Lifestyle factors and risk of cardiovascular diseases

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ABSTRACT

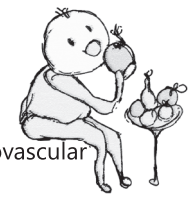
Background
Lifestyle factors have been shown to influence the incidence of fatal and non-fatal cardiovascular diseases (CVD). A healthy diet, being physically active, moderate alcohol consumption and not smoking are associated with a lower CVD risk. In addition to these lifestyle factors, recent research suggests that poor sleep may also be a risk factor of CVD. In this thesis, we focused on a Mediterranean style diet, specific leisure time physical activities, and sleep duration and quality as risk factors of CVD.

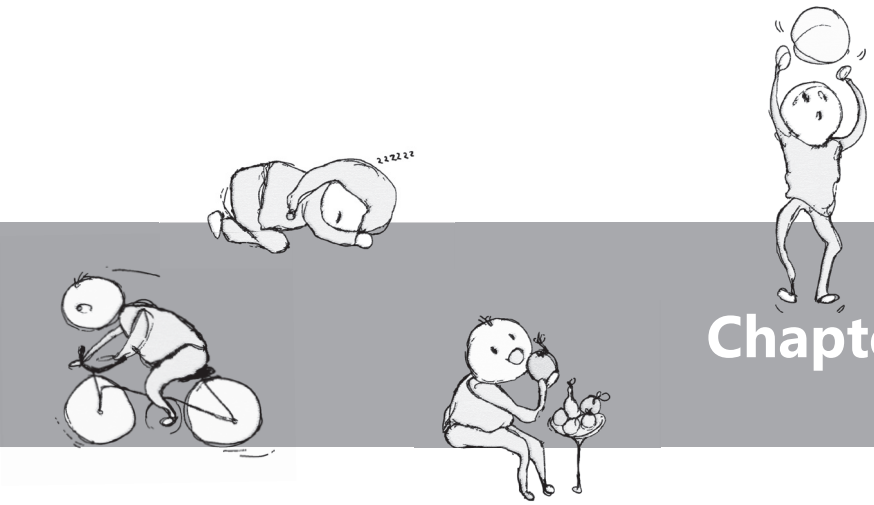
Methods
Our analyses are based on the prospective Doetinchem Cohort Study (n~3,400), the Monitoring Project on Risk Factors for Chronic Diseases (MORGEN) Study (n~20,400) and the Dutch contribution to the European Prospective Investigation into Cancer and Nutrition (EPIC-NL) (n~34,700). These studies included men and women aged 20–65 years when examined between 1993 and 1997. Diet was assessed with the validated EPIC food frequency questionnaire and operationalized with the Mediterranean Diet Score (MDS, range: 0–9). Physical activity was estimated with the validated EPIC physical activity questionnaire, with an emphasis on different leisure time activities. In addition, information was collected on duration and quality of sleep by two questions. Cardiovascular morbidity and mortality were ascertained through linkage with national registers. Multivariable Cox models were used to estimate the strength of the associations and 95% confidence intervals.

Results
During 12 years of follow-up, 206 CVD cases occurred in the Doetinchem Cohort Study, 1,486 cases in the MORGEN Study and 4,881 cases in the EPIC-NL Study. In the study on diet, a two unit increment in MDS was associated with a 22% lower risk of fatal CVD, and a 5% lower risk of total CVD. For specific CVDs, a 14% lower risk of myocardial infarction, a 12% lower risk of stroke, and a 26% lower risk of pulmonary embolism was observed. The MDS was not related to incident angina pectoris, transient ischemic attack and peripheral arterial disease. The use of multiple measurements of the MDS increased the strength of the associations with CVD and narrowed the confidence intervals. For leisure time physical activity, we showed that cycling was associated with an 18% lower risk of total CVD, sports with a 26% lower risk, and those who both cycled and performed sports had a 34% lower risk. Walking and gardening were not associated with CVD risk. Short sleep duration was associated with a 15% higher risk of total CVD, whereas long sleep duration and sleep quality separately were not associated. Short sleepers with a poor sleep quality had a 63% higher risk of total CVD compared to those with a normal sleep duration and good sleep quality. Finally, the combination of a healthy diet, sufficient physical activity, moderate alcohol consumption and non-smoking was associated with a 57% lower risk of composite CVD and a 67% lower risk of fatal CVD. The addition of sufficient sleep duration to these four traditional healthy lifestyle factors resulted in a 65% lower risk of composite CVD and an 83% lower risk of fatal CVD.

Conclusions
In this thesis, we showed that the strength of the association between dietary patterns and CVD incidence is likely underestimated because most studies used only the baseline measurement of diet. Furthermore, leisure time physical activities should be of at least moderate intensity to contribute to lower CVD risk. We also observed that sufficient sleep is a factor that should be taken into consideration in the prevention of CVD, in combination with a healthy diet, sufficient physical activity, moderate alcohol consumption and not smoking. Our results underscore the importance of a healthy lifestyle for CVD prevention.

CONTENTS





Chapter 1

General introduction

Cardiovascular diseases (CVD) are the second cause of death in The Netherlands. In 2010, 29% of deaths was due to CVD; 21,154 women and 18,581 men died from CVD (**Figure 1.1**).[1] Although CVD mortality rates in The Netherlands are continuously decreasing, the relatively low CVD mortality is accompanied by a high CVD incidence and prevalence. In 2007, there were 648,300 prevalent cases of coronary heart disease and 191,000 prevalent cases of stroke. In that year, 82,100 incident cases of coronary heart disease cases occurred and 35,600 incident cases of stroke.[2] Because of this large number of prevalent and incident cases, primary and secondary prevention of CVD is from a public health perspective of utmost importance.

Lifestyle factors and CVD risk

Evidence is strong that lifestyle factors influence the incidence of fatal and non-fatal CVD. Adhering to a healthy diet, being physically active, non-smoking and moderate alcohol consumption are associated with a low CVD risk.[3] Stampfer et al. estimated that over 70% of stroke cases and over 80% of coronary heart disease cases may be prevented or postponed by a healthy lifestyle.[4] In addition to the lifestyle factors mentioned, more recent research showed that poor sleep may also be an independent risk factor of CVD.[5,6]

Figure 1.1 Age standardized trends in total CVD mortality in The Netherlands (per 100,000), by gender. Year of standardization is 2010 (source: Statistics Netherlands/ Netherlands Heart Foundation).[1]

Mediterranean style diet and CVD risk

In the 1960's, Keys et al. showed in the Seven Countries Study that the population of the Greek island of Crete had a low rate of coronary heart disease and a long life expectancy. The traditional Mediterranean diet in Crete was considered to be largely responsible for this.[7] This diet is often defined as the dietary pattern found in the olive growing areas of the Mediterranean region, during the late 1950s and early 1960s. This was a period when the consequences of World War II had been overcome, but the fast-food culture had not yet invaded the area.[8] The diet is characterized by the use of olive oil as the principal component of fat; relatively high consumption of fruits, vegetables, fish, whole grains, legumes, and nuts; low consumption of meat and dairy; and moderate alcohol consumption with meals.[9,10] It is often seen as a prototype of a healthy dietary pattern. A recent meta-analysis by Sofi et al. showed that each two-unit increment of a nine-point score measuring the adherence to a Mediterranean style diet, was associated with a 10% lower incidence of CVD.[11]

CVD consists of various diseases. Each has a different etiology and ideally should be investigated individually in relation to dietary patterns. We hypothesized that adherence to Mediterranean style diets is inversely related to all of the following CVD endpoints: myocardial infarction, angina pectoris, stroke, transient ischemic attack, peripheral arterial disease, and pulmonary embolism, but that the strength of these inverse relationships differs.

> *In chapter 2 we describe the association of a Mediterranean style diet, as a prototype of a healthy diet with different types of incident CVD.*

The strength of the association between a Mediterranean style diet and CVD may be underestimated since in most cohort studies only a single measurement of dietary intake is available. Multiple measurements of dietary intake over a longer period of time can theoretically strengthen the observed associations because averaging multiple measurements reduces random measurement error and multiple measurements over a longer period better represent long-term dietary patterns.[12,13] Studies on the effect of repeatedly estimated dietary patterns on the association with CVD have not been carried out so far. We hypothesized that multiple measurements of diet would increase the strength of the association with CVD compared to the baseline measurement.

> *In chapter 3 we report the strength of the association between a Mediterranean style diet and CVD incidence, comparing three repeated measurements to the baseline measurement.*

Physical activity and CVD risk

Many epidemiological studies have shown a clear inverse association between physical activity and CVD.[14,15] A recent meta-analysis included 21 prospective studies with more than 650,000

adults initially free from CVD. Among men, a 24% lower risk of total CVD (RR: 0.76 [95% CI: 0.70–0.82]) and among women a 27% lower risk (RR: 0.73 [0.68–0.78]) was observed for high, compared to a low level of leisure time physical activity. Also dose-response relationships were observed.[16] In The Netherlands, as in many countries worldwide, physical activities of at least moderate intensity (at least 4.0 MET) during at least 30 minutes a day on at least 5 days a week are recommended.[17] In 2011, 58% of the Dutch adult population adhered to this guideline.[18]

The Dutch are among the most physically active populations in Europe and worldwide.[19,20] Of nine European countries, people from The Netherlands dedicated most time to non-occupational physical activity.[19] Furthermore, of the 16 countries – amongst others Australia, USA, China, Brazil, Canada, New Zealand and European countries – included in a recent review, the Dutch were the second most active with regard to walking or cycling to work.[20] It was recently estimated that worldwide 5.8% (95% CI: 3.2–7.8) of the burden of disease from coronary heart disease could be prevented if all would adhere to the physical activity guidelines.[21] For The Netherlands it was estimated that 3.0% (95% CI: -0.1–7.1) of coronary heart disease cases could be prevented. This preventable proportion is relatively low compared to some other countries; in the USA, for instance, it has been estimated to be 6.7%.[21]

Much less is known about the association of specific types of leisure time physical activity in relation to CVD. In this thesis we focused on walking and activities of at least moderate intensity, in particular gardening, cycling and sports. Although walking at an average speed (3.5 METs) does not classify as 'moderate intense' activity in The Netherlands, this type of physical activity has repeatedly been associated with a lower risk of CVD.[22-24] Though gardening on average has a moderate intensity (4.0 MET), studies on gardening and CVD risk are lacking. Cycling, a frequently used means of transportation in The Netherlands, contributes substantially to the activity level of Dutch people. It was associated with a 9–28% lower risk of CVD in previous studies.[25] It should be noted that none of these investigations were conducted in a population where cycling is a common means of transportation. The protective association of sports with CVD has been shown repeatedly.[26-30] We hypothesized that walking, gardening, cycling, and sports are inversely related to CVD incidence but that the strengths of these inverse relationships differ.

In chapter 4 we describe the results of a study on the relation between specific types of leisure time physical activity (walking, gardening, cycling and sports) and CVD incidence.

Sleep and CVD risk

How much sleep does a human being need? This question has intrigued researchers for decades. Already in 1964, Hammond reported in a cohort study including 461,440 men that those sleeping about 7 hours per night had a lower death rate than men who slept either

more or less hours. A recent meta-analysis by Cappuccio et al. incorporated 15 cohort studies, and showed that short sleep duration was associated with a 48% greater risk of CHD and a 15% higher risk of stroke, but it was not associated with total CVD.[6] Also for long compared to normal sleep duration they reported a 38% higher risk of CHD, a 65% higher risk of stroke, and a 41% higher risk of total CVD.[6]

Not only the duration but also the quality of sleep may affect health. Sleep quality is an important factor in the physiologic recovery of the body during sleep.[31] This raises the question whether short sleepers who rise every morning feeling rested, are at the same cardiovascular risk as short sleepers who wake up still feeling tired. It could be that the first group did not need more hours to sleep because they slept 'efficiently'. Chandola et al. showed in the Whitehall II cohort that the negative effect of short sleep duration on CHD risk was greatest among those with sleep disturbance.[32] We therefore hypothesized that short sleep duration may be adequate for those with a good sleep quality, but not for those with a poor sleep quality.

In chapter 5 we describe the association of sleep duration and sleep quality with CVD incidence.

Combined lifestyle factors and CVD risk

Besides diet,[11] physical activity,[16] and sleep,[6] also smoking[33,34] and alcohol consumption[35] are strongly associated with CVD. The Nurses Health Study showed that, compared to never smokers, current smokers had a four times higher risk, and former smokers had a 50% higher risk of fatal and non-fatal CHD combined.[36] Similar results were observed in other studies.[34,37,38] Streppel et al. observed in Dutch men that cigarette smoking reduced the number of disease-free life-years by 5.8 years, and cigar or pipe smoking by 5.2 years.[39] For alcohol consumption, a recent meta-analysis by Ronksley et al. showed a U-shaped association with fatal CVD, an L-shaped association with fatal and non-fatal CHD, and a J-shaped association with fatal and non-fatal stroke.[35] Moderate alcohol consumption was also associated with a 2.3 year longer life expectancy at age 50 compared to never drinking.[40] Given the already available strong evidence for smoking and drinking in relation to CVD risk and survival, we did not investigate these associations separately in this thesis.

The lifestyle factors diet, physical activity, smoking and alcohol consumption tend to cluster in individuals.[41-47] In the MORGEN-Study about 20% of the population had at least three out of four unhealthy lifestyle factors.[41] To take clustering of lifestyle factors into account, several studies investigated different combinations of lifestyle factors in relation to CVD incidence.[4,48-57] Hazard ratios for the highest compared with the lowest degrees of adherence to a healthy lifestyle ranged from 0.24 to 0.35 for fatal CVD.[48-51,54-56] Only two studies included sleep duration as a lifestyle factor.[48,54] Both studies were performed in Asian populations and assessed the associations of the combination of sleep and the other four lifestyle factors with CVD, without providing insight

into the additional impact of sleep over the other healthy lifestyle factors.[48,54] We hypothesized that – in addition to a healthy diet, sufficient physical activity, moderate alcohol consumption, and non-smoking – sufficient sleep duration further lowers the risk of CVD.

In chapter 6 we describe whether sufficient sleep duration – in addition to a healthy diet, sufficient physical activity, moderate alcohol consumption, and non-smoking – contributed to lower CVD incidence.

Study populations

Figure 1.2 summarizes the characteristics of the populations used for the analyses presented in this thesis. The EPIC-NL cohort is the Dutch contribution to the European Prospective Investigation into Cancer and Nutrition (EPIC) and consists of the Monitoring Project on Risk Factors for Chronic Diseases (MORGEN) and the PROSPECT cohort. Baseline data of the 40,011 participants in the EPIC-NL cohort were collected between 1993 and 1997. The MORGEN cohort consists of 22,654 men and women aged 20–65 years who were recruited through random population sampling in three towns in The Netherlands (Amsterdam, Maastricht and Doetinchem).[58] In the MORGEN cohort the participants from Doetinchem had already been examined five years before, and were subsequently examined every five years as part of the Doetinchem Cohort Study.[59] About 4,000 persons participated in the last three rounds of this study and the fifth examination round has just been finished.

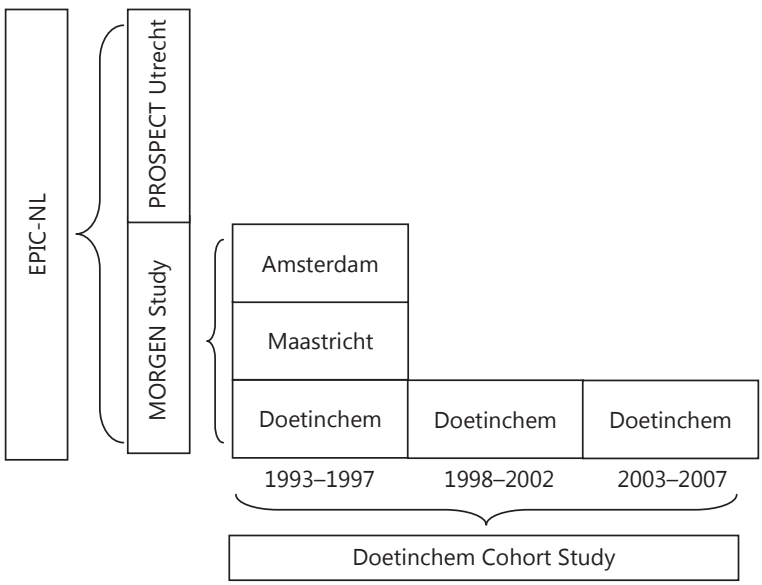


Figure 1.2 Study populations in this thesis.

Outline

Though much is known about the relation between lifestyle factors and risk of CVD, details regarding the nature of this relation are lacking. This thesis provides more information on some of these details. We investigated the associations of a Mediterranean style diet with different subgroups of CVD (chapter 2), and the additional value of repeated measurements of this diet in estimating the strength of the association with CVD incidence (chapter 3). With respect to physical activity, we studied specific types of leisure time physical activity in relation to CVD incidence (chapter 4). In addition, we studied sleep duration and sleep quality in relation to CVD incidence (chapter 5), and also the contribution to lower CVD risk of sufficient sleep duration – in addition to a healthy diet, sufficient physical activity, moderate alcohol consumption, and non-smoking (chapter 6). Finally, we discussed the results of the different studies with an emphasis on methodological aspects and public health implications (chapter 7).

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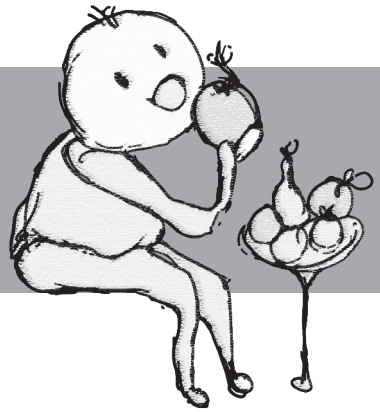

Chapter 2

Mediterranean style diet and 12-year incidence of cardiovascular diseases. The EPIC-NL cohort study

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ABSTRACT

Background: A recent meta-analysis showed that a Mediterranean style diet may protect against cardiovascular diseases (CVD). Studies on disease-specific associations are limited. We evaluated the Mediterranean Diet Score (MDS) in relation to incidence of total and specific CVDs.

Methods: The EPIC-NL Study is a cohort of 40,011 men and women aged 20–70 years, examined between 1993 and 1997, with 10–15 years of follow-up. Diet was assessed with a validated food frequency questionnaire and the MDS was based on the daily intakes of vegetables, fruits, legumes and nuts, grains, fish, fatty acids, meat, dairy, and alcohol. Cardiovascular morbidity and mortality were ascertained through linkage with national registries. Cox proportional hazards models were used to estimate hazard ratios (HRs) and 95% confidence intervals (CI) adjusted for age, sex, cohort, smoking, physical activity, total energy intake, and educational level.

Results: In 34,708 participants free of CVD at baseline, 4,881 CVD events occurred, and 487 persons died from CVD. A two unit increment in MDS (range: 0–9) was inversely associated with fatal CVD (HR: 0.78; 95% CI: 0.69–0.88), total CVD (HR: 0.95 [0.91–0.98]), myocardial infarction (HR: 0.86 [0.79–0.93]), stroke (HR: 0.88 [0.78–1.00]), and pulmonary embolism (HR: 0.74 [0.59–0.92]). The MDS was not related to incident angina pectoris, transient ischemic attack and peripheral arterial disease.

Conclusion: Better adherence to a Mediterranean style diet was more strongly associated with fatal CVD than with total CVD. Disease specific associations were strongest for incident myocardial infarction, stroke and pulmonary embolism.

INTRODUCTION

For a long time, the focus of nutritional research was on nutrients. However, interactions, synergy and correlations among nutrients will influence their bioavailability and absorption.[1,2] Therefore, investigating the relation of single nutrients with cardiovascular diseases (CVD) is not sufficient, and also individual foods and food patterns need to be studied in relation to CVD incidence.

The traditional Mediterranean diet is a prototype of a healthy diet and is associated with a low risk of CVD.[3] Key features of a traditional Mediterranean diet are the use of olive oil as the principal component of fat; relatively high consumption of fruit, vegetables, fish, whole grains, legumes, and nuts; low consumption of meat and dairy; and moderate alcohol consumption with meals.[4,5]

Controlled trials showed that a Mediterranean style diet was associated with favorable changes in body weight, body mass index, systolic and diastolic blood pressure, fasting plasma glucose, total cholesterol and high-sensitivity C-reactive protein, which may all affect CVD incidence.[6] In a secondary prevention trial in myocardial infarction patients, a Mediterranean style diet enriched with alpha-linolenic acid was associated with a 73% lower risk of cardiac death and non-fatal myocardial infarction compared with a prudent diet.[7,8] Also, in a cohort study on the Mediterranean diet and survival among Greek cardiac patients, a higher adherence to the Mediterranean diet was strongly inversely associated with cardiac death.[9]

A recent meta-analysis showed that each two unit increment in the MDS was associated with a 10% lower incidence of CVD.[10] The meta-analysis combined studies with different fatal and nonfatal CVD enpoints.[10] The recently published prospective cohort study by Gardener et al. observed an association of the MDS with fatal CVD, but not with incident myocardial infarction or ischemic stroke.[11] These results suggest that a Mediterranean style diet may affect the risk of specific CVD differently. Gardener et al. concluded that studies on Mediterranean style diets in relation to specific CVD endpoints are needed.[11] Associations of Mediterranean style diets with angina pectoris, transient ischemic attack, peripheral arterial disease, or pulmonary embolism have not yet been investigated.

The purpose of the present study was to investigate a Mediterranean style diet, as a prototype of a healthy diet, in association with incident fatal CVD, total CVD, and with specific CVD. The secondary aim was to explore which of the components of the MDS contributed most to these associations by assessing the effect of alternately excluding components of the MDS.

