial infarction differed significantly for heme iron intake (table 1) and percentage of high equivalent income.

When adjusted for age and sex, subjects with a serum ferritin level above 200 µg/l tended to have a risk of 1.82 (95% CI, 0.90 - 3.69; $p = 0.096$) for myocardial infarction compared to those with serum ferritin levels equal or below 200 µg/l. Further adjustment for body mass index, packyears smoked, income, and alcohol intake only marginally altered the risk of myocardial infarction (OR = 1.81, 95% CI 0.88 - 3.74, $p = 0.108$). To evaluate whether there was a graded association between serum ferritin levels and risk of myocardial infarction, serum ferritin tertiles were examined. Age- and sex-adjusted odds ratio (OR) for the highest compared to the lowest tertile was 1.26 (95% CI 0.98 - 1.64; $p_{trend} = 0.070$) and was 1.28 (95% CI 0.98 - 1.67; $p_{trend} = 0.066$) in the multivariate adjusted model (table 2). Inclusion of subjects with elevated CRP or erythrocyte sedimentation rate gave smaller estimates for subjects with ferritin levels above 200 µg/l (age- and sex-adjusted OR = 1.46; 95% CI 0.75-2.80) indicating misclassification of iron status when subjects with signs of inflammation were not excluded from analysis. No association with risk of myocardial infarction was observed for tertiles of serum iron, serum transferrin, or total dietary iron (table 2). For

Table 3: Odds ratio and 95% confidence intervals (CI) for myocardial infarction for tertiles of serum ferritin*f*

	No MI	No controls	Tertiles			P value
			1 (Lowest)†	2	3 (Highest)	for trend
smoking status‡						
current	11	18	1.00	0.39 (0.07-1.66)	2.62 (1.12-9.10)	0.047
former	29	46	1.00	1.63 (0.88-3.13)	1.56 (1.01-2.48)	0.046
non-smoker	20	48	1.00	0.80 (0.36-1.70)	1.00 (0.65-1.54)	0.975
hypertensionξ						
present	42	68	1.00	0.96 (0.55-1.66)	1.23 (0.88-1.74)	0.219
not present	18	44	1.00	1.02 (0.46-2.31)	1.26 (0.76-2.12)	0.353
hypercholesterolemiaλ						
present	33	50	1.00	1.17 (0.65-2.10)	1.43 (0.99-2.11)	0.056
not present	27	62	1.00	1.03 (0.56-1.90)	1.17 (0.80-1.74)	0.410
diabetes						
present	12	22	1.00	3.35 (0.91-19.92)	2.41 (1.12-7.67)	0.027
not present	47	85	1.00	0.95 (0.60-1.49)	1.11 (0.81-1.50)	0.541

f adjusted for age, sex, body mass index, packyears smoked, equivalent household income (five categories), and alcohol intake (five categories)

† reference category

‡ adjusted for age, sex, body mass index, equivalent household income (five categories), and alcohol intake (five categories)

ξ systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure≥ 90 and/or use of antihypertensive medication

λ serum cholesterol ≥ 6.5 mmol/l

dietary heme iron a significantly increased risk of myocardial infarction was observed for the highest compared to the lowest tertile of heme iron intake in an age- and sex-adjusted model (OR = 2.79; 95% CI 1.01- 8.13, p_{trend} = 0.047). Multivariate adjustment including dietary variables that could potentially confound the association between heme iron and myocardial infarction resulted in an OR of 4.01 (95% CI 1.17 - 15.87; p_{trend} = 0.031). Further adjustment by dietary antioxidants (ß-carotene, vitamin C, and vitamin E) did not materially alter the risk estimate.

Stratification by smoking status, hypercholesterolemia, and diabetes showed modification of the association between serum ferritin and myocardial infarction (table 3). Risk of myocardial infarction was more pronounced in the case of hypercholesterolemia (OR = 1.43; 95% CI 0.99 - 2.11; p_{trend} = 0.056 for the highest versus lowest tertile of serum ferritin) or diabetes (OR = 2.50; 95% CI 1.15 - 8.05; p_{trend} = 0.020). Both, current and former smoking considerably increased the risk of myocardial infarction in association with elevated serum ferritin concentrations (table 3).

DISCUSSION

In this study in an elderly Dutch population, elevated serum ferritin levels were associated with increased risk of myocardial infarction. It was most pronounced in current and former smokers and in those with diabetes. Serum iron, transferrin, and dietary total iron were not associated with myocardial infarction. High heme iron intake, however, was significantly associated with increased myocardial infarction risk.

Studies investigating whether iron status can be considered a cardiovascular risk factor presented conflicting results as recently reviewed[20,21]. This is not unexpected as none of the parameters advocated - hemoglobin, hematocrit, serum iron, transferrin, transferrin saturation, total iron binding capacity, or ferritin - accurately reflects body iron[22]. Because serum ferritin concentrations are directly proportional to intracellular ferritin concentrations, it is considered to be the best clinical measure of body iron stores[22] and the most feasible to use in epidemiological studies[23].

However, so far only few studies used serum ferritin levels to examine whether body iron stores are associated with cardiovascular disease. Serum ferritin levels are known to increase in response to inflammation. To circumvent a confounding effect of inflammation on serum ferritin levels we excluded subjects with C-reactive protein levels greater 6 g/l from analysis.

Previous evidence for an increased risk of myocardial infarction with elevated serum ferritin levels has come from the Kuopio Ischemic Heart Disease Study following 1931 men for on average three years[1]. Men with serum ferritin levels ≥ 200 µg/l had a 2.2-fold (95% CI 1.2 - 4.0; p <0.01) risk of myocardial infarction compared with men with lower serum ferritin levels after adjustment for other risk factors. This association was stronger in men with serum low density lipoprotein concentrations of 5.0 mmol/l or more than in others. Extendend follow-up after a mean follow-up period of five years confirmed these previous findings[24]. In 847 Austrian men and women aged 40 to 79 years, Kiechl et al.[2] examined the relation between sonographically assessed carotid atherosclerosis and body iron stores. Ferritin was observed to be one of the strongest indicators of presence of carotid artery disease (OR = 1.54 per 100 µg/l serum ferritin; p<0.001) in men and women aged 40 to 59. Again, a synergistic effect between hypercholesterolemia and serum ferritin levels was observed. Five-year follow-up showed that serum ferritin was also a strong risk predictor of overall progression of atherosclerosis and of incident cardiovascular disease and death. Risk of atherosclerosis and cardiovascular disease was modified by serum low-density lipoprotein cholesterol. Changes in iron stores during the follow-up period modified the atherosclerosis risk in that a lowering was beneficial and further iron accumulation unfavourable[3]. Further studies relating serum ferritin levels to carotid intima media thickness[4], presence of atherosclerosis[26], myocardial infarction[6,7], or coronary heart disease[8,27] were not supportive of an association of body iron stores with cardiovascular disease. However, studies investigating the effect of blood donation or phlebotomy, resulting in a considerable decrease of serum ferritin levels, on cardiovascular disease risk corroborate the finding of decreased risk. Meyers et al.[5], comparing cardiovascular event rates in whole blood donors to non-donors, demonstrated blood donation to be associated with reduced risk of cardiovascular events (crude OR = 0.50, 95% CI 0.38 - 0.66) after 5-8 years of follow-up. Benefit of donation was confined to non-smoking males (adjusted OR 0.67; 95% CI 0.45 - 0.99),

limited to blood donation in the most recent three years, and was observed to be greater in non-smoking men with serum LDL cholesterol levels >160 mg/dl. Among 2682 Finnish men blood donation was prospectively associated with a risk reduction of myocardial infarction by 86%[4]. However, a likely self-selection of healthier persons as blood donors should be considered. Examination of the effect of phlebotomy on the oxidation resistance of serum lipoproteins in 14 men with raised serum ferritin concentrations demonstrated significantly decreased maximal oxidation and increased lag time to start of oxidation[28].

We observed risk of myocardial infarction to be confined to current and former smokers and to be more pronounced in subjects with diabetes or serum cholesterol above 6.5 mmol/l (table 4). The effects of iron stores on atherogenesis were found to be more pronounced in smokers[3] and synergistic effects between serum ferritin and serum cholesterol or low-density lipoprotein cholesterol have been reported as discussed above[1-3]. This indicates that ferritin may adversely affect coronary heart disease risk in the presence of other risk factors that may increase the formation of free radicals thus accelerating atherogenesis by stimulation of low-density lipoprotein oxidation[29,30]. Basic research has provided strong evidence that low-density lipoprotein (LDL) oxidation plays an important role in the pathogenesis of atherosclerosis and cardiovascular disease. Oxidized LDL causes lipid accumulation in macrophages and foam cell formation[31,32] and has been shown to be cytotoxic to many cell types and chemotactic for monocyte macrophages. Lipid peroxidation of LDL can be enhanced by metal-catalyzed reactions resulting in highly reactive hydroxyl radicals. Superoxide anions produced by oxidative stress and reducing agents have been found to be capable of mobilizing iron from ferritin[29,30].

In the current study we observed dietary heme iron to be associated with increased risk of myocardial infarction. Increased risk of non-fatal myocardial infarction or fatal coronary heart disease with heme iron intake has also been reported in the Health Professional Study[33]. Observations among Seventh Day Adventists in whom meat consumption 6 or more times per week was associated with increased risk of fatal ischemic heart disease[34] are furthermore supportive of a possible role of heme iron in coronary heart disease. Results suggestive of a role of heme iron in lipid peroxidation come furthermore from a nested case-control study showing a positive association

between blood hemoglobin concentration and the titer of autoantibodies against malondialdehyde-modified LDL[35] and from the ability of hemin to very efficiently promote LDL oxidation in vitro[36].

In conclusion, we observed elevated serum ferritin levels to be associated with increased risk of myocardial infarction in our elderly population. Elevated risk was most evident in current or former smokers and diabetic subjects suggesting that ferritin may adversely affect coronary heart disease risk in the presence of other risk factors. Possibly those factors in interaction with elevated body iron stores may accelerate atherogenesis by stimulating oxidation of low-density lipoproteins.

REFERENCES

1. Salonen JT, Nyyssönen K, Korpela H et al. High stored iron levels are associated with excess risk of myocardial infarction in eastern Finnish men. *Circulation* 1992; **86**:803-811.
2. Kiechl S, Aichner F, Gerstenbrand F et al. Body iron stores and presence of carotid atherosclerosis: Results from the Bruneck Study. *Arterioscler Thromb* 1994; **14**:1625-1630.
3. Kiechl S, Willeit J, Egger G et al. Body iron stores and the risk of carotid atherosclerosis. Prospective results from the Bruneck Study. *Circulation* 1997; **96**:3300-3307.
4. Tuomainen TP, Salonen R, Nyyssönen K et al. Cohort study of the relation between donating blood and risk of myocardial infarction in 2682 men in eastern Finland. *BMJ* 1997; **314**:793-794.
5. Meyers DG, Strickland D, Maloley PA et al. Possible association of a reduction in cardiovascular events with blood donation. *Heart* 1997; **78**:188-193.
6. Stampfer MJ, Grodstein F, Rosenberg I et al. A prospective study of plasma ferritin and risk of myocardial infarction in US physicians. *Circulation* 1993; **87**:688. [Abstract].
7. Magnusson MK, Sigfusson N, Sigvaldason H et al. Low iron-binding capacity as a risk factor for myocardial infarction. *Circulation* 1994; **89**:102-108.
8. Mänttäri M, Manninen V, Huttunen JK et al. Serum ferritin and ceruloplasmin as coronary risk factors. *Eur Heart J* 1994; **15**:1599-1603.
9. Sempos TC, Looker AC, Gillum RF et al. Body iron stores and the risk of coronary heart disease. *N Engl J Med* 1994; **330**:1119-1124.
10. Liao Y, Cooper RS, McGee DL. Iron status and coronary heart disease: negative findings from the NHANES I Epidemiologic Follow-Up Study. *Am J Epidemiol* 1994; **139**:704-712.
11. Morrison HI, Semenciw RM, Mao Y et al. Serum iron and risk of fatal acute myocardial infarction. *Epidemiology* 1994; **5**:243-246.
12. Baer DM, Tekawa IS, Hurley LB. Iron stores are not associated with acute myocardial infarction. *Circulation* 1994; **89**:2915-2918.

13. Reunanen A, Takkunen H, Knekt P et al. Body iron stores, dietary iron intake and coronary heart disease mortality. *J Intern Med* 1995; **238**:223-230.
14. Corti M-C, Guralnik JM, Salive ME et al. Serum iron level, coronary artery disease, and all-cause mortality in older men and women. *Am J Cardiol* 1997; 120-127.
15. Hofman A, Grobbee DE, De Jong PTVM et al. Determinants of disease and disability in the elderly: The Rotterdam Elderly Study. *Eur J Epidemiol* 1991; 7:403-422.
16. WHO: International Statistical Classification of Diseases and Related Health Problems, 10th revision, 1992. Geneva, Vol 1.
17. Voedingsraad: Nederlands Voedingsstoffenbestand (NEVO). 1993. The Hague, The Netherlands: Voorlichtingsbureau voor de Voeding (Dutch food composition table).
18. Monsen ER, Hallberg L, Layrisse M et al. Estimation of available dietary iron. *Am J Clin Nutr* 1978; **31**:134-141.
19. Klipstein-Grobusch K, den Breeijen JH, Goldbohm RA et al. Dietary assessment in the elderly: validation of a semiquantitative food frequency questionnaire. *Eur J Clin Nutr* 1998; **52**:588-596.
20. Sempos CT, Looker AC, Gillum RF. Iron and heart disease: The epidemiologic data. *Nutr Rev* 1996; **54**:73-84.
21. Corti M-C, Gaziano M, Hennekens CH. Iron status and risk of cardiovascular disease. *Ann Epidemiol* 1997; 7:62-68.
22. Cook JD, Lipschitz DA, Miles LEM et al. Serum ferritin as a measure of iron stores in normal subjects. *Am J Clin Nutr* 1974; **27**:681-687.
23. Beaton GH, Corey PN, Steele C: Conceptual and methodological issues regarding the epidemiology of iron deficiency and their implications for studies of the functional consequences of iron deficiency. *Am J Clin Nutr* 1989; **50**:575-588.
24. Salonen JT, Nyyssönen K, Salonen R. Body iron stores and the risk of coronary heart disease [letter]. *N Engl J Med* 1994; **331**:1159-1160.
25. Moore M, Folsom AR, Barnes RW, Eckfeldt JH. No association between serum ferritin and asymptomatic carotid atherosclerosis. The Atherosclerosis Risk in Communities (ARIC) Study. *Am J Epidemiol* 1995; **141**:719-723.
26. Rauramaa R, Väisänen S, Mercuri M et al. Association of risk factors and body iron status to carotid atherosclerosis in middle-aged eastern Finnish men. *Eur Heart J* 1994; **15**:1020-1027.
27. Aronow WS, Ahn C: Three-year follow-up shows no association of serum ferritin levels with incidence of new coronary events in 577 persons aged 62 years. *Am J Cardiol* 1996; **78**:678-679.
28. Salonen JT, Korpela H, Nyyssönen K et al. Lowering of body iron stores by blood letting and oxdiation resistance of serum lipoproteins: a randomized cross-over trial in male smokers. *J Intern Med* 1995; **237**:161-168.
29. de Silva DM, Aust SD. Ferritin and ceruloplasmin in oxidative damage: review and recent findings. *Can J Physiol Pharmacol* 1993; **71**:715-720.
30. Reif DW. Ferritin as a source of iron for oxidative damage. *Free Radical Biol Med* 1992; **12**:417-427.

31. Steinberg D, Parthasarathy S, Carew TE et al. Beyond cholesterol: Modifications of low density lipoprotein that increase its atherogenecity. *N Engl J Med* 1989; **320**:915-924.
32. Witztum JL. The oxidation hypothesis of atherosclerosis. *Lancet* 1994; **344**:793-795.
33. Ascherio A, Willett WC, Rimm EB et al. Dietary iron intake and risk of coronary disease among men. *Circulation* 1994; **89**:969-974.
34. Snowdon DA, Phillips RL, Fraser GE. Meat consumption and fatal ischemic heart disease. *Prev Med* 1984; **13**:490-500.
35. Salonen JT, Ylä-Herttuala S, Yamamoto R et al. Autoantibody against oxidised LDL and progression of carotid atherosclerosis. *Lancet* 1992; **339**:883-887.
36. Balla G, Jacob HS, Eaton JW et al. Hemin: a possible physiological mediator of low density lipoprotein oxidation and endothelial injury. *Arterioscler Thromb* 1991; **11**:1700-1711.

3.3

Dietary iron and risk of myocardial infarction

ABSTRACT

Free iron has been implicated in lipid peroxidation and ischemic myocardial damage, and it has been suggested that iron is an independent risk factor for myocardial infarction (MI). The authors investigated whether dietary iron is associated with increased risk of fatal and nonfatal MI in the Rotterdam Study, a community-based prospective cohort study among 7983 elderly subjects in Rotterdam, The Netherlands. The study sample consisted of 4802 participants who at baseline had no known history of myocardial infarction and for whom dietary data were available. From 1990 to 1996 124 subjects experienced MI. No association was observed between total iron intake and risk of MI adjusted for age and sex (relative risk highest versus lowest tertile 0.89; 95 percent confidence interval 0.55-1.45; p_{trend} 0.640). Heme iron intake was positively associated with risk of MI (RR highest versus lowest tertile 1.83; 95 percent confidence interval 1.16-2.91; p_{trend} 0.008) after adjustment for age and sex and persisted after multivariate adjustment (RR 1.86; 95 percent confidence interval 1.14-3.09; p_{trend} 0.010). Distinction between fatal and non-fatal cases of MI indicated that the association of heme iron to MI was more pronounced in fatal cases. The results suggest that high dietary heme iron intake is related to increased risk of myocardial infarction and that it may specifically affect the rate of fatality of myocardial infarction.

INTRODUCTION

Because of the importance of oxidized low density lipoprotein in atherosclerosis[1,2], the potential for iron to act as catalyst in biological oxidative damage[3,4], and ecological observations of coronary artery disease and iron status[5], a potential role of iron in coronary heart disease seems plausible. So far the strongest supporting evidence for iron as a coronary heart disease risk factor stems from a cohort study of eastern Finnish men[6,7], where high levels of serum ferritin and dietary iron were positively associated with incidence of myocardial infarction. Most subsequent prospective studies investigating whether iron status or dietary iron intake are associated with increased risk of myocardial infarction or coronary heart disease could not support the hypothesis that high body iron stores increase the risk of coronary heart disease[8-13], though Ascherio et al.[4], reported an increased risk of non-fatal myocardial infarction or fatal coronary heart disease with intake of heme iron. However, measures of iron status such as serum iron or transferrin saturation as applied in most of these studies are generally not considered to accurately reflect body iron stores[15]. Most studies did not discriminate between non-fatal and fatal myocardial infarction, although iron may be directly involved in the myocardial injury caused by ischemia and reperfusion. Indirect evidence that iron is involved in reperfusion injury after an ischemic event has been provided by results of animal experiments showing that free radicals are generated after restoration of blood flow to ischemic myocardium[16], contributing to the subsequent myocardial injury[17]. Experiments with an iron chelator[18,19] or iron-overloading[20] in animals emphasize this notion. It has subsequently been suggested that elevated iron stores may have an adverse effect on the survival after a myocardial infarction[21]. In the current study we investigated whether dietary iron and heme iron are associated with the incidence and case fatality of myocardial infarction in the cohort of the Rotterdam Study.

MATERIAL AND METHODS

Study population

The Rotterdam Study is a community based prospective cohort study of 7983 persons (response rate 78 percent) aged 55 years and over, living in Ommoord, an urban district in Rotterdam, the Netherlands. The aim of the study is to investigate the incidence of and the risk factors for chronic and disabling cardiovascular, neurodegenerative,

locomotor, and ophthalmic diseases as described elsewhere[22]. The study was approved by the Medical Ethics Committee of the Erasmus University, and written informed consent was obtained from all participants. Follow-up for coronary heart disease mortality started after the baseline survey in 1990. For the present analysis follow-up information until April 1996 was available. Of the 5159 subjects with dietary data, 173 subjects experienced a myocardial infarction during the follow-up period. Because of possible changes in dietary pattern, subjects with history of myocardial infarction at baseline (n = 357) were excluded from analysis. This resulted in 4802 subjects for the current analysis. Of these, 124 cases of first fatal or non-fatal myocardial infarction occurred.

Case ascertainment

The follow-up period comprised 3 to 7 (mean 4) years. With respect to the vital status of participants, information was obtained at regular intervals from the municipal health service in Rotterdam. Information on fatal and non-fatal endpoints was obtained from the general practitioners working in the study district of Ommoord. All possible events reported by the general practitioners were verified by research physicians from the Rotterdam Study through records of the participating general practitioners and medical specialists. Cause and circumstances of death were obtained by using a questionnaire for general practitioners and by scrutinizing information from hospital discharge records in cases of admittance or referral, shortly after a death was reported by the municipal health service or the general practitioner. Overall, follow-up information was available for 94 percent of the cohort.

Classification of fatal and non-fatal events was based on the International Classification of Diseases (ICD), 10th edition[23]. For the present analysis cases of first non-fatal myocardial infarction or fatal myocardial infarction (ICD-10 codes I21-I24) were selected. All events were independently classified by two research physicians. If there was disagreement, a consensus was reached in a special session. Finally, all these events were verified by a cardiovascular disease expert. In case of discrepancies, the judgment by this expert was considered definite.

Measurements

Information on current health status, medical history, drug use, education, income, and smoking behavior was obtained with a computerized questionnaire during a home

interview. Height and weight were measured, and body mass index (weight in kg/height in m^2) was calculated as a measure of obesity. Sitting blood pressure was measured on the right upper arm with a random-zero sphygmomanometer. The average of two measurements was used in the analysis. Hypertension was defined as a systolic blood pressure of at least 140 mm Hg and/or diastolic blood pressure of at least 90 mm Hg and/or current use of antihypertensive drugs for indication of hypertension. A venepuncture was performed and serum total and high density lipoprotein (HDL) cholesterol concentrations were determined by an automated enzymatic procedure. If serum cholesterol levels were at least 6.5 mmol/l or higher, subjects were considered to be hypercholesterolemic. All participants except for those using antidiabetic medication, were given a 37.5 percent oral glucose solution (75 g of glucose) in a nonfasting state. Venous glucose levels were measured before and after two hours. Non-insulin-dependent diabetes was diagnosed if at least one measurement was 11.1 mmol/l or more or if the participant used antidiabetic medication.

Dietary assessment

A semiquantitative food frequency questionnaire (SFFQ) completed during baseline aimed at assessing habitual food intake during the past year. The questionnaire included 170 food items in 13 food groups and questions about dietary habits, supplementation, and prescribed diets. SFFQ data were converted to nutrient intake using the computerized Dutch food composition table[24]. Heme iron intake was estimated to be equal to 40% of the iron found in meat, fish, and poultry[25]. Data for ß-carotene and tocopherol were updated by use of an additional database by the Netherlands Institute of Public Health and Environmental Protection (YCJ Vollebregt, EJM Feskens, unpublished observations, 1993). Intake through nutritional supplements was not considered since information from brand labels were not recorded with sufficient accuracy. The validity of the SFFQ was assessed in a subsample of 80 men and women aged 55 to 75 years. Nutrient intakes estimated from the SFFQ were compared with estimated nutrient intakes of 15 days of food records collected over a one-year period[26]. The ability of the SFFQ to adequately rank subjects according to their dietary intake was demonstrated by Pearson's correlation coefficients (0.5 to 0.9 for crude data) and a high degree of classification into the same or adjacent quintile (75.8 percent for crude data). Pearson's correlation coefficient between food records and SFFQ for iron was 0.67.

Table 1: Association of various baseline factors adjusted for age and sex to energy-adjusted total and heme iron intake for the cohort of the Rotterdam Study, Rotterdam, the Netherlands, from 1990-1996

	Tertile of energy adjusted iron intake			Tertile of energy adjusted heme iron intake		
	1 (Lowest)	2	3 (Highest)	1 (Lowest)	2	3 (Highest)
Mean body mass index (kg/m^2)	25.9	26.3	26.4	25.4	26.3	26.9
Current smokers (%)	28.5	24.7	21.9	23.0	23.0	28.1
Hypertension* (%)	48.3	49.8	47.7	46.1	49.0	50.0
Hypercholesteremia† (%)	51.7	49.2	48.9	45.6	50.2	53.0
Diabetes (%)	9.0	8.9	9.7	7.2	8.9	11.5
Mean alcohol intake (g/d)	13.1	11.8	9.5	10.1	11.1	12.7
Antioxidative vitamin supplements‡ (%)	11.1	10.9	11.6	13.0	10.2	10.7
Mean energy-adjusted						
ß-carotene (mg/d)	1.16	1.39	1.70	1.43	1.43	1.46
Vitamin C (mg/d)	97	111	125	112	112	112
Vitamin E (mg/d)	12.3	13.0	13.6	13.2	13.3	12.6
Total fat (g/d)	81.9	79.7	77.3	77.2	79.4	81.6
Saturated fat (g/d)	32.9	30.9	29.3	30.2	30.8	31.7
Cholesterol (mg/d)	228	227	227	203	224	251

* systolic blood pressure ≥ 140 and/or diastolic blood pressure ≥ 90 and/or use of antihypertensive medication

† serum cholesterol ≥ 6.5 mmol/l

‡ use of either ß-carotene, vitamine E, vitamin C, or multivitamin supplements

Table 2: Relative risk of myocardial infarction and 95 percent confidence intervals according to intake of energy-adjusted tertiles of iron and heme iron for the cohort of the Rotterdam Study, Rotterdam, the Netherlands, 1990-1996

Variable	Tertiles of energy-adjusted intake			P value for trend
	1 (Lowest)§	2	3 (Highest)	
iron				
No. of MI	38	40	46	
iron intake (mg/d)	< 10.6	10.6 - 12.4	> 12.4	
Relative risk (95% CI*)				
age and sex adjusted	1	0.97 (0.61 - 1.54)	0.89 (0.55 - 1.45)	0.640
multivariate adjusted†	1	1.05 (0.66 - 1.68)	1.11 (0.67 (1.87)	0.787
heme iron				
No. of MI	29	37	58	
heme iron intake (mg/d)	< 0.70	0.70 - 1.00	> 1.00	
Relative risk (95% CI*)				
age and sex adjusted	1	1.23 (0.75 - 2.03)	1.83 (1.16 - 2.91)	0.008
multivariate adjusted‡	1	1.23 (0.75 - 2.06)	1.86 (1.14 - 3.09)	0.010

§ reference category

* confidence interval

† adjusted for age, sex, body mass index, packyears smoked, equivalent houshold income (five categories), education (five categories) and alcohol intake (five categories), categories of energy-adjusted ß-carotene, vitamin C, vitamin E, use of antioxidative vitamin supplements

‡ additionally adjusted for categories of energy-adjusted fat, saturated fat, and cholesterol.

Adjustment for sex, age, total energy intake[27], and for within-person variability in daily intake[28] resulted in a correlation coefficient for iron of 0.44.

Data Analysis

The association between energy-adjusted dietary iron and heme iron intake and risk of myocardial infarction was examined by multivariate logistic regression. Energy-adjusted dietary intake of iron and heme iron was categorized into tertiles, and risk of myocardial infarction in the middle and highest tertile was compared to risk in the lowest tertile. The

initial analysis was adjusted for age and sex. The analyses were subsequently adjusted for body mass index, pack-years smoked, equivalent household income (five categories), highest education attained (five categories), and alcohol intake (five categories). In addition, dietary energy-adjusted intake of ß-carotene, vitamin E, vitamin C, total fat, saturated fat, and cholesterol were considered. Use of antioxidative vitamin supplements defined as use of either ß-carotene, vitamin C, vitamin E, or multivitamin supplements was also taken into account. Each association was expressed as a relative risk with 95 percent confidence interval (CI). Results were considered statistically different at the two-sided 0.05 alpha-level. Two-sided p-values were calculated. Statistical analysis was performed using SAS® (Release 6.11; SAS Institute, Cary, NC, USA).

RESULTS

The mean intake of energy-adjusted total iron was 9.3 mg/d in the lowest and 14.3 mg/d in the highest tertile. For energy-adjusted heme iron, figures were 0.48 mg/d in the lowest and 1.36 mg/d in the highest tertile. Since sex and age were not equally distributed across tertiles of total and heme iron intake, baseline factors and selected nutrient intakes presented in table 1 were adjusted for sex and age. Percentage of current smokers decreased across tertiles of iron, whereas intake of ß-carotene and vitamin C increased and total fat and alcohol intake decreased. For heme iron intake an increase in the prevalence of hypercholesterolemia and total fat and cholesterol intake across tertiles was observed. The percentage of current smokers was considerably higher in the highest tertile of heme iron intake. Iron supplementation was negligible in this elderly cohort (0.2 percent) and was not related to dietary iron intake.

When adjusted for age and sex, intake of total iron was not associated with risk of myocardial infarction. Intake of heme iron was significantly associated with an increased risk of myocardial infarction (table 2). The relative risk for the highest compared to the lowest tertile was 1.83 (95 percent confidence interval 1.16-2.91; p_{trend} 0.008). After adjustment for body mass index, pack-years smoked, equivalent household income, education, and alcohol intake the observed association with heme iron remained (relative risk 1.84, 95 percent confidence interval 1.16-3.00; p_{trend} 0.008). Adjustment by dietary factors and cholesterol and subsequently for intake

of Table 3: Relative risk of myocardial infarction and 95 percent confidence intervals according to intake of energy-adjusted tertiles of heme iron for the cohort of the Rotterdam Study, Rotterdam, the Netherlands, 1990-1996

Variable	Tertiles of energy-adjusted heme iron intake			P value for trend
	1 (Lowest)*	2	3 (Highest)	
Hypercholesterolemia				
multivariate adjusted†				
present (cases = 74)	1	1.59 (0.82 - 3.21)	2.39 (1.26 - 4.75)	0.008
absent (cases = 50)	1	0.89 (0.41 - 1.91)	1.27 (0.64 - 2.61)	0.060
Hypertension				
multivariate adjusted†				
present (cases = 77)	1	1.34 (0.71 - 2.56)	2.10 (1.16 - 3.92)	0.014
absent (cases = 47)	1	1.10 (0.49 - 3.53)	1.51 (0.71 - 3.38)	0.260
Smoking status				
multivariate adjusted ‡				
current (cases = 40)	1	0.81 (0.26 - 2.47)	2.67 (1.17 - 6.86)	0.008
ex/non-smoker (cases = 84)	1	1.38 (0.79 - 2.45)	1.45 (0.82 - 2.62)	0.216
Diabetes				
multivariate adjusted†				
present (cases = 21)	1	1.81 (0.34 - 13.37)	5.40 (1.41 - 35.55)	0.012
absent (cases = 100)	1	1.18 (0.70 - 2.00)	1.46 (0.87 - 2.48)	0.148

* reference category

† adjusted for age, sex, body mass index, packyears smoked, equivalent houshold income (five categories), education (five categories) and alcohol intake (five categories).

‡ adjusted for age, sex, body mass index, equivalent houshold income (five categories), education (five categories), and alcohol intake (five categories).

ß-carotene, vitamin E, vitamin C, and use of antioxidative vitamin supplements did not materially alter the risk estimate (table 2). On repeat of the analysis without applying any exclusion criteria, the relative risk was somewhat attenuated but still significantly

elevated with 1.68 (95 percent confidence interval 1.10-2.60; p_{trend} 0.017) for the highest compared to the lowest tertile of heme iron intake.

Table 3 presents the multivariate adjusted relative risks of myocardial infarction according to presence of other risk factors for myocardial infarction - hypertension, hypercholesterolemia, cigarette smoking, diabetes - and heme iron intake. For all risk factors investigated the increased risk of myocardial infarction was primarily observed in the presence of these risk factors. Relative risk estimates for fatal (n = 30) and non-fatal (n = 94) cases of first myocardial infarction and heme iron intake are presented in table 4. Multivariate age- and sex-adjusted risk for the top compared to the bottom tertile of heme iron was considerably elevated for fatal cases with 3.77 (95 percent confidence interval 1.22-14.20; p_{trend} 0.033). For non-fatal cases relative risk was 1.59 (95 percent confidence interval 0.93-2.76; p_{trend} 0.064).

Table 4: Relative risk of fatal and non-fatal myocardial infarction and 95 percent confidence intervals according to intake of energy-adjusted tertiles of heme iron for the cohort of the Rotterdam Study, Rotterdam, the Netherlands, from 1990-1996

Variable	Tertiles of energy-adjusted heme iron intake			P value for trend
	1 (Lowest)*	2	3 (Highest)	
No. of non-fatal MI	25	23	46	
Relative risk (95% CI)				
age and sex adjusted	1	0.86 (0.48 - 1.53)	1.57 (0.95 - 2.65)	0.053
multivariate adjusted†	1	0.86 (0.48 - 1.55)	1.59 (0.93 - 2.76)	0.064
No. of fatal MI	4	13	13	
Relative risk (95% CI)				
age and sex adjusted	1	3.41 (1.20 - 12.19)	3.56 (1.23 - 12.99)	0.029
multivariate adjusted†	1	3.66 (1.26 - 13.25)	3.77 (1.22 - 14.20)	0.033

* reference category

† adjusted for age, sex, body mass index, packyears smoked, equivalent houshold income (five), education (five categories), alcohol intake (five categories), categories of energy-adjusted ß-carotene, vitamin C, vitamin E, fat, saturated fat, and cholesterol, and use of antioxidative vitamin supplements

DISCUSSION

In the prospective cohort of the Rotterdam Study, we found no evidence for a positive association between total iron intake and risk of myocardial infarction. Heme iron intake, however, was positively associated with risk of myocardial infarction. Associations were strongest in those with presence of other risk factors for myocardial infarction.The results indicated that the association of heme iron to myocardial infarction was more pronounced in fatal than non-fatal cases of first myocardial infarction.

Prior to interpretation of these results some methodological issues should be considered. Potential bias due to incomplete follow-up was unlikely to occur due to the high follow-up rate (94 percent) achieved. Case ascertainment was facilitated because general practitioners working in the study district of Ommoord covered 85% of the cohort and records were linked to the municipal health service in Rotterdam. Since all events were classified independently by two research physicians and in the case of disagreement by a medical expert in the field of cardiovascular disease, inaccuracies of coding of diagnosis was minimized. Because subjects with a previous diagnosis of myocardial infarction may have altered their diet as a consequence of disease, those reporting at baseline that they had been hospitalized for myocardial infarction were excluded from analysis.

Confounding by dietary fats and cholesterol is of particular concern since these factors are likely to be correlated with heme iron (age- and sex-adjusted Spearman correlation's between heme iron and total fat, saturated fat, and cholesterol were 0.14, 0.10, and 0.34 [for all $p < 0.0001$], respectively). We attempted to address this point by inclusion of these variables in the multivariate model. However, adjustment for these factors did not materially alter the risk estimate. Since heme iron is largely derived from meat and meat products, the observed association between heme iron intake and myocardial infarction may be attributed to other factors from meat apart from fat or cholesterol or to unidentified lifestyle habits associated with meat intake. We attempted to adjust for lifestyle factors such as smoking and alcohol intake, but we cannot exclude presence of residual confounding. However, it seems unlikely that a confounder could explain that the observed association between heme iron and myocardial infarction was most marked in the presence of hypertension,

hypercholesterolemia, diabetes, or cigarette smoking. In fact, this is highly compatible with the hypothesis of a promoting role of iron on LDL cholesterol oxidation. Furthermore, a confounder could not explain the difference in findings with respect to fatal and non-fatal events. Considering this, it seems unlikely that confounding accounts for the observed association between heme iron and myocardial infarction, although we cannot conclusively dismiss presence of residual confounding.

We found heme and not total iron intake associated with myocardial infarction. There are several reasons to separate dietary heme iron from nonheme iron as a potential risk factor for coronary diseases. Iron status is regulated as a result of both absorption and bioavailability. Absorption of dietary iron is in general determined by two major factors, the rate of erythropoiesis and the size of the body's iron stores. Bioavailability is dependent both on the amount of meat in the diet since heme iron is well absorbed, and on the balance in the diet between promoters of nonheme iron absorption (e.g. ascorbic acid, meat, fish) and inhibitors of iron absorption (e.g. phytates, soy protein, polyphenols)[29]. The mechanisms of absorption of non-heme and heme iron differ markedly. At physiological levels, iron uptake is mediated by a series of receptors and binding proteins, whereas heme iron is taken up by the mucosal cells as such, therefore the iron in these cells is not exposed to the effects of the many ligands in the diet that inhibits iron absorption. For this reasons, only a slight association of total iron with risk of coronary heart disease would be expected in a generally well nourished population.

Studies investigating the association between dietary iron and coronary heart disease have yielded inconsistent results. Whereas some researchers observed no association between dietary iron intake and coronary heart disease[9,13], Ascherio et al.[14] reported a decreased risk of non-fatal myocardial infarction or fatal coronary heart disease with total dietary iron intake. Intake of heme iron, however, was, as in the present analysis, associated with an increased risk of non-fatal myocardial infarction or fatal coronary heart disease. After multivariate adjustment the inverse association with total iron and the direct association of heme iron intake were attenuated, though the association of heme iron of non-fatal myocardial infarction or fatal coronary heart disease remained statistically significant. Among Eastern Finnish men, in contrast, a direct association of total iron with risk of myocardial infarction was observed[6]. For each milligram of

daily iron intake, the authors reported an increment of 5% in the myocardial infarction risk. They also reported a weak association of meat intake with risk of myocardial infarction. Since men in the Kuopio study reported on average a daily meat intake of 150 g[29] the reported observation probably reflects the predominant contribution of heme iron to total iron intake in the population under study. Observations among Seventh Day Adventists in whom frequency of meat consumption (6 or more times compared to less than once per week) was associated with increased risk of fatal ischemic heart disease[31] are furthermore supportive of a possible role of heme iron in coronary heart disease.

A plausible explanation for an effect of iron is by stimulating oxidation of LDL cholesterol through catalyzing production of tissue-damaging free radicals[3,4]. Basic research has provided strong evidence that LDL cholesterol oxidation plays an important role in the pathogenesis of atherosclerosis and cardiovascular disease. Oxidized LDL cholesterol causes lipid accumulation in macrophages and foam cell formation[1,32] and has been shown to be cytotoxic to many cell types and chemotactic for monocyte macrophages. Lipid peroxidation of LDL cholesterol can be enhanced by metal-catalyzed reactions resulting in highly reactive hydroxyl radicals. Because of the considerable iron-binding capacity of the carrier protein transferrin, free low molecular weight iron cannot be detected in plasma under physiologic conditions. Iron is stored in the ferrite form that is bound to apoferritin, a protein widely distributed throughout the body. To exert its catalyzing activity, the ferrite ion must first be released from the protein moeity and reduced to the ferrous form. Superoxide anions produced by oxidative stress and reducing agents have been found to be capable of mobilizing iron from ferritin[3,4]. Recently hemin has also been shown to very efficiently promote LDL cholesterol oxidation in vitro[33]. Lack of an amplifying effect of lipid-peroxidation on iron-deficient 'heme' (protoporphyrin IX) in vitro underlines this finding. Further results suggesting that heme iron plays a role in lipid peroxidation comes furthermore from the results of a nested case-control study showing a positive association between blood hemoglobin concentration and the titer of autoantibodies against malondialdehyde-modified LDL cholesterol[34]. The promoting effect of hemin on LDL cholesterol oxidation may thus be an additional plausible explanation for observed association of heme iron intake with risk of myocardial infarction in the current study.

Stratification by risk factors for myocardial infarction such as hypercholesterolemia, hypertension, cigarette smoking, and diabetes showed that the risk was most marked when any of these risk factors was present (table 3). This indicates that iron may adversely affect coronary heart disease risk only in the presence of other risk factors. Previous observations stress this view: relative risk for myocardial infarction associated with dietary heme iron intake was found to be stronger in subjects with history of diabetes or smoking[14] and synergistic associations of serum ferritin and elevated serum LDL cholesterol concentrations with myocardial infarction[6] or carotid atherosclerosis[35] were observed.

Experiments with iron-chelators[18] or iron-overloading in animals[20] provided indirect evidence that iron is involved in reperfusion injury after an ischemic insult. During ischaemia the amount of iron in the low molecular weight pool was observed to increase[36] and iron accumulated in the perfusate when the circulation was restored[37]. Possibly entailed iron-catalyzed redox reactions within the cardiomyocyte are sufficient to elicit contractile dysfunction and lethal cell disruption[38]. This possibility suggests that iron-catalyzed reactions involving partially reduced oxygen might exacerbate reperfusion injury, which would imply that high iron stores may affect the fatality rate of myocardial infarction apart from an effect on the incidence of myocardial infarction[21]. The more pronounced association between heme iron intake and risk of myocardial infarction observed in fatal than non-fatal cases of first myocardial infarction support this hypothesis, even though the number of fatal cases in the current study was small.

In summary, our results suggest that high dietary heme iron intake increases the risk of myocardial infarction in the presence of other risk factors and may specifically affect its fatality.

REFERENCES

1. Steinberg D, Parthasarathy S, Carew TE, et al. Beyond cholesterol: modifications of low density lipoprotein that increases its atherogenecity. *N Engl J Med* 1989; 320:915-924.
2. Aviram M. Modified forms of low density lipoprotein and atherosclerosis. *Atherosclerosis* 1993; 98:1-9.
3. de Silva DM, Aust SD. Ferritin and ceruloplasmin in oxidative damage: review and recent

findings. *Can J Physiol Pharmacol* 1993; **71**:715-720.

4. Reif DW. Ferritin as a source of iron for oxidative damage. *Free Radical Biol Med* 1992; **12**:417-427.
5. Lauffer RB. Iron stores and the international variation in mortality from coronary artery disease. *Med Hypotheses* 1990; **35**:96-102.
6. Salonen JT, Nyyssönen K, Korpela H et al. High stored iron levels are associated with excess risk of myocardial infarction in eastern Finnish men. *Circulation* 1992; **86**:803-811.
7. Salonen JT, Nyyssönen K, Salonen R. Body iron stores and risk of coronary heart disease [letter] *N Engl J Med* 1994; **331**:1159-1160.
8. Mänttäri M, Manninen V, Huttunen JK et al. Serum ferritin and ceruloplasmin as coronary risk factors. *Eur Heart J* 1994; **15**:1599-1603.
9. Liao Y, Cooper RS, McGee DL. Iron status and coronary heart disease: negative findings from the NHANES I Epidemiologic Follow-Up Study. *Am J Epidemiol* 1994; **139**:704-712.
10. Magnusson MK, Sigfusson N, Sigvaldason H et al. Low iron-binding capacity as a risk factor for myocardial infarction. *Circulation* 1994; **89**:102-108.
11. Sempos TC, Looker AC, Gillum RF et al. Body iron stores and the risk of coronary heart disease. *N Engl J Med* 1994; **330**:1119-1124.
12. Baer DM, Tekawa IS, Hurley LB. Iron stores are not associated with acute myocardial infarction. *Circulation* 1994; **89**:2915-2918.
13. Reunanen A, Takkunen H, Knekt P et al. Body iron stores, dietary iron intake and coronary heart disease mortality. *J Intern Med* 1995; **238**:223-230.
14. Ascherio A, Willett WC, Rimm EB et al. Dietary iron intake and risk of coronary disease among men. *Circulation* 1994; **89**:969-974.
15. Cook JD, Lipschitz DA, Miles LEM et al. Serum ferritin as a measure of iron stores in normal subjects. *Am J Clin Nutr* 1974; **27**;681-687.
16. Bolli R, Patel BS, Jeroudi MO et al. Demonstration of free radical generation in 'stunned' myocardium of intact dogs with the use of the spin trap α-phenyl-N-tert-butyl-nitrone. *J Clin Invest* 1988; **82**:476-485.
17. McCord JM. Oxygen-derived free radicals in post-ischemic tissue injury. *N Engl J Med* 1985; **312**:159-163.
18. Williams RE, Zweier JL, Flaherty JT. Treatment with desferoxamine during ischemia improves functional and metabolic recovery and reduces reperfusion-induced oxygen radical generation in rabbit hearts. *Circulation* 1991; **83**:1006-1011.
19. van der Kraaij AMM, van Eijk HG, Koster JF. Prevention of post-ischemic cardiac injury by the orally active iron chelator 1,2-dimethyl-3-hydroxy-4-pyridone (LI) and the antioxidant (+)-cyanidol-3. *Circulation* 1989; **80**:158-164.
20. van der Kraaij AMM, Mostert LJ, van Eijk HG et al. Iron-load increases the susceptibility of rat hearts to oxygen reperfusion damage. *Circulation* 1988; **78**:442-449.
21. Koster JF, Sluiter W. Is increased tissue ferritin a risk factor for atherosclerosis and ischemic heart disease? *Br Heart J* 1995; **73**:208.

22. Hofman A, Grobbee DE, De Jong PTVM et al. Determinants of disease and disability in the elderly: The Rotterdam Elderly Study. *Eur J Epidemiol* 1991; 7:403-422.
23. WHO. International Statistical Classification of Diseases and Related Health Problems, 10th revision. Geneva, Vol 1, 1992.
24. Food and Nutrition Council. Dutch food composition table (NEVO). The Hague, Netherlands: Voorlichtingsbureau voor de Voeding, 1993 (in Dutch)
25. Monsen ER, Hallberg L, Layrisse M et al. Estimation of available dietary iron. *Am J Clin Nutr* 1978; **31**:134-141.
26. Klipstein-Grobusch K, den Breeijen JH, Goldbohm RA et al. Dietary assessment in the elderly: validation of a semiquantitative food frequency questionnaire. *Eur J Clin Nutr* 1998; **52**:588-596.
27. Willett WC, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. *Am J Epidemiol* 1986; **124**:17-27.
28. Beaton GH, Milner J, Corey P et al. Source of variance in 24-hour dietary recall data: implications for nutrition study design and interpretation. *Am J Clin Nutr* 1983; **32**:2546-2559.
29. Bothwell TH. Overview and mechanisms of iron regulation. *Nutr Rev* 1995; 9:237-245.
30. Ihanainen M, Salonen R, Seppänanen R et al. Nutrition data collection in the Kuopio Ischaemic Heart Disease Risk Factor Study: Nutrient intake of middle-aged eastern Finnish men. *Nutr Res* 1989; 9:597-604.
31. Snowdon DA, Phillips, RL, Fraser GE. Meat consumption and fatal ischemic heart disease. *Prev Med* 1984; **13**:490-500.
32. Witztum JL. The oxidation hypothesis of atherosclerosis. *Lancet* 1994; **344**:793-795.
33. Balla G, Jacob HS, Eaton JW et al. Hemin: a possible physiological mediator of low density lipoprotein oxidation and endothelial injury. *Arterioscler Thromb* 1991; **11**:1700-1711.
34. Salonen JT, Ylä-Herttuala S, Yamamoto R et al. Autoantibody against oxidised LDL and progression of carotid atherosclerosis. *Lancet* 1992; **399**:883-887.
35. Kiechl S, Willeit J, Egger G et al. Body iron stores and risk of carotid atherosclerosis: Prospective results from the Bruneck Study. *Circulation* 1997; **96**:3300-3307.
36. Voogd A, Sluiter W, van Eijk HG et al. Low molecular weight iron and the oxygen paradox in isolated rat hearts. *J Clin Invest* 1992; **90**:2050-2055.
37. Nohl H, Stolze K, Napetsching S, et al. Is oxidative stress primarily involved in reperfusion injury of the ischemic heart? *Free Radic Biol Med* 1991; **11**:581-588.
38. Janero DR, Hreniuk D, Sharif HM. Hydrogen peroxide induced oxidative stress to the mammalian heart-muscle cell (cardiomyocyte): Nonperoxidative purine and pyrimidine nucleotide depletion. *J Cell Physiol* 1993; **155**:494-504.