METHODS

Study population

The EPIC-NL cohort is the Dutch contribution to the European Prospective Investigation into Cancer and Nutrition (EPIC) and consists of the Monitoring Project on Risk Factors for Chronic

Diseases (MORGEN) and the PROSPECT cohort. Baseline data of the 40,011 participants were collected between 1993 and 1997. The MORGEN cohort consists of 22,654 men and women aged 20–65 years who were recruited through random population sampling in three Dutch towns (Amsterdam, Maastricht and Doetinchem).[12] The PROSPECT cohort included 17,357 women aged 50–70 years, who participated in a breast cancer screening program in the province of Utrecht.

We excluded participants with prevalent CVD or type 2 diabetes based on self-report and hospital admission data (n=1,401), women who were pregnant at baseline (n=140), those with extremely low or high reported energy intakes (i.e., those in the lowest and highest 0.5% of the ratio of energy intake over basal metabolic rate) (n=373), those with no information on dietary intake or any of the covariates (n=855), as well as those with no information on vital status or cardiovascular events (n=2,534). In total 34,708 participants remained for the analyses.

Ethics statement

All participants signed an informed consent form prior to study inclusion. The study complies with the Declaration of Helsinki and was approved by two medical ethics committees.[12]

Mediterranean style diet

Dietary intake was assessed by a validated[13,14] food frequency questionnaire (FFQ) which contained questions on the habitual frequency of consumption of 178 food items during the year preceding enrollment. Additional information was obtained on consumption frequency for different sub-items, and preparation methods. Colored photographs were used to estimate portion sizes of 28 food items.[13,14] Reproducibility and validity were assessed in 121 men and women.[13,14] Median twelve-month reproducibility of 16 food groups was 0.71 for men and 0.77 for women, ranging from resp. 0.45 and 0.63 for fish to 0.83 and 0.92 for alcoholic beverages.[13] The median validity of these 16 food groups, with 12 monthly 24-hour recalls as reference, was 0.61 for men and 0.53 for women, ranging from 0.32 for fish in men and 0.31 for vegetables in women to 0.74 (men) and 0.87 (women) for alcoholic beverages.[13]

We operationalized the concept of a healthy diet with the modified Mediterranean Diet Score (MDS) defined by Trichopoulou et al.[5] For the composition of this score, values of 0 or 1 were assigned to each nutritional component using the sex-specific medians as cut-off values. For vegetables, fruits, legumes and nuts combined, grains, fish and seafood combined, and the ratio of unsaturated to saturated fatty acids, intakes equal to or above the median were assigned a value of 1, and for intakes below that median a value of 0. For meat and for dairy products the scoring was inverted. We dichotomized alcohol consumption into non-users and users, because of the relatively low levels of alcohol consumption in this population. We did not define an upper limit for alcohol consumption, due to the low levels of alcohol consumption in the

present population, and called the alcohol consumption of our population moderate. A value of 1 was assigned for consuming at least one drink per month and a value of 0 for consuming less than 1 drink per month. The modified MDS could take a value from 0 (minimal adherence) to 9 (maximal adherence)[5] and associations with CVD were assessed in categories 0–2, 3–4, 5–6 and 7–9 and by two unit increment.

Ascertainment of fatal and nonfatal CVD events

Participants were followed to the first nonfatal cardiovascular event, death, emigration, or were censored at January 1st 2008. Morbidity data were provided by the National Medical Registry (NMR) using the Dutch Hospital Discharge data. Eighty-eight percent of the hospital admissions could uniquely be linked to an individual on the basis of sex, date of birth, and postal code.[15] In a validation study conducted in a subsample of this population, the Hospital Discharge data was compared with that of a detailed clinical registry.[16] A sensitivity of 84% was observed for acute myocardial infarction hospital admissions.[16] We obtained vital status through linkage with municipal population registries. Subsequently, primary (underlying) and secondary causes of death were obtained through linkage with data from 'Statistics Netherlands'.[12] In a study in which causes of death were coded again two years after initial coding, agreement ranged from 77–89% for CVD.[17]

Incidence was defined as both fatal and nonfatal events unless otherwise specified. For fatal and nonfatal incidence combined, only first events were taken into account. For instance, when a person experienced a myocardial infarction followed by a pulmonary embolism, this person was censored after the myocardial infarction. For fatal CVD, no exclusions were made due to previous nonfatal events during follow-up. Total CVD was coded as ICD9[18] codes 390–459 and 798. Myocardial infarction was coded as 410–412 and 414, angina pectoris as 413, stroke as 430–434, and 436, transient ischemic attack as 435, peripheral arterial disease as 440–444, and pulmonary embolism as 415.1. The remaining CVD codes were coded as other CVD. Composite CVD consisted of fatal CVD, plus nonfatal first myocardial infarction and stroke. Causes of death after 1996 were coded according to the corresponding ICD10[19] codes.

Covariates

A self-administered general questionnaire provided information on educational level, smoking status, and physical activity.[12] Educational level was operationalized as low (lower vocational training or primary school), medium (secondary school and intermediate vocational training), or high (higher vocational training or university) and cigarette, cigar or pipe smoking as current, former or never. Physical activity was assessed using the validated[20] EPIC questionnaire and dichotomized according to the Cambridge Physical Activity Index (CPAI) into (moderately) active and (moderately) inactive.[21] In the first year of the MORGEN study (1993, 14.2% of the

study population), physical activity was not assessed with the EPIC questionnaire. The missing physical activity data were imputed using single imputation as previously described (SPSS MVA procedure).[22]

Statistical analysis

Statistical analyses were performed using SAS 9.2 software (SAS Institute, INC., Cary, NC). Participants' characteristics were calculated by sex and cohort as means (standard deviation) or medians (inter-quartile range) for continuous variables, and as percentages for categorical variables. Cox proportional hazards models were used to estimate hazard ratios (HRs) and 95% confidence intervals (CI). The proportional hazard assumption was fulfilled according to the graphical approach and according to Schoenfeld residuals. Two consecutive models were used to assess the associations between the MDS and the CVD endpoints. The first model was adjusted for age, sex and cohort. The second model was additionally adjusted for smoking, physical activity, total energy intake, and educational level. We did not include BMI, blood pressure or serum cholesterol in our analyses, since we consider these intermediates in the association between a Mediterranean style diet and CVD. By adding interaction terms to the models, we assessed interaction on a multiplicative scale for age, sex, and cohort in the association between the MDS and the different cardiovascular endpoints. We also explored which of the components of the MDS contributed most to the associations for those CVD that were related to the MDS at $p<0.10$. For this, we alternately excluded one component of the MDS while adjusting for the excluded component. This reduced the ten point score (0–9) to a nine point score (0–8). To preserve comparability between the ten point and the nine point scores, we multiplied the logarithm of the estimated nine mortality ratios by 9/10 before exponentiating them.[23]

To minimize the possibility that dietary habits had changed in response to development of intermediate symptoms (e.g. hypertension) during follow-up (reversed causation), the analyses were repeated after exclusion of persons with an event in the first two years of follow-up.

RESULTS

During 10–15 years of follow-up (mean 11.8 years) 4,881 CVD events occurred, and 487 persons died from CVD. **Table 2.1** shows the distribution of participants' characteristics and dietary intake stratified by sex and cohort. 25% of the population was male. Mean age at baseline was 43 years for men, 42 years for women in MORGEN and 58 years for women in PROSPECT. Dietary intake was similar in women in MORGEN and PROSPECT, with the exception of fruit consumption, which was higher in PROSPECT.

After adjustment for all confounders, each two unit increment in MDS was associated with a 22% lower incidence of fatal CVD (HR: 0.78; 95% CI: 0.69–0.88), a 5% lower incidence of total

CVD (HR: 0.95 [0.91–0.98]), and a 15% lower incidence of composite CVD (HR: 0.85 [0.80–0.91]) (**Table 2.2**). For specific CVD, statistically significant associations were observed for incident myocardial infarction (HR: 0.86 [0.79–0.93]), stroke (HR: 0.88 [0.78–1.00]), and pulmonary embolism (HR: 0.74 [0.59–0.92]). The strength of the association of the MDS with ischemic (HR: 0.86 [0.72–1.01]) did not differ from that with hemorrhagic stroke (0.87 [0.60–1.09]). The

Table 2.1 Participants' characteristics at baseline and CVD incidence, by sex and cohort, the EPIC-NL Study

	Men (MORGEN)	Women (MORGEN)	Women (PROSPECT)
n	8,764	10,537	15,407
Age (years) [a]	43 (11)	42 (11)	58 (6)
Education (% low)[b]	32	35	45
Smoking (%)[c]	38	35	23
Physically active (%)	71	68	67
Alcohol consumption (%, ≥1 glass per month)	91	77	78
Alcohol (gram/day)[a]	12 (3–25)	3 (0–11)	4 (1–13)
Energy intake (kcal/day) [a]	2,518 (2,139–2,987)	1,932 (1,635–2,265)	1,763 (1,508–2,046)
Vegetables (gram/day)[a]	103 (77–134)	113 (86–147)	123 (95–158)
Fruit (gram/day)[a]	117 (51–186)	124 (78–239)	224 (119–295)
Legumes (gram/day)[a]	15 (8–24)	12 (6–20)	12 (6–20)
Nuts (gram/day)[a]	7 (2–16)	4 (2–10)	4 (1–8)
Grains (gram/day)[a]	250 (188–319)	184 (142–234)	147 (115–184)
Fish and seafood (gram/day)[a]	8 (3–14)	7 (3–14)	8 (3–16)
Unsaturated fatty acids (gram/day)[a]	58 (47–71)	44 (36–54)	36 (29–44)
Saturated fatty acids (gram/day)[a]	41 (33–50)	32 (25–39)	28 (22–34)
Dairy and dairy products (gram/day)[a]	353 (196–578)	346 (196–548)	414 (267–597)
Meat products (gram/day)[a]	141 (105–179)	102 (63–131)	87 (54–118)
Fatal CVD (n/%)[d]	136/1.6	70/0.7	281/1.8
Incident CVD (n/%)[d]	1,202/13.7	1,078/10.2	2,601/16.9
Composite CVD (n/%)[d,e]	548/6.3	317/3.0	900/5.8
Incident MI (n/%)[d]	390/4.5	184/1.8	496/3.2
Incident AP (n/%)[d]	116/1.3	70/0.7	193/1.3
Incident stroke (n/%)[d]	93/1.1	95/0.9	260/1.7
Incident TIA (n/%)[d]	32/0.4	32/0.3	90/0.6
Incident PAD (n/%)[d]	102/1.2	48/0.5	135/0.9
Incident PE (n/%)[d]	25/0.3	35/0.3	71/0.5
Incident other CVD (n/%)[d]	450/5.1	621/5.9	1,376/8.9

CVD, cardiovascular diseases; MI, myocardial infarction; AP, angina pectoris; TIA, transient ischemic attack; PAD, peripheral arterial disease; PE, pulmonary embolism.

[a] Numbers are given as mean (sd) or as median (interquartile range); [b] Low educational level: lower vocational training or primary school; [c] Cigarette, cigar or pipe; [d] See method section for ICD codes; [e] Composite of fatal CVD, nonfatal myocardial infarction and nonfatal stroke.

Tabel 2.2 Hazard ratios (95% CI) of specific CVD by MDS category and by two unit increment in MDS, The EPIC-NL Study

	MDS				
	0–2	3–4	5–6	7–9	By two unit increment
Persons at risk, n	2,469	12,249	14,832	5,158	34,708
Person years total CVD	28,026	139,539	170,208	58,924	396,697
Fatal CVD, n	62	206	178	41	487
Model 1[a]	1.00 (ref)	0.67 (0.50–0.89)	0.51 (0.38–0.69)	0.36 (0.24–0.54)	0.72 (0.64–0.81)
Model 2[b]	1.00 (ref)	0.72 (0.54–0.96)	0.60 (0.44–0.80)	0.44 (0.30–0.66)	0.78 (0.69–0.88)
Incident CVD, n	438	1,820	1,973	650	4,881
Model 1[a]	1.00 (ref)	0.84 (0.75–0.93)	0.77 (0.69–0.85)	0.75 (0.66–0.84)	0.90 (0.87–0.94)
Model 2[b]	1.00 (ref)	0.87 (0.79–0.97)	0.84 (0.75–0.93)	0.84 (0.75–0.96)	0.95 (0.91–0.98)
Composite CVD[c], n	186	699	684	196	1,765
Model 1[a]	1.00 (ref)	0.75 (0.64–0.88)	0.63 (0.53–0.74)	0.54 (0.44–0.66)	0.80 (0.75–0.84)
Model 2[b]	1.00 (ref)	0.80 (0.68–0.94)	0.72 (0.61–0.85)	0.65 (0.53–0.80)	0.85 (0.80–0.91)
Incident MI, n	106	428	412	124	1,070
Model 1[a]	1.00 (ref)	0.79 (0.64–0.98)	0.64 (0.52–0.79)	0.57 (0.44–0.74)	0.79 (0.74–0.86)
Model 2[b]	1.00 (ref)	0.85 (0.69–1.05)	0.74 (0.60–0.92)	0.70 (0.54–0.92)	0.86 (0.79–0.93)
Incident AP, n	20	138	171	50	379
Model 1[a]	1.00 (ref)	1.37 (0.86–2.20)	1.44 (0.91–2.29)	1.25 (0.74–2.10)	1.02 (0.90–1.17)
Model 2[b]	1.00 (ref)	1.44 (0.90–2.31)	1.59 (1.00–2.54)	1.44 (0.85–2.43)	1.08 (0.95–1.23)

Incident stroke, n	46	174	177	51	448
Model 1[a]	1.00 (ref)	0.77 (0.55–1.06)	0.68 (0.49–0.94)	0.59 (0.39–0.87)	0.83 (0.74–0.93)
Model 2[b]	1.00 (ref)	0.82 (0.59–1.13)	0.77 (0.55–1.07)	0.70 (0.47–1.05)	0.88 (0.78–1.00)
Incident TIA, n	13	59	55	27	154
Model 1[a]	1.00 (ref)	0.92 (0.51–1.68)	0.75 (0.41–1.37)	1.10 (0.57–2.13)	0.98 (0.80–1.20)
Model 2[b]	1.00 (ref)	0.94 (0.51–1.71)	0.77 (0.42–1.43)	1.15 (0.58–2.25)	1.00 (0.81–1.23)
Incident PAD, n	32	111	109	33	285
Model 1[a]	1.00 (ref)	0.68 (0.46–1.01)	0.56 (0.38–0.84)	0.51 (0.31–0.83)	0.79 (0.68–0.92)
Model 2[b]	1.00 (ref)	0.77 (0.52–1.14)	0.73 (0.49–1.09)	0.74 (0.45–1.21)	0.91 (0.78–1.06)
Incident PE, n	19	46	52	14	131
Model 1[a]	1.00 (ref)	0.49 (0.29–0.84)	0.47 (0.28–0.79)	0.37 (0.18–0.73)	0.77 (0.62–0.95)
Model 2[b]	1.00 (ref)	0.47 (0.28–0.81)	0.43 (0.25–0.74)	0.33 (0.16–0.67)	0.74 (0.59–0.92)
Incident other CVD, n	206	873	1,011	357	2,447
Model 1[a]	1.00 (ref)	0.86 (0.74–1.01)	0.85 (0.73–0.99)	0.88 (0.74–1.04)	0.97 (0.93–1.02)
Model 2[b]	1.00 (ref)	0.88 (0.76–1.03)	0.88 (0.76–1.03)	0.93 (0.78–1.11)	0.99 (0.94–1.05)

CVD, cardiovascular diseases; MI, myocardial infarction; AP, angina pectoris; TIA, transient ischemic attack; PAD, peripheral arterial disease; PE, pulmonary embolism.
[a] Model 1: analyses adjusted for age, sex and cohort.
[b] Model 2: model 1 + smoking, physical activity, energy intake and educational level.
[c] Composite of fatal CVD, nonfatal myocardial infarction and nonfatal stroke.

Table 2.3 Hazard ratios (95% CI) associated with two unit increment in MDS and after alternate exclusion of each of its components[a,b]

MDS	Fatal CVD	Incident CVD	Composite CVD	Incident MI	Incident stroke	Incident PE
Total	0.78 (0.69–0.88)	0.95 (0.91–0.98)	0.85 (0.80–0.91)	0.86 (0.79–0.93)	0.88 (0.78–1.00)	0.74 (0.59–0.92)
Minus vegetables	0.79 (0.70–0.88)	0.94 (0.90–0.97)	0.86 (0.81–0.92)	0.86 (0.80–0.93)	0.90 (0.80–1.02)	0.71 (0.57–0.89)
Minus fruits	0.78 (0.70–0.87)	0.95 (0.91–0.98)	0.87 (0.82–0.93)	0.88 (0.82–0.95)	0.91 (0.81–1.03)	0.76 (0.62–0.95)
Minus legumes and nuts	0.77 (0.69–0.87)	0.94 (0.91–0.97)	0.83 (0.78–0.89)	0.84 (0.78–0.91)	0.86 (0.76–0.97)	0.73 (0.59–0.92)
Minus grains	0.81 (0.72–0.90)	0.96 (0.93–1.00)	0.87 (0.82–0.92)	0.87 (0.81–0.94)	0.91 (0.81–1.02)	0.79 (0.64–0.98)
Minus fish and seafood	0.81 (0.72–0.91)	0.95 (0.92–0.98)	0.87 (0.82–0.92)	0.86 (0.79–0.93)	0.92 (0.82–1.04)	0.81 (0.65–1.00)
Minus fatty acid ratio	0.81 (0.72–0.92)	0.94 (0.90–0.98)	0.84 (0.79–0.89)	0.84 (0.77–0.91)	0.85 (0.75–0.96)	0.82 (0.65–1.03)
Minus dairy products	0.79 (0.70–0.88)	0.94 (0.91–0.98)	0.85 (0.80–0.90)	0.86 (0.79–0.92)	0.85 (0.76–0.95)	0.75 (0.61–0.93)
Minus meat products	0.79 (0.71–0.89)	0.96 (0.93–1.00)	0.88 (0.83–0.93)	0.89 (0.83–0.96)	0.91 (0.82–1.02)	0.73 (0.59–0.90)
Minus alcohol	0.82 (0.74–0.92)	0.97 (0.93–1.00)	0.89 (0.84–0.94)	0.91 (0.84–0.98)	0.90 (0.80–1.01)	0.75 (0.61–0.93)

CVD, cardiovascular diseases; MI, myocardial infarction; PE, pulmonary embolism.

[a] Originally estimated logarithms of hazard ratios were multiplied by 9/10 and then exponentiated to correct for nine point scale.

[b] Hazard ratio (95% CI) adjusted for age, sex, cohort, smoking, physical activity, energy intake, educational level, and excluded component.

association with pulmonary embolism was not linear: the largest decrease in HR was between the MDS categories '0–2' and '3–4'. Also, the association with pulmonary embolism was stronger for men (HR: 0.39 [0.23–0.67] per two unit increment) than for women (HR 0.84 [0.65–1.07]) (p for interaction: <0.01). Adherence to a Mediterranean style diet was not related to incident angina pectoris, transient ischemic attack and peripheral arterial disease. We observed no other interactions with age, sex (except for pulmonary embolism) or cohort. Exclusion of cases in the first two years of follow-up hardly changed our results (data not shown).

In general, alternately excluding one of the components of the MDS did not materially change our results (**Table 2.3**). The exclusion of alcohol from the MDS had the largest impact on the associations of MDS with CVD, in particular for incident fatal CVD, total CVD, composite CVD and myocardial infarction. Excluding fish and seafood or the fatty acid ratio attenuated the association for pulmonary embolism most.

DISCUSSION

This study showed that a Mediterranean style diet was inversely associated with total CVD and more strongly so with fatal CVD. Inverse associations were also observed for composite CVD, myocardial infarction, stroke and pulmonary embolism. The MDS was not related to incident angina pectoris, transient ischemic attack and peripheral arterial disease. Alternate exclusion of components of the MDS showed that alcohol contributed most to the inverse association between MDS and CVD.

In the present study, a MDS of 7–9 was associated with a 56% lower incidence of fatal CVD compared to a MDS of 0–2, with a 16% lower incidence of total CVD and a 35% lower incidence of composite CVD. Comparison of our results with those of other investigations is hampered by differences in definition of Mediterranean style diet and of CVD endpoints. In previous studies, the MDS varied in definition of the components. Also, adherence to a Mediterranean style diet was categorized in various ways, ranging from two, to five categories of adherence.[10] The definition of CVD also varied among studies, with various combinations of ICD codes and inclusion of fatal or nonfatal CVD events. Taken together, results of previous studies showed that high compared to low adherence to a Mediterranean style diet was associated with a 20[24] to 40%[25,26] lower incidence of fatal CVD and a 20[25] to 25%[11] lower incidence of composite CVD. These results are in line with those in the present study.

The association of the MDS with fatal CVD was stronger than with total CVD. A stronger association with fatal CVD may be due to the probabilistic linkage of the non-fatal Hospital Discharge data causing more misclassification than for fatal events. This may have resulted in weaker associations for nonfatal CVD. Furthermore, our results showed that adding 'softer' endpoints such as transient ischemic attack, angina pectoris and peripheral arterial disease to the composite of 'hard' endpoints reduced the strength of the associations considerably.

Therefore, in discussing the strength of associations with different CVD endpoints, the definition of the latter is of utmost importance.

For the highest compared to the lowest MDS category we observed a 30% lower incidence of myocardial infarction and for each two unit increment a 14% lower incidence. This was in line with a recent cohort study in 2,568 men and women in the United States by Gardener et al. who observed a 39% lower incidence of myocardial infarction for the highest compared to the lowest MDS category, and for each 1 unit increment a 6% lower incidence, though these associations were non-significant due to the small sample size.[11] Our results are also consistent with those observed in other cohort studies, in which high compared to low adherence to a Mediterranean style diet was associated with a 30–40% lower incidence of coronary heart disease,[25-29] although coronary heart disease incidence was defined differently in the various studies.

The inverse association between the MDS and stroke incidence in the present study (highest compared to lowest category HR: 0.70 [0.47–1.05] and for a two unit increment HR: 0.88 [0.78–1.00]) is in agreement with the results Fung et al. obtained in the Nurses Health Study.[25] They observed a 13% lower incidence of stroke for those in the highest compared to the lowest quintile of adherence to a Mediterranean style diet.[25] The results of the cohort studies published so far, including ours (results not shown), showed similar results for ischemic and hemorrhagic stroke.[11,25]

We observed that the MDS was inversely associated with the incidence of pulmonary embolism. This association was stronger for men than for women. Diet is hypothesized to affect venous thromboembolism, and thereby pulmonary embolism, by altering levels of haemostatic and fibrinolytic factors.[30] To our knowledge other studies did not investigate diet in relation to pulmonary embolism before. However, we could compare our results with those of three large cohort studies investigating the relation of diet with venous thromboembolism. The results of these studies were inconsistent.[31-33] Therefore, our results for pulmonary embolism need confirmation by other prospective cohort studies.

In the present study, the MDS was not statistically significantly related to incident angina pectoris, transient ischemic attack and peripheral arterial disease. To our knowledge these associations have not been investigated earlier. These three diseases are 'softer' endpoints than e.g. myocardial infarction and stroke. This may have resulted in more misclassification[16] which may have diluted the associations of the MDS with these endpoints.

The associations of the MDS with fatal CVD, incident CVD, composite CVD, and myocardial infarction attenuated most when excluding alcohol from the MDS. Excluding fish and seafood or the fatty acid ratio attenuated the association with pulmonary embolism most. No previous study on the MDS in relation to CVD assessed the effect of alternately excluding components of the MDS. Trichopoulou et al. observed for the association between MDS and all-cause mortality also most attenuation after excluding alcohol consumption from the MDS.[23] Previous

studies on a Mediterranean style diet assessing the contribution of its individual components to CVD incidence showed inconsistent results with respect to which component was strongest associated to CVD.[11,27,29]

We studied the adherence to a Mediterranean style diet in a Dutch population. The Dutch diet is characterized by a low consumption of plant foods and fish and by a high consumption of animal foods compared to the traditional Mediterranean diet.[34] However, similar associations of a Mediterranean style diet with CVD were observed in Mediterranean, Northern European and in American populations.[10] This implies that, at different levels of adherence, a Mediterranean style diet is beneficial in relation to cardiovascular health. Furthermore, our associations were robust since in sensitivity analyses, including only whole grain cereals in the component 'cereals', or only moderate to high fat meat products and dairy products in the components 'meat products' and 'dairy products', hardly changed the results (results not shown). Also additionally adjusting the components of the MDS for energy intake (density method) or exclusion of cases in the first two years of follow-up hardly changed our results (results not shown).

Some limitations of our study need to be addressed. Dietary intake was self-reported using a validated food-frequency questionnaire (FFQ). This questionnaire had a good reproducibility, although the validity of vegetable (Spearman correlation coefficients: 0.38 for men and 0.31 for women) and fish (0.32 for men and 0.37 for women) consumption is of concern.[13,14] In addition, diet was assessed only once and may have changed during follow-up, resulting in non-differential misclassification that may have attenuated the observed associations. Furthermore, our study is a prospective cohort study in which adherence to MDS was not randomized. Therefore, residual confounding cannot be ruled out. With regard to the cardiocascular follow-up, 'hard' endpoints, like myocardial infarction or stroke, are easier to diagnose than 'softer' endpoints like angina pectoris, transient ischemic attack or peripheral arterial disease. Also, 'hard' endpoints are more likely to be treated in the hospital than 'softer' ones, and thus monitored in the Hospital Discharge Registries.[16] In a validation study comparing the Hospital Discharge data to that of a cardiology information system, sensitivity was considerably larger for acute myocardial infarction (84%) than for unstable angina pectoris (53%).[16] Therefore, misclassification is likely smaller for the 'hard' events, which may have resulted in stronger associations.

The present study also has advantages. EPIC-NL is a large prospective cohort study, especially designed to study associations between diet and chronic diseases, and included both men and women from the general population, with a broad age range and a long follow-up period. Because of the detailed cardiovascular follow-up data and the large sample size, we were able to investigate associations of a Mediterranean style diet with specific CVD.

In conclusion, the present study showed that better adherence to a Mediterranean style diet was more strongly associated with fatal CVD than with total CVD. Disease specific associations were strongest for incident myocardial infarction, stroke and pulmonary embolism.

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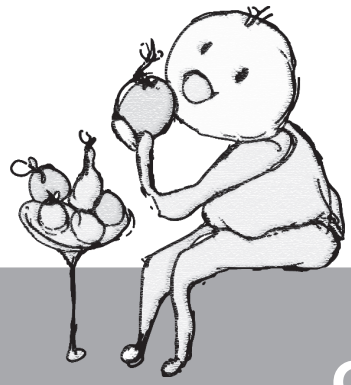

Chapter 3

Effect of using repeated measurements of a Mediterranean style diet on the strength of the association with 12-year cardiovascular disease incidence. The Doetinchem Cohort Study

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ABSTRACT

Background: In cohort studies, often only the baseline measurement of dietary intake is available. This may underestimate the strength of the association with cardiovascular diseases (CVD).

Objective: To compare the strength of the association of a Mediterranean style diet with CVD using the baseline measurement of diet versus three repeated measurements over a 10-year period.

Design: We used dietary and lifestyle data of three rounds of the Doetinchem Cohort Study. Diet was assessed with a 178 item validated food frequency questionnaire and operationalized with the Mediterranean Diet Score (MDS) ranging from 0–9. Cox proportional hazards models were used to estimate hazard ratios (HRs) and 95% confidence intervals (CI), for baseline MDS and updated mean MDS in association with fatal CVD and a composite of fatal CVD, non-fatal myocardial infarction and stroke. All analyses were adjusted for age, sex, smoking, sports, total energy intake, and educational level.

Results: 206 composite CVD events occurred, of which 38 were fatal. Baseline MDS was associated with a 23% lower risk (HR: 0.77 [95% CI: 0.53–1.11]) and the updated mean with a 35% lower risk (HR: 0.65 [0.43–0.97]) of composite CVD, for an MDS of 5.5–9 compared to an MDS of 0–3.5. For fatal CVD, baseline MDS was associated with a 13% lower risk (HR: 0.87 [0.36–2.07]) and the updated mean with a 56% lower risk (HR: 0.44 [0.19–1.05]).

Conclusion: The strength of the association between a Mediterranean style diet and CVD incidence is likely underestimated because most studies so far used only the baseline measurement.

INTRODUCTION

In many cohort studies, often only the baseline measurement of dietary intake is available to estimate the strength of the association of dietary patterns with cardiovascular diseases (CVD). Using only the baseline measurements may underestimate the true association of a dietary pattern with CVD. Multiple measurements of dietary intake over a longer period could strengthen the observed associations for two reasons. Firstly, averaging multiple measurements reduces random measurement error.[1,2] Secondly, multiple measurements over a longer period of time better represent long-term dietary intake since they take changes in dietary intake into account.[1]

Several studies investigated the effect of repeated measurements of single foods or nutrients on the strength of associations.[1,3-5] However, to the best of our knowledge, no previous study investigated the effect of measuring dietary patterns multiple times on the strength of the association with CVD. Two studies of the Nurses Health cohort used multiple measurements to estimate long-term dietary patterns in relation to breast cancer[6] and (cause specific) mortality[7]. In a sensitivity analysis they observed 'similar associations'[6] or 'similar but weaker associations'[7] for baseline measurements compared to multiple measurements of dietary patterns, though the strength of these associations was not reported. Also in a study on fish consumption in relation to major chronic diseases in the Health Professionals Follow Up cohort 'no appreciable difference' was reported between baseline intake only and five measurements of fish intake over 16 years based on a sensitivity analysis.[3] Three studies on nutrients and foods dealt with the issue of multiple measurements versus the baseline measurement.[1,4,5] One study was on alcohol consumption in relation to all cause mortality and coronary heart disease, one on tomato products and lycopene in relation to prostate cancer, and one on dietary fat and coronary heart disease.[1,4,5] These studies generally observed stronger associations for multiple measurements than for the baseline measurement only.[1,4,5] Overall, the studies carried out so far suggest that the effect of multiple measurements was stronger for nutrients than for foods and dietary patterns.

Besides improvements in dietary exposure measurement, also covariates may be better classified with multiple measurements. Though Hu et al. noted that the use of baseline or updated covariates yielded similar results in a study on dietary fat and coronary heart disease,[1] for some covariates recent exposure may be etiologically more relevant than baseline exposure, because effects are expected on the short-term instead of on the long-term. For example, Kenfield et al. noted that associations of former and current smoking with cardiovascular mortality were stronger when updated information was used.[8] Therefore, adjustment for smoking may improve when updated information is used. Assuming that associations are attenuated after better adjustment for confounders, we hypothesized that adjustment for recent confounders would result in weaker effect estimates for the associations between dietary patterns and CVD.

This paper describes the association of the Mediterranean Diet Score (MDS) with fatal CVD and a composite of fatal CVD, non-fatal myocardial infarction and stroke, comparing only the baseline measurement of the diet with three repeated measurements. In addition, we explored the impact of adjustment for most recent measurements of confounders compared to adjustment for the baseline measurement of confounders only.

SUBJECTS AND METHODS

Study population

The Doetinchem Cohort Study is a longitudinal cohort study of a general population sample from the town of Doetinchem, located in a rural area in the eastern part of the Netherlands. Ethical approval for this study was obtained from the Medical Ethics Committee TNO, Zeist, the Netherlands. During the first examination round (1987–1991) 12,405 men and women aged 20 to 59 years were examined. For the second round (1993–1997), a random two-third of those who were measured in the first round were re-invited. For the third (1998–2002) and fourth (2003–2007) round, all persons who participated in the second round were re-invited, excluding those who emigrated, actively withdrew from the study or had died. As of the second round, a validated[9,10] food-frequency questionnaire was used. Therefore, these data were used as baseline for the present study. The response rates for the measurement rounds ranged from 75% to 79%. More details of the Doetinchem Cohort Study are described elsewhere.[11]

To compare results of the baseline MDS with those of repeated measurements, we excluded persons who did not participate in all measurement rounds before censoring or getting an event. Furthermore, we excluded participants with prevalent CVD or type 2 diabetes, women who were pregnant, those with extremely low or high reported energy intakes (i.e., those in the lowest and highest 0.5% of the ratio of energy intake over estimated basal metabolic rate), people reporting to be on a diet prescribed by a doctor or a dietician, those with no information on dietary intake or any of the covariates, as well as those with no information on vital status or cardiovascular events. A total of 3,417 persons remained for the analyses on composite CVD and 3,357 persons for the analyses on fatal CVD.

Mediterranean Diet Score

Dietary intake was assessed by a validated[9,10] food frequency questionnaire (FFQ) which contained questions on the habitual frequency of consumption of 178 food items during the year preceding enrollment. Additional information was obtained on consumption frequency for different sub-items, and preparation methods. Colored photographs were used to estimate portion sizes of 28 food items.[9,10] Reproducibility and validity were assessed in 121 men and women.[9,10] The median

12-months reproducibility (Spearman correlation coefficients) of 16 food groups was 0.71 for men and 0.77 for women, ranging from resp. 0.45 and 0.63 for fish to 0.83 and 0.92 for alcoholic beverages.10 In a validation study with 12 monthly 24-hour recalls, the median relative validity of these 16 food groups was 0.61 for men and 0.53 for women, ranging from 0.32 for fish in men and 0.31 for vegetables in women to 0.74 (men) and 0.87 (women) for alcoholic beverages.[10]

We operationalized the concept of a healthy diet with the modified Mediterranean Diet Score (MDS) defined by Trichopoulou et al.[12] For the composition of this score, values of 0 or 1 were assigned to each nutritional component of the MDS using sex-specific medians. We used the median intakes at baseline as cut-off values for all subsequent measurement rounds. For vegetables, fruits, legumes & nuts, grains, fish & seafood, and the ratio of unsaturated to saturated fatty acids, intakes equal to or above the baseline median were assigned a value 1, and intakes below the median a value 0. As the traditional Mediterranean diet is characterized by a low intake of meat and dairy products, the scoring of these products was inverted. We dichotomized alcohol consumption into consumers and non-consumers, because of the relatively low level of alcohol consumption in this population. At baseline, 22% consumed more than two glasses of alcohol per day. A value of 1 was assigned for consuming at least one drink per month (users) and a value of 0 for consuming less than 1 drink per month (non-users). The MDS could take a value from 0 (minimal adherence) to 9 (maximal adherence)[12] and associations with CVD were assessed for the categories 0–3.5, >3.5–5.5, and >5.5–9.

Ascertainment of fatal and nonfatal CVD events

Participants were followed to the first nonfatal cardiovascular event, death, emigration, or were censored at January 1st 2008. Morbidity data were provided by the National Medical Register (NMR) using the Dutch Hospital Discharge data. Eighty-eight percent of the hospital admissions can uniquely be linked to an individual on the basis of sex, date of birth, and postal code.[13] We obtained vital status through linkage with municipal population registers. Subsequently, primary and secondary causes of death were obtained through linkage with data from 'Statistics Netherlands'.[14]

Composite CVD was defined as fatal CVD (ICD9[15] codes 390–459 and 798), plus nonfatal myocardial infarction (410–412 and 414) and stroke (430–434, and 436). Causes of death after 1996 were coded according to the corresponding ICD10[16] codes. For nonfatal myocardial infarction and stroke, only first events were taken into account. For fatal CVD, no exclusions were made because of previous nonfatal events.

Covariates

A self-administered general questionnaire provided information on age, sex, educational level, smoking status, and sports participation.[14] Educational level was operationalized as low (lower

vocational training or primary school), medium (secondary school and intermediate vocational training), or high (higher vocational training or university). Current cigarette smoking and sports participation (≥4 MET) were operationalized as yes or no.

Statistical analyses

Statistical analyses were performed using SAS 9.3 software (SAS Institute, INC., Cary, NC). Participants' characteristics were presented by measurement round as means (standard deviation) or medians (inter-quartile range) for continuous variables, and as percentages for categorical variables.

Cox proportional hazards models were used to estimate hazard ratios (HRs) and 95% confidence intervals (CI) for the relation of baseline MDS and the updated mean of the MDS with composite and fatal CVD incidence using all measurement rounds preceding the event or censoring. The proportional hazard assumption for baseline MDS in relation to composite and fatal CVD was fulfilled according to the graphical approach and according to Schoenfeld residuals. To calculate updated means of the MDS and of total energy intake, we calculated the means of these variables at the time of each consecutive measurement round from baseline until the first event or censoring.[1] The associations between the MDS and the CVD endpoints were adjusted for age, and sex. In the second model associations were additionally adjusted for baseline smoking, sports, total energy intake, and educational level. In the third model associations were additionally adjusted for the most recent levels of the same non-dietary covariates and the updated mean of total energy intake. To adjust for the most recent smoking (yes, no) and sports participation (yes, no) data we updated the information at each measurement round.[1] We did not include BMI, blood pressure or serum cholesterol in our analyses, since we consider these to be intermediates in the association between a Mediterranean style diet and CVD.

3

RESULTS

Table 3.1 shows the distribution of the participants' characteristics by measurement round. Participants were on average 45 years old at baseline, and 49% were men. At baseline, 27% of the participants were current smokers, which decreased to 20% after 10 years. At all measurement rounds, more than half of the participants performed sports. Total energy intake decreased from 2,200 kcal per day at baseline to 2,050 kcal per day after 10 years. Especially the consumption of grains, dairy, and meat products was lower at later rounds than at baseline. Median fruit consumption increased over 10 years from 125 to 172 grams per day and median fish consumption from 8 to 13 grams per day. Although median MDS was 5 at all three measurement rounds, 55% of the participants showed a change of at least 2 MDS points between two subsequent rounds. The Spearman correlation coefficients were 0.47 for MDS at baseline and five years later, and 0.39 for MDS at baseline and ten years later.

Table 3.1 Population characteristics Doetinchem Cohort Study, by measurement round

	Round 2 1993–1997	Round 3 1998–2002	Round 4 2003–2007
n	3,417	3,353	3,328
Age (y)[a]	45 (10)	50 (10)	55 (10)
Sex (% men)	49	49	49
Education (% low)[b]	51	48	47
Current smokers (%)	27	24	20
Alcohol users (%)	86	86	84
Sports participation (% yes)	54	55	58
Energy intake (kcal/day)	2,202 (1,863–2,629)	2,137 (1,820–2,543)	2,053 (1,745–2,411)
Mediterranean Diet Score[a]	5 (4–6)	5 (4–6)	5 (4–6)
MDS components			
Vegetables (gram/day)[a]	107 (83–134)	106 (83–135)	105 (81–134)
Fruit (gram/day)[a]	125 (85–242)	126 (81–243)	172 (89–247)
Legumes (gram/day)[a]	14 (8–23)	14 (7–21)	12 (6–20)
Nuts (gram/day)[a]	7 (2–14)	7 (3–15)	7 (2–15)
Grains (gram/day)[a]	210 (161–263)	205 (158–262)	197 (155–251)
Fish and seafood (gram/day)[a]	8 (3–14)	9 (4–16)	13 (7–18)
Dairy and dairy products (gram/day)[a]	418 (267–605)	395 (258–586)	366 (230–552)
Meat and meat products (gram/day)[a]	118 (84–149)	113 (78–144)	106 (73–136)
Unsaturated fatty acids (gram/day)[a]	50 (40–62)	48 (40–60)	47 (38–57)
Saturated fatty acids (gram/day)[a]	37 (30–45)	35 (28–43)	32 (26–39)
Alcohol consumption (gram/day)[a]	7 (1–17)	8 (2–19)	8 (2–20)

[a] Numbers are given as mean (sd) or as median (interquartile range); [b] Low educational level: lower vocational training or primary school.

During 10–15 years of follow-up (mean 12 years) 206 composite CVD events occurred, of which 38 were fatal. In **Table 3.2** the results for MDS in relation to CVD are shown, using baseline confounders for the model with baseline MDS and the most recent confounders for the model with the updated mean of the MDS. Since the associations were not linear, we describe the regression coefficients of the MDS-category of 5.5–9 compared to 0–3.5. Baseline MDS was associated with a 23% lower risk (HR: 0.77 [0.53–1.11]) of composite CVD and the updated mean with a 35% lower risk (HR: 0.65 [0.43–0.97]). For fatal CVD, baseline MDS was associated with a 13% lower risk (HR: 0.87 [0.36–2.07]) and the updated mean with a 56% lower risk (HR: 0.44 [0.19–1.05]).

Table 3.3 shows the influence of different ways to adjust the associations between the updated mean MDS and CVD for confounders. Compared to a model with only age and sex as covariates, additional adjustment for smoking, sports, energy intake and educational level resulted in weaker associations. Adjustment for the most recent levels of the confounders resulted in a

Table 3.2 Hazard ratios (95% CI) of baseline and updated mean Mediterranean Diet Score in relation to composite and fatal CVD. The Doetinchem Cohort Study

	MDS		
	0–3.5	3.5–5.5	5.5–9
Composite CVD			
Persons at risk, n	673	1,541	1,203
Cases, n	52	87	67
Baseline MDS	1.00 (ref)	0.72 (0.51–1.02)	0.77 (0.53–1.11)
Updated mean MDS	1.00 (ref)	0.68 (0.46–1.00)	0.65 (0.43–0.97)
Fatal CVD			
Persons at risk, n	664	1,513	1,180
Cases, n	9	16	13
Baseline MDS	1.00 (ref)	0.80 (0.35–1.82)	0.87 (0.36–2.07)
Updated mean MDS	1.00 (ref)	0.40 (0.17–0.93)	0.44 (0.19–1.05)

All analyses are adjusted for age, sex, smoking, sports, energy intake, and educational level. Analyses with baseline MDS as exposure were adjusted for the baseline levels of the confounders. Analyses with the updated mean MDS were adjusted for the most recent level of the confounders.

3

Table 3.3 Hazard ratios (95% CI) of baseline and updated mean Mediterranean Diet Score in relation to composite and fatal CVD; *different ways of adjustment*. The Doetinchem Cohort Study

	MDS		
	0–3.5	3.5–5.5	5.5–9
Composite CVD			
Persons at risk, n (round 2)	673	1,541	1,203
Cases, n (round 2)	52	87	67
Updated mean MDS			
Model 1	1.00 (ref)	0.66 (0.45–0.96)	0.58 (0.39–0.86)
Model 2	1.00 (ref)	0.70 (0.48–1.03)	0.68 (0.45–1.02)
Model 3	1.00 (ref)	0.68 (0.46–1.00)	0.65 (0.43–0.97)
Fatal CVD			
Persons at risk, n (round 2)	664	1,513	1,180
Cases, n (round 2)	9	16	13
Updated mean MDS			
Model 1	1.00 (ref)	0.40 (0.17–0.92)	0.43 (0.18–1.00)
Model 2	1.00 (ref)	0.43 (0.19–1.00)	0.50 (0.21–1.20)
Model 3	1.00 (ref)	0.40 (0.17–0.93)	0.44 (0.19–1.05)

Model 1: adjusted for age and sex;
Model 2: Model 1 + baseline smoking, sports, energy intake, and educational level;
Model 3: Model 1 + most recent smoking, most recent sports, updated mean energy intake, and educational level.