CHAPTER 4

Antioxidants in coronary heart disease

4.1

Serum carotenoids and atherosclerosis. The Rotterdam Study

ABSTRACT

High circulating levels of carotenoids have been thought to exhibit a protective function in the development of atherosclerosis. We investigated whether aortic atherosclerosis was associated with lower levels of the major serum carotenoids in serum – α-carotene, β-carotene, ß-cryptoxanthin, lutein, lycopene, and zeaxanthin – in a subsample of the elderly population of the Rotterdam Study. Aortic atherosclerosis was assessed by presence of calcified plaques of the abdominal aorta. The case-control analysis comprised 108 subjects with aortic atherosclerosis and 109 age- and sex-matched controls.

In an age- and sex-adjusted logistic regression model, serum lycopene was inversely associated with the risk of atherosclerosis. The odds ratio for the highest compared to the lowest quartile of serum lycopene was 0.55 (95% CI 0.25 - 1.22; $p_{trend} = 0.13$). Multivariate adjustment did not appreciably alter these results. Stratification by smoking status indicated that the inverse association between lycopene and aortic calcification was most evident in current and former smokers (OR = 0.35; 0.13 - 0.94; $p_{trend} = 0.04$). No association with atherosclerosis was observed for quartiles of serum concentrations of α-carotene, β-carotene, β-cryptoxanthin, lutein, and zeaxanthin.

In conclusion, this study provides evidence for a modest inverse association between levels of serum lycopene and presence of atherosclerosis, the association being most pronounced in current and former smokers. Our findings suggest that lycopene may play a protective role in the development of atherosclerosis.

INTRODUCTION

As reviewed by Steinberg et al.[1] the oxidation of LDL may be an important step in atherogenesis, promoting the development of foam cells and endothelial injury that lead to the formation of organized arterial plaques. Evidence that LDL oxidation occurs in vivo and contributes to the clinical manifestations of atherosclerosis supports the oxidative modification hypothesis. The link between oxidation of LDL and atherogenesis is hypothesized to be the basis for a beneficial effect of antioxidants on the incidence of subclinical and clinical coronary artery disease[2]. Observational studies suggest that dietary carotenoids exert a protective effect on incidence and mortality of coronary heart disease[3-7], whereas results from supplement intervention trials[8-12] have shown few beneficial and some deleterious effects of ß-carotene supplementation with respect to cardiovascular disease. Studies focusing on carotenoids in serum, plasma, or adipose tissue yielded, in general, consistent results concerning a protective effect of carotenoids on risk of coronary heart disease[13-19]. The antioxidant potential of ß-carotene to scavenge free radicals[20] and its ability to inhibit LDL oxidation[21,22] support this hypothesis. Observations that other carotenoids, namely lycopene, lutein, and zeaxanthin, can also quench singlet oxygen, a potential initiator of lipid peroxidation give further support[20,22]. Data on carotenoids and atherosclerosis are scarce. In the ARIC-Study dietary provitamin A carotenoids were observed to be inversely related to carotid intima-media thickness in older male participants[23] and subsequently to the presence of carotid artery plaques[24]. High ß-cryptoxanthin and lutein/zeaxanthin serum levels were both associated with lower odds of asymptomatic atherosclerosis[25], whereas, in contrast, no association between carotenoid plasma concentrations and carotid intima media thickness and presence of plaques in the common carotides in an elderly population was found[26].

In the current study we investigated whether atherosclerosis as assessed by presence of calcified plaques in the abdominal aorta was associated with levels of the major carotenoids in serum - α-carotene, β-carotene, β-cryptoxanthin, lutein, lycopene, and zeaxanthin - in the population-based Rotterdam Study.

SUBJECTS & METHODS

Study population

The Rotterdam Study is a community based prospective cohort study of 7983 persons (response rate 78%) aged 55 years and over, living in Ommoord, an urban district in Rotterdam, the Netherlands. The aim of the study is to investigate the incidence of and the risk factors for chronic and disabling diseases as described previously[27]. The study was approved by the Medical Ethics Committee of Erasmus University, and written informed consent was obtained from all participants. The present case-control analysis comprised a sample of the Rotterdam Study without a self-reported history of myocardial infarction, stroke, percutaneous transluminal coronary angioplasty, or coronary bypass operation at baseline. Hundred and nine subjects with moderate/severe atherosclerosis measured by presence of plaque of the abdominal aorta and 109 controls without atherosclerosis frequency-matched on age (5-year categories) and sex were selected. For one subject serum for analysis of carotenoids was missing, resulting in a final sample of 108 cases and 109 controls.

Measurements

Information on current health status, medical history, drug use, education, income, and smoking behaviour was obtained with a computerized questionnaire during a home interview. Height and weight were measured, and body mass index (weight in kg/height in m^2) was calculated as a measure of obesity. Sitting blood pressure was measured on the right upper arm with a random-zero sphygmomanometer. The average of two measurements was used in the analysis. A venepuncture was performed and serum total and high density lipoprotein (HDL) cholesterol concentrations were determined by an automated enzymatic procedure. Use of supplement preparations was ascertained during the home interview. None of the subjects reported use of ß-carotene supplements, but some reported use of vitamin A (n = 1), vitamin E (n = 2), vitamin C (n = 6) , or multivitamins (n = 8). Since taking these supplements increases the serum concentrations of any of these antioxidants[28], we combined use of any of these supplements for data analysis.

Assessment of atherosclerosis

A lateral radiographic film of the abdomen was made from a fixed distance while the participant was seated. Atherosclerosis was diagnosed off-line by detecting calcified deposits in the abdominal aorta, as described previously[29]. Calcification was judged present when linear densities were seen in an area parallel and interior to the lumbar spine (L1-L4). Atherosclerosis was classified as no, mild, moderate, or severe calcification, according to the length of the involved area (< 1, 2.5 - 4.9 cm, 5 - 9.9 cm, ≥ 10 cm, respectively). These calcified deposits have been shown to represent intimal atherosclerosis[30] and have been associated with cardiovascular risk factors[29,31] and cardiovascular disease at several sites[32-34]. Cases for the current analysis comprised subjects with moderate and severe atherosclerosis, controls comprised subjects without atherosclerosis.

Serum carotenoids

Frozen sera collected at baseline examination and preserved at -80°C for on average 5.7 years for cases and 5.8 years for controls were used to determine serum concentrations of α-carotene, β-carotene, β-cryptoxanthin, lutein, lycopene, and zeaxanthin. Serum carotenoids appeared to be stable during long-term freezer storage at -70°C[35]. Sera from cases and controls were analyzed in the same run. For carotenoid analyses one volume of sample was mixed with one volume of α-tocopheryl acetate (0.1 mg/ml in ethanol) as internal standard, and deproteinized. Carotenoids were then extracted with two volumes of n-hexane. Half of the hexane layer was evaporated to dryness under nitrogen and redissolved in 0.2 to 1.0 of solvent (acetonitrile/methanol/methylenchloride, 90;5;5, v/v/v). Fifty µl were then injected onto a Hyperchrome stainless steel column filled with Nucleosil 120 3C18 (Applied Science Group, Emmen, The Netherlands), and eluted with acetonitrile/methanol/methylenchloride/ammonium acetate 0.05 mol/l (900:50:40:10, v/v/v/v). Carotenoids were detected by absorption at 450 nm and α-tocopheryl acetate at 286 nm. Peak areas were calibrated against standard solution of the respective carotenoids. The procedure was carried out in a room with special yellow light, free from daylight.

Data analysis

Associations of the serum carotenoids with risk factors for atherosclerosis were evaluated by use of Spearman's correlation coefficient and for categorical variables by

Table 1: Spearman correlation coefficients between serum carotenoids and age, waist-to-hip ratio, serum cholesterol, cigarette packyears smoked and alcohol intake for 217 subjects of the Rotterdam Study, the Netherlands

	Serum											
	α-carotene (μmol/l)		β-carotene (μmol/l)		β-cryptoxanthin (μmol/l)		lutein (μmol/l)		lycopene (μmol/l)		zeaxanthin (μmol/l)	
	r	p	r	p	r	p	r	p	r	p	r	p
Variables												
Age (years)	0.15	(0.023)	0.10	(0.128)	0.05	(0.482)	0.17	(0.012)	-0.32	(< 0.001)	-0.13	(0.041)
Waist-to-hip ratio	-0.23	(< 0.001)	-0.31	(< 0.001)	-0.28	(< 0.001)	- 0.26	(0.007)	-0.18	(0.007)	-0.17	(0.014)
Serum cholesterol (mmol/l)	0.11	(0.102)	0.12	(0.089)	0.14	(0.039)	0.24	(< 0.001)	-0.08	(0.225)	0.14	(0.030)
Cigarette packyears smoked	-0.17	(0.012)	-0.14	(0.037)	-0.24	(< 0.001)	-0.15	(0.032)	-0.11	(0.093)	-0.06	(0.368)
Alcohol intake (g/d)	-0.19	(0.006)	-0.22	(0.001)	-0.26	(< 0.001)	-0.09	(0.200)	0.11	(0.101)	-0.13	(0.050)

chi-square statistics. Student's t-test was used to assess whether cases and controls of atherosclerosis differed by mean levels of serum α-carotene, β-carotene, cryptoxanthin, lutein, lycopene, and zeaxanthin. Serum carotenoids were categorized into quartiles; the risk of atherosclerosis according to quartile of serum carotenoids was assessed with multivariate logistic regession. Analyses were initially adjusted for age, sex, serum cholesterol level, and season of blood draw, and were subsequently adjusted for pack-years smoked, waist-to-hip ratio, and alcohol intake. Stratified analysis was performed to evaluate whether risk estimates were modified by smoking status as suggested by other studies[15,17]. Associations are expressed as odds ratios with 95% confidence interval (CI). Results were considered statistically different at the two-tailed alpha-level of 0.05. Statistical analysis was performed using SAS® release 6.12 (SAS Institute, Inc, Cary, North Carolina, USA).

RESULTS

Correlation coefficients between serum carotenoids and age, waist-to-hip ratio, serum cholesterol, cigarette packyears smoked, and alcohol intake are shown in table 1. Age was significantly associated with all serum carotenoids except β-cryptoxanthin and β-carotene. Except for lycopene, serum carotenoid levels were significantly higher in women compared to men. Serum concentrations of α-carotene, β-carotene, zeaxanthin, and lutein differed significantly across seasons. Supplement users had significantly higher mean serum concentration levels of α-carotene, β-carotene, and zeaxanthin than subjects who did not use supplements. All carotenoids except lutein with lycopene were significantly correlated with each other. Correlation coefficients were mostly between 0.3 and 0.5; stronger correlations were found between β-cryptoxanthin and zeaxanthin ($r = 0.52$, $p < 0.0001$), lutein and zeaxanthin ($r = 0.56$, $p < 0.0001$) and α-carotene and β-carotene ($r = 0.72$, $p < 0.0001$).

Subjects with and without atherosclerosis differed significantly by body mass index, systolic blood pressure, presence of hypertension and diabetes (table 2). Among cases of atherosclerosis, more subjects currently smoked. Lycopene, α-carotene, β-carotene, and β-cryptoxanthin levels were observed to be lower in cases than controls of atherosclerosis.

Table 2: Means (SD) and distribution (%) of baseline characteristics between subjects with and without calcified aortic atherosclerosis; the Rotterdam Study

	Atherosclerosis			
	no		yes	
Variables	n = 109		n = 108	
Men (%)	43.1		40.7	
Age (years)	66.3	(7.4)	67.3	(7.1)
Serum α-carotene (μmol/l)	0.052	(0.039)	0.047	(0.030)
Serum β-carotene (μmol/l)	0.301	(0.216)	0.290	(0.152)
Serum cryptoxanthin (μmol/l)	0.477	(0.426)	0.469	(0.333)
Serum lutein (μmol/l)	0.390	(0.180)	0.393	(0.178)
Serum lycopene (μmol/l)	0.134	(0.094)	0.119	(0.092)
Serum zeaxanthin (μmol/l)	0.066	(0.036)	0.065	(0.036)
Body mass index (kg/m^2)	25.9	(3.9)	26.9	(3.4)[1]
Waist-to-hip ratio	0.90	(0.08)	0.91	(0.08)
Serum cholesterol (mmol/l)	6.65	(1.17)	6.90	(1.18)
HDL-serum cholesterol (mmol/l)	1.37	(0.41)	1.36	(0.37)
Diastolic blood pressure (mmHg)	74	(11)	75	(11)
Systolic blood pressure (mm Hg)	135	(21)	143	(20)[2]
Hypertension* (%)	14.7		30.6[2]	
Diabetes (%)	1.9		7.4[1]	
Current smokers (%)	24.8		28.7	
High alcohol intake† (%)	20.2		19.4	

Values are means (standard error) unless otherwise indicated

[1] p≤0.05

[2] p≤0.005

* systolic blood pressure ≥ 160 mm Hg and/or diastolic blood pressure ≥ 95 mm Hg and/or use of antihypertensive medication

† alcohol intake > 20g/d in women or >30 g/d in men

Table 3: Odds ratio and 95% confidence intervals (CI) for atherosclerosis according to quartiles of serum carotenoids, the Rotterdam Study, The Netherlands

	Quartiles				P value for
Variable	**1 (Lowest)***	**2**	**3**	**4 (Highest)**	**trend**
α-carotene					
mean level (μmol/l)	< 0.028	0.028 - 0.039	0.040 - 0.063	> 0.063	
No cases of atherosclerosis	27	28	31	22	
OR (95% CI)					
adjusted for age and sex	1.00	1.41 (0.66 - 3.03)	1.31 (0.62 - 2.75)	0.80 (0.36 - 1.73)	0.616
multivariate adjusted§	1.00	1.56 (0.71 - 3.47)	1.49 (0.69 - 3.24)	0.90 (0.40 - 2.04)	0.843
β-carotene					
mean level (μmol/l)	< 0.184	0.184 - 0.263	0.264 - 0.367	> 0.367	
No cases of atherosclerosis	27	27	28	26	
OR (95% CI)					
adjusted for age and sex	1.00	0.96 (0.45 - 2.04)	1.10 (0.51 - 2.36)	0.90 (0.41 - 1.95)	0.906
multivariate adjusted§	1.00	1.06 (0.47 - 2.37)	1.24 (0.56 - 2.78)	1.00 (0.43 - 2.32)	0.899
cryptoxanthin					
mean level (μmol/l)	< 0.214	0.214 - 0.364	0.365 - 0.583	> 0.583	
No cases of atherosclerosis	21	31	31	25	
OR (95% CI)					
adjusted for age and sex	1.00	2.02 (0.94 - 4.40)	2.41 (1.08 - 5.49)	1.40 (0.63 - 3.11)	0.413
multivariate adjusted§	1.00	2.17 (0.99 - 4.89)	2.69 (1.17 - 6.37)	1.47 (0.63 - 3.49)	0.334

table 3 continued

Variable	Quartiles				P value for trend
	1 (Lowest)*	2	3	4 (Highest)	
lutein					
mean level (μmol/l)	< 0.281	0.281 - 0.380	0.381 - 0.505	> 0.505	
No cases of atherosclerosis	26	27	29	26	
OR (95% CI)					
adjusted for age and sex	1.00	1.08 (0.51 - 2.32)	1.22 (0.57 - 2.63)	0.93 (0.42 - 2.03)	0.938
multivariate adjusted§	1.00	1.09 (0.50 - 2.37)	1.30 (0.58 - 2.91)	0.96 (0.42 - 2.20)	0.964
lycopene					
mean level (μmol/l)	< 0.058	0.058 - 0.103	0.104 - 0.166	> 0.166	
No cases of atherosclerosis	32	28	25	23	
OR (95% CI)					
adjusted for age and sex	1.00	0.79 (0.37 - 1.69)	0.65 (0.30 - 1.42)	0.55 (0.25 - 1.22)	0.126
multivariate adjusted§	1.00	0.89 (0.41 - 1.95)	0.78 (0.33 - 1.71)	0.66 (0.29 - 1.49)	0.284
zeaxanthin					
mean level (μmol/l)	< 0.046	0.046 - 0.062	0.063 - 0.084	> 0.084	
No cases of atherosclerosis	29	31	24	24	
OR (95% CI)					
adjusted for age and sex	1.00	1.16 (0.55 - 2.46)	0.73 (0.34 - 1.55)	0.88 (0.41 - 1.90)	0.493
multivariate adjusted§	1.00	1.15 (0.54 - 2.47)	0.75 (0.34 - 1.65)	0.80 (0.35 - 1.78)	0.400

*reference category

§ multivariate adjusted: adjusted for age, sex, serum cholesterol, season, waist-to-hip ratio, pack-years smoked, alcohol intake

Table 4: Odds ratio and 95% confidence intervals (CI) for atherosclerosis for quartiles of serum lycopene stratified by smoking status, the Rotterdam Study, the Netherlands

Variable	Quartiles				P value for trend
	1 (Lowest)*	2	3	4 (Highest)	
smoking status					
non-smokers (30 cases/39 controls)					
OR (95% CI)					
adjusted for age and sex	1.00	0.92 (0.22 - 3.81)	1.02 (0.21 - 4.96)	1.48 (0.36 - 6.35)	0.543
multivariate adjusted§	1.00	0.95 (0.20 - 4.52)	1.04 (0.17 - 6.53)	1.19 (0.23 - 6.34)	0.798
ever smokers (78 cases/70 controls)					
OR (95% CI)					
adjusted for age and sex	1.00	0.34 (0.12 - 0.91)	0.29 (0.11 - 0.73)	0.35 (0.13 - 0.94)	0.036
multivariate adjusted§	1.00	0.32 (0.11 - 0.89)	0.34 (0.12 - 0.91)	0.43 (0.14 - 1.25)	0.139

* reference category

§ multivariate adjusted: adjusted for age, sex, season, serum cholesterol, waist-to-hip ratio, pack-years smoked and alcohol intake

In a logistic regression model adjusted for age and sex there was a non-significant inverse association of quartiles of serum lycopene to risk of atherosclerosis. The odds ratio for the highest compared to the lowest level of serum lycopene was 0.55 (95% CI 0.25 - 1.22; $p_{trend} = 0.13$). Quartiles of serum concentrations of α-carotene, β-carotene, lutein, and zeaxanthin were not associated with atherosclerosis (table 3). For the second and third quartile of serum β-cryptoxanthin, an increased risk of aortic calcification was observed (table 3). Further adjustment for season and serum cholesterol, and subsequently waist-to-hip ratio, pack-years smoked, and alcohol intake did not appreciably alter these results (table 3).

Stratification by smoking status indicated that the inverse association observed for serum lycopene with aortic calcification was most evident in ever smokers. The age- and sex-adjusted odds ratio for the highest compared to the lowest quartile of serum lycopene was 0.35 (0.13 - 0.94; $p_{trend} = 0.04$) for ever smokers. Multivariate adjustment only slightly altered the risk estimates (table 4).

DISCUSSION

High circulating levels of carotenoids have been presumed to protect against atherosclerosis before its clinical manifestation[14]. We tested this hypothesis by investigating whether high levels of the major carotenoids in serum – α-carotene, β-carotene, β-cryptoxanthin, lutein, lycopene, and zeaxanthin – were associated with decreased risk of aortic atherosclerosis assessed by presence of calcified plaques of the abdominal aorta. Serum levels of lycopene were inversely associated with aortic calcification. The association was most pronounced in current and former smokers. No association with risk of aortic calcification for the serum carotenoids α-carotene, β-carotene, β-cryptoxanthin, lutein, and zeaxanthin was observed.

We recognize that there are some sources of potential bias in our study. Presence of calcifications in the abdominal aorta was used as a proxy for generalized atherosclerosis in the current study. These calcifications have been shown previously to be associated with atherosclerosis in other arterial vessels[36] and with cardiovascular disease risk factors[29,31]. They have been shown to predict coronary heart disease[34] and cardiovascular mortality[32,33]. Validity of roentgenographic assessment of aortic

calcification for assessment of atherosclerosis was demonstrated by comparison with necropsy material[30] and comparison of radiographs with CT images of calcified aortas showed that calcifications seen on the radiographs were within the vascular wall of the aorta in almost all cases[29].

We used single blood specimen to determine serum carotenoids. Given the biologic variation of the actual carotenoid concentration, possibly reflecting variations in dietary intake and imprecision in the measurement technique, a single serum specimen may not be able to provide a precise estimate of typical serum carotenoid concentrations over time[37]. This could potentially attenuate the relationship between carotenoid concentrations and presence of atherosclerosis towards the null. To compensate for the reduction in statistical power resulting from intrasubject variability in serum carotenoids our sample size (n = 217) may have been too small. However, lack of power does not explain why the observed association between serum lycopene concentrations and aortic atherosclerosis turns out to be stronger than those for the other carotenoids.

Looking at atherosclerosis adds to previous studies on coronary heart disease with respect to asymptomatic disease. We observed a consistent trend of a decreased risk for aortic calcification with elevated serum lycopene levels, but no association for α-carotene, β-carotene, lutein, or zeaxanthin. The observed positive association of the second and third quartile of β-cryptoxanthin with atherosclerosis was unexpected and cannot be explained. Few other studies have focused on carotenoids and atherosclerosis and so far results are inconsistent. Provitamin A carotenoid intake has been reported to be inversely associated with presence of carotid intima media thickness in men aged 55 years and older[23] and in a subsequent analysis with carotid artery plaques[24]. In an elderly French population no association of plasma carotenoids with common carotid intima media thickness or with presence of carotid plaques was observed[26]. Iribarren et al.[25], however, observed serum ß-cryptoxanthin and lutein/zeaxanthin levels to be inversely related to the extent of asymptomatic carotid atherosclerosis, and increases of serum α-carotene and lycopene to be associated with nonsignificantly lower odds of being a case. In our study on serum carotenoids and atherosclerosis only elevated serum lycopene levels were nonsignificantly associated with decreased risk of aortic atherosclerosis. Supportive of a beneficial effect of

lycopene in cardiovascular disease are furthermore results from the EURAMIC study, where elevated adipose tissue levels of lycopene were associated with decreased risk of myocardial infarction[18,19].

The observed association with lycopene in the current study was most evident in current and former smokers. Interaction between carotenoids and cardiovascular disease was reported in several epidemiologic studies[3,15,17,25]. Street et al.[15] observed low serum lycopene levels to be associated with subsequent myocardial infarction only in smokers and Kritchevsky et al.[24] observed the strongest evidence for a protective effect of dietary provitamin A carotenoids in female smokers. In both studies the carotenoid effect on smoking did not seem to depend on the intensity of smoking. In contrast, the effect observed for lycopene in EURAMIC was strongest among non-smokers, though it was also evident in former and current smokers[18]. Cigarette smoke contains an abundance of free radical species which could be expected to deplete lipid-phase micronutrients such as carotenoids. Exposing human plasma to the gas phase of cigarette smoke led to depletion of most of the lipophilic antioxidants. The most susceptible of the carotenoids to cigarette smoke-induced depletion were lycopene followed by lutein + zeaxanthin and ß-carotene[38]. This might explain the observed increased risk of cardiovascular diseases with low carotenoid levels in ever smokers in this and other studies[15,17].

In the current study only serum lycopene but not ß-carotene was inversely associated with a decreased risk of atherosclerosis. Factors related to measurement and the interaction between different foodstuffs or nutrients may explain why we see an effect only in lycopene, but additional studies on carotenoids are needed to further clarify this. For instance, some variability in antioxidant activity among the carotenoids has been observed in vitro; e.g. lycopene exhibits superior antioxidant capability compared with ß-carotene and lutein[39]. Given its ability to quench singlet oxygen and scavenge peroxyl radicals, lycopene is considered to be among the most efficient single oxygen quenchers of the natural carotenoids[39,40]. In addition, tomato lycopene has been shown to inhibit low density lipoprotein oxidation in vitro[41], a finding supporting its possible role in the prevention of cardiovascular disease. However, whether the observed association between serum lycopene and atherosclerosis can be attributed to lycopene itself or whether lycopene is merely a marker of active phytochemicals found in

lycopene-rich foods such as tomatoes and tomato products remains to be elucidated.

In conclusion, this study provides evidence for a modest inverse association between levels of serum lycopene levels and presence of aortic atherosclerosis in a population-based study. The associations were most pronounced in former and current smokers. Our findings suggest that serum carotenoids with a strong antioxidant activity such as lycopene may be important in the prevention of atherosclerosis.