HR of 0.65 (0.43–0.97) for the association between updated mean MDS and composite CVD, while adjustment for the baseline level of the confounders resulted in a HR of 0.68 (0.45–1.02). For fatal CVD, adjustment for the most recent levels of the confounders resulted in a HR of 0.44 (0.19–1.05), while adjustment for baseline level of the confounders resulted in a HR of 0.50 (0.21–1.20).

DISCUSSION

This study showed that the updated mean of three measurements of a Mediterranean style diet over 10 years compared with the baseline measurement resulted in stronger associations and smaller confidence intervals for composite and fatal CVD. In contrast to our hypothesis, using the most recent levels of potential confounders did not attenuate the strength of the associations further.

We observed stronger associations of a Mediterranean style diet in relation to CVD for the updated mean of three measurements over 10 years compared to using the baseline measurement only. Other cohort studies on dietary patterns[6,7] or food groups[3] observed in sensitivity analyses 'similar' or 'similar but weaker' associations when using the baseline instead of the updated mean of dietary intake. Because these studies provided only the effect estimates of the updated mean and not of the baseline measurements, we were not able to compare the results quantitatively. In the present study, the updated mean reduced random measurement error and therefore resulted in less attenuation of the regression coefficients resulting in stronger associations. It is also possible that the updated mean, which reflects long-term diet, is etiologically more relevant than the most remote (baseline) measurement of the diet. We observed larger differences between the results for baseline compared to updated MDS in association with fatal CVD than for composite CVD. This may be explained by less misclassification for fatal than for non-fatal CVD cases.[17]

We observed only minor differences in the associations of MDS with CVD endpoints when the most recent levels of confounders were used instead of baseline values only. This may be due to the limited changes in smoking and sports behaviour in our population. Only 13% of the total population changed smoking habits during 10 years of follow-up, and 36% changed their sports behaviour. Daily energy intake changed during follow-up, however, adjustment for daily energy intake hardly attenuated our associations. Hu et al. also noted that the use of baseline or updated covariates yielded similar results.[1]

Although median MDS remained stable in the present study, individuals changed their level of adherence to the MDS in the different rounds. About 55% of the participants had a change of at least two MDS points between subsequent rounds. Using only baseline MDS likely resulted in misclassification of dietary intake of these people. The Spearman correlation coefficient was 0.47 for the MDS at baseline and after five years, and 0.39 for the MDS at baseline and after

ten years. These are low correlations compared to the study of Sijtsma et al. who observed correlation coefficients around 0.6 for time periods of 7–20 years for an a-priori diet score of the 'whites' in the CARDIA-study.[18] Correlation coefficients between repeated measurements of dietary patterns observed in the CARDIA study were similar to those of serum cholesterol and blood pressure.[18,19]

We quantified for the first time the effect of updated mean measurements of a dietary pattern score on the strength of the association with CVD compared to the baseline measurement. A limitation of our study is that the results for fatal CVD were based on only 38 cases, resulting in large and overlapping confidence intervals. Therefore, our study should be repeated in other cohorts. The present study also has advantages. The food frequency questionnaires, as well as the questions on the covariates remained the same during 10 years of follow-up. In addition, our study was performed in a general population sample.

In conclusion, the present study showed that the use of multiple measurements of the Mediterranean Diet Score increased the strength of the associations with composite and fatal CVD and narrowed the confidence intervals of these associations. Therefore, it is likely that the association has been underestimated because most studies so far used only the baseline measurement.

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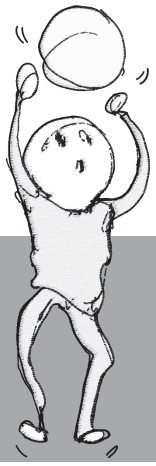

Chapter 4

Cycling and sports, but not walking, are associated with 10-year cardiovascular disease incidence. The MORGEN Study

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ABSTRACT

Background: Physical activity is inversely related to cardiovascular diseases. However, the type of activities that contribute most to these beneficial effects remain unclear. For this reason, we investigated self-reported leisure time physical activities in relation to fatal/non-fatal cardiovascular disease incidence.

Design: The Dutch Monitoring Project on Risk Factors for Chronic Diseases (MORGEN Study), carried out between 1993 and 1997, is a prospective cohort study of over 23,000 men and women aged 20–65 years from the general Dutch population.

Methods: From 1994 until 1997 physical activity was assessed with a questionnaire in 7,451 men and 8,991 women who were followed for an average of 9.8 years. Cox Proportional Hazards models were used adjusting for age, sex, other physical activities, smoking, alcohol consumption and educational level.

Results: Almost the entire study population (97%) was engaged in walking, about 75% in regular cycling and about half the population in sports or gardening. Cycling (HR: 0.82; 95% CI: 0.71–0.95) and sports (HR: 0.74; 95% CI: 0.64–0.87) were both inversely related to cardiovascular disease incidence, whereas walking and gardening were not. For sports ($p<0.001$), but not for cycling ($p=0.06$), we observed a dose–response relationship with respect to cardiovascular disease incidence. Engaging in both cycling and sports resulted in an even greater risk reduction (HR: 0.64; 95% CI: 0.52–0.77).

Conclusions: In this relatively active population, types of activities of at least moderate intensity, such as cycling and sports, were associated with lower CVD incidence, whereas activities of lower intensity, such as walking and gardening, were not.

INTRODUCTION

Over the past few decades, many epidemiological studies have demonstrated a clear inverse association between physical activity and cardiovascular diseases (CVD).[1] In a recent meta-analysis, including almost 900,000 participants, physical activity was associated with a 35% lower risk of fatal CVD when comparing active with inactive individuals.[1] Early studies focused mainly on occupational physical activity and total physical activity. Over the years, however, interest has shifted towards daily leisure time physical activities, such as walking and cycling, because of a wider range of activities that are also easier to modify than occupational physical activities. Therefore leisure time physical activities make a suitable target for public health promotion.

Several investigations have observed associations between leisure time physical activity and CVD.[2,3] However, it remains unclear what specific type of activities contribute most to these beneficial effects, since few studies have addressed the impact of different types of activities on CVD.[4-6] For this reason, we aimed at investigating specific types of leisure time physical activity (walking, gardening, cycling and sports) in relation to CVD incidence in a population-based sample of the Dutch population aged 20–65 years at baseline. We evaluated dose–response relationships and the impact of combinations of different types of activities.

METHODS

Study population

The MORGEN Study was carried out in the Netherlands between 1993 and 1997 in 10,448 men and 12,652 women. Random sex–age stratified samples were drawn from municipal population registers in three towns in the Netherlands (Doetinchem, Maastricht and Amsterdam). The average response rate in the MORGEN Study was 45%.[7]

From 1994 until 1997 physical activity was assessed with an identical questionnaire for 8,142 men and 9,778 women. Exclusion criteria for the present analyses were no informed consent for follow-up of vital status (n=1,017), pregnancy at baseline (n=106), prevalent myocardial infarction or stroke (n=342) and missing data on physical activity (n=13). After these exclusions, 7,451 men and 8,991 women remained for the present analyses.

Measurements

Questionnaires

Physical activity was assessed with a validated[8] physical activity questionnaire designed for the "European Prospective Investigation into Cancer and nutrition" (EPIC) including questions on occupational and leisure time physical activity. The question on cycling included cycling towards

and from work and in leisure time as an example and the question on walking additionally included shopping as an example. For the MORGEN Study, the questionnaire was extended with two open-ended questions on type, frequency, and duration of sports, and one open-ended question on other strenuous activities.

A Dutch reproducibility and validity study showed that the EPIC questionnaire was suitable for ranking participants according to their physical activity level.[8] The 11-month reproducibility (Spearman correlation coefficients) of the questionnaire for men yielded: 0.57 for walking, 0.47 for cycling, and 0.71 for gardening and for women, 0.57, 0.72, and 0.67, respectively.[8] Spearman correlation coefficients relative to a three-day activity diary, repeated four times, were, for men: 0.32 for walking, 0.46 for cycling, and 0.43 for gardening and odd jobs and for women, 0.33, 0.48, and 0.47, respectively.[8]

In general, health effects may be expected from at least moderate intensity physical activity,[9] which in the Netherlands has been defined as at least 4.0 metabolic equivalents (METs).[10] Although walking (3.5 METs) does not classify as 'moderate intensity' in the Netherlands, this activity has repeatedly been shown to be associated with reduced risk of CVD.[11-13] Moreover, walking is an important leisure time activity in the Netherlands, both in terms of frequency and duration. Therefore, we investigated walking (Ainsworth code: 17250[14]; 3.5 METs), gardening (Ainsworth code: 08245[14]; 4.0 METs), cycling (Ainsworth code: 02010[14]; 7.0 METs), and sports (≥4.0 METs) as specific types of leisure time physical activity possibly related to CVD.

Time spent on occupational physical activities (continuous) of at least moderate intensity was included as confounder. Gardening, cycling and sports were classified as 'yes', or 'no'. Since 97% of all participants reported at least some walking, participants who reported <3.5 hr/wk (30 minutes per day) were used as the reference category for walking. To study dose–response relationships, we divided cycling and sports into three categories (0 hr/wk, >0–3.5 hr/wk, and ≥3.5 hr/wk).

Educational level was classified as low (lower vocational training or primary school), medium (secondary school and intermediate vocational training), or high (higher vocational training or university). Smoking was categorized as yes or no, and alcohol consumption as none, moderate (male: ≤2 glasses / female: ≤1 glass per day), or high (male: >2 glasses / female: >1 glass per day). Self-reported CVD risk factor medication was defined as using cholesterol lowering medication or antihypertensive agents.

Physical examination

Weight and height were measured to the nearest 0.1 kg and 0.5 cm without shoes. Body Mass Index (BMI) was calculated as weight in kilograms (minus one kilogram for light clothing) divided by height in meters squared. BMI was classified as normal (<25 kg/m^2), overweight (25–30 kg/m^2), or obese (≥30 kg/m^2). Non-fasting plasma total cholesterol and HDL cholesterol were determined in the Lipid Reference Laboratory of the University Hospital Dijkzigt in Rotterdam using standardized enzymatic methods.[15] Systolic blood pressure was recorded twice with a

Random Zero Sphygmomano-meter at the appearance of sounds (first-phase Korotkoff) in sitting position.[15] The average of the two blood pressure measurements was included in current analyses.

Ascertainment of fatal and non-fatal events

Information on mortality and morbidity follow-up was available up to January 1st 2006. Vital status was identified using the municipal population register with a loss-to-follow-up below 0.1%. Cause of death was obtained from Statistics Netherlands and morbidity data were provided by the national hospital discharge register (HDR). On the national level, at least 88% of the hospital admissions can be uniquely linked to a single person.[16] In a validation study with an approximate 33% overlap of participants from our study, a high sensitivity (84%) and a positive predictive value (97%) were observed for CHD hospital admissions.[17]

Fatal (primary or secondary cause of death) and non-fatal CVD were defined according to ICD-9[18], codes 410–414, 415.1, 427.5, 428, 430–438, 440–444, 798.1, 798.2 and 798.9 and corresponding ICD-10[19] codes for the fatal cases after 1996.

Statistical analyses

Cox Proportional Hazards models were used to estimate hazard ratios and 95 percent confidence intervals. Participants were either followed up to the first non-fatal cardiovascular event, death, emigration, or were censored at January 1st 2006.

Three consecutive models were used. The first model was adjusted for age and sex. The second model was additionally adjusted for hours per week spent on physical activities (both occupational and leisure time) other than the one under study, lifestyle factors (smoking and alcohol consumption), and educational level. Since biological risk factors such as BMI, total and HDL cholesterol, and systolic blood pressure are possible intermediates in the association between physical activity and CVD, we additionally adjusted for these factors in a third model. Since no difference occurred in the hazard ratios, we will report only the hazard ratios of the second model. There was no interaction with age (younger or older than 50 years), nor with sex and the proportional hazard assumption was satisfied. To study dose–response relationships, we performed tests for linear trend using the median values of the respective category as continuous parameters. Analyses were performed using SAS 9.1 software (SAS Institute, INC., Cary, NC).

RESULTS

Baseline characteristics of the study population are shown in **Table 4.1**. Participants were, on average, 41.9 ± 11.2 years old at baseline, and 45% were male. Walking and cycling contributed the most to the time spent on leisure time physical activities. Almost the entire study population was engaged in walking (97%), about 75% in regular cycling and about half of the population

in gardening or sports. Intensities of sports ranged from 4.0 to 12.0. **Table 4.2** shows the 10 most frequently reported sports for men and women. During 9.8 years of follow-up, 362 fatal and non-fatal CVD events occurred in women, compared to 561 in men.

Table 4.1 Characteristics of participants in the MORGEN Study, 1994–1997[a,b]

	Men (n=7,451)	Women (n=8,991)
Demographic factors		
Age (years)	42.4 (11.1)	41.6 (11.4)
Education (%)[c]		
Low	41.9	50.9
Medium	30.2	26.9
High	28.0	22.2
Leisure time physical activity (%)		
Walking (3.5 MET)	96.4	96.5
Gardening (4.0 MET)	54.5	51.7
Cycling (7.0 MET)	74.7	78.0
Sports (≥4.0 MET)	42.5	41.1
Lifestyle factors (%)		
Current smoking	35.2	35.5
Alcohol[d]		
Never/former	8.5	18.2
Moderate	56.9	57.5
High	34.6	24.2
Biological risk factors		
BMI (kg/m^2)	25.4 (3.6)	24.7 (4.3)
Total cholesterol (mmol/l)	5.3 (1.1)	5.2 (1.0)
HDL cholesterol (mmol/l)	1.2 (0.3)	1.5 (0.4)
Systolic blood pressure (mmHg)	125 (15)	118 (16)
Self-reported CVD risk factor medication (%)[e]	5.2	5.4
Cardiovascular cases (n)		
Fatal CVD	66	28
Coronary heart disease	38	10
Stroke	7	9
Non-fatal CVD	526	346
Coronary heart disease	340	196
Myocardial infarction	116	61
Angina pectoris	117	71
Stroke	77	63

BMI, body mass index; HDL, high-density lipoprotein; CVD, cardiovascular disease.
[a] With consent for follow-up and free from baseline CVD, pregnancy and without missing data on physical activity, n=16,442; [b] Data presented as mean (sd); [c] low (lower vocational training or primary school), medium (secondary school and intermediate vocational training), high (higher vocational training or university); [d] Moderate (male: ≤2 glasses / female: ≤1 glass per day), high (male: >2 glasses / female: >1 glass per day); [e] Cholesterol-lowering and/or antihypertensive medication.

Table 4.2 Ten most frequent reported sports for men and women in the MORGEN Study

Sport	Ainsworth code (2000)[a]	Intensity (MET)[a]	Ainsworth description[a]	n men or women	% men or woman
Men					
soccer	15610	7	soccer, casual, general	690	9
tennis	15675	7	tennis, general	624	8
swimming	18310	6	swimming, leisurely, not lap swimming, general	412	6
jogging	12020	7	jogging, general	401	5
running	12150	8	running	363	5
aerobic fitness	2060	5.5	health club exercise, general	352	5
volleyball	15710	4	volleyball	240	3
squash	15650	12	squash	195	3
badminton	15030	4.5	badminton, social singles and doubles, general	177	2
snowboarding	19160	6	skiing, downhill, moderate effort, general	152	2
Women					
swimming	18310	6	swimming, leisurely, not lap swimming, general	995	11
aerobics	3015	6.5	aerobic dancing, general	742	8
tennis	15675	7	tennis, general	640	7
fitness for strength	2030	6	calisthenics, home exercise, light or moderate effort	435	5
gymnastics	15300	4	gymnastics, general	420	5
aerobic fitness	2060	5.5	health club exercise, general	371	4
jogging	12020	7	jogging, general	245	3
volleyball	15710	4	volleyball	230	3
snowboarding	19160	6	skiing, downhill, moderate effort, general	200	2
mountain walking	17210	6	walking, 3.5 mph, uphill	181	2

[a] This information was derived from Ainsworth et al.[14]

Walking or gardening were not associated with CVD incidence. Inverse associations with CVD incidence were observed for cycling (HR: 0.82; 95% CI: 0.71–0.95) and sports (HR: 0.74; 95% CI: 0.64–0.87) (**Table 4.3**). Cycling for up to 3.5 hr/wk was protective (HR: 0.82; 95% CI: 0.69–0.97) compared to not cycling, but cycling for 3.5 hr/wk or more did not give additional protection (p for trend=0.06) (**Figure 4.1**). The dose–response relationship was more pronounced for sports than for cycling. Engaging in sports for up to 3.5 hr/wk resulted in a 23% lower risk for CVD incidence (HR: 0.77; 95% CI: 0.65–0.91) and in a 34% (HR: 0.66; 95% CI: 0.50–0.88) lower risk when more than 3.5 hr/wk were spent on sports, compared to not engaging in sports (p for trend <0.001).

Engaging in both cycling and sports increased the protective effect of these separate types of activities on CVD incidence (**Figure 4.2**). The HR for engaging in cycling, but not in sports, was 0.83 (95% CI: 0.70–0.97), for sports, but no cycling, 0.73 (95% CI: 0.53–0.99), and for both cycling and sports 0.64 (95% CI: 0.52–0.77).

Table 4.3 Hazard ratios of walking, gardening, cycling and sports in relation to CVD incidence in participants aged 20–65 years at baseline in the MORGEN Study, 1994–1997[a]

		CVD						
		n at risk	n cases	Person years	Model 1[b]		Model 2[c]	
Walking	<3.5 hr/wk	3,464	196	33,503	1.00	--	1.00	--
	≥3.5 hr/wk	12,978	727	124,169	1.07	(0.91–1.25)	1.06	(0.91–1.25)
Gardening	No	7,735	397	73,960	1.00	--	1.00	--
	Yes	8,707	526	83,712	0.90	(0.79–1.03)	0.94	(0.83–1.08)
Cycling	No	3,862	290	36,547	1.00	--	1.00	--
	Yes	12,580	633	121,125	0.74	(0.65–0.86)	0.82	(0.71–0.95)
Sports[d]	No	9,581	672	91,461	1.00	--	1.00	--
	Yes	6,861	251	66,211	0.67	(0.58–0.77)	0.74	(0.64–0.87)

[a] Participants were followed up to January 1st 2006; [b] Adjusted for age (continuous) and sex; [c] Additionally adjusted for other physical activities (both occupational and leisure) than the one under study, current smoking (yes, no), alcohol consumption (never/former, moderate, or high) and educational level (low, medium, or high); [d] Sports ≥4.0 METs.

DISCUSSION

In this relatively active population, cycling and sports were both inversely associated with CVD incidence, but walking and gardening were not. For sports, but not for cycling, a dose–response relationship was observed with CVD incidence. Furthermore, the results of our study suggest that engaging in both cycling and sports was strongly related to CVD incidence.

Our results showed that specific leisure time physical activities of higher intensity, such as cycling and sports, were inversely associated with CVD incidence in contrast to activities of lower intensity, such as walking and gardening. Moderate to vigorous intensity physical activity has been hypothesized to reduce blood pressure, improve the blood lipid profile, endothelial function, lower systemic inflammation, and to have an anti-thrombotic effect and will thereby lower the damage due to atherosclerosis of the cardiac, cerebral, and peripheral blood vessels.[20,21] Since adjustments for blood pressure, HDL and total cholesterol in our investigation had no influence on the associations of the activities with CVD incidence, these factors cannot explain our results. We were not able to asses the effects of systemic inflammation, endothelial function, and the antithrombotic effect on our results.

Several large prospective investigations and a recent meta-analysis observed inverse associations between walking and CVD[11-13] but we did not find an association. A possible explanation could be that in our investigation the validity of walking is lower than for the other activities, which

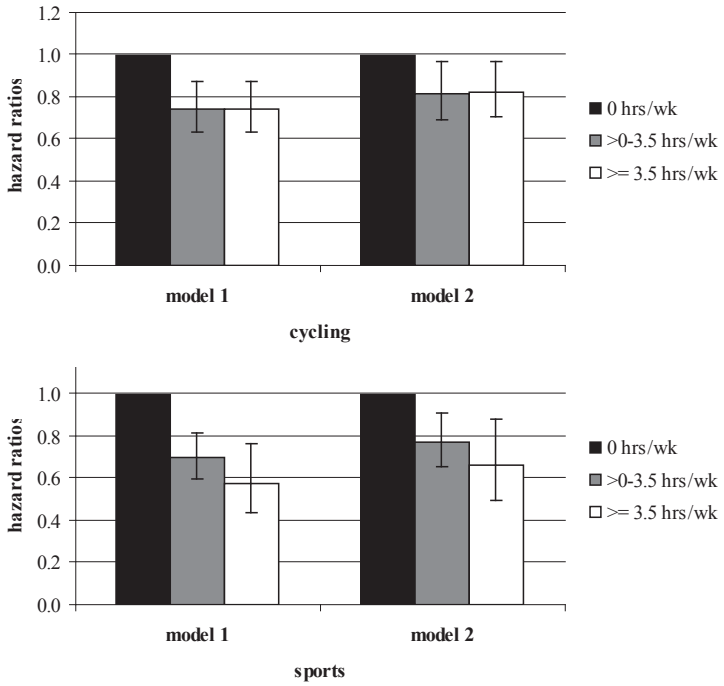

Figure 4.1 Dose–response relationship between sports, cycling and CVD incidence.
Model 1 was adjusted for age (continuous) and sex; model 2 was additionally adjusted for other physical activities (both occupational and leisure) than the one under study, current smoking (yes, no), alcohol consumption (never/former, moderate, or high) and educational level (low, medium, or high).

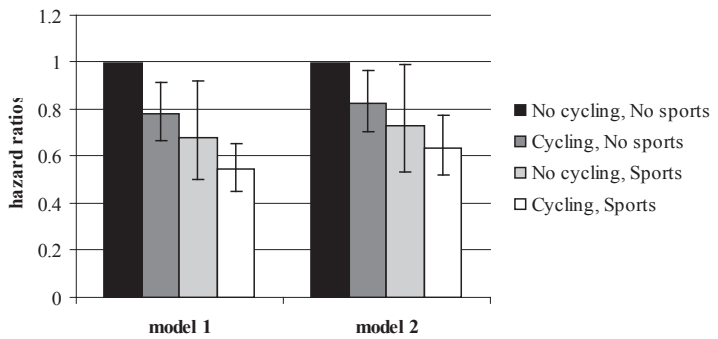

Figure 4.2 Hazard ratios of CVD incidence for the combination of cycling and sports with no cycling and sports as reference category.
Model 1 was adjusted for age (continuous) and sex; model 2 was additionally adjusted for other physical activities (both occupational and leisure) than the one under study, current smoking (yes, no), alcohol consumption (never/former, moderate, or high), and educational level (low, medium, or high).

might have attenuated an existing association. However, activities of lower intensity generally have lower reproducibility and validity than activities of higher intensity.[13] Therefore, the lower validity of walking cannot fully explain the lack of association in the present study where others have observed an association. The most likely explanation for not finding an association is that almost everybody walked. Only 3% of our population did not walk at all, and 21% walked less than 3.5 hours per week or 30 minutes per day. The mean walking duration among those who reported any walking in the present study was 13.6 ± 12.2 hrs/wk and the median 9.0 (interquartile range: 4.0–20.0) hrs/wk. One of the reasons for this high frequency and duration of walking could be that walking was assessed in a broad sense e.g. not only taking a walk but also walking for transportation, shopping and walking at work.[4,6] In a population with a walking pattern comparable to the current population, no association with CVD was observed,[5] which suggests that walking lowers risk in relatively inactive populations only.

Consistent with our finding, Andersen et al. observed a 38% lower risk of all cause mortality for cycling on average three hours per week compared to not cycling.[22] We found only two studies investigating the independent association between cycling and CVD.[5,6] These investigations did not find any associations, which might be due to the limited number of participants cycling a substantial amount of time. The protective association we observed for sports in relation to CVD is consistent with the literature.[4,5,23-25]

This study had some limitations. First, physical activity was recorded only at baseline. Second, as in most other prospective studies, our results were based on self-reported physical activity. Although our questionnaire was shown to be reproducible and valid,[8] recall and social desirability bias may have occurred. Especially for a low intensity activity as walking recall bias is possible. Both limitations could have resulted in bias towards the null hypothesis. A major strength of the present study is the large study population of relatively young (20–65 years) and active men and women from the Netherlands. The Netherlands is a country with a relatively high level of physical activity compared to other countries.[26,27] Moreover, we were able to study cycling in detail, since cycling is a frequently used means of transportation in the Netherlands. Another strength is the extensive physical activity questionnaire which made it possible to address several individual leisure time physical activities simultaneously. Only few studies have reported multiple separate types of activities in relation to CVD. Furthermore, the comprehensive data collection made it possible to adjust for several confounders.

We conclude that in this relatively active population, types of activities of at least moderate intensity, such as cycling and sports, were associated with lower CVD incidence, whereas activities of lower intensity, such as walking and gardening, were not. Engaging in both cycling and sports gave the greatest risk reduction, and may therefore be a good way to prevent CVD.

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Chapter 5

Sleep duration and sleep quality in relation to 12-year cardiovascular disease incidence. The MORGEN Study

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ABSTRACT

Study objectives: We studied sleep duration and sleep quality in relation to cardiovascular disease (CVD) incidence.

Design/setting: Dutch population-based cohort study.

Participants: 20,432 men and women aged 20–65 y with no history of CVD.

Interventions: N/A

Measurements: Sleep duration and sleep quality were assessed by a self-administered questionnaire. Morbidity data, vital status, and causes of death were obtained through linkage with several national registries. Hazard ratios (HRs) and 95% confidence intervals (95% CIs) were calculated using Cox proportional hazards models.

Results: During 10–15 years of follow-up, 1,486 CVD and 1,148 coronary heart disease (CHD) events occurred. Short sleepers (≤6 h) had a 15% higher risk of total CVD (HR: 1.15; 95% CI: 1.00–1.32) and a 23% higher risk of CHD (HR: 1.23; 95% CI: 1.04–1.45) compared to normal sleepers (7 h) after adjustment for all confounders. Additional adjustment for intermediate biological risk factors attenuated these relative risks to 1.11 (0.97–1.27) for total CVD and to 1.19 (1.00–1.40) for CHD. Short sleepers with poor sleep quality had a 63% higher risk of CVD (HR: 1.63; 95% CI: 1.21–2.19) and a 79% higher risk of CHD incidence (HR: 1.79; 95% CI: 1.24–2.58) compared to normal sleepers with good sleep quality, after adjustments for all confounders. We observed no associations between long sleep duration (≥9 h) and CVD or CHD incidence.

Conclusions: Short sleepers, especially those with poor sleep quality, have an increased risk of total CVD and CHD incidence. Future investigations should not only focus on sleep duration, but should also take sleep quality into account.

INTRODUCTION

Cohort studies reported conflicting results for the association of short sleep duration with the occurrence of cardiovascular diseases (CVD). In a recent meta-analysis incorporating 15 studies by Cappuccio et al.,[1] short compared to normal sleep duration was not related to total CVD (RR: 1.03 [95% CI 0.93–1.15]), though it was related to coronary heart disease (CHD) (RR: 1.48 [1.22–1.80]) and stroke (RR: 1.15 [1.00–1.31]). For long compared to normal sleep duration, Cappuccio et al reported an RR of 1.41 (1.19–1.68) for total CVD, 1.38 (1.15–1.66) for CHD, and 1.65 (1.45–1.87) for stroke.[1]

The relation between short sleep duration and CVD incidence could be due to an effect of short sleep on intermediate biological CVD risk factors such as BMI, blood lipids, high blood pressure, and prevalence of diabetes.[2-4] The association between long sleep duration and CVD incidence may be explained by long sleep duration being an early symptom of disease and preceding clinical diagnoses.[2] Also, sleep quality may modify the association between sleep duration and CVD. Sleep quality is an important factor in the physiologic recovery of the body during sleep, and good sleep quality may prevent CVD.[5] Therefore, short and long sleep duration may be adequate for those with good sleep quality but not for those with poor sleep quality. Chandola et al. were the first to demonstrate that the association between short sleep duration and CHD was strongest among those with sleep disturbance.[6]

The purpose of the present study was to investigate the association between short and long sleep duration and total CVD and CHD incidence, independent of lifestyle related factors, subjective health and educational level. We investigated mediation by biological CVD risk factors by adjusting for these factors. In a subsample of our population, we explored the combined associations of sleep duration and sleep quality with CVD and CHD incidence.

METHODS

Study population

The MORGEN Study (Monitoring Project on Risk Factors and Chronic Diseases in the Netherlands) was carried out in the Netherlands between 1993 and 1997 in 23,033 participants (10,422 men and 12,611 women). Random sex- and age-stratified samples were drawn from municipal population registers in 3 towns in the Netherlands (Doetinchem, Maastricht, and Amsterdam). The average response rate in the MORGEN Study was 45%.[7] We excluded participants with prevalent CVD based on self-report and hospital admission data, women who were pregnant at baseline, those with no information on sleep duration, sleep quality, or any of the covariates, as well as those with no follow-up of vital status or of cardiovascular events. In total 20,432 participants 9,217 men and 11,215 women remained for our analyses. The Medical Ethics

Committee of the Netherlands Organization for Applied Scientific Research (TNO) approved the study protocol and all participants signed an informed consent form.

Sleep duration and sleep quality

Information on sleep duration and on sleep quality was obtained by a self-administered questionnaire. The average sleep duration was assessed by asking "How many hours of sleep do you usually get per 24-hour period?" with answer categories "5 hours or less," "6 hours," "7 hours," "8 hours," and "9 hours or more." Short sleep duration was defined as sleeping ≤6 h, normal sleep duration as 7 (reference group) or 8 h, and long sleep duration as ≥9 h.

Information on sleep quality was obtained only in the first 2 years of baseline measurements (1993 and 1994; n=8,341) as part of the Amsterdam Biographical Questionnaire on neuroticism.[8] It was assessed by asking "Do you usually rise rested?" with answer categories "yes," "don't know," or "no". "Good sleep quality" was defined as "yes" for rising rested and "poor sleep quality" was defined as "no" for rising rested. We excluded those answering "don't know" (n=1,658, 20%) from the analyses regarding sleep quality. Thus 6,683 participants remained for our analyses on sleep quality.

Ascertainment of non-fatal and fatal CVD and CHD events

After enrolment in the MORGEN Study, the participants were followed for the occurrence of non-fatal and fatal CVD and CHD by linkage with registries. Morbidity data were provided by the National Medical Registry (NMR) using the Dutch Hospital Discharge Diagnosis Database (HDR).Vital status was obtained through linkage with the municipal population registries. Subsequently, primary and secondary causes of death were obtained through linkage with "Statistics Netherlands." Information on morbidity and mortality follow-up was available up to January 1, 2008.[7] Non-fatal and fatal CVD were defined according to ICD-9[9] codes 410– 414, 415.1, 427.5, 428, 430–438, 440–444, 798.1, 798.2, and 798.9; or ICD-10[10] codes G45, I21–I26, I46, I50, I60–I67, I69 I70–I74, R96 for the fatal cases after 1996. CHD was defined as ICD9 codes 410–414 or ICD10 codes I20–I25.

Covariates

Information on educational level, current smoking, physical activity, alcohol and coffee consumption, subjective health and CVD risk factor medication were assessed by a self-administered questionnaire. Educational level was assessed as the highest level of education reached. Smoking was assessed as "yes," "no," or "former." Information on physical activity was assessed with the validated[11] and extended[12] EPIC questionnaire during the period 1994–1997. We included cycling and sports as covariates, since in our population only those two activities were inversely associated with CVD incidence.[12] Subjective health was assessed by a self-

administered questionnaire by asking "How do you rate your health in general?" with five answer categories ranging from "excellent" to "bad." Self-reported CVD risk factor medication was defined as using cholesterol lowering medication or antihypertensive agents. Prevalent cases of diabetes were identified through linkage with the Dutch HDR (1990–97) and by self-report. Cases detected by either of these methods were verified by consulting medical records of general practitioners.[7] Standardized measurements of weight, height, and systolic and diastolic blood pressure were obtained, and blood was drawn by trained personnel during a visit to the municipal health service. Non-fasting plasma glucose and total and HDL cholesterol were measured with commonly used methods. A more detailed description of these measurements has previously been published by Beulens et al.[7]

Statistical analyses

Statistical analyses were carried out using SAS statistical software version 9.2 (SAS Institute, INC., Cary, NC). Participants' characteristics are presented as mean ± SD for continuous variables, or percentages for categorical variables, in strata of sleep duration categories. Participants were followed up to the first non-fatal cardiovascular event, death, emigration, or were censored at January 1, 2008, whichever came first. Cox proportional hazards models were used to estimate hazard ratios (HRs) and 95% confidence intervals (95% CIs) of total CVD and CHD incidence in sleep categories. Participants with a normal sleep duration of 7 h were considered as the reference group. The Cox proportional hazard assumption was fulfilled according to the graphical approach and according to Schoenfeld residuals.

To assess sleep duration and sleep quality in relation to total CVD and CHD incidence, four models were used. The first model was adjusted for age and sex. In the second model we additionally included current smoking (yes, no), alcohol consumption (male: none, >0–2, >2 glasses per day/ female: none, >0–1, >1 glass per day) and coffee consumption (<6, ≥6 cups a day) in the model. In the third model we additionally included educational level (low [lower vocational training or primary school], medium [secondary school and intermediate vocational training], or high [higher vocational training or university]) and subjective health (bad-reasonable, good-excellent). In the fourth model we assessed mediation by biological risk factors, by additionally adjusting for BMI (continuously), total-/HDL cholesterol ratio, systolic blood pressure (continuously), CVD risk factor medication (yes, no), and prevalent type 2 diabetes (yes, no).

To test for interaction in the association between sleep duration and total CVD with age (younger or older than 50 years), sex, current smoking, hypertension, BMI categories (<25, 25–30, ≥30 kg/ m^2) and sleep quality, we added interaction terms to models adjusted for age and sex. Only the interaction term between sleep quality and sleep duration was statistically significant at $\alpha<0.05$.

To minimize the possibility that sleeping habits have changed in response to subclinical disease, the analyses were repeated after exclusion of cases in the first 2 years of CVD follow-up. Additionally,

sensitivity analyses were performed in which participants with underweight (BMI <18.5; n=327), prevalent diabetes (n=189), and those with CVD risk factor medication (n=1,015) were excluded.

RESULTS

During 10–15 years of follow-up (mean 11.9 years) 1,486 total CVD events and 1,148 CHD events occurred. In total 3,387 (17%) participants were short sleepers, and 1,480 (7%) long sleepers. At baseline, short sleepers were generally older, more likely to be male, and had a less favorable risk profile than normal sleepers (**Table 5.1**). Long sleepers were of the same age, less likely to be male, had less favorable levels of lifestyle factors, and similar levels of biological risk factors compared to normal sleepers.

After adjustment for relevant confounders (age, sex, lifestyle factors, subjective health, and educational level; model 3), short sleepers had a 15% higher risk of total CVD incidence (HR: 1.15; 95% CI: 1.00–1.32) and a 23% higher risk of CHD incidence (HR: 1.23; 95% CI: 1.04–1.45) compared to normal sleepers (**Table 5.2**). In the subgroup of participants with information on physical activity (n=16,183), additional adjusting for cycling (yes, no) and sports (yes, no)[12] did not further attenuate the association (results not shown). Adjustment for intermediate biological risk factors (BMI, total-/HDL cholesterol ratio, systolic blood pressure, CVD risk factor medication, and prevalent type 2 diabetes; model 4) diminished the relative risks to 1.11% for total CVD incidence (HR: 1.11; 95% CI: 0.97–1.27) and to 1.19% for CHD incidence (HR: 1.19 [1.00–1.40]). Long sleep duration was not associated with total CVD incidence (HR: 0.94; 95% CI: 0.76–1.16; model 3), although long sleepers tended to have a lower risk of CHD incidence (HR: 0.77; 95% CI: 0.58–1.02; model 3).

In a subgroup of 6,683 participants data was available on sleep quality. After adjustment for age and sex, participants with poor sleep quality had a 22% (HR: 1.22; 95% CI: 1.02–1.44) higher risk of CVD and a 34% (HR: 1.34; 95% CI: 1.08–1.66) higher risk of CHD incidence than those with good sleep quality (**Table 5.3**). After additional adjustment for sleep duration, lifestyle factors, subjective health, and educational level, this higher risk was no longer statistically significant (HR CVD: 1.05; 95% CI: 0.87–1.26/ HR CHD: 1.18; 95% CI: 0.94–1.49). Interactions between sleep duration and sleep quality in relation to CVD and to CHD were significant (both $p<0.05$). After adjustment for all relevant confounders and compared to participants with a normal sleep duration and good sleep quality, those with short sleep duration or with poor sleep quality were not at higher risk of CVD or CHD incidence, whereas those with both short sleep duration and poor sleep quality had a 63% higher risk of CVD (HR: 1.63; 95% CI: 1.21–2.19) and a 79% higher risk of CHD incidence (HR: 1.79; 95% CI: 1.24–2.58) (**Figure 5.1**). Among short sleepers, in our full model (adjusted for age, sex, smoking, alcohol consumption, coffee, subjective health, and educational level) we observed an HR of 1.32 (95% CI: 0.95–1.84) for poor compared to good quality in relation to total CVD and an HR of 1.57 (95% CI: 1.05–2.36) for CHD.

Table 5.1 Baseline characteristics of men and women; the MORGEN Study, 1993–1997

	Sleep duration			
	≤6 h (n=3,387)	7 h (n=8,216)	8 h (n=7,349)	≥9 h (n=1,480)
Demographic factors				
Age (years)[a]	44.3 (10.3)	41.7 (10.6)	41.5 (11.8)	41.4 (12.9)
Sex (male, %)	54	50	38	32
Education (%)				
Low	54	44	49	57
Lifestyle factors (%)				
Current cigarette smoking	40	35	33	35
Cycling [b]	70	80	79	72
Sports[b]	36	46	43	36
Alcohol				
None	15	10	15	23
Moderate[c]	52	58	60	54
Coffee consumption (≥6 cups a day)	44	39	31	28
General health (%)				
Good subjective health	82	92	91	79
Sleep quality[d]				
Good	39	53	58	44
Don't know	21	21	19	16
Poor	40	26	24	40
Biological risk factors				
BMI (kg/m^2)[a]	25.7 (4.1)	24.9 (3.8)	24.8 (3.9)	24.9 (4.3)
Overweight (25–30 kg/m^2, %)	39	35	33	34
Obesity (≥30 kg/m^2, %)	14	9	9	11
Total cholesterol (mmol/L)[a]	5.4 (1.1)	5.2 (1)	5.3 (1.1)	5.3 (1.1)
HDL cholesterol (mmol/L)[a]	1.3 (0.4)	1.4 (0.4)	1.4 (0.4)	1.4 (0.4)
Glucose (mmol/L)[a]	5.4 (1.5)	5.2 (1.2)	5.2 (1.3)	5.3 (1.5)
Systolic blood pressure (mm Hg)[a]	123 (17)	121 (16)	120 (16)	120 (16)
Hypertension (%)[e]	23	18	17	18
Self-reported CVD risk factor medication (%)[f]	7	5	5	6
Diabetes mellitus type 2 (%)	1.4	0.6	0.8	1.4
Cardiovascular events				
Non-fatal CVD (n/%)[g]	309/9.2	531/6.5	456/6.3	97/6.7
Non-fatal CHD (n/%)[h]	209/6.2	345/4.3	260/3.6	51/3.5
Fatal CVD (n/%)[g]	42/1.2	58/0.7	64/0.9	13/0.9
Fatal CHD (n/%)[h]	25/0.7	25/0.3	33/0.5	5/0.3

[a] Numbers are given as mean (SD); [b] Numbers are based on baseline data from 1994–1997; [c] Moderate alcohol consumption: ≤2 glasses per day for men and ≤1 glass for women; [d] Numbers are based on baseline data from 1993 and 1994; [e] Systolic blood pressure ≥ mm Hg and/ or diastolic blood pressure ≥90 mm Hg and/or usage of antihypertensive medication; [f] Cholesterol lowering and/or antihypertensive medication; [g] CVD were defined according to ICD-9 codes 410–414, 415.1,427.5, 428, 430–438, 440–444, 798.1, 798.2 and 798.9 and corresponding ICD-10 codes for the fatal cases after 1996; [h] CHD was defined according to ICD-9 codes 410–414 and corresponding ICD-10 codes for the fatal cases after 1996.

Table 5.2 Hazard ratios (95% CI) of incident total CVD and CHD by sleep duration category; the MORGEN Study, 1993–1997

	Sleep duration			
	≤6 h	7 h	8 h	≥9 h
Persons at risk, n	3,387	8,216	7,349	1,480
Incident total CVD, n	331	563	488	104
Person years	39,738	97,980	87,967	17,468
Model 1[a]	1.25 (1.09–1.43)	1.00 (ref)	0.99 (0.88–1.12)	1.04 (0.84–1.28)
Model 2[b]	1.20 (1.05–1.37)	1.00 (ref)	0.98 (0.87–1.11)	1.00 (0.81–1.23)
Model 3[c]	1.15 (1.00–1.32)	1.00 (ref)	0.97 (0.86–1.10)	0.94 (0.76–1.16)
Model 4[d]	1.11 (0.97–1.27)	1.00 (ref)	0.95 (0.84–1.08)	0.96 (0.77–1.18)
Incident CHD, n	227	361	280	55
Person years	40,185	98,969	88,929	17,715
Model 1[a]	1.33 (1.13–1.57)	1.00 (ref)	0.89 (0.76–1.04)	0.85 (0.64–1.14)
Model 2[b]	1.29 (1.09–1.53)	1.00 (ref)	0.88 (0.75–1.03)	0.82 (0.61–1.09)
Model 3[c]	1.23 (1.04–1.45)	1.00 (ref)	0.87 (0.74–1.02)	0.77 (0.58–1.02)
Model 4[d]	1.19 (1.00–1.40)	1.00 (ref)	0.85 (0.73–1.00)	0.78 (0.58–1.04)

[a] Model 1: analyses adjusted for age and sex;
[b] Model 2: model 1 + smoking, alcohol, and coffee;
[c] Model 3: model 2 + subjective health and educational level;
[d] Model 4: model 3 + BMI, total-/HDL cholesterol ratio, systolic blood pressure, CVD risk factor medication, and prevalence of type 2 diabetes.

The exclusion of participants with a CVD event during the first 2 years of follow-up did not attenuate our results; neither did exclusion of participants with prevalent type 2 diabetes, with underweight, or those with CVD risk factor medication.

5

DISCUSSION

For short sleepers, we observed a 15% higher risk of total CVD and a 23% higher risk of CHD compared to normal sleepers after adjustment for relevant confounders. These associations could partially be explained by intermediate biological risk factors. Compared to participants with a normal sleep duration and good sleep quality, those with both short sleep duration and poor sleep quality had a 63% higher risk of total CVD incidence and a 79% higher risk of CHD incidence. Long sleepers did not have an increased risk of total CVD or CHD incidence.