REFERENCES

1. Steinberg D, Parthasarathy S, Carew TE et al. Beyond cholesterol: modifications of low-density lipoprotein that increase its atherogenicity. *N Engl J Med* 1989; **320**:915-924.
2. Diaz MN, Frei B, Vita JA et al. Antioxidants and atherosclerotic heart disease. *N Engl J Med* 1997; **337**:408-416.
3. Rimm EB, Stampfer MJ, Ascherio A et al. Vitamin E consumption and the risk of coronary heart disease in men. *N Engl J Med* 1993; **328**:1450-1456.
4. Knekt P, Reunanen A, Järvinen R et al. Antioxidant vitamin intake and coronary mortality in a longitudinal population study. *Am J Epidemiol* 1994; **139**:1180-1189.
5. Gaziano JM, Manson JE, Branch LG et al. A prospective study of consumption of carotenoids in fruits and vegetables and decreased cardiovascular mortality in the elderly. *Ann Epidemiol* 1995; **5**: 255-260.
6. Pandey DK, Shekelle R, Selwyn BJ et al. Dietary vitamin C and ß-carotene and risk of death in middle-aged men. The Western Electric Study. *Am J Epidemiol* 1995; **142**:1269-1278.
7. Kushi LH, Folsom AR, Prineas RJ et al. Dietary antioxidant vitamins and death from coronary heart disease in postmenopausal women. *N Engl J Med* 1996; **334**:1156-1162.
8. The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study Group. The effect of vitamin E and beta-carotene on the incidence of lung cancer and other cancers in male smokers. *N Engl J Med* 1994; **330**:1029-1035.
9. Hennekens CH, Buring JE, Manson JE et al. Lack of effect of long-term supplementation with beta-carotene on the incidence of malignant neoplasms and cardiovascular disease. *N Engl J Med* 1996; **334**:1145-1149.
10. Omenn, GS, Goodman GE, Thornquist MD et al. Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. *N Engl J Med* 1996; **334**:1150-1155.
11. Greenberg ER, Baron JA, Karagas MR et al. Mortality associated with low plasma concentration of beta-carotene and the effect of oral supplementation. *JAMA* 1996; **275**: 699-703.
12. Rapola JM, Virtamo J, Ripatti S et al. Randomised trial of alpha-tocopherol and ß-carotene supplements on incidence of major coronary events in men with previous myocardial infarction. *Lancet* 1997; **349**:1715-1720.

13. Riemersma RA, Wood DA, Macinytre CCA et al. Risk of angina pectoris and plasma concentrations of vitamins A, C, and E, and carotene. *Lancet* 1991; **337**:1-5.
14. Morris DL, Kritchevsky SB, Davis CE. Serum carotenoids and coronary heart disease. The Lipid Research Clinics Coronary Primary Prevention Trial and Follow-up Study. *JAMA* 1994; **272**:1439-1441.
15. Street DA, Comstock GW, Salkeld RM et al. Serum antioxidants and myocardial infarction. Are low levels of carotenoids and α-tocopherol risk factors for myocardial infarction? *Circulation* 1994; **90**:1154-1161.
16. Greenberg ER, Baron JA, Karagas MR et al. Mortality associated with low plasma concentration of beta-carotene and the effect of oral supplementation. *JAMA* 1996; **275**:699-703.
17. Kardinaal AFM, Kok FJ, Ringstad J et al. Antioxidants in adipose tissue and risk of myocardial infarction: The EURAMIC Study. *Lancet* 1993; **342**:1379-1384.
18. Kohlmeier L, Kark JD, Gomez-Gracia E et al. Lycopene and myocardial infarction risk in the EURAMIC Study. *Am J Epidemiol* 1997; **146**:618-626.
19. Gómez-Aracena J, Sloots L, Garcia-Rodriguez A et al. Antioxidants in adipose tissue and myocardial infarction in a Mediterranean area. The EURAMIC Study in Malaga. *Nutr Metab Cardiovasc Dis* 1997; **7**:376-382.
20. Krinksy NI. Antioxidant functions of carotenoids. *Free Rad Biol Med* 1989; **7**:617-635.
21. Jialal I, Norkus P, Cristol L et al. Beta-carotene inhibits the oxidative modification of low-density lipoprotein. *Biochim Biophys Acta* 1991; **1086**:134-138.
22. Di Mascio P, Kaiser S, Sies H. Lycopene as the most efficient biological carotenoid singlet oxygen quencher. *Arch Biochem Biophys* 1989; **274**:532-538.
23. Kritchevsky SB, Shimakawa T, Tell GS et al. Dietary antioxidants and carotid artery wall thickness. *Circulation* 1995; **92**:2142-2150.
24. Kritchevsky SB, Tell GS, Shimakawa T et al. Provitamin A carotenoid intake and carotid artery plaques: the Atherosclerosis Risk in Communities Study. *Am J Clin Nutr* 1998; **68**:726-733.
25. Iribarren C, Folsom AR, Jacobs DR et al. Association of serum vitamin levels, LDL susceptibility to oxidation, and autoantibodies against MDA-LDL with carotid atherosclerosis: a case control study. *Arterioscler Thromb Vasc Biol* 1997; **17**:1171-1177.
26. Bonithon-Kopp C, Coudray C, Berr C et al. Combined effects of lipid peroxidation and antioxidant status on carotid atherosclerosis in a population aged 59-71 y: the EVA Study. *Am J Clin Nutr* 1997; **65**:121-127.
27. Hofman A, Grobbee DE, de Jong PTVM et al. Determinants of disease and disability in the elderly: The Rotterdam Elderly Study. *Eur J Epidemiol* 1991; **7**:403-422.
28. Jacques PF, Halpner AD, Blumberg JB. Influence of combined antioxidant nutrient intakes on their plasma concentrations in an elderly population. *Am J Clin Nutr* 1995; **62**:1228-1233.
29. Witteman JCM, Grobbee DE, Valkenburg HA et al. A J-shaped relation between change in diastolic blood pressure and progression of aortic atherosclerosis. *Lancet* 1994; **343**:504-507.
30. Hyman JB, Epstein FH. A study of the correlation between roentgenographic and postmortem calcification of the aorta. *Am Heart J* 1954; **47**:540-543.

31. Witteman JCM, Grobbee DE, Valkenburg HA et al. Cigarette smoking and the development and progression of aortic atherosclerosis. A 9-year population based follow-up study in women. *Circulation* 1993; **88**:2156-2162.
32. Eggen DA. Relationship of calcified lesions to clinically significant atherosclerotic lesions. *Ann NY Acad Sci* 1968; **149**:752-767.
33. Witteman JCM, Kok FJ, van Saase JLCM et al. Aortic calcification as a predictor of cardiovascular mortality. *Lancet* 1986;ii:1120-1122.
34. Witteman JCM, Kannel WB, Wolf PA et al. Aortic calcified plaques and cardiovascular disease (The Framingham Study). *Am J Cardiol* 1990; **66**:1060-1064.
35. Comstock GW, Norkus EP, Hoffman SC et al. Stability of ascorbic acid, carotenoids, retinol, and tocopherols in plasma stored at -70°C for 4 years. *Cancer Epidemiol, Biomarker & Prev* 1995; **4**:505-507.
36. Bots ML, Witteman JCM, Grobbee DE. Carotid intima-media thicknesss in elderly women with and without atherosclerosis of the abdominal aorta. *Atherosclerosis* 1993; **102**:99-105.
37. Cantilena LR, Stukel TA, Greenberg ER et al. Diurnal and seasonal variation of five carotenoids measured in human serum. *Am J Clin Nutr* 1992; **55**:659-663.
38. Handelman GJ, Packer L, Cross CE. Destruction of tocopherols, carotenoids, and retinol in human plasma by cigarette smoke. *Am J Clin Nutr* 1996; **63**:559-565.
39. Di Mascio P, Murphy ME, Sies H. Antioxidant defense systems: the role of carotenoids, tocopherols and thiols. *Am J Clin Nutr* 1991; **53**:194S-200S.
40. Conn PF, Schalch W, Truscott TG. The singlet oxygen and carotenoid interaction. *J Photochem Photobiol B* 1991; **11**:41-47.
41. Fuhrman B, Ben-Yaish L, Attias J et al. Tomato lycopene and ß-carotene inhibit low density lipoprotein oxidation and this effect depends on the lipoprotein vitamin E content. *Nutr Metab Cardiovasc Dis* 1997; **7**:433-443.

4.2

Dietary antioxidants and peripheral artery disease. The Rotterdam Study

ABSTRACT

The aim of this study was to examine cross-sectionally whether energy-adjusted dietary antioxidant intake - ß-carotene, vitamin C, and vitamin E - was associated with peripheral artery disease in the Rotterdam Study. The study population consisted of 4460 subjects aged 55-94 years without previous cardiovascular disease at baseline for whom dietary data assessed by a semiquantitative food frequency questionnaire were available. Peripheral artery disease was defined as a right or left ankle-arm pressure index lower than or equal to 0.9 and was present in 626 subjects (228 men, 398 women). In age-adjusted logistic regression analyses, vitamin C intake was significantly inversely associated with peripheral artery disease in women (relative risk = 0.65; 95 percent confidence interval 0.45-0.84; p_{trend} 0.001; highest versus lowest quartile of vitamin C intake), whereas in men ß-carotene (relative risk = 0.68; 95% CI 0.45-1.02; p_{trend} 0.054; highest versus lowest quartile of ß-carotene intake) and vitamin E intake (relative risk = 0.61, 95% CI 0.40-0.92; p_{trend} 0.017; highest versus lowest quartile of vitamin E intake) were inversely associated with peripheral artery disease. After adjustment by body mass index, smoking status, education, income, and alcohol intake the significantly inverse association of vitamin C in women with peripheral artery disease remained (relative risk = 0.66, 95% CI 0.48 - 0.91, p_{trend} 0.007 highest versus lowest quartile of vitamin C intake). Stratification by smoking status indicated that current or former smokers benefit most of elevated dietary antioxidant intake suggesting that a diet high in fruit and vegetables may be in particular beneficial in the prevention of peripheral atherosclerosis in ever smokers.

INTRODUCTION

Observational studies have indicated that dietary antioxidants may exert a protective effect on incidence and mortality of cardiovascular disease[1-9] and suggest that antioxidative vitamins may be associated with reduced atherosclerosis[10-12]. Peripheral artery disease, reflecting the presence of atherosclerotic vessel wall abnormalities of the arteries of the lower extremities, is considered to be a good indicator of asymptomatic atherosclerosis[13,14]. Peripheral artery disease occurs commonly in the elderly[14-16] and presence of peripheral artery disease has been shown to be a strong predictor of subsequent morbidity and mortality[15,17,18]. The aim of the present study was to investigate whether dietary antioxidants are associated with presence of peripheral artery disease.

SUBJECTS AND METHODS

Study population

The Rotterdam Study is a community based prospective cohort study of 7983 persons (response rate 78%) aged 55 years and over, living in Ommoord, an urban district in Rotterdam, the Netherlands. The aim of the study is to investigate the incidence of and the risk factors for chronic and disabling diseases as described previously[19]. The study was approved by the Medical Ethics Committee of the Erasmus University, and written informed consent was obtained from all participants. Dietary assessment was conducted in 5646 participants. Two hundred and twelve subjects were excluded because they did not fully cooperate during the interview and the dietitian considered their reported diets unreliable. This resulted in 5434 subjects (2225 men and 3209 women) with dietary data. Blood pressure of the lower extremities was measured in 4900 subjects with complete dietary data. A total of 733 subjects (288 men, 445 women) had peripheral artery disease in one or both extremities. Among subjects with peripheral artery disease were 107 subjects with a self-report of myocardial infarction, cerebrovascular accident, bypass surgery, or percutaneous transluminal coronary angioplasty and among those without peripheral artery disease were 333 subjects with any of these conditions. Because they might intentionally have changed their diets after having experienced a cardiovascular event, they were not included in the analysis. This resulted in a study sample of 4460 subjects (626 subjects with and 3834 subjects without peripheral artery disease) for the current analysis.

Measurements

All subjects were first visited at their home. Information on current health status, medical history, drug use, education, income, and smoking behaviour was obtained by a computerized questionnaire, which included the Dutch version of the Rose cardiovascular questionnaire[20]. The home visit was followed by two visits at the research centre. During those visits several cardiovascular risk factors were determined. Height and weight were measured, and body mass index (weight in kg/height in m^2) was calculated as a measure of obesity. Sitting blood pressure was measured twice at the right upper arm with a random-zero sphygmomanometer; the average of two measurements was used. Systolic blood pressure at the ankles (posterior tibial artery) was measured in supine position with an adult size cuff just above the malleoli, and a 8 Mhz Doppler transducer[21]. The ankle-arm pressure index (ABPI) is the ratio of the systolic blood pressure at the ankle to the systolic blood pressure at the arm. Peripheral artery disease was defined as a right or left ankle-arm pressure index lower than or equal to 0.9[22]. A venepuncture was performed and serum total and high density lipoprotein (HDL) cholesterol concentrations were determined by an automated enzymatic procedure.

Dietary assessment

The semiquantitative food frequency questionnaire (SFFQ) completed during baseline aimed at assessing habitual food intake during the past year and included 170 food items in 13 food groups and questions about dietary habits, supplementation, and prescribed diets. SFFQ data were converted to nutrient intake using the computerized Dutch Food Composition Table[23]. Data for ß-carotene, retinol, and tocopherol were updated by use of an additional database by the Netherlands Institute of Public Health and Environmental Protection (Vollebregts & Feskens, unpublished observations, 1993). Nutrient intake through nutritional supplements was not considered since brand labels were not recorded with sufficient accuracy.

The validity of the SFFQ was assessed in a subsample of 80 men and women aged 55 to 75 years. Nutrient intake estimated from the SFFQ was compared with estimated nutrient intake of in total 15 days of food records collected over a one-year period[24]. The ability of the SFFQ to rank subjects adequately according to their dietary intake was demonstrated by Pearson's correlation coefficients of 0.4 to 0.8 adjusted for sex, age, total energy intake, and for within-person variability in daily intake[25], and a high degree of classification into the same or adjacent quintile (76.8% for energy-adjusted data).

Data Analysis

Differences in mean levels of dietary antioxidants and risk factors for peripheral artery disease between subjects with and without peripheral artery disease were assessed by analysis of variance, respectively by chi-square statistics for categorical variables. The association between dietary antioxidant intake and peripheral artery disease was examined primarily by multivariate logistic regression. Energy-adjusted nutrient intakes were derived by adding the median nutrient intake to the residuals from regression analysis of nutrient intake on energy[28]. Energy-adjusted dietary antioxidant intake from food sources was categorized into quartiles, and risk of peripheral artery disease of the upper quartiles was compared to risk in the lowest quartile. The initial analysis examined associations adjusted for age and was furthermore adjusted for body mass index, smoking status, equivalent household income (five categories), highest education attained (five categories), and alcohol intake (five categories). The models were fit separately for men and women. The associations between quartiles of energy-adjusted intake of ß-carotene, vitamin C, and vitamin E and risk of peripheral artery disease were expressed as relative risk with 95% confidence intervals. Results were considered statistically different at the two-tailed 0.05 alpha-level. Statistical analysis was performed using SAS® (Release 6.11; SAS Institute, Cary, NC, USA).

RESULTS

Mean age of the 1720 men and 2740 women was 66.6 years (SD 7.3) and 67.5 years (7.9 SD), respectively. Compared to the total population of the Rotterdam Study for whom dietary data were available (n = 5434) the study population did not differ in characteristics such as age, body mass index, blood pressure, income, level of education, or nutrient intake. Vitamin supplements containing either ß-carotene, vitamin C, or vitamin E were used by 9.0% of the male and 13.8% of the female study population.

Peripheral artery disease was present in 13.3% of the men and 14.5% of the women. Subjects with peripheral artery disease were on average significantly older than those without peripheral artery disease. Analysis was thus subsequently adjusted for age. Table 1 depicts differences between subjects with and without peripheral artery disease stratified by sex. Risk factors for peripheral artery disease - smoking, elevated blood

Table 1: Age-adjusted means (SE) and distributions (%) of baseline characteristics for men and women with and without peripheral artery disease*

	Men					Women				
	PAD not present n = 1492		PAD present n = 228		p†	PAD not present n = 2342		PAD present n = 398		p†
Variables										
Age (years)	66.0	0.18	70.7	0.56	< 0.001	66.8	0.16	71.3	0.45	< 0.001
Body mass index (kg/m^2)	25.8	0.07	25.3	0.19	0.028	26.7	0.08	26.7	0.20	0.848
Waist-to-hip ratio	0.95	0.002	0.96	0.004	0.245	0.86	0.002	0.86	0.004	0.874
Systolic blood pressure (mm Hg)	137	0.55	145	1.42	< 0.001	137	0.42	147	1.04	< 0.001
Diastolic blood pressure (mmHg)	75	0.29	75	0.77	0.845	7.3	0.23	75	0.56	0.006
Serum cholesterol (mmol/l)	6.32	0.03	6.42	0.07	0.232	6.84	0.02	7.05	0.06	0.001
HDL-serum cholesterol (mmol/l)	1.24	0.01	1.20	0.02	0.133	1.46	0.01	1.41	0.02	0.006
Packyears smoked	1.88	9.6	225	11.9	0.004	68.3	2.55	95.6	6.20	< 0.001
Obesity (%)	6.7		7.3		0.804	19.2		18.1		0.845
Hypertension*f* (%)	21.1		33.0		0.001	22.6		36.0		0.001
Hypercholesterolemia‡ (%)	38.5		46.1		0.043	58.7		64.2		0.023
Diabetes (%)	7.5		14.2		0.001	8.0		13.5		0.001
Current smokers (%)	27.2		50.1		0.001	17.1		28.8		0.001
High alcohol intake¥ (%)	32.1		37.3		0.100	10.4		11.5		0.462
University education (%)	15.2		19.2		0.187	4.4		5.0		0.888
Higher incomeλ (%)	13.7		15.1		0.993	8.7		8.0		0.310
Antioxidative supplementation (%)	8.5		11.7		0.122	13.2		16.9		0.072
Diet (%)	8.2		13.7		0.007	13.3		19.8		0.001

... table 1 continued

Variables	Men					Women				
	PAD not present n = 1492		PAD present n = 228		p†	PAD not present n = 2342		PAD present n = 398		p†
Energy-adjusted intake of										
ß-carotene (mg/d)	1.50	0.02	1.43	0.04	0.143	1.41	0.02	1.37	0.04	0.367
vitamin C (mg/d)	108	1.32	106	3.43	0.494	117	1.13	109	2.78	0.006
vitamin E (mg/d)	14.3	0.15	13.2	0.40	0.014	11.9	0.10	12.2	0.24	0.202
total fat (g/d)	88.6	0.39	88.2	1.00	0.744	70.2	0.25	71.0	0.61	0.208
saturated fat (g/d)	33.8	0.20	34.3	0.51	0.423	27.9	0.13	27.9	0.33	0.970
cholesterol (mg/d)	246	1.77	252	4.61	0.210	208	1.14	211	2.81	0.290

* subjects with self-reports of myocardial infarction, cerebrovascular accident, bypass operation, or percutaneous transluminal angioplasty were excluded from analysis

‡ if serum cholesterol ≥ 6.5 mmol/l
ƒ if systolic blood pressure ≥ 160 mm Hg and/or diastolic blood pressure ≥ 95 mm Hg and/or use of antihypertensive medication
¥ alcohol intake > 20 g/d
λ equivalent household income > 3000 Hfl

pressure, elevated serum cholesterol levels, and presence of diabetes were significantly more pronounced in males and females with peripheral artery disease than in those without peripheral artery disease (table 1). In female subjects a significantly lower intake of dietary vitamin C with presence of peripheral artery disease was seen, in male cases intake of vitamin E was significantly lower.

In men and women ß-carotene intake was inversely associated with age (men $r = -0.13$, $p < 0.001$; women $r = -0.12$, $p < 0.001$). In addition, a weak inverse correlation of vitamin C with age ($r = -0.05$, $p = 0.036$) was observed in men. Vitamin C and ß-carotene intake were highly correlated in both sexes (men $r = 0.39$, $p < 0.001$; women $r = 0.36$, $p < 0.001$), whereas correlations between ß-carotene and vitamin E, and between vitamin C and vitamin E were rather modest (range 0.12 - 0.19; $p < 0.001$). Dietary fat and cholesterol intake were inversely associated with ß-carotene, vitamin C and vitamin E with the exception of total fat and polyunsatured fat that were positively correlated with vitamin E intake. Only weak associations of antioxidative vitamins with measures of body composition, blood pressure, and serum cholesterol were seen for both sexes. Current smoking was associated with lower dietary intake of energy-adjusted ß-carotene (both sexes $p < 0.0001$), vitamin C (both sexes $p < 0.0001$) and vitamin E (men $p < 0.0001$).

In women, dietary vitamin C was inversely associated with age-adjusted risk of peripheral artery disease. The relative risk for the highest compared to the lowest quartile was 0.65 (95% CI 0.45-0.84, p_{trend} 0.001). Multivariate adjustment did not change risk estimates and further adjustment by ß-carotene and vitamin E intake and use of antioxidative vitamin supplements only slightly altered risk estimates (data not shown). Dietary intake of ß-carotene or vitamin E was not associated with risk of peripheral artery disease in women. In men, dietary ß-carotene and vitamin E were inversely associated with age-adjusted risk of peripheral artery disease. The relative risk for the highest compared to the lowest quartile was 0.68 (95% CI 0.45-1.02, p_{trend} 0.054) for ß-carotene and 0.61 (95% CI 0.40-0.92, p_{trend} 0.017) for vitamin E. After adjustment for body mass index, smoking status, education, income, and alcohol intake, point estimates were considerably decreased (table 2).

Table 2: Relative risk and 95% CI for peripheral artery disease according to quartiles of energy-adjusted dietary antioxidants* among 1720 men and 2740 women of the Rotterdam Study

	Peripheral artery disease				
	Men (228/1492)			Women (398/2342)	
Dietary intake of energy-adjusted	**RR[1]**	**RR[2]**	**Dietary intake of energy-adjusted**	**RR[1]**	**RR[2]**
β-carotene (mg/d)			**β-carotene (mg/d)**		
< 1.10	1.00	1.00	< 0.98	1.00	1.00
1.10 - 1.41	0.97 (1.07 - 1.11)	1.03 (0.70 - 1.52)	0.98 - 1.30	0.79 (0.59 - 1.07)	0.85 (0.57 - 1.15)
1.41 - 1.77	0.68 (0.45 - 1.14)	0.73 (0.48 - 1.11)	1.30 - 1.67	0.72 (0.53 - 0.98)	0.78 (0.66 - 1.07)
> 1.77	0.68 (0.45 - 1.02)	0.79 (0.51 - 1.14)	> 1.67	0.81 (0.59 - 1.10)	0.92 (0.065 - 1.28)
p_{trend}	0.054	0.137	p_{trend}	0.106	0.427
vitamin C (mg/d)			**vitamin C (mg/d)**		
< 73	1.00	1.00	< 80	1.00	1.00
73 - 100	0.76 (0.51 - 1.14)	0.82 (0.55 - 1.24)	80 - 108	0.80 (0.60 - 1.07)	0.85 (0.60 - 1.15)
100 - 132	0.83 (0.56 - 1.24)	1.00 (0.66 - 1.52)	108 - 142	0.72 (0.53 - 0.97)	0.80 (0.58 - 1.09)
> 132	0.86 (0.57 - 1.28)	1.09 (0.71 - 1.67)	> 142	0.65 (0.45 - 0.84)	0.67 (0.48 - 0.93)
p_{trend}	0.536	0.548	p_{trend}	0.002	0.017
vitamin E (mg/d)			**vitamin E (mg/d)**		
< 10.0	1.00	1.00	< 8.7	1.00	1.00
10.0 - 13.3	0.80 (0.54 - 1.17)	0.85 (0.57 - 1.26)	8.7 - 11.4	1.33 (0.98 - 1.81)	1.42 (1.04 - 1.95)
13.3 - 17.2	0.73 (0.49 - 1.08)	0.84 (0.56 - 1.26)	11.4 - 14.5	1.06 (0.77 - 1.45)	1.13 (0.81 - 1.57)
> 17.2	0.61 (0.40 - 0.92)	0.70 (0.46 - 1.06)	> 14.5	1.28 (0.84 - 1.74)	1.41 (1.03 - 1.95)
p_{trend}	0.017	0.110	p_{trend}	0.319	0.122

* subjects with a self-reported history of MI, cerebrovascular accident, coronary bypass operation, and PTCA are excluded from the analyses
† reference category
RR[1] adjusted for age
RR[2] adjusted for age, BMI, number of packyears smoked, education, income, alcohol intake, and energy-adjusted ß-carotene, vitamin C, and vitamin E; use of antioxidative vitamin supplements

Risk of peripheral artery disease was significantly increased in men and women following a prescribed diet. Multivariate adjusted risk estimates were 1.88 (95% CI 1.19-2.91, p= 0.006) for men and 1.62 (95% CI 1.22-2.13, p<0.001) for women. Subsequent exclusion of subjects reporting a precribed diet (n = 544), however, only marginally altered risk estimates (data not shown).