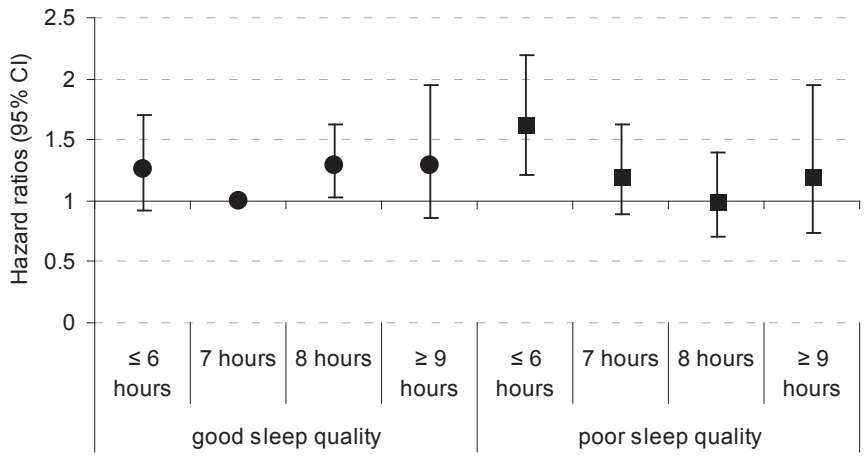
The finding that short sleep duration was associated with increased total CVD[2,13-19] and CHD incidence[1,2,13,15,16,18,20,21] has previously been reported. Epidemiological studies showed that short sleep duration was associated with higher incidence of overweight, obesity, and hypertension, with higher levels of blood pressure, total cholesterol, hemoglobin A (1c), and

Table 5.3 Hazard ratios (95% CI) of incident CVD and CHD by sleep duration and sleep quality; the MORGEN Study, 1993 and 1994[a]

	Sleep duration				Sleep quality	
	≤6 h	7 h	8 h	≥9 h	Good	Poor
Persons at risk, n	1,082	2,669	2,466	466	4,332	2,351
Incident CVD, n	131	198	211	48	384	204
Model 1[b]	1.49 (1.19–1.85)	1.00 (ref)	1.18 (0.97–1.43)	1.36 (0.99–1.86)	1.00 (ref)	1.22 (1.02–1.44)
Model 2[c]	1.44 (1.15–1.80)	1.00 (ref)	1.18 (0.97–1.44)	1.33 (0.96–1.82)	1.00 (ref)	1.17 (0.98–1.39)
Model 3[d]	1.34 (1.07–1.68)	1.00 (ref)	1.14 (0.94–1.39)	1.17 (0.85–1.62)	1.00 (ref)	1.05 (0.87–1.26)
Model 4[e]	1.26 (1.01–1.58)	1.00 (ref)	1.12 (0.92–1.36)	1.19 (0.86–1.64)	1.00 (ref)	1.04 (0.87–1.26)
Incident CHD, n	86	132	125	28	237	134
Model 1[b]	1.45 (1.11–1.91)	1.00 (ref)	1.05 (0.82–1.34)	1.16 (0.77–1.75)	1.00 (ref)	1.34 (1.08–1.66)
Model 2[c]	1.38 (1.05–1.82)	1.00 (ref)	1.05 (0.82–1.35)	1.12 (0.74–1.69)	1.00 (ref)	1.29 (1.03–1.60)
Model 3[d]	1.29 (0.98–1.71)	1.00 (ref)	1.03 (0.80–1.32)	1.01 (0.67–1.52)	1.00 (ref)	1.18 (0.94–1.49)
Model 4[e]	1.19 (0.90–1.58)	1.00 (ref)	1.01 (0.79–1.29)	1.00 (0.66–1.51)	1.00 (ref)	1.19 (0.95–1.50)

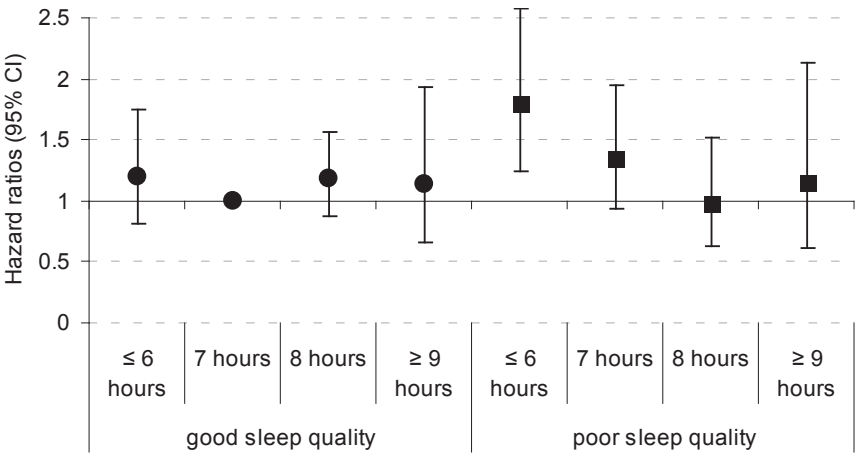
[a] These are the first 2 years of baseline measurement. This is therefore a subpopulation;
[b] Model 1: analyses adjusted for age and sex;
[c] Model 2: model 1 + sleep duration or sleep quality;
[d] Model 3: model 2 + smoking, alcohol, coffee, subjective health, and educational level;
[e] Model 4: model 3 + BMI, total-/HDL cholesterol ratio, systolic blood pressure, CVD risk factor medication, and prevalence of type 2 diabetes.

(a) CVD



# at risk	539	1,797	1,750	246	543	872	716	220
# cases	59	131	166	28	72	67	45	20

b) CHD



# at risk	539	1,797	1,750	246	543	872	716	220
# cases	37	86	98	16	49	84	45	12

Figure 5.1 Hazard ratios (95% CI) of incident CVD (a) and CHD (b) by sleep duration and sleep quality, adjusted for age, sex, smoking, alcohol, coffee, subjective health and educational level; the MORGEN Study, 1993 and 1994.

triglycerides.[2-4] Also, blood pressure declines to its lowest levels during nighttime sleep when the parasympathetic activity is highest.[22] Consequently, the arteries of people with short sleep duration benefit the least from this positive effect. Indeed, part of the association was explained by adjusting for BMI, total-/HDL cholesterol ratio, systolic blood pressure, and prevalence of type 2 diabetes. Unfortunately, we were unable to assess the effects of hemoglobin A (1c), triglycerides, or nocturnal blood pressures on our results.

In our study, long sleep duration was not associated with CVD and tended to be protective for CHD (HR: 0.77; 95% CI: 0.58–1.02). The latter result was not observed in earlier research. Cappuccio et al. reported in their meta-analysis of long compared to normal sleep duration, a RR of 1.41 (95% CI: 1.19–1.68) for total CVD and 1.38 (95% CI: 1.15–1.66) for CHD.[1] However, our trend towards a protective association between long sleep duration and CVD is consistent with the results of King et al., who observed that the longer the (actigraphically measured) sleep duration, the lower the 5-year incidence of coronary artery calcifications.[23]

We observed that short sleepers did not have a higher risk of CVD or CHD incidence compared to normal sleepers when both had good sleep quality. Only short sleepers with poor sleep quality had a 63% higher risk of CVD and a 79% higher risk of CHD. When looking within short sleepers only, poor compared to good sleep quality increased the risk of CHD. The results also suggest an increased risk of total CVD, but this association did not reach statistical significance. This is consistent with our findings presented in **Figure 5.1**. In line with our results, Chandola et al. observed in the Whitehall II cohort that the association of short sleep (≤6 h) with CHD risk was greatest among those who reported some sleep disturbance.[6] These findings support the hypothesis that for some people, sleep duration of 6 hours or less may be adequate for the restorative physiologic processes accompanied by sleep, but not for others.[6] Therefore, sleep quality should be taken into account in addition to sleep duration when investigating sleep.

We obtained data on non-fatal CVD and CHD incidence through linkage with the Dutch Hospital Discharge Diagnosis Database (HDR). In a validation study, with an approximate 33% overlap of participants from our study, the HDR was compared with the detailed clinical registry of cardiovascular patients of the Cardiology department of Maastricht University Hospital.[24] A high sensitivity (84%) and positive predictive value (97%) were observed for CHD hospital admissions.[24] Causes of death were obtained through linkage with "Statistics Netherlands" in the present study. In a study in which causes of death were coded again 2 years after initial coding, agreement ranged from 77% to 89% for cardiovascular diseases.[25] If non-fatal or fatal CVD or CHD cases in our study have been missed, this is unlikely to be related to sleep duration and will therefore not have biased our results.

Our results must be considered in the context of the inherent limitations of research on sleep duration. As in most other epidemiological studies, a self-reported single survey item was used to assess sleep duration and sleep quality. It was not feasible to obtain more detailed and objective measures of sleep, such as actigraphic or polysomnographic measures, from such a large

population.[6] Reported sleep durations may represent time spent in bed rather than physiologic sleep duration.[26] Lauderdale et al. observed that actigraphically measured sleep duration was shorter than self-reported questionnaire sleep duration (6.06 h vs. 6.65 h on a weekday).[27] This measurement error may have biased our results since it is related to age, sex, and subjective sleep quality.[28] Sleep quality was assessed in the first two years of the study. The question on sleep quality was dropped due to space limitations when in the third year additional questions on other topics were added to the questionnaire. Since sample selection has not changed over the years, it is unlikely that this group is different from the people measured in subsequent years. We therefore do not suspect any selection bias. Sleep quality was assessed as rising rested which is an indirect measure of sleep quality. As in other studies assessing sleep quality by questionnaire, this question was not validated. However, those not rising rested are likely to have poor sleep quality, even detecting people with micro-arousals or disturbances in sleep phases who might not even know they have poor sleep quality. These people only experience not rising rested in the morning. Moreover, we did not have the power to analyze the "don't know" category of the rising rested question separately. Therefore, we excluded those answering "don't know" from our analyses pertaining to sleep quality. Finally, data on physical activity, which could be an important confounder, were available for only 77% of the participants. Multivariate analyses with and without adjustment for physical activity yielded similar risk estimates for sleep duration and CVD incidence. Therefore, residual confounding by physical activity is not a major issue in the present study.

A major strength of the present study was the comprehensive data collection from a large study population that allowed us to adjust for a large number of potential confounders. Another advantage was that we could investigate intermediate risk factors and explore the interaction of sleep duration with sleep quality in relation to CVD and CHD incidence.

In conclusion, we observed that short sleep duration was associated with total CVD and CHD incidence, whereas long sleep duration was not. This association could partially be explained by intermediate biological CVD risk factors. The strongest association with CVD and CHD incidence was found in short sleepers with poor sleep quality. Future investigations should not only focus on sleep duration, but should also take sleep quality into account.

5

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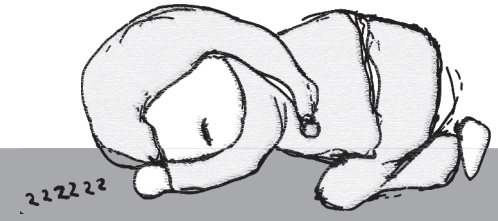

Chapter 6

Sufficient sleep duration contributes to lower cardiovascular disease risk in addition to four traditional lifestyle factors. The MORGEN Study

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ABSTRACT

Background: The contribution of sufficient sleep duration to lower CVD risk in addition to sufficient physical activity, a healthy diet, moderate alcohol consumption, and non-smoking has not been investigated yet.

Design: The MORGEN Study is a prospective cohort study including 8,128 men and 9,759 women aged 20–65 years, free of CVD at baseline.

Methods: Sufficient physical activity (≥3.5 hrs/wk cycling or sports), a healthy diet (Mediterranean Diet Score ≥5), moderate alcohol consumption (≥1 beverage/month), non-smoking, and sufficient sleep duration (≥7 hours) were assessed by self-administered questionnaires between 1994 and 1997. Cardiovascular morbidity and mortality were ascertained through linkage with national registers. Hazard ratios (HRs) and preventable proportions were calculated adjusted for age, sex, and educational level.

Results: During 10 to 14 years of follow-up, 607 composite CVD events (fatal CVD, nonfatal myocardial infarction and stroke) occurred, of which 129 were fatal. Those with the four traditional healthy lifestyle factors had a 57% lower risk of composite CVD (HR: 0.43 [95% CI: 0.31–0.59]) and a 67% lower risk of fatal CVD (HR: 0.33 [0.16–0.68]) compared with those with none or one healthy lifestyle factor. Sleeping sufficiently in addition to the four traditional lifestyle factors resulted in a 65% lower risk of composite CVD (HR: 0.35 [0.23–0.52]), and an 83% lower risk of fatal CVD (HR: 0.17 [0.07–0.43]).

Conclusions: Sufficient sleep and adherence to all four traditional healthy lifestyle factors was associated with lower CVD risk. When sufficient sleep duration was added to the traditional lifestyle factors, the risk of CVD was further reduced.

INTRODUCTION

Physical activity,[1,2] diet,[3,4] alcohol consumption,[5,6] and smoking[7-9] have all been individually associated with incident cardiovascular diseases (CVD). Since these lifestyle factors tend to cluster in individuals,[10] different combinations of these factors in relation to incident CVD have been investigated.[11-21] When investigating scores of healthy lifestyle factors, the highest compared with the lowest adherence to a healthy lifestyle was associated with hazard ratios (HRs) ranging from 0.24 to 0.35 for fatal CVD.[11-14,17-19] Similarly, these HRs ranged from 3.12 to 8.17 for scores representative of unhealthy lifestyle factors.[15,16]

Most studies investigating combinations of lifestyle factors have included physical activity, diet or dietary components, alcohol consumption, smoking and body mass index (BMI) or waist circumference.[11,12,14-18,21-25] Recently, it has been shown that poor sleep duration is an independent risk factor for CVD.[26,27] So far, only two studies have included sleep duration as a lifestyle factor.[11,17] Both studies were performed in Asian populations and assessed the combination of all lifestyle factors with CVD, without providing insight into the additional impact of sleep outside of the other healthy lifestyle factors.

The purpose of the present study was to examine whether sufficient sleep duration, in addition to sufficient physical activity, a healthy diet, moderate alcohol consumption, and non-smoking, further reduces CVD incidence.

METHODS

Study population

The Monitoring Project on Risk Factors for Chronic Diseases (MORGEN Study) was carried out in the Netherlands between 1993 and 1997 with 10,448 men and 12,652 women. Municipal population registers were used as sampling frames to draw random sex- and age-stratified samples in three cities in the Netherlands (Doetinchem, Maastricht, and Amsterdam). The response rate in the MORGEN Study was 45%.[28]

We excluded participants examined in 1993 due to the use of a different physical activity questionnaire (n=5,213), persons with prevalent CVD or type 2 diabetes based on self-report and hospital discharge data (n=613), women who were pregnant at baseline (n=115), persons with extremely low or high reported energy intakes (i.e., those in the lowest and highest 0.5% of the ratio of energy intake over basal metabolic rate) (n=166) and those with no information on one or more of the lifestyle factors or covariates (n=683), as well as those with no information on vital status or cardiovascular events (n=1,671). After these exclusions, 6,672 men and 7,967 women remained for our analyses. There was no meaningful difference in age and sex between those included and those excluded; however, those excluded had a slightly lower educational level.

Lifestyle

Physical activity

Physical activity was assessed with a validated questionnaire[29] developed for the "European Prospective Investigation into Cancer and Nutrition" (EPIC). Cycling was assessed according to the number of hours spent per week, separately for summer and winter, and the average number of hours was included in our analyses. For the MORGEN Study, the EPIC questionnaire was extended with two open-ended questions on type, frequency, and duration of sports per week. The recommendation in the Netherlands is 30 minutes of moderate to vigorous physical activity per day for 5 to 7 days a week.[30] Therefore, spending 3.5 hours or more per week on cycling plus sports (≥4 MET) was considered sufficient physical activity and less than 3.5 hours per week, insufficient physical activity.

Diet

Dietary intake was assessed with a validated food frequency questionnaire,[31,32] including questions on the habitual frequency of consumption of 178 food items during the year preceding enrollment. Additional information was obtained regarding the frequency of consumption of different sub-items and the preparation methods. Colored photographs were used to estimate portion sizes of 28 food items.[31,32] We operationalized the concept of a healthy diet with the modified Mediterranean Diet Score (MDS) defined by Trichopoulou et al.[33] For the composition of this score, values of 0 or 1 were assigned to each nutritional component. For vegetables, fruits, legumes and nuts, grains, fish and seafood, and the ratio of unsaturated to saturated fatty acids, intakes equal to or above the sex-specific median were assigned a value of 1, and intakes below that median a value of 0. As the traditional Mediterranean diet is characterized by a low intake of meat and dairy products, the scoring of these products was inverted. The MDS could result in a value from 0 (minimal adherence) to 8 (maximal adherence). We classified an MDS of 5 or higher as a healthy diet and the remainder as an unhealthy diet.

Alcohol consumption

Alcohol consumption was also assessed with the food frequency questionnaire[32] and dichotomized in non-consumption (<1 glass per month) and moderate consumption (≥1 glass per month). Because of the relatively low level of alcohol consumption in the MORGEN population, consumption of alcohol was considered moderate and therefore a healthy lifestyle factor for CVD. Of the total population, 23% consumed more than two glasses of alcohol per day. Although we did not define an upper limit for alcohol consumption, this is, of course, necessary in establishing public health guidelines.[21]

Smoking

Information on smoking was obtained by a self-administered questionnaire.[28] Since CVD risk swiftly declines after smoking cessation, never or past smoking (non-smoking) was classified as a healthy lifestyle factor and current smoking as an unhealthy lifestyle factor.

Sleep duration

Information on sleep duration was obtained by a self-administered questionnaire. The usual sleep duration was assessed by the question "How many hours of sleep do you usually get per 24-hour period?". We showed in a previous study that short (≤6 hours) compared with normal (7–8 hours) sleep duration increased CVD risk, whereas long (≥9 hours) sleep duration did not.[26] Therefore, we classified sleep duration of ≥7 hours as sufficient and the remainder as insufficient.

Ascertainment of fatal and nonfatal CVD events

Participants were followed to the first nonfatal cardiovascular event, death, or emigration or were censored at January 1, 2008. Morbidity data were provided by the National Medical Register (NMR) with the Dutch Hospital Discharge data. Eighty-eight percent of the hospital admissions can be linked uniquely to an individual on the basis of sex, date of birth, and postal code.[34] We obtained vital status through linkage with municipal population registers. Subsequently, primary and secondary causes of death were obtained through linkage with data from 'Statistics Netherlands'.[28]

Composite CVD was defined as fatal CVD (ICD9[35] codes 390–459 and 798), plus nonfatal myocardial infarction (410–412 and 414) and stroke (430–434, and 436). Causes of death after 1996 were coded according to the corresponding ICD10[36] codes. For nonfatal myocardial infarction and stroke, only first events were taken into account. For fatal CVD, no exclusions were made because of previous nonfatal events.

Covariates

Information on age, sex and the highest educational level achieved was obtained by questionnaire.[28] Educational level was classified as low (lower vocational training or primary school), medium (secondary school and intermediate vocational training), or high (higher vocational training or university). To calculate BMI, weight was measured to the nearest 0.1 kg and height to the nearest 0.5 cm.[28] Non-fasting plasma total cholesterol and high density lipoprotein (HDL) cholesterol were determined in the Lipid Reference Laboratory of the University Hospital Dijkzigt in Rotterdam by standardized enzymatic methods.[28] Systolic blood pressure was recorded twice with a Random Zero Sphygmomano-meter at the appearance of sounds (first-phase Korotkoff) in sitting position.[28] The average of the two blood pressure measurements was included in current analyses.

Statistical analyses

Statistical analyses were performed with SAS 9.3 software (SAS Institute, Inc., Cary, NC). Participants' characteristics are presented as means (standard deviation) for continuous variables and as percentages (total number) for categorical variables. We used Cox proportional hazards models to estimate HRs and 95% confidence intervals (CI) for (combinations of) lifestyle factors in relation to composite and fatal CVD. We assessed these associations in categories with none or one healthy lifestyle factor as the reference category and continuously per healthy lifestyle factor. We assessed interaction on a multiplicative scale for age, sex, educational level and BMI with the number of healthy lifestyle factors in relation to composite and fatal CVD at $p<0.10$. The proportional hazard assumption was fulfilled according to the graphical approach and according to Schoenfeld residuals. We performed our analyses separately for the four traditional lifestyle factors (five-point score: 0–4) and for the traditional lifestyle factors plus sleep duration (six-point score: 0–5). In order to compare the six-point score to the five-point score, the six-point score was recalculated to the five-point score by multiplying the logarithm of the estimated HRs by 6/5 before exponentiating them. To estimate the proportion of cases that could theoretically be prevented or postponed by adherence to a healthier lifestyle, we calculated the preventable proportion with the equation developed by Wahrendorf.[37] All analyses shown in the tables were adjusted for age, sex and highest educational level achieved. In a sensitivity analysis, we additionally adjusted the analyses for systolic blood pressure, BMI, and the ratio of serum total cholesterol to HDL cholesterol. In another sensitivity analysis, CVD cases in the first two years of follow-up were excluded. A p-value of 0.05 was considered statistically significant.

RESULTS

During 10 to 14 years of follow-up (mean, 12 years), 607 composite CVD events occurred, of which 129 were fatal. **Table 6.1** shows the distribution of participants' characteristics at baseline and CVD incidence stratified by sex. Participants were on average 42 years of age at baseline, and 46% were male. Men were slightly better educated than women. Of the total population, 52% were sufficiently physically active, and 37% consumed a healthy diet. Among men, 91% consumed alcohol, compared with 78% of women. About 65% of the population were non-smokers, and 80% of the men and 86% of the women slept a sufficient number of hours. The distribution of the number of healthy lifestyle factors was similar for both men and women. Of the total population, 6% adhered to none or one healthy lifestyle factor, and 12% adhered to all five healthy lifestyle factors.

Table 6.2 shows the associations of the single lifestyle factors with composite and fatal CVD after adjustment for age, sex, educational level, and with and without mutual adjustments for the other lifestyle factors. With mutual adjustments, non-smoking was strongly inversely associated

Table 6.1 Participants' characteristics at baseline and CVD incidence by sex, MORGEN Study 1994–1997[a]

	Men n=6,672	Women n=7,967
Age (years, mean, [sd])	42 (11)	41 (11)
Educational level		
Low	42 (2,777)	51 (4,039)
Medium	31 (2,038)	27 (2,159)
High	28 (1,857)	22 (1,769)
Sufficient physical activity[b]	52 (3,436)	53 (4,222)
Healthy diet[c]	37 (2,489)	37 (2,986)
Alcohol consumption[d]	91 (6,068)	78 (6,182)
Intake among consumers (median [q1–q3])[e]	14 (6–28)	6 (2–14)
Non-smoking[f]	66 (4,379)	65 (5,172)
Sufficient sleep duration[g]	80 (5,346)	86 (6,880)
Number of healthy lifestyle factors[h]		
0–1	5 (331)	6 (472)
2	19 (1,272)	20 (1,623)
3	33 (2,174)	33 (2,685)
4	32 (2,129)	29 (2,271)
5	11 (766)	12 (943)
Composite CVD[i]	6 (384)	3 (223)
Fatal CVD	1.3 (87)	0.5 (42)

[a]All data are shown as '% (n)' unless otherwise indicated; [b] Sufficient physical activity was defined as ≥3.5 hrs/wk cycling and sports; [c] A healthy diet was defined as a Mediterranean Diet Score of 5–8 (range 0–8); [d] Alcohol consumption was defined as ≥ one glass per month; [e] Grams/ day; [f] Non-smoking was defined as never or past smoking; [g] Sufficient sleep duration was defined as ≥7 hrs; [h] Sufficient physical activity, a healthy diet, moderate alcohol consumption, non-smoking, and sufficient sleep duration; [i] Composite of fatal CVD plus nonfatal myocardial infarction and stroke.

with composite CVD (HR: 0.57 [0.48–0.67]), as were sufficient sleep duration (HR: 0.78 [0.65–0.94]) and moderate alcohol consumption (HR: 0.79 [0.63–0.98]). Non-smoking (HR: 0.61 [0.43–0.86]) and sufficient sleep duration (HR: 0.57 [0.39–0.83]) were both strongly inversely associated with fatal CVD. For sufficient physical activity, a healthy diet, and moderate alcohol consumption the associations with composite and fatal CVD were all inverse, although mostly not statistically significant. Without mutual adjustments, most associations were slightly stronger, especially for diet and physical activity.

The association between the number of healthy lifestyle factors and composite and fatal CVD was non-linear, with the largest decrease in risk for two healthy lifestyle factors compared to

Table 6.2 Hazard ratios (95% CI) of lifestyle factors for composite and fatal CVD, MORGEN Study 1994–1997

	Composite CVD n=607		Fatal CVD n=129	
	Model 1[a]	Model 2[b]	Model 1[a]	Model 2[b]
Physical activity[c]				
Insufficient	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
Sufficient	0.80 (0.68–0.94)	0.87 (0.74–1.03)	0.67(0.47–0.95)	0.74 (0.52–1.06)
Diet[d]				
Unhealthy	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
Healthy	0.84 (0.71–1.00)	0.88 (0.74–1.05)	0.68 (0.46–1.01)	0.73 (0.50–1.08)
Alcohol consumption[e]				
No	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
Yes	0.81 (0.65–1.00)	0.79 (0.63–0.98)	0.70 (0.44–1.10)	0.70 (0.44–1.10)
Smoking[f]				
Yes	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
No	0.56 (0.48–0.66)	0.57 (0.48–0.67)	0.57 (0.40–0.82)	0.61 (0.43–0.86)
Sleep duration[g]				
Insufficient	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
Sufficient	0.76 (0.63–0.91)	0.78 (0.65–0.94)	0.55 (0.38–0.80)	0.57 (0.39–0.83)

[a] Model 1: Adjusted for age, sex, educational level; [b] Model 2: model 1 plus mutually adjusted for the other lifestyle factors; [c] Sufficient physical activity was defined as ≥3.5 hrs/wk cycling and sports; [d] A healthy diet was defined as a Mediterranean Diet Score of 5–8 (range 0–8); [e] Alcohol consumption was defined as ≥ one glass per month; [f] Non-smoking was defined as never or past smoking; [g] Sufficient sleep duration was defined as ≥7 hrs.

none or one (**Table 6.3**). Adherence to all four traditional healthy lifestyle factors was associated with a 57% lower risk of composite CVD (HR: 0.43 [0.31–0.59]), and a 67% lower risk of fatal CVD (HR: 0.33 [0.16–0.68]) compared with adherence to none or one. After addition of sufficient sleep duration to adherence to all four traditional healthy lifestyle factors – thus comparing five to none or one healthy lifestyle factor – the CVD risk was even lower: HR: 0.35 (0.23–0.52) for composite CVD and HR: 0.17 (0.07–0.43) for fatal CVD. The preventable proportions showed that, if all participants adopted all four traditional healthy lifestyle factors, 36% of the composite CVD cases and 46% of the fatal CVD cases could theoretically be prevented or postponed. The percentage of composite CVD cases that would theoretically be prevented or postponed by adherence to all five lifestyle factors remained 36%. However, for fatal CVD, this percentage increased from 46% to 57%.

There were no statistically significant interactions for age, sex, educational level, and BMI in any of the associations. Exclusion of the first two years of follow-up slightly attenuated the results.

Table 6.3 Hazard ratios (95% CI) and preventable proportions of incident composite and fatal CVD by the number of healthy lifestyle factors, The MORGEN Study 1994–1997

	Number of healthy lifestyle factors[a]							
	0, 1	2	3	4	5	Per healthy lifestyle factor	Preventable proportion (+1 healthy lifestyle factor, %)[b]	Preventable proportion (all healthy lifestyle factors, %)[c]
Traditional healthy lifestyle factors								
Persons at risk, n	2,861	4,934	4,858	1,986	-	140,639		
Composite CVD[e]								
N cases	173	213	170	51	-	607		
Person years	32,817	56,796	56,252	22,999	-	164,944		
Model 1[d]	1.00 (ref)	0.69 (0.57–0.85)	0.55 (0.44–0.68)	0.43 (0.31–0.59)	-	0.76 (0.70–0.83)	22	36
Fatal CVD								
N cases	40	48	32	9	-	129		
Person years	33,675	57,837	57,114	23,240	-	167,838		
Model 1[d]	1.00 (ref)	0.68 (0.44–1.03)	0.44 (0.28–0.71)	0.33 (0.16–0.68)	-	0.68 (0.57–0.82)	29	46

Table 6.3 continues on next page

Table 6.3 ***Continued***

	Number of healthy lifestyle factors[a]							
	0, 1	2	3	4	5	Per healthy lifestyle factor	Preventable proportion (+1 healthy lifestyle factor, %)[b]	Preventable proportion (all healthy lifestyle factors, %)[c]
Traditional healthy lifestyle factors plus sufficient sleep duration								
Persons at risk, n	803	2,895	4,832	4,400	1,709	14,639		
Composite CVD[e]								
N cases	61	162	197	145	42	607		
Person years	9,146	33,177	55,752	50,953	19,838	164,943		
Model 1[d]	1.00 (ref)	0.72 (0.54–0.97)	0.54 (0.40–0.72)	0.43 (0.32–0.58)	0.35 (0.23–0.52)	0.73 (0.66–0.79)[f]	20	36
Fatal CVD								
N cases	18	39	36	30	6	129		
Person years	9,444	33,947	56,752	51,669	20,054	167,837		
Model 1[d]	1.00 (ref)	0.59 (0.34–1.03)	0.33 (0.19–0.59)	0.30 (0.17–0.54)	0.17 (0.07–0.43)	0.61 (0.51–0.74)[f]	31	57

[a] Four traditional healthy lifestyle factors: sufficient physical activity, healthy diet, moderate alcohol consumption, and non-smoking. Five healthy lifestyle factors: previous plus sufficient sleep duration; [b] Proportion of the cases that could have been prevented if all participants adopted one additional healthy lifestyle factor; [c] Proportion of the cases that could have been prevented if all participants adopted all healthy lifestyle factors; [d] Model 1: adjusted for age, sex, and educational level; [e] Composite of fatal CVD plus nonfatal myocardial infarction and stroke; [f] Six point score was recalculated to five point score by multiplying the logarithm of the estimated HRs by 6/5 before exponentiating them.

DISCUSSION

In this study, the combination of sufficient physical activity, a healthy diet, moderate alcohol consumption, and non-smoking was associated with a 57% lower risk of composite CVD and a 67% lower risk of fatal CVD compared with adherence to none or one of these healthy lifestyle factors. The addition of sufficient sleep to adherence to all four traditional healthy lifestyle factors resulted in a 65% lower risk of composite CVD and an 83% lower risk of fatal CVD. If all participants adhered to all five healthy lifestyle factors, 36% of composite CVD and 57% of fatal CVD could theoretically be prevented or postponed.

Some limitations of the study need to be addressed. All lifestyle factors were self-reported. We have no data on the reproducibility and validity of sleep duration. It was not feasible to obtain more detailed and objective measures of sleep, such as actigraphic or polysomnographic measures, from such a large population.[38] Self-reported sleep durations may represent time spent in bed rather than physiologic sleep duration. Lauderdale et al. observed that actigraphic-measured sleep duration was about 10% shorter than self-reported sleep duration.[39] This measurement error may have biased our results, since it is related to age, and sex.[40] Median reproducibility for physical activity,[29] dietary intake,[31,32] and alcohol consumption[32] ranged from 0.5 for cycling to 0.9 for alcohol consumption, and median relative validity ranged from 0.5 for cycling and food groups to 0.8 for alcohol consumption. All lifestyle factors were assessed only once and may have changed during follow-up, resulting in non-differential misclassification that may have attenuated our associations.

Our study has several strengths. The MORGEN Study is a large, prospective cohort study that includes both men and women from the general population, with a broad age range and a long follow-up period. We are the first to investigate whether the addition of sleep duration to the four traditional healthy lifestyle factors contributed to an association with CVD.

Cardiovascular follow-up was obtained by linkage to several national registers. In a validation study conducted in a subsample of this population, a high sensitivity was observed for acute myocardial infarction hospital admissions (84%) comparing the Dutch Hospital Discharge data with that of a detailed clinical register.[41] In a study in which causes of death were coded again two years after initial coding, the agreement was 89% for myocardial infarction, 79% for stroke and 92% for total CVD.[42] Despite the relatively high level of agreement, non-differential misclassification of cardiovascular morbidity and mortality may have attenuated our results.

Comparison with the results of other studies was hampered by differences between studies in definition and operationalisation of lifestyle factors and CVD events. For instance, we did not include BMI or waist circumference as a lifestyle factor, because we considered those to be outcomes of lifestyle factors and intermediates in the association between lifestyle factors and CVD. In this study, the highest level of adherence to the four traditional lifestyle factors compared with the lowest was associated with a 57% lower risk of composite CVD. When Stampfer et al.[21]

compared five healthy lifestyle factors (non-smoking, sufficient physical activity, desirable BMI, moderate alcohol consumption, healthy diet) with none or one, they observed a 75% lower risk of a composite of CVD. We observed a 67% lower risk for fatal CVD when comparing the highest to the lowest adherence to the four traditional lifestyle factors which is comparable to the 65–76% lower risk in other investigations.[12-14,18,19] Despite differences between studies, adherence to the four traditional healthy lifestyle factors was consistently associated with a lower CVD risk of at least 50%.

We observed that sufficient sleep duration was independently and strongly associated with a lower risk of composite CVD and an even lower risk of fatal CVD. Additional adjustments for the intermediate factors systolic blood pressure, BMI and the ratio of serum total to HDL cholesterol slightly attenuated the associations. In a recent meta-analysis by Cappuccio et al. that incorporated 15 cohort studies, short sleep duration was associated with a greater risk of fatal and non-fatal coronary heart disease (RR: 1.48 [1.22–1.80]), and stroke (RR: 1.15 [1.00–1.31]), but not total CVD (HR: 1.03 [0.93–1.15]).[27] Short sleep duration has also been associated with a higher incidence of overweight, obesity and hypertension and with higher levels of blood pressure, total cholesterol, hemoglobin A (1c) and triglycerides.[43] Mechanisms include changes in circulating levels of leptin and ghrelin that, in turn, increase appetite, caloric intake, reduce energy expenditure and facilitate the development of obesity and impaired glycemic control.[44] Also, low-grade inflammation is activated as a result of short sleep duration.[44] Although we cannot exclude depressive symptoms, sleep apnea or psychological stress as partial explanations for the observed associations,[45] the mechanisms are consistent with the hypothesis that short sleep duration is directly associated with CVD risk.

In conclusion, sufficient sleep duration was independently associated with both composite and fatal CVD. Also, the combination of sufficient physical activity, a healthy diet, moderate alcohol consumption and non-smoking was associated with a substantially lower risk of composite and fatal CVD. Although the risk was low in persons adhering to the four traditional healthy lifestyle factors, the addition of sufficient sleep duration to these factors further reduced CVD risk. Our results suggest that the addition of sufficient sleep duration to the traditional lifestyle factors increases the percentage of fatal CVD cases that could be prevented or postponed from 46% to 57%. If replicated in other studies, the public-health impact of sufficient sleep duration, in addition to the traditional healthy lifestyle factors, could be substantial.

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6

Chapter 7

General discussion

The focus of the research described in this thesis was on the Mediterranean style diet, aspects of leisure time physical activity and sleep in relation to CVD in the general population. The associations of these three lifestyle factors with CVD incidence were studied for each factor separately, and also in combination with smoking and alcohol consumption. In this chapter, we discuss the main findings (**Table 7.1**), some methodological considerations and the effects of these lifestyle factors on CVD risk factors in intervention studies. We conclude with a discussion of the potential public health implications of the associations of these lifestyle factors with CVD.

MEDITERRANEAN STYLE DIET

Main findings for a Mediterranean style diet and CVD risk

- The Mediterranean Diet Score (MDS) was more strongly inversely associated with fatal CVD than with total CVD.
- The MDS was inversely related to incident myocardial infarction, stroke and pulmonary embolism.
- The use of multiple measurements of the MDS increased the strength of the inverse associations with composite and fatal CVD and narrowed the confidence intervals of the estimated HRs.

Interpretation of the Mediterranean Diet Score in the Northern European context

In this thesis, we used the modified MDS defined by Trichopoulou et al. to operationalise a healthy dietary pattern.[1] In this score, values of 0 or 1 were assigned to vegetables, fruits, legumes and nuts combined, grains, fish and seafood combined, the ratio of unsaturated to saturated fatty acids, meat and dairy based on sex-specific medians as cut-off points. Alcohol consumption was dichotomized into non-users (<1 drink/ month) and users. The MDS can take a value from 0 (minimal adherence) to 9 (maximal adherence).[1]

Some of the components of the MDS[1] may not be optimally defined to quantify a healthy diet. For instance, within the MDS no distinction is made between whole grains and refined grains. With regards to fish and seafood, no differentiation is made between fatty fish and lean fish. For meat and milk products no distinction is made between low, medium or high saturated fat content. This shows that the MDS is a relatively crude indicator of a healthy dietary pattern.

In the traditional Mediterranean diet, alcohol was consumed in moderate amounts with meals. Trichopoulou, defined a 'moderate' amount as 5–25 gram alcohol per day for women and 10–50 gram per day for men.[1] In the MORGEN population, only 8% of women and 7% of men

consumed more than this amount. We observed in the MORGEN Study a lower CVD risk for all alcohol users compared to non-users (results not shown) which is compatable to literature.[2] Therefore, we dichotomized alcohol consumption into non-users (<1 drink/ month) and users.

A high score on the MDS in our study population, may not necessarily represent a high adherence to the traditional Mediterranean diet. As described above, the scoring system is based on levels of intake of broad food groups that are dichotomized into a low or high level of consumption based on population specific medians. In The Netherlands, as in other Northern European countries, olive oil, vegetables, fruits, legumes, nuts, are less frequently consumed than in the traditional Mediterranean diet.[2] Hence, in the Dutch population even the highest adherence to the MDS, does not resemble the traditional Mediterranean diet.[2]

In addition to the difference in population specific medians, also the content of the general food groups in Northern European countries differs from that of the traditional Mediterranean diet. For instance, in the Mediterranean, almonds are usually eaten,[3] whereas in our population mainly peanuts were consumed. Because both legumes and nuts are consumed in relatively small quantities in Northern European populations, Trichopoulou combined legumes and nuts into one category.[1] However, legumes and nuts differ in beneficial properties. For instance, nuts are a rich source of polyunsaturated fatty acids whereas legumes are not. Furthermore, olive oil consumption is low in non-Mediterranean populations. Therefore, olive oil was replaced by the ratio of unsaturated to saturated fatty acids in the MDS of Trichopoulou.[1] Plant oils used in Northern Europe, such as sunflower oil are high in polyunsaturated fatty acids and olive oil are rich in monounsaturated fatty acids. Both oils are characterized by a high unsaturated to saturated fatty acid ratio. The effects of monounsaturated fatty acids on blood lipids hardly differ from those of polyunsaturated fatty acids.[4,5] Therefore, the ratio of unsaturated to saturated fatty acids can be used for both Mediterranean and Northern European populations.

In conclusion, in the present study diet was operationalized with a relatively crude dietary pattern score. The food groups included in the MDS consist of different foods, and are eaten in different quantities in both Northern European and Mediterranean countries. Despite these differences between the traditional Mediterranean and a Northern European diet, the ranking of people based on the basic foods in Non-Mediterranean countries are likely to be valid since observed health benefits are similar in Mediterranean as in non-Mediterranean countries.[6] This suggests that the health-enhancing properties of what has been labeled the Mediterranean diet is not necessarily based on Mediterranean foods or on absolute intakes.[3] Rather, the Mediterranean diet is a prototype of a healthy diet.

Table 7.1 Main findings on the relations of different lifestyle factors with CVD in prospective cohort studies

Chapter	Study population	Lifestyle factors	Main results	Hazard ratio
2	EPIC-NL study; n=34,708 ♀ + ♂; 20–70 years	Mediterranean style diet	For each two-unit increment in MDS	
			Total CVD (n=4,881):	0.95 (0.91–0.98)
			Fatal CVD (n=487):	0.78 (0.69–0.88)
			Composite CVD[a] (n=1,765):	0.85 (0.80–0.91)
			MI (n=1,070):	0.86 (0.79–0.93)
			Stroke (n=448):	0.88 (0.78–1.00)
			PE (n=131):	0.74 (0.59–0.92)
3	Doetinchem Study; n=3,417/ 3,357 ♀ + ♂ for composite CVD/ fatal CVD	Mediterranean style diet, three repeated measurements over 10 years	For high versus low adherence to MDS[b]	
			Composite CVD[a] (n=206)	
			Baseline MDS:	0.77 (0.53–1.11)
			Updated mean MDS:	0.65 (0.43–0.97)
			Fatal CVD (n=38)	
			Baseline MDS:	0.87 (0.36–2.07)
			Updated mean MDS:	0.44 (0.19–1.05)
4	MORGEN study; n=16,442 ♀ + ♂; 20–65 years	Cycling and sports	Total CVD (n=923)	
			Cycling: yes vs. no:	0.82 (0.71–0.95)
			Sports: yes vs. no:	0.74 (0.64–0.87)
			>0–3.5 hr/wk vs. no:	0.77 (0.65–0.91)
			>3.5 hr/wk vs. no:	0.66 (0.50–0.88) (p for trend <0.001)
			Both cycling and sports versus no cycling and no sports:	0.64 (0.52–0.77)

5	MORGEN study; n=20,432 ♀ + ♂; 20–65 years	Sleep duration	Total CVD (n=1,486)	
			Short (≤6 h) vs. normal (7 h) duration:	1.15 (1.00–1.32)
			Long (≥9 h) vs. normal duration:	0.94 (0.76–1.16)
	n=6,683 (sleep quality)	Sleep duration + sleep quality	Short duration and poor quality versus normal duration and good quality:	1.63 (1.21–2.19)
6	MORGEN study; n=14,639 ♀ + ♂; 20–65 years	Sleep duration in combination with traditional lifestyle factors: dietary pattern, physical activity, alcohol consumption and smoking	Composite CVD[a] (n=607)	
			Sufficient (≥7 h) versus insufficient (≤6 h) sleep duration:	0.78 (0.65–0.94)
			Four traditional healthy lifestyle factors versus zero or one:	0.43 (0.31–0.59)
			Four traditional healthy lifestyle factors + sufficient sleep duration versus zero or one:	0.35 (0.23–0.52)
			Fatal CVD (n=129)	
			Sufficient versus insufficient sleep duration:	0.57 (0.39–0.83)
			Four traditional healthy lifestyle factors versus zero or one:	0.33 (0.16–0.68)
			Four traditional healthy lifestyle factors + sufficient sleep duration versus zero or one:	0.17 (0.07–0.43)

MDS = Mediterranean Diet Score, (total) CVD = (total) cardiovascular diseases, MI = myocardial infarction, PE = pulmonary embolism.
[a] Composite of fatal CVD, nonfatal myocardial infarction and nonfatal stroke; [b] Low adherence: MDS ≤3.5, high adherence: MDS ≥5.5.