Risk of peripheral artery disease was considerably modified by smoking status (tables 3,4). In general, inverse associations were stronger among smokers compared to never smokers. In men high levels of dietary ß-carotene were significantly (relative risk = 0.59, 95% CI 0.29-1.17; p_{trend} 0.042; highest versus lowest quartile) and high levels of vitamin C and vitamin E were nonsignificantly associated with age-adjusted risk of peripheral artery in current smokers. Multivariate adjustment only slightly altered risk estimates. For men who never smoked increased risk of PAD was observed with vitamin C intake (table 3), but numbers were small and confidence limits were wide. In women, this association was significant for vitamin C intake among former (relative risk = 0.54, 95% CI 0.29-0.99; p_{trend} 0.029 for the highest versus the lowest quartile) and among current female smokers (relative risk = 0.48, 95% CI 0.23-0.95; p_{trend} 0.039 for the highest versus the lowest quartile) in a multivariate adjusted model (table 4).

DISCUSSION

In the present cross-sectional study we investigated whether energy-adjusted dietary ß-carotene, vitamin C, and vitamin E were associated with peripheral artery disease in the elderly cohort of the Rotterdam Study. In women, we observed vitamin C to be inversely associated with risk of peripheral artery disease, whereas in men ß-carotene and vitamin E were inversely associated with risk of peripheral artery disease. Stratification by smoking status indicated that current or former smokers benefit most of elevated dietary antioxidant intake.

Interpretation of our findings should be preceeded by methodological considerations. Since our results are based on cross-sectional analysis, the basis for causal inference is inherently limited. Timing of dietary intake with respect to the development of peripheral artery disease is not known, but one might expect that recent dietary changes would not reflect the dietary milieu under which peripheral artery disease developed. For most subjects peripheral artery disease is asymptomatic, therefore we do not expect changes as

Table 3: Relative risk and 95% confidence intervals for peripheral artery disease for quartiles of energy-adjusted antioxidant intake stratified by smoking status among 1720 men of the Rotterdam Study

Intake quartile	ß-carotene		vitamin C		vitamin E	
	RR^1 (95% CI)	RR^2 (95% CI)	RR^1 (95% CI)	RR^2 (95% CI)	RR^1 (95% CI)	RR^2 (95% CI)
Never smoked						
1	1.00	1.00	1.00	*-f*	1.00	1.00
2	0.83 (0.09 - 7.86)	0.90 (0.09 - 9.82)	1.63 (0.21- 12.86)	-	1.26 (0.18 - 11.05)	1.51 (0.19 - 14.67)
3	1.60 (0.23 - 14.49)	1.08 (0.12 - 12.31)	1.55 (0.23- 10.52)	-	0.64 (0.07 - 6.07)	0.47 (0.04 - 4.79)
4	2.43 (0.32 - 24.45)	1.71 (0.18-20.30)	3.10 (0.33- 29.38)	-	1.08 (0.15 - 9.81)	1.22 (0.16 - 11.60)
p_{trend}	0.316	0.592	0.032	-	0.895	0.890
Former smokers						
1	1.00	1.00	1.00	1.00	1.00	1.00
2	1.57 (0.88 - 2.82)	1.04 (0.57 - 1.92)	1.03 (0.56 - 1.90)	1.04 (0.57 - 1.92)	0.88 (0.48 - 1.61)	0.86 (0.47 - 1.57)
3	1.02 (0.56 - 1.88)	0.95 (0.51 - 1.78)	0.92 (0.50 - 1.72)	0.95 (0.51 - 1.78)	1.10 (0.63 - 1.94)	1.08 (0.61 - 1.92)
4	1.22 (0.68 - 2.22)	1.45 (0.81 - 2.67)	1.39 (0.78 - 2.51)	1.45 (0.81 - 2.67)	0.82 (0.45 - 1.48)	0.79 (0.43 - 1.44)
p_{trend}	0.895	0.240	0.304	0.240	0.680	0.621
Current smokers						
1	1.00	1.00	1.00	1.00	1.00	1.00
2	0.74 (0.42 - 1.29)	0.75 (0.43 - 1.32)	0.66 (0.36 - 1.17)	0.65 (0.35 - 1.17)	0.80 (0.45 - 1.40)	0.82 (0.46 - 1.43)
3	0.53 (0.28 - 0.97)	0.50 (0.27 - 0.92)	1.07 (0.60 - 1.90)	1.12 (0.62 - 2.01)	0.62 (0.33 - 1.15)	0.63 (0.33 - 1.17)
4	0.59 (0.29 - 1.17)	0.56 (0.27 - 1.12)	0.51 (0.25 - 0.99)	0.51 (0.25 - 1.00)	0.59 (0.30 - 1.12)	0.62 (0.31 - 1.18)
p_{trend}	0.042	0.027	0.185	0.210	0.060	0.090

* subjects with a self-reported history of MI, cerebrovascular accident, coronary bypass operation, and PTCA were excluded from the analyses
† reference category
RR^1 adjusted for age
RR^2 adjusted for age, body mass index, smoking status (two variables), education (five categories), income (five categories), alcohol intake (five categories)
f because of an empty cell in the reference category no risk estimates are given

Table 4: Relative risk and 95% confidence intervals for peripheral artery disease for quartiles of energy-adjusted antioxidant intake stratified by smoking status among 2740 women of the Rotterdam Study

Intake quartile	ß-carotene		vitamin C		vitamin E	
	RR^1 (95% CI)	RR^2 (95% CI)	RR^1 (95% CI)	RR^2 (95% CI)	RR^1 (95% CI)	RR^2 (95% CI)
Never smoked						
1	1.00	1.00	1.00	1.00	1.00	1.00
2	0.80 (0.52 - 1.21)	0.80 (0.52 - 1.22)	0.73 (0.48 - 1.13)	0.73 (0.48 - 1.13)	1.60 (1.01 - 2.55)	1.59 (1.01 - 2.54)
3	0.85 (0.56 - 1.30)	0.85 (0.55 - 1.30)	0.75 (0.48 - 1.16)	0.76 (0.49 - 1.17)	1.23 (0.77 - 1.98)	1.20 (0.75 - 1.93)
4	0.84 (0.53 - 1.31)	0.82 (0.52 - 1.29)	0.74 (0.48 - 1.16)	0.76 (0.49-1.18)	1.72 (1.11 - 2.71)	1.70 (1.09 - 2.67)
P_{trend}	0.472	0.430	0.225	0.270	0.056	0.068
Former smokers						
1	1.00	1.00	1.00	1.00	1.00	1.00
2	0.63 (0.35 - 1.12)	0.63 (0.35 - 1.12)	0.92 (0.52 - 1.62)	0.95 (0.53 - 1.69)	0.82 (0.46 - 1.45)	0.80 (0.45 - 1.43)
3	0.46 (0.24 - 0.85)	0.45 (0.23 - 0.83)	0.70 (0.38 - 1.29)	0.69 (0.37 - 1.27)	0.66 (0.36 - 1.21)	0.65 (0.35 - 1.20)
4	0.72 (0.40 - 1.29)	0.71 (0.39 - 1.28)	0.55 (0.29 - 1.00)	0.54 (0.29 - 0.99)	0.73 (0.40 - 1.33)	0.73 (0.39 - 1.32)
P_{trend}	0.142	0.131	0.036	0.029	0.887	0.220
Current smokers						
1	1.00	1.00	1.00	1.00	1.00	1.00
2	1.09 (0.60 - 2.00)	1.05 (0.57 - 1.93)	1.06 (0.59 - 1.18)	1.08 (0.60 - 1.94)	1.74 (0.93 - 3.30)	1.79 (0.95 - 3.43)
3	0.91 (0.48 - 1.69)	0.91 (0.48 - 1.71)	0.85 (0.47 - 1.52)	0.78 (0.42 - 1.42)	1.46 (0.77 - 2.80)	1.49 (0.77 - 2.89)
4	1.08 (0.57 - 2.02)	1.11 (0.58 - 2.11)	0.59 (0.29 - 1.14)	0.48 (0.23 - 0.95)	1.46 (0.76 - 2.82)	1.04 (0.98 - 1.10)
P_{trend}	0.980	0.890	0.127	0.039	0.362	0.323

* subjects with a self-reported history of MI, cerebrovascular accident, coronary bypass operation, and PTCA were excluded from the analyses
† reference category
RR^1 adjusted for age
RR^2 adjusted for age, body mass index, smoking status (two variables), education (five categories), income (five categories), alcohol intake (five categories)

a result of the presence of disease. We excluded subjects with symptomatic disease assuming that they might intentionally have changed their diets after having experienced a cardiovascular event. In addition, we evaluated whether exclusion of subjects reporting a prescribed diet modified risk estimates. However, only minor alterations were observed, suggesting that recent dietary changes were not a major source of bias in the present study.

Few other studies have focused on the association of dietary antioxidants and peripheral artery disease. In a cross-sectional analysis of dietary antioxidants with peripheral atherosclerosis lower dietary intakes of vitamins E, C, and A were observed to be significantly associated with lower ankle-arm index in men but not in women in the ARIC Study[29]. Cross-sectional data from the Edinburgh Artery Study showed lower vitamin E intake to be associated with decreased ankle-arm index[30], whereas case-control data from the same study showed no association of alpha-tocopherol and beta-carotene with intermittent claudication. Among cases of intermittent claudication, however, a lower intake of vitamin C was observed[31]. In the current study we found dietary vitamin C to be significantly inversely associated with peripheral artery disease in women and ß-carotene and vitamin E nonsignificantly inversely associated with peripheral artery disease in men in a multivariate model. Whether this may be partly attributable to a different food pattern compared to women remains to be elucidated.

Smokers are at a high risk of developing atherosclerosis, though the mechanisms are not yet fully understood. Cigarette smoke contains vast amounts of free radicals that could directly initiate and propagate the process of lipid peroxidation. An imbalance between prooxidants and antioxidants, linked to decreased smoke related antioxidative capacity and increased free radical generation, might render smokers more prone to peroxidative stress[32]. Several studies examining antioxidants and cardiovascular disease observed an interaction between dietary antioxidants and smoking. This interaction has not been observed uniformly in epidemiological studies, but several studies have seen fairly strong associations in current or former smokers[10,14,33]. In the current study we observed that among men current smokers benefit most of elevated dietary antioxidant intake. Among women this was less clear for current smokers which might be due to relatively small numbers. Differences in smoking history between men and women might be a possible explanation for these observations. In

conclusion, our results suggest that a diet high in fruit and vegetables may be beneficial in the prevention of peripheral atherosclerosis in particular in ever smokers.

REFERENCES

1. Riemersma RA, Wood DA, Macintyre CCA et al. Risk of angina pectoris and plasma concentrations of vitamins A, C, and E and carotene. *Lancet* 1991; **337**:1-5.
2. Stampfer MJ, Hennekens CH, Manson JE et al. Vitamin E consumption and the risk of coronary disease in women. *N Engl J Med* 1993; **328**:1444-1449.
3. Rimm EB, Stampfer MJ, Ascherio A et al.Vitamin E consumption and the risk of coronary heart disease in men. *N Engl J Med* 1993; **328**:1450-1456.
4. Knekt P, Reunanen A, Järvinen R et al. Antioxidant vitamin intake and coronary mortality in a longitudinal population study. *Am J Epidemiol* 1994; **139**:1180-1189.
5. Gaziano JM, Manson JE, Branch LG et al. A prospective study of consumption of carotenoids in fruits and vegetables and decreased cardiovascular mortality in the elderly. *Ann Epidemiol* 1995; **5**:255-260.
6. Pandey DK, Shekelle R, Selwyn BJ et al. Dietary vitamin C and ß-carotene and risk of death in middle-aged men. The Western Electric Study. *Am J Epidemiol* 1995; **142**:1269-1278.
7. Kushi LH, Folsom AR, Prineas RJ et al. Dietary antioxidant vitamins and death from coronary heart disease in postmenopausal women. *N Engl J Med* 1996; **334**:1156-1162.
8. Losonczy KG, Harris TB, Havlik RJ. Vitamin E and vitamin C supplement use and risk of all-cause and coronary heart disease mortality in older persons: the Established Populations for Epidemiologic Studies of the Elderly. *Am J Clin Nutr* 1996; **64**:190-96.
9. Sahyoun NR, Jaques PF, Russell RM. Carotenoids, vitamins C and E, and mortality in an elderly population. *Am J Epidemiol* 1996; **144**:501-511.
10. Klipstein-Grobusch K, Geleijnse JH, den Breeijen JH et al. Dietary antioxidants and risk of myocardial infarction in the elderly: The Rotterdam Study. *Am J Clin Nutr* 1999; **69**:261-266.
11. Kritchevsky SB, Shimakawa T, Tell GS et al. Dietary antioxidants and carotid artery wall thickness. *Circulation* 1995; **92**:2142-2150.
12. Bonithon-Kopp C, Coudray C, Berr C et al. Combined effects of lipid peroxidation on carotid atherosclerosis in a population aged 59-71 y: The EVA Study. *Am J Clin Nutr* 1997; **65**:121-127.
13. Hodis HN, Mack WJ, LaBree L et al. Serial coronary angiographic evidence that antioxidant vitamin intake reduces progression of coronary artery atherosclerosis. *JAMA* 1995; **273**:1849-1854.
14. Kritchevsky SB, Tell GS, Shimakawa T et al. Provitamin A carotenoid intake and carotid artery plaques: the Atherosclerosis Risk in Communities Study. *Am J Clin Nutr* 1998; **68**:726-733.
15. Schroll M, Munck O. Estimation of peripheral artery disease by ankle blood pressure measurements in a population of 60 year old men and women. *J Chronic Dis* 1981; **34**:261-269.

16. Fowkes FGR, Houseley E, Cawood EHH et al. Edinburgh Artery Study: prevalence of asymptomatic and symptomatic peripheral arterial disease in the general population. *Int J Epidemiol* 1991; **20**:384-392.
17. Newman AB, Sutton-Tyrell K, Vogt MT et al. Morbidity and mortality in hypertensive adults with low ankle/arm blood pressure index. *JAMA* 1993; **270**:465-469.
18. Curb JD, Masaki K, Rodriguez BL et al. Peripheral artery disease and cardiovascular risk factors in the elderly. *Arterioscler Thromb Vasc Biol* 1995; **15**:1495-1500.
19. Vogt MT, Cauley JA, Newman AB et al. Decreased ankle/arm blood pressure index and mortality in elderly women. *JAMA* 1993; **270**:465-469.
20. Newman AB, Shemanski L, Manolio TA et al. Ankle arm index as a predictor of cardiovascular disease in the cardiovascular health study. *Circulation* 1996; **94**:I-144.
21. Hofman A, Grobbee DE, De Jong PTVM et al. Determinants of disease and disability in the elderly: The Rotterdam Elderly Study. *Eur J Epidemiol* 1991; **7**:403-422.
22. Rose GA, Blackburn H, Gillum RF. Cardiovascular survey methods. World Health Organization, Geneva, Switzerland, 1982.
23. Bots ML, Hofman A, Grobbee DE. Common carotid intima-media thickness and lower extremtiy arterial atherosclerosis. *Arterioscl Thromb* 1994; **14**:1885-91.
24. Vogt MT, Wolfson SK, Kuller LH. Lower extremity arterial disease and the ageing process. *J Clin Epidemiol* 1992; **268**:1287-91.
25. Food and Nutrition Council. Dutch food composition table (NEVO). 1993. The Hague, Netherlands: Voorlichtingsbureau voor de Voeding, (in Dutch).
26. Klipstein-Grobusch K, den Breeijen JH, Goldbohm RA et al. Dietary assessment in the elderly: validation of a semiquantitative food frequency questionnaire. *Eur J Clin Nutr* 1998, **52**:588-596.
27. Beaton GH, Milner J, Corey P et al. Sources of variance in 24-hour dietary recall data: implications for nutrition study design and interpretation. *Am J Clin Nutr* 1979 **32**:2546-2559.
28. Willett WL, Stampfer MJ. Total energy intake: implications for epidemiologic analysis. *Am J Epidemiol* 1986; **124**:17-27.
29. Zheng Z-J, Folsom AR, Kritchevsky SB et al. Associations of dietary antioxidants with peripheral atherosclerosis in middle-aged men and women: The Atherosclerosis Risk in Communities ARIC. Study. *Circulation* 1996; **94**:I-144.
30. Donnan PT, Thomson M, Fowkes FGR et al. Diet as a risk factor for peripheral artery disease in the general population: The Edinburgh Artery Study. *Am J Clin Nutr* 1993; **57**:917-21.
31. Leng GC, Horrobin DF, Fowkes FGR et al. Plasma essential fatty acids, cigarette smoking, and dietary antioxidants in peripheral artery disease. A population-based case-control study. *Arterioscler Thromb* 1994; **14**:471-478.
32. Mezetti A, Lapenna D, Pierdomenico S et al. Vitamins E, C, and lipid peroxidation in plasma and arterial tissue of smokers and non-smokers. *Atherosclerosis* 1995; **112**:91-99.
33. Street DA, Comstock GW, Salkeld RM et al. Serum antioxidants and myocardial infarction. Are low levels of carotenoids and α-tocopherol risk factors for myocardial infarction? *Circulation* 1994; **90**:1154-1161.

4.3

Dietary antioxidants and risk of myocardial infarction in the elderly. The Rotterdam Study

ABSTRACT

Epidemiological studies have shown dietary antioxidants to be inversely correlated with coronary heart disease (CHD). In the current observational study we investigated whether ß-carotene, vitamin C, and vitamin E from food sources and supplements were related to risk of myocardial infarction (MI) in an elderly population. The study sample consisted of 4802 participants of the Rotterdam Study free of MI at baseline for whom dietary data assessed by a semiquantitative food frequency questionnaire were available. During a four year follow-up period 124 subjects had a first MI. The association between energy-adjusted ß-carotene, vitamin C, and vitamin E and risk of MI was examined by multivariate logistic regression. Risk of MI for the highest compared to the lowest tertile of ß-carotene intake was 0.55 (95% CI: 0.34,0.83; p_{trend} 0.013) adjusted for age, sex, body mass index, packyears smoked, income, education, alcohol intake, intakes of energy-adjusted vitamin C and vitamin E, and use of antioxidative vitamin supplements. If ß-carotene intake from food sources and supplements was combined, the inverse relation with risk of MI was slightly more pronounced. Stratification by smoking status indicated that the association was most evident in current and former smokers. No association with risk of MI was observed for dietary vitamin C and vitamin E.

In conclusion, the results of this observational study in the elderly population of the Rotterdam Study are supportive of the hypothesis that high levels of dietary ß-carotene may protect against CHD. We did not observe an association between vitamin C or vitamin E and myocardial infarction.

INTRODUCTION

Several epidemiological studies have shown dietary antioxidants to be inversely associated with coronary heart disease[1-9]. The antioxidants ß-carotene, tocopherol, and vitamin C have been implicated to prevent or slow down the atherosclerotic process by inhibiting low-density-lipoprotein (LDL) oxidation. The most consistent and reliable association has been seen with vitamin E, either with supplementation or with relatively high levels of dietary intake. Beta-carotene and vitamin C intake have been less clearly associated with a reduced risk[10]. Most epidemiological studies on dietary antioxidants and risk of CHD focused on middle-aged populations. In the current study we investigated whether dietary intake of the antioxidants ß-carotene, vitamin C, and vitamin E from food and supplemental sources was related to risk of myocardial infarction in an elderly population.

SUBJECTS AND METHODS

Study population

The Rotterdam Study is a community based prospective cohort study of 7983 persons (response rate 78%) aged 55 years and over, living in Ommoord, an urban district in Rotterdam, the Netherlands. The aim of the study is to investigate the incidence of and the risk factors for chronic and disabling cardiovascular, neurodegenerative, locomotor, and ophthalmic diseases as described elsewhere[11]. The study was approved by the Medical Ethics Committee of the Erasmus University, and written informed consent was obtained from all participants. Follow-up for coronary heart disease mortality started after the baseline survey in 1990, and until April 1996 follow-up information was available for 94% of the cohort. Of the 5159 subjects with dietary data, 173 subjects had a myocardial infarction (MI) during the follow-up period. Because of possible changes in dietary pattern, subjects with a previously known MI at baseline (n = 357) were excluded from analysis. This resulted in 4802 subjects for the current analysis. During an average follow-up time of 4 years, 124 cases of first fatal or non-fatal myocardial infarction occurred.

Case Ascertainment

The follow-up period, starting at the baseline examination and lasting until April 1996 for the present analysis, comprised 3 to 7 (mean 4) years. With respect to the vital status

of participants information was obtained at regular intervals from the municipal health service in Rotterdam. Information on fatal and non-fatal endpoints was obtained from the general practitioners (GPs) working in the study district of Ommoord. Information on fatal and non-fatal endpoints for 85% of the cohort was obtained from GPs in the study district of Ommoord, who regularly report such information to the data center of the Rotterdam Study. All possible events reported by the GPs were verified by research physicians from the Rotterdam Study through records of the participating practitioners and medical specialists. In April 1996, the medical records of participants with GPs from outside the Ommoord area, contributing around 15% of the cohort, were checked by research physicians and of all possible events additional information for coding was collected. Cause and circumstances of death were obtained by questionnaire from the GP and by scrutinizing information from hospital discharge records in case of admittance or referral shortly after reporting of death by the municipal health service or the GP. Overall, follow-up information was available for 94% of the cohort.

Classification of fatal and non-fatal events was based on the International Classification of Diseases, 10th edition[12]. For the present analysis cases of first non-fatal myocardial infarction or fatal myocardial infarction (ICD-10:I21-I24) were selected. All events were classified independently by two research physicians. If there was disagreement, a consensus was reached in a special session. Finally, all these events were verified by a medical expert in the field of cardiovascular disease. In case of discrepancies the judgment by this expert was considered definite.

Measurements

Information on current health status, medical history, drug use, education, income, and smoking behaviour was obtained with a computerized questionnaire during a home interview. Height and weight were measured, and body mass index (weight in kg/height in m^2) was calculated as a measure of obesity. Sitting blood pressure was measured in the right upper arm with a random-zero sphygmomanometer; the average of two measurements was used. Hypertension was defined as a systolic blood pressure of at least 140 mm Hg and/or diastolic blood pressure of at least 90 mm Hg, and/or current use of antihypertensive drugs. A venepuncture was performed and serum total and high density lipoprotein (HDL) cholesterol concentrations were determined by an automated enzymatic procedure. Subjects were classified as hypercholesteremic if serum cholesterol levels were at least 6.5 mmol/l or higher.

Dietary assessment

The semiquantitative food frequency questionnaire (SFFQ) completed during baseline aimed at assessing habitual food intake during the past year and included 170 food items in 13 food groups and questions about dietary habits, supplementation, and prescribed diets. The dietary assessment consisted of a simple self-administered questionnaire that was completed at home (completion time < 20 min) and a subsequent structured interview with a trained dietitian (allocated time < 20 min). The structured interview was based on the contents of the completed questionnaire and was conducted during the subjects' second visit at the study center. SFFQ data were converted to nutrient intake using the computerized Dutch Food Composition Table[13]. Data for ß-carotene, retinol, and tocopherol were updated by use of an additional database by the Netherlands Institute of Public Health and Environmental Protection (unpublished observation, 1993). Intake through nutritional supplements was not considered since brand labels were not recorded with sufficient accuracy.

The validity of the SFFQ was assessed in a subsample of 80 men and women aged 55 to 75 years. Nutrient intake estimated from the SFFQ was compared with estimated nutrient intake of in total 15 days of food records collected over a one-year period[14]. The ability of the SFFQ to rank subjects adequately according to their dietary intake was demonstrated by Pearson's correlation coefficients of 0.4 to 0.8 adjusted for sex, age, total energy intake[15] and for within-person variability in daily intake[16], and a high degree of classification into the same or adjacent quintile (76.8 % for energy-adjusted data).

Data Analysis

All analyses were performed for men and women combined. The association between dietary antioxidants and risk of myocardial infarction was examined primarily by multivariate logistic regression. Because vitamins can be ingested from both foods and supplements, exposure to each vitamin was studied in two ways: first, dietary antioxidant intake from food sources was categorized into tertiles, and risk of myocardial infarction in the highest and middle tertile was compared to risk in the lowest tertile. The initial analysis examined associations adjusted for age and sex. The analyses were furthermore adjusted for body mass index, packyears smoked, equivalent household income (five categories), highest education attained (five categories), and alcohol intake (five categories). Supplement preparations containing ß-carotene, vitamin C or vitamin E were combined and added to the model as separate variable. This allowed investigation

of the relation between dietary antioxidants and the risk of MI unconfounded by supplemental antioxidant vitamin intake. Second, in order to study the effects of combined intakes of antioxidants from food sources and supplement preparations, users of ß-carotene, vitamin C, vitamin E, or multivitamin supplements were categorized into the highest tertile of the correspondent dietary intake. Age- and sex-adjusted risk of MI according to tertile of intake was investigated and the models were subsequently adjusted for the above mentioned factors except for supplemental antioxidant intake.

The associations between tertiles of energy-adjusted ß-carotene, vitamin E, and vitamin C with and without supplemental intake and risk of MI were expressed as odds ratio with 95% confidence intervals. Results were considered statistically different at the two-sided 0.05 alpha-level. Statistical analysis was performed using SAS® (Release 6.11; SAS Institute, Cary, NC, USA).

RESULTS

Mean age of the 1856 men and 2946 women was 67.0 years (SD 7.3) and 67.9 years (SD 8.0), respectively. Compared to the general population of the Rotterdam Study for whom dietary data were available (n=5434) the study population for the present analysis did not differ in characteristics such as age, BMI, blood pressure, income, level of education or nutrient intake. Vitamin supplements containing either ß-carotene, vitamin C, or vitamin E were used by 11.8% of the study population.

Details of the association between the intake of energy-adjusted antioxidants and selected baseline factors are presented in table 1. Since sex and age were not equally distributed across tertiles of dietary antioxidant intake, adjustment for age and sex was performed. Mean energy-adjusted dietary intakes of ß-carotene were 0.84 mg/d in the lowest and 2.11 mg/d in the highest tertile, of vitamin C 63 mg/d in the bottom tertile and 170 mg/d in the top tertile and of vitamin E 18.5 mg/d and 7.8 mg/d, respectively. The percentage of hypercholesterolemic subjects was higher in the lowest tertile compared to the upper two tertiles of ß-carotene intake. Percentage of current smokers declined across tertiles of ß-carotene. A similar decline was observed for percentage of current smokers across tertiles of vitamin C and vitamin E (table 1). In addition, mean

Table 1: Levels of various baseline factors in tertiles of energy-adjusted ß-carotene, vitamin C, and vitamin E from food intake (adjusted for age and sex)

	Tertile of energy adjusted dietary ß-carotene intake			
Energy-adjusted ß-carotene intake (mg/d)	**1 (Lowest) (n = 1601) < 1.13**	**2 (n = 1601) 1.13 - 1.57**	**3 (Highest) (n = 1600) > 1.57**	**p-value for trend**
Body mass index (kg/m^2)	26.0	26.4	26.3	0.011
Current smokers (%)	29.1	25.9	19.5	0.015
Hypertension[1] (%)	48.4	48.3	49.0	0.610
Hypercholesteremia[2] (%)	52.5	48.0	49.0	0.063
Diabetes (%)	9.5	9.33	9.0	0.631
Alcohol intake (mg/d)	12.1	10.9	10.9	0.021
Antioxidative vitamin supplements[3] (%)	11.0	11.5	11.3	0.651
Energy-adjusted vitamin C intake (mg/d)	93.8	108.7	132.8	<0.001
Energy-adjusted vitamin E intake (mg/d)	11.8	13,0	14.2	<0.001

	Tertile of energy adjusted vitamin E intake			
Energy-adjusted vitamin E intake (mg/d)	**1 (Lowest) (n = 1601) < 10.2**	**2 (n = 1601) 10.2 - 14.2**	**3 (Highest) (n = 1600) > 14.2**	**p-value for trend**
Body mass index (kg/m^2)	26.3	26.3	26.1	0.323
Current smokers (%)	27.6	25.4	21.7	0.007
Hypertension[1] (%)	48.6	48.5	48.6	0.330
Hypercholesteremia[2] (%)	51.3	48.8	49.5	0.212
Diabetes (%)	8.0	9.8	9.7	0.170
Alcohol intake (g/d)	14.1	10.5	9.7	<0.001
Antioxidative vitamin supplements[3] (%)	10.3	11.9	11.5	0.577
Energy-adjusted ß-carotene intake (mg/d)	1.27	1.45	1.59	<0.001
Energy-adjusted vitamin C intake (mg/d)	104.6	112.3	118.6	<0.001

table 1 continued

	Tertile of energy adjusted dietary vitamin C intake			
	1 (Lowest) (n = 1601)	2 (n = 1601)	3 (Highest) (n = 1600)	p-value for
Energy-adjusted vitamin C intake (mg/d)	**< 87**	**87 - 126**	**> 126**	**trend**
Body mass index (kg/m^2)	26.0	26.2	26.5	0.003
Current smokers (%)	31.4	22.8	20.0	0.001
Hypertension[1] (%)	47.3	49.9	48.5	0.381
Hypercholesteremia[2] (%)	52.0	49.9	47.5	0.786
Diabetes (%)	10.2	9.6	7.9	0.052
Alcohol intake (g/d)	12.0	11.6	10.3	0.002
Antioxidative vitamin supplements[3] (%)	11.6	10.9	11.3	0.482
Energy-adjusted ß-carotene intake (mg/d)	1.19	1.44	1.71	<0.001
Energy-adjusted vitamin E intake (mg/d)	12.3	13.0	13.7	<0.001

[1] systolic blood pressure ≥ 140 and/or diastolic blood pressure ≥ 90 and/or use of antihypertensive medication
[2] serum cholesterol ≥ 6.5 mmol/l
[3] use of either ß-carotene, vitamin E, vitamin C, or multivitamin supplements

alcohol intake was highest for the lowest tertiles of dietary antioxidant intake. Although dietary antioxidant intake generally increased across tertiles of the other antioxidants, the increase of ß-carotene and vitamin C with tertiles of vitamin E was very modest. Spearman correlation coefficient was highest between ß-carotene and vitamin C ($r = 0.36$, $p < 0.0001$) and lowest between vitamin C and vitamin E ($r = 0.12$, $p < 0.0001$).

Beta-carotene from food sources was inversely related to risk of myocardial infarction after adjustment for age and sex (table 2). The relative risk for the highest compared to the lowest tertile was 0.59 (95% CI: 0.34,0.86, p_{trend} 0.009). After additional adjustment for body mass index, packyears smoked, equivalent household income, education, and alcohol intake, the observed association remained (table 2). Further adjustment for vitamin E and vitamin C did not alter the observed association. To evaluate whether the observed association of ß-carotene from food sources was independent of antioxidative vitamin supplements, antioxidant vitamin supplement use

Table 2: Risk of myocardial infarction and 95% confidence intervals according to energy-adjusted tertiles of dietary ß-carotene, vitamin C, and vitamin E

Variable	Tertiles of energy-adjusted dietary intake			P value for trend
	1 (Lowest)* (n = 1601)	2 (n = 1601)	3 (Highest) (n = 1600)	
ß-carotene level				
No. of MI	53	41	30	
energy-adjusted ß-carotene intake (mg/d)	< 1.13	1.13 - 1.57	> 1.57	
Relative risk (95% CI)				
age and sex adjusted	1.00	0.74 (0.48 - 1.12)	0.55 (0.34- 0.86)	0.009
multivariate adjusted I[1]	1.00	0.74 (0.48 - 1.12)	0.57 (0.36 - 0.91)	0.017
multivariate adjusted II[2]	1.00	0.72 (0.47 - 1.10)	0.55 (0.34 - 0.83)	0.013
vitamin C level				
No. of MI	47	41	36	
energy-adjusted vitamin C intake (mg/d)	< 87	87 - 126	> 126	
Relative risk (95% CI)				
age and sex adjusted	1.00	0.89 (0.58 - 1.37)	0.84 (0.54 - 1.30)	0.446
multivariate adjusted I[1]	1.00	0.94 (0.58 - 1.37)	0.88 (0.56 - 1.38)	0.581
multivariate adjusted II[2]	1.00	1.01 (0.65 - 1.56)	1.05 (0.65 - 1.67)	0.856
vitamin E level				
No. of MI	33	49	42	
energy-adjusted vitamin E intake (mg/d)	< 10.2	10.2 - 14.2	> 14.2	
Relative risk (95% CI)				
age and sex adjusted	1.00	1.41 (0.90 - 2.23)	1.05 (0.66 - 1.69)	0.916
multivariate adjusted I[1]	1.00	1.40 (0.90 - 2.22)	1.07 (0.67 - 1.73)	0.836
multivariate adjusted II[2]	1.00	1.52 (0.97 - 2.42)	1.21 (0.75 - 1.98)	0.528
multivariate adjusted II[2]	1.00	1.01 (0.65 - 1.56)	1.05 (0.65 - 1.67)	0.856

* reference category

[1]multivariate adjusted I adjusted for age, sex, BMI, packyears smoked, equivalent household income (five categories, education (five categories), and alcohol intake (five categories)

[2]multivariate adjusted II additionally adjusted categories of energy-adjusted ß-carotene, vitamin C, and vitamin E, use of multivitamin supplements

Table 3: Tertiles of energy-adjusted dietary ß-carotene intake and risk of myocardial infarction (95% confidence interval) according to smoking status

Variable	Tertiles of energy-adjusted dietary ß-carotene intake			P value for trend
	1 (Lowest)*	2	3 (Highest)	
energy-adjusted dietary ß-carotene (mg/d)	**< 1.13**	**1.13 - 1.57**	**> 1.57**	
smokers (n = 1133)	1.00	0.87 (0.42 - 1.83)	0.45 (0.17 - 1.10)	0.058
former smokers (n = 1984)	1.00	0.53 (0.27 - 1.01)	0.32 (0.14 - 0.66)	0.002
non-smokers (n = 1685)	1.00	1.00 (0.39 - 2.55)	1.68 (0.65 - 4.39)	0.299

* reference category

[1] multivariate adjusted model adjusted for age, sex, body mass index, packyears smoked, equivalent household income (5 cat.), education (5 cat.), and alcohol intake (5 cat.), cat. of energy-adjusted vitamin C, vitamin E and antioxidative supplements

(dietary ß-carotene, vitamin C, vitamin E or multivitamin supplements) was included in the multivariate model. For users of antioxidant vitamin supplements the RR was 0.49 (95% CI: 0.21,0.99; p = 0.008) compared to non-users. The relative risk of the highest compared to the lowest tertile of ß-carotene intake was 0.55 (95% CI: 0.34,0.83; p = 0.013). Further adjustment for dietary fats and cholesterol did not change the observed association. For ß-carotene combined from food sources and supplements the age- and sex-adjusted RR was 0.49 (95% CI: 0.31,0.86, p_{trend} 0.019) for the highest compared to the lowest tertile of ß-carotene consumption. Multivariate adjustment led only to slight changes in the point estimate (highest compared to lowest tertile: RR = 0.50; 95% CI, 0.31 - 0.82, p_{trend} = 0.006).

The association between dietary ß-carotene and the risk of MI differed significantly according to baseline smoking status. We observed a significant inverse association between ß-carotene and risk of MI among former smokers and a nonsignificant one among current smokers (table 3). Interaction between ß-carotene and smoking status was not observed. These associations where comparable when ß-carotene from food and supplements were combined (table 3). We did not observe an association for tertiles of vitamin C intake from food sources or food sources and supplements

combined with risk of MI in an age- and sex-adjusted model (table 2). Also no association with MI was observed for tertiles of vitamin E. Stratification by smoking status did not alter these results.

DISCUSSION

In the elderly cohort of the Rotterdam Study we observed an inverse association of ß-carotene intake with risk of myocardial infarction independent of the use of antioxidative vitamin supplement use. The association observed was most evident in former and current smokers. Vitamin C or vitamin E from food sources or from food sources and vitamin supplements combined were not associated with risk of MI.

Before interpreting these results some methodological issues should be considered. Potential bias due to incomplete follow-up was unlikely to occur due to the high follow-up rate (94%) achieved. Ascertainment of cases was facilitated through the close cooperation with GPs working in the study district of Ommoord and linkage to the municipal health service in Rotterdam. Since all events were classified independently by two research physicians and subsequently by a cardiovascular disease expert, inaccuracies in coding of diagnosis was minimized. Because subjects with a previous diagnosis of myocardial infarction may have altered their diet as a consequence of the disease, subjects reporting hospitalization for myocardial infarction at baseline were excluded from the analysis. Certain methodological issues may weaken the observed association between dietary antioxidant vitamins and risk of disease. A serious limitation of studies addressing diet are inaccuracies in measurement of intake. Random misclassification of dietary habits may decrease the ability to detect associations between diet and disease as do changes in dietary habits during the follow-up period. Weak correlation between vitamin intake and bioavailability may have further diluted the observed associations. Insufficient heterogeneity of dietary intake in the population may make it more difficult to detect diet-disease associations. In the current study contrasts in the range of dietary intake of the lowest to the highest tertile were modest for ß-carotene, vitamin E, and vitamin C, i.e. between-person variation in the current study was modest. In addition, ß-carotene, vitamin E, and vitamin C supplements were only used by a small proportion of the population. Figures were 0.6% for ß-carotene, 1.4% for vitamin E, and 5.7% for vitamin C. Inclusion of

supplemental dietary intake somewhat strengthened the association between ß-carotene intake and risk of MI.

Observational studies have indicated that ß-carotene may exert a protective effect on incidence and mortality of coronary heart disease, though the evidence is not conclusive[10]. Studies focusing on plasma or adipose tissue concentration of ß-carotene, however, yielded in general consistent results. Biologically plausible mechanisms such as the antioxidant potential to scavenge free radicals[21] and the ability to inhibit LDL oxidation[22] supported these findings. In middle-aged populations, subjects with low plasma concentration of ß-carotene were found to have an elevated risk for angina pectoris[1] and of MI[23], while the latter appeared to be limited to smokers with low ß-carotene serum levels. Risk of MI was also considerably elevated in current smokers with low ß-carotene adipose tissue concentrations[24]. In contrast, an inverse association between total plasma carotenoids and risk of fatal coronary heart disease and non-fatal MI in hyperlipidemic men was present in non-smokers[25]. Studies focusing on the elderly showed either no association between carotenoid concentrations and dietary intake and risk of coronary heart disease[7] or an inverse association between carotene-containing fruit and vegetables and the risk of cardiac death due to MI and CHD[5]. In the current study, we observed a significant protective effect of increased ß-carotene intake with risk of MI in the elderly population of the Rotterdam Study that was most evident in current and former smokers (table 3).

The effects of ß-carotene found in our study and other observational studies are at odds with recent findings of large supplement intervention trials for chronic disease prevention[26-30]. These trials reported either no effect of ß-carotene supplementation on the incidence of cardiovascular disease[27] or a slight increase in cardiovascular mortality[26,28-30]. However, only one of these trials was designed to address cardiovascular disease in particular[27]. To explain the disparity between results obtained by observational and intervention studies several reasons were proposed. Administration may have been too late in the cancerogenic or atherogenic process (a protective effect of ß-carotene may be most beneficial in an early phase of the cancerogenic or atherosclerotic process) and inadequate duration and dosage of supplement use has been discussed[31,32]. Adverse effects of supplemental ß-carotene on plasma concentrations of other carotenoids have been reported[33,34] and there is limited

evidence that ß-carotene could function as a prooxidant at higher concentrations[31]. The associations found, however, may not be ascribed to ß-carotene exclusively. Possibly, ß-carotene could only be a marker for some other substance in ß-carotene containing foods or to dietary patterns and lifestyle behaviour closely linked to a diet rich in vegetables and fruit that is associated with reduced risk of coronary heart disease. A habitual diet rich in ß-carotene-containing products may thus also protect against coronary heart disease among the elderly.

In the current study, we did not observe an association of dietary vitamin C and risk of myocardial infarction. Our results are in line with several other studies that did not find vitamin C to be associated with a protective effect as summarized by Jha et al.[10]. In studies in the elderly, some investigators observed an inverse association of high dietary vitamin C intake and high plasma levels[7] with cardiovascular disease, whereas others observed no association with dietary and plasma vitamin C[17] or use of vitamin C supplements[9]. Results of a Finnish study linking vitamin C deficiency to increased risk of MI[18] suggest that suboptimal vitamin C concentrations elevate the risk of MI. These findings are supported by experimental data showing that detectable lipid peroxidation starts only after all ascorbate has been completely used up[19]. The most consistent and reliable effect of antioxidants and fatal and nonfatal cardiovascular disease in middle-aged populations in the literature has been seen with vitamin E. The relations have been seen both with supplementation and with relatively high levels of dietary intake[1,4,8,20] that are sustained for two or more years[2,3]. The relative risk reduction for various cardiovascular end points ranged from 31% to 65%[10]. In the current study, however, we did not observe an association between dietary vitamin E and MI. This is consistent with most studies investigating the association between dietary and plasma vitamin E and risk of heart disease mortality in the elderly[7]. Only one study observed an inverse association of vitamin E supplementation on coronary heart disease risk in the elderly[9].

In summary, we found an inverse association of high dietary ß-carotene intake on risk of myocardial infarction in an elderly population. Whether this association may be ascribed to ß-carotene exclusively, to a diet rich in ß-carotene containing products, or to dietary patterns and lifestyle behaviour closely linked to a diet rich in vegetables and fruit remains to be elucidated.

REFERENCES

1. Riemersma RA, Wood DA, Macinytre CCA et al. Risk of angina pectoris and plasma concentrations of vitamins A, C, and E and carotene. *Lancet* 1991; **337**:1-5.
2. Stampfer MJ, Hennekens CH, Manson JE et al. Vitamin E consumption and the risk of coronary disease in women. *N Engl J Med* 1993; **328**:1444-1449.
3. Rimm EB, Stampfer MJ, Ascherio A et al. Vitamin E consumption and the risk of coronary heart disease in men. *N Engl J Med* 1993; **328**:1450-1456.
4. Knekt P, Reunanen A, Järvinen R et al. Antioxidant vitamin intake and coronary mortality in a longitudinal population study. *Am J Epidemiol* 1994; **139**:1180-1189.
5. Gaziano JM, Manson JE, Branch LG et al. A prospective study of consumption of carotenoids in fruits and vegetables and decreased cardiovascular mortality in the elderly. *Ann Epidemiol* 1995; **5**:255-260.
6. Pandey DK, Shekelle R, Selwyn BJ et al. Dietary vitamin C and ß-carotene and risk of death in middle-aged men. The Western Electric Study. *Am J Epidemiol* 1995; **142**:1269-1278.
7. Sahyoun NR, Jaques PF, Russell RM: Carotenoids, vitamins C and E, and mortality in an elderly population. *Am J Epidemiol* 1996; **144**:501-511.
8. Kushi LH, Folsom AR, Prineas RJ et al. Dietary antioxidant vitamins and death from coronary heart disease in postmenopausal women. *N Engl J Med* 1996; **334**:1156-1162.
9. Losonczy KG, Harris TB, Havlik RJ: Vitamin E and vitamin C supplement use and risk of all-cause and coronary heart disease mortality in older persons: the Established Populations for Epidemiologic Studies of the Elderly. *Am J Clin Nutr* 1996; **64**:190-196.
10. Jha P, Flather M, Lonn E et al. The antioxidant vitamins and cardiovascular disease. A critical review of epidemiologic and clinical trial data. *Ann Intern Med* 1995; **123**:860-872.
11. Hofman A, Grobbee DE, De Jong PTVM et al. Determinants of disease and disability in the elderly: The Rotterdam Elderly Study. *Eur J Epidemiol* 1991; **7**:403-422.
12. WHO (1992). International Statistical Classification of Diseases and Related Health Problems, 10th revision. Geneva, Vol 1.
13. Voedingsraad: Nederlands Voedingsstoffenbestand (NEVO). 1993. The Hague, The Netherlands: Voorlichtingsbureau voor de Voeding (Dutch food composition table).
14. Klipstein-Grobusch K, den Breeijen JH, Goldbohm RA et al. Dietary assessment in the elderly: validation of a semiquantitative food frequency questionnaire. *Eur J Clin Nutr* 1998; **52**:588-596.
15. Willett WC, Stampfer MJ: Total energy intake: implications for epidemiologic analysis. Am J *Epidemiology* 1986; **124**:17-27.
16. Beaton GH, Milner J, Corey P et al. Source of variance in 24-hour dietary recall data: implications for nutrition study design and interpretation. *Am J Clin Nutr* 1979; **32**:2546-2559.
17. Gale CR, Martyn CN, Winter P et al. Vitamin C and risk of death from stroke and coronary heart disease in a cohort of elderly people. *BMJ* 1995; **310**:1563-1566.
18. Nyyssönen K, Parvianinen MT, Salonen R et al. Vitamin C deficiency and risk of myocardial infarction: prospective population study of men from eastern Finland. *BMJ* 1997; **314**:634-638.

19. Retsky KL, Frei B. Vitamin C prevents metal ion-dependent initiation and propagation of lipid peroxidation in human low-density lipoprotein. *Biochim Biophys* 1995; **1257**:279-287.
20. Stephens NG, Parsons A, Schofield PM et al. Randomised controlled trial of vitamin E in patients with coronary disease: Cambridge Heart Antioxidant Study (CHAOS). *Lancet* 1996; **347**:781-86.
21. Krinksy NI Antioxidant functions of carotenoids. *Free Rad Biol Med* 1989; **7**:617-635.
22. Jilial I, Norkus EP, Cristol L. ß-carotene inhibits the oxidative modification of low-density lipoprotein. *Biochim Biophys Acta* 1991; **1086**:134-138.
23. Street DA, Comstock GW, Salkeld RM et al. Serum antioxidants and myocardial infarction. Are low levels of carotenoids and α-tocopherol risk factors for myocardial infarction? *Circulation* 1994; **90**:1154-1161.
24. Kardinaal AF, Kok FJ, Ringstad J et al. Antioxidants in adipose tissue and risk of myocardial infarction. *Lancet* 1993; **342**:1379-1384.
25. Morris DL, Kritchevsky SB, Davis CE. Serum carotenoids and coronary heart disease. The Lipid Research Clinics Coronary Primary Prevention Trial and Follow-up Study. *JAMA* 1994; **272**:1439-1441.
26. Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study Group. The effect of vitamin E and beta-carotene on the incidence of lung cancer and other cancers in male smokers. *N Engl J Med* 1994; **330**:1029-1035.
27. Hennekens CH, Buring JE, Manson JE et al. Lack of effect of long-term supplementation with beta-carotene on the incidence of malignant neoplasms and cardiovascular disease. *N Engl J Med* 1996; **334**:1145-1149.
28. Omenn, GS, Goodman GE, Thornquist MD et al. Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascuar disease. *N Engl J Med* 1996; **334**:1150-1155.
29. Greenberg ER, Baron JA, Karagas MR et al. Mortality associated with low plasma concentration of beta-carotene and the effect of oral supplementation. *JAMA* 1996; **275**:699-703.
30. Rapola JM, Virtamo J, Ripatti S et al. Randomised trial of alpha-tocopherol and ß-carotene supplements on incidence of major coronary events in men with previous myocardial infarction. *Lancet* 1997; **349**:1715-1720.
31. Rautalahti M, Albanes D, Virtamo J et al. Beta-carotene did not work: aftermath of the ABTC study. *Cancer Letters* 1997; **114**:235-236.
32 .Mayne ST. Beta-carotene, carotenoids, and disease prevention in humans. *FASEB J* 1996; **10**: 690-701.
33. Micozzi MS, Brown ED, Edwards BK et al. Plasma carotenoid response to chronic intake of selected foods and ß-carotene supplements in men. *Am J Clin Nutr* 1992; **55**:1120-1125.
34. Goodman GE, Thornquist M, Kestin M et al. The association between participant characteristics and serum concentrations of ß-carotene, retinol, retinyl palmitate, and alpha-tocopherol among participants in the Carotene and Retinol Efficacy Trial (CARET) for prevention of lung cancer. *Cancer Epidemiol., Biomarkers & Prev* 1996; **5**:815-821.

CHAPTER 5

General Discussion

PRO- AND ANTIOXIDANTS IN ATHEROSCLEROSIS AND CORONARY HEART DISEASE

Free iron - a catalyst of the production of free radicals - has been implicated in ischemic myocardial damage and lipid peroxidation. Plausible hypotheses as to how free iron may accelerate the progression of atherosclerosis or contribute to myocardial injury after an ischemic event have been generated from basic research. Direct evidence that high iron stores or high iron intake increase the incidence of coronary heart disease in humans, however, is limited. So far the strongest supporting evidence for iron as a coronary heart disease risk factor stems from a cohort study of eastern Finnish men, where high levels of serum ferritin and dietary iron were positively associated with incidence of myocardial infarction[1]. Furthermore, serum ferritin was observed to be one of the strongest indicators of presence and progression of carotid artery disease[2,3] and blood donation, depleting iron stores in blood donors, was associated with reduced risk of myocardial infarction[4] and cardiovascular disease[5]. However, subsequent studies investigating whether iron status or dietary iron intake are associated with increased risk of myocardial infarction or coronary heart disease have not provided consistent results[6-15]. The results presented in the current thesis suggest that elevated serum ferritin (Chapter 3.2) and high dietary heme iron intake (Chapter 3.3) are associated with increased risk of myocardial infarction. Most studies did not discriminate between non-fatal and fatal myocardial infarction, although iron may be directly involved in the myocardial injury caused by ischemia and reperfusion. Indirect evidence that iron is involved in reperfusion injury after an ischemic event has been provided by results of animal experiments showing that free radicals are generated after restoration of blood flow to ischemic myocardium[16], contributing to the subsequent myocardial injury[17]. Experiments with an iron chelator[18,19] or iron-overloading[20] in animals emphasize this notion. It has subsequently been suggested that elevated iron stores may have an adverse effect on the survival after a myocardial infarction[21]. Our observation of a considerably more pronounced association between heme iron intake and fatal myocardial infarction may be supportive of this hypothesis, even though the number of fatal cases in the current study was small (Chapter 3.3).

Besides iron, copper and ceruloplasmin have been suggested to be independent risk factors for coronary heart disease[8,22-26]. Recent experimental studies demonstrating the ability of ceruloplasmin to oxidatively modify low-density lipoprotein[27,28] seem to underline this idea. However, the question has been raised whether elevated

ceruloplasmin is not merely an indicator of inflammation given its acute-phase protein property. Our results suggest that a substantial part of the increased risk of myocardial infarction associated with elevated levels of serum ceruloplasmin may be attributed to inflammation processes (Chapter 3.1), supporting the conception that serum ceruloplasmin may reflect low-level inflammation involved in the coronary heart disease process. The remaining elevated risk may be due to other properties of ceruloplasmin, like its pro-oxidant activity. Since serum ferritin is regarded as an indicator of chronic inflammation, we aimed to circumvent the problem of misclassification of iron status with respect to disease by a priori exclusion of subjects with high levels of C-reactive protein, respectively elevated erythrocyte sedimentation rate from analysis (Chapter 3.2). This provision, however, may not have ruled out the possibility that our results on serum ferritin and myocardial infarction may at least to a certain extent reflect low-level inflammation involved in the coronary heart disease process.

Observational studies have indicated that dietary antioxidants may exert a protective effect on incidence and mortality of cardiovascular disease[29-37] and suggest that antioxidative vitamins may be able to reduce atherosclerosis[38-40]. The most consistent, reliable, published beneficial effects of antioxidants on fatal and nonfatal cardiovascular diseases in middle-aged populations have been observed with vitamin E[29-32,35]. Beta-carotene and vitamin C intake have been less clearly associated with a reduced risk[41,42]. Recent findings from supplement intervention trials only partly support these observations. Vitamin E supplementation in subjects with previous cardiovascular disease was reported to be associated with less coronary artery lesion progression[43] and reduced risk of cardiovascular death and non-fatal myocardial infarction[41], but also with non-significantly increased risk of CHD[44] and CVD deaths[43]. For vitamin C no effect of supplementation on reduction of progression in coronary artery lesion[40] was seen and for ß-carotene either no effect on the incidence of cardiovascular disease[44] or a slight increase in cardiovascular mortality[45-47] was reported. Further results from ongoing intervention trials testing antioxidants[48-50] in cardiovascular disease prevention may be able to help clarifying associations between antioxidants and cardiovascular disease. The results presented in Chapter 4.3 of this thesis are supportive of a beneficial effect of dietary ß-carotene intake on risk of myocardial infarction in an elderly population. For dietary vitamin C and vitamin E,

however, no beneficial effect on risk of myocardial infarction was observed. Using a nested case-control design, we subsequently investigated whether serum carotenoids were associated with aortic atherosclerosis assessed by presence of calcified plaques in the abdominal aorta. Our results suggest that among the major serum carotenoids, lycopene may play a protective role in the development of atherosclerosis. For concentrations of serum α-carotene, β-carotene, β-cryptoxanthin, lutein, or zeaxanthin no association with presence of aortic atherosclerosis was observed (Chapter 4.1). Notwithstanding the objections that different mechanisms may operate on the development of aortic atherosclerosis and myocardial infarction, one might have expected to observe serum ß-carotene to be inversely associated with aortic atherosclerosis. A possible explanation for the absence of such a relationship might be that the effect observed for dietary ß-carotene on myocardial infarction risk may be attributed rather to dietary lycopene or to some other substance in foods containing this carotenoid or to dietary patterns and lifestyle behaviour closely linked to a diet rich in vegetables and fruit associated with reduced risk of coronary heart disease.

A common condition among the elderly is peripheral artery disease. Cross-sectional analysis allowed us to investigate whether elevated dietary antioxidant intake was associated with a lower prevalence of peripheral artery disease (Chapter 4.2). We observed sex-specific risks: dietary ß-carotene and vitamin E were inversely associated with peripheral artery disease in men, whereas dietary vitamin C was inversely associated with peripheral artery disease in women. The association of vitamin C with peripheral artery disease in women remained after multivariate adjustment. Possibly the difference between men and women is partly explained by differences in smoking history. Stratification by smoking status indicated that current or former smokers benefit most of elevated dietary antioxidant intake.

METHODOLOGICAL CONSIDERATIONS

Exposure measurement

Dietary assessment

A serious limitation of studies addressing diet are inaccuracies in measurement of intake. Use of food composition tables will invariably introduce further measurement error. This is due to the assumption that the nutrient content of a specific food is approximately constant, non-random sampling of foods for the chemical analysis, errors in the chemical analysis themselves, incorrect use of values from other food composition tables, and missing data[51]. For iron and the antioxidant (pro)vitamins, the errors are probably larger than for macronutrients, because the variation in the vitamin and mineral content of foods is generally much greater than the variation in macronutrient content of foods[51], and also because many values, and especially those for vitamins in food composition tables are determined with less reliable analytical techniques than those currently available[52].

Results of studies on diet and disease are often difficult to interpret unless the method used to measure diet has been validated in a population reasonably similar to that being investigated. Without documentation of validity, null associations could simply be due to lack of variation in dietary exposure in the study population or the inability of the dietary assessment method to detect existing differences in diet. Dietary assessment in the cohort of the Rotterdam Study was conducted by a semiquantitative food frequency questionnaire (SFFQ) adapted to the elderly. Adaptation of the SFFQ aimed at completion of the dietary assessment within a limited time-frame and at ease in administration. This was achieved by use of a two-step approach consisting of a simple self-administered foodlist-based questionnaire that was completed at home (completion time: 20 minutes) and a subsequent structured interview with a dietitian (time allocated: 20 minutes) based on the contents of the completed questionnaire (Chapter 2.2). Validity of the modified SFFQ was assessed in a subsample of 80 men and women (55 - 75 years) of the Rotterdam Study. Multiple food records (FR) collected over a one year period served as reference method. 24-h urine urea was used as indirect marker for protein intake. For evaluation of presence of gross underreporting of energy we applied the Goldberg cut-off[53]. Measures of concordance used to assess the ability of the SFFQ to adequately rank nutrients encompassed the correlation coefficient, cross-classification by quintile category, the difference between means, and the ratio of

SFFQ to FR (Chapter 2.1). Our results indicated relatively good validity and were similar to results in other elderly[54-62] and middle-aged[63-76] populations.

Dietary assessment is often assumed to reflect long-term intake. The time period addressed by the SFFQ applied in the elderly cohort of the Rotterdam Study was one year as for most other (S)FFQs. For the original version of the SFFQ Goldbohm et al.[77] determined the 5-year reproducibility, indicating the potential of a single SFFQ measurement to rank subjects according to nutrient intake. Since elderly people tend to have fairly definite likes and dislikes, it is likely that the diet of an older person is more uniform compared to a younger person. Thus, use of a single dietary assessment as a proxy for long-term dietary intake seems to be justified. However, if with prolonged follow-up dietary exposure tends to change over time and if recent exposure is relevant to risk of disease, misclassification of dietary exposure increases over time. Repeated assessment of diet during the follow-up period would allow for a more accurate measure of diet during different time periods.

Conscious or unconscious underreporting can have a profound effect on the quality of the dietary data. Results of various studies employing different methodologies and investigating different populations demonstrated that underestimation of energy intake seem to be a common problem of questionnaire or recording based measurement instruments[78-80]. Overweight, female sex, age, attained education are among the variables discussed to independently predict underreporting. For the elderly cohort of the Rotterdam Study we observed a significant association between age and the ratio of energy intake to estimated basal metabolic rate (EI/BMR) suggesting underreporting to be present more often in younger subjects. EI/BMR was furthermore observed to be significantly inversely associated with body mass index independent of age (Chapter 2.2). Some studies reported that some food items are particularly susceptible to be underreported[81-83]. If certain items are related to disease, reporting bias may lead to differential misclassification of dietary intake and generate misleading associations between dietary exposure and the disease under study[84]. To compare nutrient intakes of subjects independent of variations in energy intake, to control for confounding, and to reduce extraneous variation[84,85] we performed analysis on energy-adjusted nutrients. Energy-adjusted nutrients were obtained by regressing individual nutrient intakes on the respective total energy intake[86].

Biochemical indicators of diet

The studies on dietary intake of pro- and antioxidants of this thesis were complemented by studies applying biochemical indicators of diet. Requirement for a valid biochemical indicator is not only sensitivity to intake, but it should also reflect the cumulative effect of diet over an extended period of time[87]. Biochemical indicators can be used as a surrogate for actual dietary intake in studies of disease occurence, as advocated in Chapter 4.1 where we investigated the association between serum carotenoids and aortic atherosclerosis. A related use of biochemical indicators is to predict disease risk, irrespective of whether the level of the biochemical measure is determined by dietary intake or other factors which is the case when tissue levels are highly regulated and poorly reflect dietary intake. Our studies on serum ferritin (Chapter 3.2) and ceruloplasmin (Chapter 3.1) are good examples for this type of studies. Serum studies are considered to be potentially more objective since they are not subject to the errors in measuring diet, e.g. under- or overreporting of foods or nutrients by participants. Still, one has to be aware that in general, biochemical measures are almost always subject to the same problems of misclassification and bias as measures of diet[87]. The power of a single measurement to predict long-term average levels or to estimate long-term dietary intake is low, if the within-person variation of the biochemical indicator of interest is large. Further issues which need to be taken into consideration are measurement variation (between-person variation), sampling of fasting or nonfasting blood, or whether the biochemical measures are affected by storage time or temperature. As for dietary measures repeated measurements will reduce non-differential error, i.e. will account for individual changes and secular trends in nutrient intake. Agreement in effect estimates between independent measures, e.g diet and biochemical indicators of diet with respect to risk allow greater confidence in the validity of both sets of results as it is the case with our results on dietary heme iron (Chapter 3.3) and serum ferritin (Chapter 3.1) with respect to risk of myocardial infarction.

Outcome measurement

Asymptomatic atherosclerosis and myocardial infarction were the outcome measurements applied in the studies of this thesis. In our studies on dietary antioxidants we have been able to investigate dietary intake, respectively serum levels with regard to both, atherosclerosis and myocardial infarction. Our results concerning a

modest inverse association of serum lycopene concentrations with calcified plaques of the aorta (asymptomatic atherosclerosis) and a significantly inverse association of dietary ß-carotene with myocardial infarction are supportive of the hypothesis that dietary antioxidants, i.e. carotenoids are beneficial in both the prevention of atherosclerosis and coronary heart disease. Myocardial infarction was the only endpoint for our studies on measures of iron status. Our results of an increased myocardial infarction risk with elevated dietary heme iron and serum ferritin concentrations are supportive of the iron hypothesis. Although we have not been able to present results on measures of iron status and atherosclerosis, recent results observing serum ferritin to be a risk factor for presence and progression of carotid artery disease[2,3] suggest iron status to be considered a risk factor in atherosclerosis and coronary heart disease.

Fatal and nonfatal outcomes are often pooled in epidemiological studies. In our follow-up study on dietary iron intake and myocardial infarction we were able to demonstrate that risk of myocardial infarction with dietary heme iron intake was considerably more pronounced in fatal than non-fatal cases. Although the sample size for this sub-analysis was small, the differing results between fatal and non-fatal cases of myocardial infarction may suggest that high iron stores may affect the fatality rate of myocardial infarction apart from an effect on the incidence of myocardial infarction[21]. Numbers in our study on serum ferritin and myocardial infarction were, however, too small to differentiate between fatal and nonfatal myocardial infarction. In future studies on iron status and coronary heart disease, respectively cardiovascular disease, it may be worthwhile to discriminate between fatal and non-fatal events.

Effect modification

Investigation of interaction may point at the existence of high risk groups for whom modification of risk factors may be particularly beneficial. Biological interaction is considered present when the joint effect of two risk factors is more, or less, than the sum or the multiple of the effects of each individual risk factor. Biological interaction indicates that two risk factors are not acting independently, but have a synergistic or antagonistic effect on the outcome. The effects of pro- and antioxidants on cardiovascular disease described in the studies of this thesis were studied in older people. A potential modifying effect of age may affect generalizability of the results of this thesis to the general population. In all studies of this thesis we observed

susceptibility to smoking status. High dietary intake of antioxidants or elevated levels of serum carotenoids were observed to be most beneficial in current or ever smokers. For the same group of subjects, high dietary heme iron intake or elevated serum ferritin levels were observed to have a harmful effect. Furthermore, in the case of dietary heme iron and serum ferritin, stratification by hypertension, hypercholesterolemia, or diabetes modified the association considerably suggesting that dietary heme iron or serum ferritin may adversely affect coronary heart disease risk only in the presence of other risk factors.

Future Research

Generally, investigation of dietary determinants of cardiovascular disease has mainly focused on single nutrients such as antioxidants. Conflicting evidence regarding the potential of single nutrients in the prevention of atherosclerosis and coronary events raises the question whether research should only be focused on nutrients. Certainly, an optimal approach to epidemiologic analyses will employ both, research on nutrients and foods, strenghtening the case for causality when an association is observed with overall intake of a nutrient and also with more than one food source of that nutrient. Conducting randomized trials on foods, such as fruit and vegetables, in addition to the supplement intervention trials currently under way may help to clarify the relation of nutrients and foods to cardiovascular disease. With respect to fruit and vegetables, until then, from a public health point of view, recommendations for a diet rich in fruit and vegetables should also be extended to the elderly population.

With respect to iron stores (e.g. serum ferritin levels) or dietary iron intake, further research should be directed towards investigation of possible harmful effects of iron on coronary heart disease in the presence of known modifiable risk factors such as cigarette smoking or hypercholesterolemia and on the effect of elevated iron stores on case fatality. Clinical trials of interventions to lower body iron stores such as blood donation for prevention of coronary events have already been suggested to clarify the role of iron in coronary heart disease[5]. Genetic susceptibility to hemochromatosis is a related issue and recent work suggests heterozygosity for the HFE Cys282Tyr polymorphism[88] to be an independent risk factor for cardiovascular mortality (D.E. Grobbee, personal communication), underlining that research on genetic susceptibility may further extend our knowledge on effects of nutrients on health and disease.

REFERENCES

1. Salonen JT, Nyyssönen K, Korpela H et al. High stored iron levels are associated with excess risk of myocardial infarction in eastern Finnish men. *Circulation* 1992; **86**:803-811.
2. Kiechl S, Aichner F, Gerstenbrand F et al. Body iron stores and presence of carotid atherosclerosis: Results from the Bruneck Study. *Arterioscler Thromb* 1994; **14**:1625-1630.
3. Kiechl S, Willeit J, Egger G et al. Body iron stores and the risk of carotid atherosclerosis. Prospective results from the Bruneck Study. *Circulation* 1997; **96**:3300-3307.
4. Tuomainen TP, Salonen R, Nyyssönen K et al. Cohort study of the relation between donating blood and risk of myocardial infarction in 2682 men in Eastern Finland. *BMJ* 1997; **314**:161-168.
5. Meyers DG, Strickland D, Maloley PA et al. Possible association of a reduction in cardiovascular events with blood donation. *Heart* 1997; **78**:188-193.
6. Stampfer MJ, Grodstein F, Rosenberg I et al. A prospective study of plasma ferritin and risk of myocardial infarction in US physicians. [Abstract]. *Circulation* 1993; **87**:688.
7. Magnusson MK, Sigfusson N, Sigvaldason H et al. Low iron-binding capacity as a risk factor for myocardial infarction. *Circulation* 1994; **89**:102-108.
8. Mänttäri M, Manninen V, Huttunen JK et al. Serum ferritin and ceruloplasmin as coronary risk factors. *Eur Heart J* 1994; **15**:1599-1603.
9. Sempos TC, Looker AC, Gillum RF et al. Body iron stores and the risk of coronary heart disease. *N Engl J Med* 1994; **330**:1119-1124.
10. Liao Y, Cooper RS, McGee DL. Iron status and coronary heart disease: negative findings from the NHANES I Epidemiologic Follow-Up Study. *Am J Epidemiol* 1994; **139**:704-712.
11. Morrison HI, Semenicw RM, Mao Y et al. Serum iron and risk of fatal acute myocardial infarction. *Epidemiology* 1994; **5**:243-246.
12. Baer DM, Tekawa IS, Hurley LB. Iron stores are not associated with acute myocardial infarction. *Circulation* 1994; **89**:2915-2918.
13. Reunanen A, Takkunen H, Knekt P et al. Body iron stores, dietary iron intake and coronary heart disease mortality. *J Intern Med* 1995; **238**:223-230.
14. Corti M-C, Guralnik JM, Salive ME et al. Serum iron level, coronary artery disease, and all-cause mortality in older men and women. *Am J Cardiol* 1997; **79**:120-127.
15. Tzonou A, Lagiou P, Trichopoulou A et al. Dietary iron and coronary heart disease risk: a study from Greece. *Am J Epidemiol* 1998; **147**:161-166.
16. Bolli R, Patel BS, Jeroudi MO et al. Demonstration of free radical generation in 'stunned' myocardium of intact dogs with the use of the spin trap α-phenyl-N-tert-butyl-nitrone. *J Clin Invest* 1988; **82**:476-485.
17. McCord JM. Oxygen-derived free radicals in post-ischemic tissue injury. *N Engl J Med* 1985; **312**:159-163.
18. Williams RE, Zweier JL, Flaherty JT. Treatment with desferoxamine during ischemia improves functional and metabolic recovery and reduces reperfusion-induced oxygen radical generation in rabbit hearts. *Circulation* 1991; **83**:1006-1011.
19. van der Kraaij AMM, van Eijk HG, Koster JF. Prevention of post-ischemic cardiac injury by the

orally active iron chelator 1,2-dimethyl-3-hydroxy-4-pyridone (LI) and the antioxidant (+)-cyanindol-3. *Circulation* 1989; **80**:158-164.

20. van der Kraaij AMM, Mostert LJ, van Eijk HG et al. Iron-load increases the susceptibility of rat hearts to oxygen reperfusion damage. *Circulation* 1988; **78**:442-449.
21. Koster JF, Sluiter W. Is increased tissue ferritin a risk factor for atherosclerosis and ischemic heart disease? *Br Heart J* 1995;**73**:208.
22. Fox PL, Mukhopadhyay C, Ehrenwald E. Structure, oxidant activity, and cardiovascular mechanisms of human ceruloplasmin. *Life Sciences* 1995; **56**:1749-1758.
23. Kok FJ, van Duijn CM, Hofman A et al. Serum copper and zinc and the risk of death from cancer and cardiovascular disease. *Am J Epidemiol* 1988; **128**:352-359.
24. Salonen JT, Salonen R, Korpela H et al. Serum copper and the risk of acute myocardial infarction: a prospective study in men in eastern Finland. *Am J Epidemiol* 1991; **134**:268-76.
25. Reunanen A, Knekt P, Aaran R-K. Serum ceruloplasmin level and the risk of myocardial infarction and stroke. *Am J Epidemiol* 1992; **136**, 1082-1090.
26. Reunanen A, Knekt P, Marniemi J et al. Serum calcium, magnesium, copper, and zinc and risk of cardiovascular death. *Eur J Clin Nutr* 1996; **50**:431-437.
27. Ehrenwald E, Chisolm GM, Fox PL. Intact human ceruloplasmin oxidatively modifies low density lipoprotein. *J Clin Invest 1994;* **93**:1493-1501.
28. Lamb DJ, Leake DS. Acidic pH enables caeruloplasmin to catalyse the modification of low-density lipoprotein. *FEBS Letters* 1994; **338**:122-126.
29. Riemersma RA, Wood DA, Macinytre CCA et al. Risk of angina pectoris and plasma concentrations of vitamins A, C, and E, and carotene. *Lancet* 1991; **337**:1-5.
30. Stampfer MJ, Hennekens CH, Manson JE et al. Vitamin E consumption and the risk of coronary disease in women. *N Engl J Med* 1993; **328**:1444-1449.
31. Rimm EB, Stampfer MJ, Ascherio A, et al.Vitamin E consumption and the risk of coronary heart disease in men. *N Engl J Med* 1993; **328**:1450-1456.
32. Knekt P, Reunanen A, Järvinen R et al. Antioxidant vitamin intake and coronary mortality in a longitudinal population study. *Am J Epidemiol* 1994; **139**:1180-1189.
33. Gaziano JM, Manson JE, Branch LG et al. A prospective study of consumption of carotenoids in fruits and vegetables and decreased cardiovascular mortality in the elderly. *Ann Epidemiol* 1995; **5**:255-260.
35. Kushi LH, Folsom AR, Prineas RJ et al. Dietary antioxidant vitamins and death from coronary heart disease in postmenopausal women. *N Engl J Med* 1996; **334**:1156-1162.
36. Losonczy KG, Harris TB, Havlik RJ. Vitamin E and vitamin C supplement use and risk of all-cause and coronary heart disease mortality in older persons: the Established Populations for Epidemiologic Studies of the Elderly. *Am J Clin Nutr* 1996; **64**:190-196.
37. Sahyoun NR, Jaques PF, Russell RM. Carotenoids, vitamins C and E, and mortality in an elderly population. *Am J Epidemiol* 1996; **144**:501-511.
38. Kritchevsky SB, Shimakawa T, Tell GS et al. Dietary antioxidants and carotid artery wall thickness. *Circulation* 1995; **92**:2142-2150.

39. Bonithon-Kopp C, Coudray C, Berr C et al. Combined effects of lipid peroxidation on carotid atherosclerosis in a population aged 59-71 y: The EVA Study. *Am J Clin Nutr* 1997; **65**:121-127.
40. Hodis HN, Mack WJ, LaBree L et al. Serial coronary angiographic evidence that antioxidant vitamin intake reduces progression of coronary artery atherosclerosis. *JAMA* 1995; **273**:1849-1854.
41. Stephens NG, Parsons A, Schofield PM et al. Randomised controlled trial of vitamin E in patients with coronary disease: Cambridge Heart Antioxidant Study (CHAOS). *Lancet* 1996; **347**:781-786.
42. Jha P, Flather M, Lonn E, Farkouth M et al. The antioxidant vitamins and cardiovascular disease. A critical review of epidemiologic and clinical trial data. *Ann Intern Med* 1995; **123**:860-872.
43. Buring JH, Hennekens CH. Antioxidants and coronary heart disease: weighing the totality of the evidence. *J Cardiovasc Risk* 1996; **3**:343-345.
44. Rapola JM, Virtamo J, Ripatti S et al. Randomised trial of alpha-tocopherol and ß-carotene supplements on incidence of major coronary events in men with previous myocardial infarction. *Lancet* 1997; **349**:1715-1720.
45. Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study Group. The effect of vitamin E and beta-carotene on the incidence of lung cancer and other cancers in male smokers. *N Engl J Med* 1994; **330**:1029-1035.
46. Omenn, GS, Goodman GE, Thornquist MD et al. Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. *N Engl J Med* 1996; **334**:1150-1155.
47. Greenberg ER, Baron JA, Karagas MR et al. Mortality associated with low plasma concentration of beta-carotene and the effect of oral supplementation. *JAMA* 1996; **275**:699-703.
48. Buring JE, Hennekens CH. Randomized trials of primary prevention of cardiovascular disease in women. An investigator's view. *Ann Epidemiol* 1994; **4**:111-114.
49. Manson JE, Gaziano JM, Spelsberg S et al. A secondary prevention trial of antioxidant vitamins and cardiovascular disease in women. *Ann Epidemiol* 1995, **5**:261-269.
50. Hercberg S, Galan P, Preziosi P et al. Background and rationale behind the SU.VI.MAX Study, a prevention trial using nutritional doses of a combination of antioxidant vitamins and minerals to reduce cardiovascular diseases and cancer. *Intern J Vit Nutr Res* 1998; **68**:3-20.
51. West CE, van Staveren WA. Food consumption, nutrient intake, and the use of food composition tables. In: Margetts BM, Nelson M: Design concepts in nutritional epidemiology. Oxford: Oxford University Press 1991.
52. Mangels AR, Holden JM, Beecher GR et al. Carotenoid content of fruits and vegetables: an evaluation of analytic data. *J Am Diet Assoc* 1993; **93**: 284-296.
53. Goldberg GR, Black AE, Jebb SA et al. Critical evaluation of energy intake data using fundamental principles of energy physiology: 1. Derivation of cut-off limits to identify under-recording. *Eur J Clin Nutr 1991;* **45**:569-581.
54. Horwarth CC. Validity of a short frequency questionnaire for estimating nutrient intake in elderly people. *Br J Nutr* 1993; **70**:3-14.
55. Nes M, Frost Andersen L, Solvoll K et al. Accuracy of a quantitative food frequency questionnaire applied in elderly Norwegian women. *Eur J Clin Nutr* 1992; **46**:809-821.

56. Rothenberg E. Validation of the food frequency questionnaire with the 4-day record method and analysis of 24-h urinary nitrogen. *Eur J Clin Nutr* 1994; **48**:725-735.
57. Mares-Perlman JA, Klein BEK, Klein R et al. A diet history questionnaire ranks nutrient intakes in middle-aged and older men and women similarly to multiple food records. *J Nutr* 1993; **123**:489-501.
58. Tjoenneland A, Overvad K, Haraldsdóttir J et al. Validation of a semiquantitative food frequency questionnaire developed in Denmark. *Int J Epidemiol* 1991;**20**, 906-912.
59. van Staveren WA, Burema J, Livingstone MBE et al. Evaluation of the dietary history method used in the SENECA study. *Eur J Clin Nutr* 1996; **50**:S47-S55.
60. Grootenhuis PA, Westenbrink S, Sie CMTL et al. A semiquantitative food frequency questionnaire for use in epidemiologic research among the elderly: Validation by comparison with dietary history. *J Clin Epidemiol* 1995; **48**: 859-868.
61. Mahalko JR, Johnson LAK, Gallagher SK et al. Comparison of dietary histories and seven-day food records in a nutritional assessment of older adults. *Am J Clin Nutr* 1985; **42**:542-533.
62. Munger RG, Folsom AR, Kushi LH et al. Dietary assessment of older Iowa women with a food frequency questionnaire: nutrient intake, reproducibility, and comparison with 24-hour dietary recall interviews. *Am J Epidemiol* 1992; **136**:192-200.
63. Goldbohm RA, van den Brandt PA, Brants HAM et al. Validation of a dietary questionnaire used in a large-scale prospective cohort study on diet and cancer. *Eur J Clin Nutr* 1994; **48**:253-265.
64. Jain M, Howe GR, Rohan T. Dietary assessment in epidemiology: comparison of food frequency and a dietary history questionnaire with a 7-day food record. *Am J Epidemiol* 1996; **143**:953-960.
65. Block G, Hartman AM, Naughton D. A reduced dietary questionnaire: development and validation. *Epidemiology* 1990;**1**:58-64.
66. Rimm EB, Giovannucci EL, Stampfer MJ et al. Reproducibility and validity of an expanded self-administered semiquantitative food frequency questionnaire among male health professionals. *Am J Epidemiol* 1992;**135**:1114-1126.
67. Willett WC, Sampson L, Stampfer MJ et al. Reproducibility and validity of a semiquantitative food frequency questionnaire. *Am J Epidemiol* 1985;**122**:51-65.
68. Pietinen P, Hartman AM, Haapa E et al. Reproducibility and validity of dietary assessment instruments. I. A self-administered food use questionnaire with a portion size picture booklet. *Am J Epidemiol* 1988;**128**: 655-666.
69. Pietinen P, Hartman AM, Haapa E et al. Reproducibility and validity of dietary assessment instruments. II. A qualitative food frequency questionnaire. *Am J Epidemiol* 1988; **128**:667-676.
70. Engle A, Lynn LL, Koury K et al. Reproducibility and comparability of a computerized, self-administered food frequency questionnaire. *Nutr Cancer* 1990; **13**:281-292.
71. Gnardellis C, Trichopoulou A, Katsouyanni K et al. Reproducibility and validity of an extensive semiquantitative food frequency questionnaire among Greek school teachers. *Epidemiology 1995;* **6**:74-77.
72. Bingham SA, Gill C, Welch A et al. Validation of dietary assessment methods in the UK arm of EPIC using weighed records, and 24-h urinary nitrogen and potassium and serum vitamin C and

carotenoids as biomarkers. *Int J Epidemiol* 1997; **26**:S137-S151.

73. Ocké MC, Bueno-de-Mesquita B, Pols MA et al. The Dutch EPIC food frequency questionnaire II. Relative validity and reproducibility for nutrients. *Int J Epidemiol* 1997; **26**:S49-S58.
74. Bohlscheid-Thomas S, Hoting I, Boeing H et al. Reproducibility and relative validity of energy and macronutrient intake of a food frequency questionnaire developed for the German part of the EPIC Project. *Int J Epidemiol* 1997; **26**:S71-S81.
75. Riboli E, Elmstahl S, Saracci R et al. The Malmö Food Study: Validity of two dietary assessment methods for measuring food intake. *Int J Epidemiol* 1997; **26**:S161-173.
76. van Liere M, Lucas F, Clavel F et al. Relative validity and reproducibility of a French dietary history questionnaire. *Int J Epidemiol* 1997; **26**:S128-S136.
77. Goldbohm RA, van't Veer P, van den Brandt PA et al. Reproducibility of a food frequency questionnaire and stability of dietary habits determined from five annually repeated measurements. *Eur J Clin Nutr* 1995; **49**:420-429.
78. Flegal KM, Larkin FA. Partitioning macronutrient intake estimates from a food frequency questionnaire. Am J *Epidemiol* 1990; **131**:1046-1058.
79. Lichtman SW, Pisarska K, Raynes Berman E et al. Discrepancy between self-reported and actual caloric intake and exercise in obese subjects. *N Engl J Med* 1992; **327**:1893-1898.
80. Lindroos A-K, Lissner L, Sjöström L. Validity and reproducibility of a self-administered dietary questionnaire in obese and non-obese subjects. *Eur J Clin Nutr* 1993;**47**:461-81.
81. Johnson RK, Goran MI, Poehlman ET. Correlates of over- and underreporting of energy intake in healthy older men and women. *Am J Clin Nutr* 1994; **59**:1286-1290.
82. Fricker J, Baelde D, Igoin-Apfelbaum L et al. Underreporting of food intake in obese 'small eaters'. *Appetite* 1992; **19**:273-283.
83. Bingham SA. The use of 24-h urine samples and energy expenditure to validate dietary assessments. *Am J Clin Nutr* 1994; **59**:227S-231S.
84. Prentice RL. Measurement error and results from analytic epidemiology - dietary fat and breast cancer. *J Nat Cancer Inst* 1996; **88**:1738-1747.
85. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. *Am J Clin Nutr* 1997; **65**:1220S-1228S.
86. Voss S, Kroke A, Klipstein-Grobusch K et al. Is macronutrient composition of dietary data affected by underreporting? Results from the EPIC- Potsdam study. *Eur J Clin Nutr* 1998; **52**:119-126.
87. Willett WC, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. *Am J Epidemiol* 1986; **124**:17-27.
88. Hunter D. Biochemical indicators of dietary intake. In: Willett W. Nutritional epidemiology. 2nd edition. 1998, Oxford University Press, New York.
89. Feder JN, Gnirke A, Thomas W et al. A novel MHC class I-like gene is mutated in patients with hereditary hemochromatosis. Nat Gen 1996; **13**:399-408.

CHAPTER 6

Summary/Samenvatting/Zusammenfassung

SUMMARY

Diet is an important aspect of lifestyle and may affect life expectancy, morbidity and mortality of older adults. This thesis focuses on the deleterious and beneficial effects of dietary pro- and antioxidant intake and its effects on the development of atherosclerosis and coronary heart disease in the elderly cohort of the Rotterdam study. The overall aim of the community based prospective Rotterdam Study is to investigate the incidence of and the risk factors for chronic and disabling diseases.

Following the introductory chapter we examined in chapter 2 of this thesis the dietary assessment method applied to the elderly population of the Rotterdam Study. Chapter 2.1 reports on the relative validity of nutrient intake estimated by an adapted semiquantitative food frequency questionnaire (SFFQ) for dietary assessment in the elderly compared to 15-d food records and protein intake estimated from protein excretion. In general, the ability of the SFFQ to adequately rank subjects according to their dietary intake was found to be comparable to results of previous validation studies in middle-aged and elderly populations. This supports the idea that age has little adverse effects on the validity of questionnaires if administered appropriately. We applied the SFFQ across a wide age range in an elderly population, and reported estimates of nutrient intake and factors possibly influencing intake (Chapter 2.2). The SFFQ facilitated collection of data on dietary habits in the elderly within a limited time frame, making the method an attractive instrument for dietary assessment in large-scale epidemiologic studies in the elderly.

Chapter 3 discusses the effects of prooxidants on coronary heart disease in the Rotterdam Study. Free iron has been implicated in lipid peroxidation and ischemic myocardial damage in the literature. In a nested case-control setting we investigated whether serum ceruloplasmin and iron parameters - ferritin, transferrin, serum iron, and dietary iron - were related to myocardial infarction. Serum ceruloplasmin was significantly associated with increased myocardial infarction risk. Taking markers of inflammation (C-reactive protein, white blood cell count) into account reduced the excess risk for myocardial infarction by 33% suggesting that part of the observed risk associated with high levels of ceruloplasmin can be attributed to its property as acute phase protein (Chapter 3.1). For serum ferritin levels above 200 µg/l we observed an odds ratio of 1.82 (95% confidence interval 0.88-3.74), whereas for serum iron,

transferrin, and total dietary iron no association with myocardial infarction was seen. Heme iron intake, however, seemed to be a coronary heart disease risk factor (Chapter 3.2). In a subsequent follow-up analysis dietary heme iron was associated with a two-fold increased risk of myocardial infarction (Chapter 3.3). Distinction between fatal and non-fatal cases showed risk of myocardial infarction to be more pronounced in fatal cases suggesting that high iron stores may affect the fatality rate of myocardial infarction.

Chapter 4 describes the effects of antioxidants on coronary heart disease. In the study described in Chapter 4.2 we examined cross-sectionally whether dietary antioxidants were associated with peripheral artery disease and found vitamin C to be inversely associated with peripheral artery disease in women, and β-carotene and vitamin E in men. Next, we investigated whether high circulating levels of the major serum carotenoids - α-carotene, β-carotene, β-cryptoxanthin, lutein, lycopene, and zeaxanthin - were associated with atherosclerosis assessed by the presence of calcified plaques in the aorta (Chapter 4.1). There was a modest inverse association between age- and sex-adjusted serum lycopene and aortic atherosclerosis, but no association was observed for concentrations of serum α-carotene, β-carotene, β-cryptoxanthin, lutein, and zeaxanthin. Finally, in the study described in Chapter 4.3 we investigated whether dietary antioxidants were related to risk of myocardial infarction. We observed dietary ß-carotene to be inversely associated with myocardial infarction. If β-carotene intake from food sources and supplements was combined, the inverse relation with risk of myocardial infarction was slightly more pronounced.

Smoking status - current or ever cigarette smoking - had a profound effect on the results of our studies on dietary and serum pro- and antioxidants and coronary heart disease. In current or ever smokers high heme iron intake or elevated serum ferritin levels had a hazardous effect on coronary heart disease risk, whereas high dietary intake of dietary antioxidants, mainly vitamin C or β-carotene, or high serum levels of lycopene showed a beneficial effect on coronary heart disease risk especially in smokers.

SAMENVATTING

Voeding maakt een belangrijk onderdeel uit van de leefstijl en is mogelijk van invloed op de levensverwachting en het voorkomen van ziekte een sterfte bij ouderen. Dit proefschrift richt zich op de schadelijke en gunstige effecten van de inneming van pro- en antioxidanten op de ontwikkeling van atherosclerose en coronaire hartziekten. Dit is onderzocht binnen het 'Erasmus Rotterdam Gezondheid en Ouderen' (ERGO) onderzoek. Het prospectieve ERGO-onderzoek (in het Engels: The Rotterdam Study) heeft als doel het ontstaan van chronische en invaliderende ziekten en de risicofactoren daarvoor binnen een algemene populatie van ouderen te bestuderen.

In hoofdstuk 2 van dit proefschrift hebben we de meting van de voedingsinname bestudeerd welke is toegepast binnen de oudere populatie van het ERGO-onderzoek. Hoofdstuk 2.1 gaat over de relatieve validiteit van de nutriëntinneming bepaald met een, voor ouderen aangepaste, semikwantitatieve voedselfrequentievragenlijst (SFFQ) ten opzichte van een 15-daagse opschrijfmethode en de uit de excretie geschatte eiwitinneming. De mate waarin de SFFQ in staat was om personen adequaat te rangschikken naar hun voedselinneming was vergelijkbaar met bevindingen uit eerdere validatie-onderzoeken in populaties van middelbare of oudere leeftijd. Dit ondersteunt het idee dat een vragenlijst ook op oudere leeftijd valide is wanneer deze op juiste wijze wordt afgenomen. De SFFQ is toegepast binnen een groep ouderen met een breed leeftijdspectrum. De geschatte inname van nutriënten in deze populatie en factoren welke deze beïnvloeden worden gerapporteerd in hoofdstuk 2.2. De SFFQ vereenvoudigt het meten van voedingsgewoonten bij ouderen in een korte tijdsperiode en vormt daarmee een aantrekkelijke voedingsmeetmethode voor grootschalig epidemiologisch ouderenonderzoek.

Hoofdstuk 3 beschrijft de rol van pro-oxidanten bij het ontstaan van coronaire hartziekten binnen het ERGO-onderzoek. Er zijn aanwijzingen in de literatuur dat vrije ijzerionen betrokken zijn bij de vetzuurperoxidatie en ischemische schade aan het hart. In een genest patiënt-controle-onderzoek zijn het serumceruloplasmine en parameters van de ijzerstatus - ferritine, transferrine en serumijzer - bestudeerd in relatie tot het optreden van een myocardinfarct. Ceruloplasmine in het serum was significant geassocieerd met een verhoogd risico op myocardinfarct. Na adjustering voor onstekingsparameters ('C-reactive protein' en aantal witte bloedcellen) nam het toegenomen risico op een myocardinfarct met 33% af, hetgeen suggereert dat de

waargenomen relatie deels verklaard kan worden door de rol van ceruloplasmine als acute-fase-eiwit (Hoofdstuk 3.1). Bij een serumgehalte van ferritine boven 200 mg/l werd een odds-ratio van 1.85 (95% betrouwbaarheidsinterval 0.88-3.74) met myocardinfarct waargenomen, terwijl voor serumijzer en transferrine geen associatie werd gevonden. Haemijzer lijkt een risicofactor voor coronaire hartziekten te zijn (Hoofdstuk 3.2). Vervolgens was in een follow-up onderzoek haemijzer in de voeding geassocieerd met een verdubbeld risico op een myocardinfarct. Dit verband bleek meer uitgesproken voor fatale dan voor niet-fatale infarcten (Hoofdstuk 3.3). Dit suggereert dat een grote ijzervoorraad in het lichaam de sterftekans na een myocardinfarct vergroot.

Hoofdstuk 4 beschrijft een onderzoek naar antioxidanten in relatie tot coronaire hartziekten. In een cross-sectioneel onderzoek, beschreven in hoofdstuk 4.2, werd de relatie tussen antioxidanten in de voeding en perifeer vaatlijden bestudeerd. Er werd een omgekeerd verband waargenomen tussen perifeer vaatlijden en inneming van vitamine C in vrouwen en β-caroteen en vitamine E in mannen. Daarna onderzochten we of hoge serumconcentraties van carotenoïden, α-caroteen, β-caroteen, β-cryptoxanthine, luteïne, lycopeen en zeaxanthine, geassocieerd waren met atherosclerose, gemeten door middel van detectie van gecalcificeerde plaques (Hoofdstuk 4.1). De aanwezigheid van atherosclerose in de aorta vertoonde een matig sterk, omgekeerd verband met het lycopeengehalte in het serum, maar niet met de serumconcentraties van α-caroteen, β-caroteen, β-cryptoxanthine, luteïne en zeaxanthine na adjustering voor leeftijd en geslacht. Tenslotte onderzochten we in hoofdstuk 4.3 of antioxidanten in de voeding een rol spelen bij het ontstaan van een myocardinfarct. De inneming van β-caroteen via de voeding verlaagde de kans op een myocardinfarct. Het beschermend effect werd iets versterkt wanneer b-caroteen uit voedingssupplementen in de analyse werd meegenomen.

Roken, zowel huidig roken als het ooit gerookt hebben van sigaretten, bleek onze bevindingen aangaande pro- en antioxidanten in relatie tot coronaire hartziekten sterk te beïnvloeden. In (ex-) rokers was het risico op coronaire hartziekten toegenomen bij een hoge inneming van haemijzer via de voeding en een verhoogd serum-ferritinegehalte, terwijl een hoge inneming van antioxidanten via de voeding met name β-caroteen en vitamine C en een hoog serumgehalte van lycopeen beschermde vooral in rokers tegen coronaire hartziekten.

ZUSAMMENFASSUNG

Ernährung ist ein wichtiger Teil des Lebensstils von dem man annimmt, daß er die Lebenserwartung, Morbidität und Mortalität älterer Menschen beeinflußt. Die vorliegende Dissertation beschäftigt sich mit nachteiligen und vorteilhaften Effekten der nutritiven Aufnahme von Pro- bzw. Antioxidantien, sowie deren Einfluß auf die Entstehung von Atherosklerose und koronarer Herzerkrankung in der Kohorte der Rotterdam-Studie. Ziel der Rotterdam-Studie ist die Untersuchung von Inzidenz und Risikofaktoren chronischer und degenerativer Erkrankungen bei älteren Personen.

Im Anschluß an das einleitende Kapitel beschäftigt sich Kapitel 2 dieser Dissertation mit der Ernährungserhebungsmethode, die bei der älteren Population der Rotterdam-Studie angewendet wurde. Kapitel 2.1 behandelt die relative Validität der Nährstoffaufnahme aus dem angepaßten semiquantitativen Ernährungshäufigkeits-fragebogen (SFFQ) im Vergleich zur Nährstoffaufnahme aus Ernährungsprotokollen (insgesamt 15 Tage), sowie zu der aus der Proteinausscheidung geschätzten Proteinaufnahme. Die Fähigkeit des SFFQ, Studienteilnehmer adäquat anhand ihrer Nährstoffaufnahme zu klassifizieren, war mit den Ergebnissen publizierter Validierungsstudien in Populationen mittleren und höheren Alters vergleichbar. Dies stützt die These, daß Alter lediglich einen geringen Effekt auf die Validität solcher Fragebögen hat, sofern diese in geeigneter Form angewendet werden. Kapitel 2.2 beschreibt die Anwendung des SFFQ über eine große Altersspanne in der älteren Population der Rotterdam-Studie, macht Angaben über die geschätzte Nährstoffaufnahme und untersucht Faktoren, die diese Nährstoffaufnahme beeinflussen. Der eingesetzte Ernährungsfragebogen erleichterte die Erhebung von Ernährungsdaten innerhalb einer vorgegebenen Zeitspanne bei älteren Personen, so daß er als adäquates Instrument für Ernährungserhebungen in groß angelegten epidemiologischen Studien betrachtet werden kann.

Kapitel 3 beschreibt die Untersuchungen zum Effekt von Prooxidantien auf koronare Herzerkrankungen in der Rotterdam-Studie. In der Literatur wurde frei verfügbares Eisen in Verbindung mit Lipidperoxidation und ischämischen Myokardschädigungen gebracht. In einer in eine prospektive Kohortenstudie eingebetteten Fall-Kontrollstudie untersuchten wir, inwiefern Coeruloplasmin und verschiedene Eisenparameter (Ferritin, Transferrin, Serumeisen und Eisenaufnahme) mit dem Risiko eines

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ABOUT THE AUTHOR

Kerstin Klipstein was born on January 4, 1967 in Sindelfingen, Germany. She graduated in 1986 from Secondary School ('Abitur') and did a Voluntary Social Year in a clinic for the addicted and a children's home.

In 1987 Kerstin commenced working towards a Diploma degree in Nutritional Sciences at Bonn University. In addition to her theoretical studies she spent practical periods in a variety of research institutions, amongst them stays at the Nutrition and Metabolism Research Group (Prof. Clandinin) at the University of Alberta in Edmonton (Canada), and the Western Human Nutrition Research Center, USDA (Dr. Keim) at San Francisco (USA). She graduated in 1993 with a thesis focusing on the prevalence of under- and malnutrition in geriatric patients in Glasgow, Scotland (Dr. Reilly, Dr Edwards, University of Glasgow; Prof. Pietrzik, University of Bonn). Consecutively she studied at Erasmus University Rotterdam, The Netherlands, towards a M.Sc. degree in Epidemiology (funded by the Netherlands Institute of Health Sciences) which she obtained in 1994.

Since then, Kerstin worked at the Department of Epidemiology at the German Institute of Human Nutrition (DIfE) in Potsdam, being involved in the launch of the East-German study branch of the European Prospective Study on Cancer and Nutrition (EPIC). In 1995 she began working on her Ph.D. thesis as a research project between the Department of Epidemiology of Erasmus University Rotterdam and the DIfE. In January 1998, her daughter was born.