Intervention studies of Mediterranean style diets on CVD risk factors and endpoints

The traditional Mediterranean diet has many beneficial properties. It has a high content of fibre, n-3 fatty acids, and antioxidants, as well as β-carotene, vitamins C and E, and polyphenols from olive oil, legumes, whole grains, fruits, and vegetables. Furthermore, the diet is low in trans fatty acids, saturated fatty acids, and high in monounsaturated fat, mainly from olive oil.[7,8] A meta-analysis of randomized controlled trials showed that a Mediterranean diet was associated with favourable changes in systolic and diastolic blood pressure, fasting plasma glucose, total cholesterol, body weight, body mass index, and high-sensitivity C-reactive protein in comparison to low-fat diets.[9] All these factors combined may be responsible for the low CVD risk in populations with a traditional Mediterranean diet.

In the Lyon Diet Heart Study, a secondary prevention trial in 423 myocardial infarction patients, a Mediterranean style diet enriched with alpha-linolenic acid followed for 27 months reduced cardiac death and non-fatal myocardial infarction by 73% compared with a prudent diet.[10,11] After 48 months – 21 months after stopping the diet – the Mediterranean style diet group still had a lower risk of recurrent CVD compared to the group with the prudent diet.[10] It should be noted that this study was stopped early for apparent benefit. This could have overestimated the protective effect of the Mediterranean diet. Therefore, other and larger intervention studies are needed before definitive statements can be made on the beneficial effect of a Mediterranean diet on CVD.

PHYSICAL ACTIVITY

Main findings for physical activity and CVD risk

- Cycling and sports were associated with a low CVD risk.
- For sports there was a dose-response relationship with CVD, but not for cycling.
- Those who both cycled and performed sports had the lowest CVD risk.
- Walking and gardening were not associated with CVD.

Methodological aspects of physical activity questionnaires

Leisure time physical activity was assessed with a validated[12] questionnaire designed for the "European Prospective Investigation into Cancer and nutrition" (EPIC). For walking (to and from work and during leisure time), cycling (to and from work and during leisure time) and gardening the number of hours per week was assessed. For sports, the EPIC questionnaire was extended

in our study with two open-ended questions on type, frequency, and duration of sports, and one open-ended question on other strenuous activities.

Physical activity questionnaires are commonly used in large cohort studies because of relatively low costs and low participant and researcher burden.[13] However, an individual's physical activity level may be misclassified based on questionnaire data due to social desirable answers by participants, recall bias[14-16] and due to the lack of information on the intensity of the activities performed. Objective methods of physical activity measurement, such as doubly labeled water, accelerometry (e.g. actigraph) and combined heart rate monitoring (e.g. ActiHeart) do not have these sources of error and bias typical for subjective measurements such as activity diaries and questionnaires. The difference in sources of error and bias may also be reflected in the weaker associations observed for subjectively measured physical activity with CVD risk factors compared to objectively measured physical activity.[17] However, objective measures do not distinguish different types and domains of physical activity and cannot cover usual activities over a longer period of time, in contrast to questionnaires. Furthermore, because of the costs, and relative burden on participants, objective measurements of physical activity are usually not feasible in large populations. Ideally, large cohort studies would complement subjective measurement methods of physical activity with an objective measurement method to estimate the intensities of the performed activities.

In this thesis, the associations of different types of activities with CVD incidence were based on a single measurement at baseline. Thus, changes in physical activity that may have occurred during follow-up were not taken into account. Such changes are likely, as was shown in the Doetinchem Cohort Study. Over a period of 10 years, 45% of the participants changed their level of physical activity, with equal numbers of people starting to adhere and stopping to adhere to the Dutch physical activity guideline.[18] Since the Doetinchem cohort constitutes about one third of the MORGEN population, these results can probably be generalized to the entire MORGEN population. Misclassification of the activities during follow-up in our study may have resulted in an underestimation of the strength of the associations with CVD.

Another source of bias is the assignment of the intensities to different activities. As in other cohort studies, we chose conservative intensity levels from the Compendium of Physical Activities of Ainsworth.[19] Intensities of activities can vary greatly. For instance, the intensity level of walking varies from 2.0 MET (walking, household) to 8.0 MET (walking, 5.0 mph) and of cycling from 4.0 MET (<10 mph) to 16.0 MET (>20 mph). For individual sports there is a similar variance in intensity. Furthermore, assigned intensities depend on the version of the Compendium of Physical Activities. The compendium is updated about every ten years and with each update activities are added, and intensity levels are modified to reflect new information about the metabolic cost of specific activities.[20] For instance, the intensity level of gardening (Ainsworth code 8245) was 4.0, 5.0 and 3.8 MET according to the Compendium of 1993, 2000 and 2011 respectively. Thus, gardening was qualified as a moderate intense activity in 1993 and 2000, whereas in 2011 it was classified as a light intense activity.

To calculate whether persons adhere to the physical activity guidelines, it would have been useful to have information on the time spent on the different activities *per day*. In the EPIC questionnaire, the duration of the activities was assessed as the average number of hours per week. As a result, no distinction can be made between one bout of 3.5 hours of physical activity on one day and seven bouts of 0.5 hours on seven separate days. However, in studying etiologic associations, not the absolute level of physical activity is of primary interest, but the ranking of participants according to their level of physical activity. Our questionnaire was considered suitable for this purpose when validated against physical activity diaries,[12] four-day heart rate monitoring,[21] sub-maximum oxygen uptake,[21] and against ActiHeart.[13]

The number of studies addressing sedentary behaviour as opposed to or in combination with physical activity is increasing.[22] We did not investigate sedentary behaviour, as this was not included in the EPIC questionnaire. Sedentary behaviour is defined as any waking behaviour characterized by an energy expenditure ≤1.5 MET while in a sitting or reclining posture.[22] Studies with questionnaires on sedentary behaviour and physical activity suggest that those who were sedentary for many hours per day are at high risk of cardiovascular disease, regardless of their level of moderate to vigorous intensity physical activity.[23-27] Because of the high frequency of sedentary behaviour in modern societies, research on the interaction of physical activities of at least moderate intensity with sedentary behaviour in the association with CVD, is of great interest.

Intervention studies of physical activity on CVD risk factors

In exercise intervention studies aerobic exercise was associated with favourable changes in systolic and diastolic blood pressure,[28,29] HDL cholesterol,[30] LDL-particle size,[31] platelet aggregation and activation,[32] insulin sensitivity in muscles,[33,34] weight, and waist circumference.[35] All these factors combined may be responsible for the low CVD risk in populations with sufficient physical activity.

Exercise intervention studies usually aim at CVD risk factors. Furthermore, the exercise is generally more intensive than habitual non-occupational physical activity and these studies do not examine the health benefits of daily life physical activities. Community intervention trials such as Hartslag Limburg,[36] generally focus on moderate intense physical activity, and provide more information on attainable health benefits in a general population. Unfortunately, these interventions usually combine physical activity with other lifestyle factors so the independent effect of physical activity can not be determined. Intervention studies of low to moderate intense physical activities showed that walking had favourable effects on diastolic blood pressure, HDL- and LDL cholesterol, body weight, BMI, and percent body fat in previously sedentary adults.[37,38] For cycling, an increase in HDL cholesterol was observed, but no significant changes in total cholesterol or triglycerides.[39] In conclusion, there is evidence for a positive effect of exercise on cardiovascular risk factors, though for the independent effects of low-moderate intense physical activities on hard CVD endpoints more research needs to be done.

SLEEP

Main findings for sleep and CVD risk

- Short sleep duration was associated with a higher risk of CVD compared to normal sleep duration.
- Long sleep duration was not associated with CVD risk.
- Poor sleep quality was not associated with CVD risk.
- Short sleepers with poor sleep quality had the highest risk of CVD compared to those with a normal sleep duration and good sleep quality.

Methodological aspects of sleep duration and sleep quality assessed by questionnaire

The average sleep duration was assessed by the question "How many hours of sleep do you usually get per 24 hour period?", with answer categories '5 hours or less', '6 hours', '7 hours', '8 hours', and '9 hours or more'. In our and many other cohort studies, self-reported sleep duration relied upon this single question. Lauderdale et al. observed that sleep duration was 10% shorter when objectively measured by actigraphy compared to self-reported by questionnaire.[40] This disagreement between subjective and actigraphic measures of sleep duration was larger for older persons and men than for younger persons and women.[41] Therefore, this measurement error may have biased our results.

We assessed sleep quality with the question "Do you usually feel rested when you rise?" from the Amsterdam Biographical Questionnaire on neuroticism.[42] 'Good sleep quality' was defined if the answer was 'yes' for rising rested, and 'poor sleep quality' if the answer was 'no'. As in other studies assessing sleep quality by questionnaire, this question was not validated. Rising rested is an indirect measure of sleep quality. People may not know that they have a poor sleep quality (for example because of micro-arousals or disturbances in sleep phases), but when they rise in the morning, they do not feel rested. Rising rested may therefore be a good proxy for sleep quality.

The association between sleep and CVD risk factors

Sleep deprivation studies are used to study the effects of short sleep on intermediate CVD endpoints. In these studies, participants are exposed to very short sleep duration (usually around 4 hours) for a short period of time. Sleep deprivation is not likely to have the same effects as 'habitual' short sleep duration on CVD risk factors. Therefore, we discuss both the results from sleep deprivation studies and from prospective epidemiological studies in this section.

The relation between short sleep duration and CVD incidence could be due to an effect of short sleep on intermediate biological CVD risk factors such as BMI, hypertension, diabetes, hypercholesterolemia, and inflammatory markers.[43-46] Short sleep duration is associated with overweight.[47] The mechanisms supportive of the obesogenic effects of short sleep, as shown in sleep deprivation studies, include upregulation of appetite by reduced leptin and elevated ghrelin levels,[48,49] lower energy expenditure, and altered glucose metabolism.[50] Furthermore, longitudinal observational studies among healthy populations showed that short sleep duration was associated with a higher risk of hypertension.[45,51] In a recent meta-analysis including 10 cohort studies, a 28% higher risk of type 2 diabetes was observed among short sleepers compared to those with a normal sleep duration.[44] Sleep plays an important role in the control of blood glucose levels, and recurrent partial sleep deprivation (four hours sleep per night) in 11 young men had unfavourable effects on carbohydrate metabolism and endocrine function.[52] Short sleep duration was also associated with poor lipid profiles. Experimental sleep restriction (three nights of four hours) significantly increased total cholesterol and LDL cholesterol levels in postmenopausal women.[53] In a longitudinal observational study, each additional hour of sleep was associated with a lower risk of a high blood cholesterol level in healthy young women, but not in men.[46] Sleep deprivation could increase the risk of dyslipidemia by increasing appetite and dietary consumption of saturated fatty acids, decreasing motivation to engage in regular physical activity, and increasing stress resulting in catecholamine induced lipolysis.[46] Finally, the CARDIA Study showed that each additional hour of (actigraphically measured) sleep duration was associated with a lower 5-year incidence of coronary artery calcifications.[54]

Besides short sleep duration, also sleep quality may be important in relation to CVD risk. In a recent meta-analysis of 13 prospective studies including 122,500 participants, insomnia – defined as difficulty of initiating or maintaining sleep or presence of restless, disturbed nights – was associated with a 45% higher risk of fatal and non-fatal CVD.[55] Artificial suppression of slow wave sleep (SWS) may resemble poor sleep quality. SWS, thought to be the most restorative sleep stage, is associated with a lower heart rate, lower blood pressure, lower sympathetic nervous activity and cerebral glucose utilization, compared with wakefulness.[52] During SWS, growth hormone is released while the stress hormone cortisol is inhibited.[52] In a trial in young healthy adults, all-night selective suppression of SWS, without any change in total sleep time, resulted in a marked decrease in insulin sensitivity without adequate compensatory increase in insulin release.[56] This supports the hypothesis that poor sleep quality increases metabolic and CVD risk.

In conclusion, there is some evidence that short sleep duration and poor sleep quality have unfavourable effects on CVD risk factors.

IMPLICATIONS FOR PUBLIC HEALTH

The results reported in this thesis underline the importance of a healthy diet, sufficient physical activity and sufficient sleep. In The Netherlands, much is to be gained with regard to adhering to a healthy lifestyle. The Dutch National Food Consumption Survey of 2007–2010 showed that only 3–14% of the Dutch adults complied with the recommendations for vegetable consumption, only 3–26% with those for fruit, and 10–33% with those for fish consumption.[57] The situation is more favourable for physical activity, 58% of the adults adhered to the Dutch physical activity guideline in 2011.[58] Average sleep duration was 8 hours for men and 8.5 hours for women aged 25–64 in 2003.[58] As far as we know, there are no representative data on prevalence of short or long sleep duration or poor sleep quality in The Netherlands. Alcohol was consumed by 84% of persons aged between 15–65 in 2008.[59] Furthermore, 25% of the Dutch population above the age 15 smoke.[60]

From a public health perspective, both the prevalence of unhealthy lifestyle factors, and the association with health outcomes are of interest. The population attributable risk (PAR) combines these two into a measure that can be interpreted as the proportion of the disease that could have been prevented if the unhealthy lifestyle factor would not have been present. In other words, the PAR gives an estimate of the reduction in CVD incidence that would be observed if the total population adhered to a healthy lifestyle, compared to the current level of the lifestyle factors.[61] This is under the assumption of a causal association between lifestyle factors and CVD incidence. For the calculation of PARs, we used the prevalence of lifestyle factors and the HRs for the associations of these lifestyle factors with 12-year CVD incidence as reported in this thesis. Subsequently, we translated PARs into the number of composite CVD cases that could have been prevented in the total population of The Netherlands – considering that 92,602 cases occurred in 2007[62,63] (**Table 7.2**).

In the MORGEN Study, we defined five healthy lifestyle factors: a healthy diet (Mediterranean Diet Score ≥5), sufficient physical activity (≥3.5 hrs/wk cycling or sports), sufficient sleep duration (≥7 hours), alcohol consumption (≥1 beverage/month), and non-smoking (Chapter 6). According to these definitions, 37% of the MORGEN cohort had a healthy diet, 52% sufficient physical activity and 83% sufficient sleep duration (**Table 7.2**). The number of composite CVD cases that could have been prevented yearly in the total population of The Netherlands by a healthy diet, or sufficient physical activity, or sufficient sleep duration were 7,300 (8%), 6,200 (7%), and 4,100 (4%), respectively. If the whole population would adhere to all three healthy lifestyle factors 13,900 (15%) cases could have been prevented or postponed. If – in addition to these three lifestyle factors – the whole population would also adhere to moderate alcohol consumption and did not smoke 33,300 (36%) CVD cases could have been prevented or postponed (**Table 7.2**). It should be noted that PARs should be interpreted with caution because they are based on point estimates with substantial confidence intervals of the prevalence of lifestyle factors and of the HRs for the association between these lifestyle factors and CVD incidence. These

Table 7.2 Public health impact of lifestyle factors on composite CVD[a]: the MORGEN Study

Lifestyle factor	Prevalence (%)	Hazard ratio	PAR (%)[b]	Number attributable to lifestyle factor(s) in The Netherlands[c]
Healthy diet[d]	37	0.88 (0.74–1.05)	8	7,300
Sufficient physical activity[e]	52	0.87 (0.74–1.03)	7	6,200
Sufficient sleep[f]	83	0.78 (0.65–0.94)	4	4,100
All three above factors	19	0.75 (0.58–0.97)	15	13,900
Moderate alcohol consumption[g]	84	0.79 (0.63–0.98)	4	3,900
Non-smoking	65	0.57 (0.48–0.67)	21	19,200
Five healthy lifestyle factors[h]	12	0.35 (0.23–0.52)[i]	36	33,300

[a] Composite of fatal CVD, nonfatal myocardial infarction and nonfatal stroke; [b] Proportion of the cases that could have been prevented or postponed if all participants adopted all healthy lifestyle factors; [c] Considering that in The Netherlands 92,602 composite CVD cases occured in 2007,[62,63] numbers were rounded to the nearest 100; [d] A healthy diet was defined as a Mediterranean Diet Score of 5–8 (range 0–8); [e] Sufficient physical activity was defined as ≥3.5 hrs/wk cycling and sports; [f] Sufficient sleep duration was defined as ≥7 hrs; [g] Alcohol consumption was defined as ≥ one glass per month; [h] Five healthy lifestyle factors: healthy diet, sufficient physical activity, sufficient sleep duration, moderate alcohol consumption, and non-smoking; [i] Hazard ratio for five compared to none or one healthy lifestyle factors

figures therefore give only an indication about the possible effect of optimalisation of lifestyle factors on CVD risk.

Since the prevalence of lifestyle factors is population specific, preventable proportions are too. In the MORGEN Study, participants were younger and healthier than the total Dutch population, and therefore the prevalence of healthy lifestyle factors may be higher.[64] Since the proportion of the MORGEN population that can improve their lifestyle is smaller than in the general Dutch population, this may have resulted in an underestimation of the PAR for the general Dutch population. Consequently, the number of CVD cases that could have been prevented or postponed may be underestimated. For instance, the Dutch National Institute for Public Health and the Environment (RIVM) concluded in their report 'Our Food Our Health' that if all Dutch people would adhere to the dietary guidelines, 38,100 fatal and non-fatal CVD cases could have been prevented or postponed (24%).[65] For adhering to the Dutch physical activity guideline the corresponding number of cases was 20,700 (13%), for alcohol consumption 16,400 cases (10%), and for non-smoking 24,000 (15%) cases.[65] These numbers are larger than those estimated based on the MORGEN cohort. This difference is due to larger PARs in 'Our Food Our Health' due to different operationalisation of a healthy lifestyle and cut-off points for a healthy level. In addition, a difference in definition of the CVD endpoints resulted in lower estimates of the numbers of cases that could have been prevented. We used a composite of fatal total CVD, non-fatal stroke and CHD, whereas in the report a composite of fatal and non-fatal CHD, stroke

and heart failure was used.[65] In other cohort studies on lifestyle factors in relation to CVD risk, preventable proportions of CVD incidence for adhering to all lifestyle factors ranged from 35% to 80%[66-80] so our estimation of 36% may be considered conservative.

CONCLUSIONS

Our results suggest that the strength of the association between dietary patterns and CVD incidence is likely underestimated in studies in which only the baseline measurement of diet was used. Furthermore, leisure time physical activities should be of at least moderate intensity to contribute to a lower CVD risk. We also observed that sufficient sleep is a factor that should be taken into consideration in CVD prevention, in combination with a healthy diet, sufficient physical activity, moderate alcohol consumption and not smoking. We estimated that about a third of CVD cases could have been prevented or postponed by adhering to a healthy lifestyle. Our results underscore the importance of a healthy lifestyle for CVD prevention.

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7

Nederlandse samenvatting (Summary in Dutch)

Dit proefschrift richt zich op de associatie tussen leefstijl en hart- en vaatziekten (HVZ). De nadruk ligt hierbij op het voedingspatroon, lichaamsbeweging en slaapgewoonten. HVZ is de verzamelnaam voor aandoeningen van het hart- en vaatstelsel, zoals hartinfarcten en beroertes. Hoewel de sterfte aan HVZ in Nederland afneemt, neemt het aantal bestaande ziektegevallen toe.

Er is in de afgelopen tientallen jaren veel onderzoek gedaan naar leefstijl in relatie tot hart- en vaatziekten. Het is bekend dat een gezond voedingspatroon, voldoende lichaamsbeweging, matige consumptie van alcohol en niet-roken het risico op HVZ kunnen verlagen met ongeveer twee derde. Onderzoek naar de associatie tussen slaap en HVZ staat nog in de kinderschoenen.

In dit proefschrift zijn de volgende vraagstellingen onderzocht:

- Wat is de associatie tussen een Mediterraan voedingspatroon en verschillende vormen van HVZ? (**Hoofdstuk 2**)
- Wordt de effectschatter van de associatie tussen een Mediterraan voedingspatroon en HVZ sterker door meerdere metingen van dit voedingspatroon te gebruiken? (**Hoofdstuk 3**)
- Wat is de associatie tussen specifieke vormen van lichaamsbeweging (wandelen, tuinieren, fietsen en sporten) en HVZ? (**Hoofdstuk 4**)
- Hoe zijn slaapduur en slaapkwaliteit geassocieerd met HVZ? (**Hoofdstuk 5**)
- Voegt voldoende slapen iets toe aan de verlaging van het risico op HVZ die al bereikt is door een gezond voedingspatroon, voldoende lichaamsbeweging, een matige consumptie van alcohol en niet-roken? (**Hoofdstuk 6**)

Om antwoord te geven op de vraagstellingen is gebruik gemaakt van de gegevens van het **EPIC-NL cohort**, het **MORGEN cohort**, en de **Doetinchem Cohort Studie**. Voor onze analyses in het **EPIC-NL cohort** (**hoofdstuk 2**) zijn bijna 35.000 mannen en vrouwen van 20–65 jaar geselecteerd. Bij deze mensen zijn in de periode 1993–1997 leefstijl- en risicofactoren gemeten. Meer dan 20.000 van die deelnemers in **EPIC-NL** waren onderdeel van het **MORGEN cohort** (**hoofdstukken 2, 4 en 5**). Voor het **MORGEN cohort** zijn aselecte steekproeven getrokken uit de bevolkingsregisters in Amsterdam, Maastricht en Doetinchem. Voor de analyses in de **Doetinchem Cohort Studie** is gebruik gemaakt van drie herhaalde metingen over een periode van 10 jaar bij ongeveer 3.400 mensen uit Doetinchem.

De mate waarin volgens het Mediterrane voedingspatroon wordt gegeten, wordt vaak gemeten met een Mediterrane voedingsscore die van 0–9 loopt. In het **EPIC-NL cohort** is onderzocht hoe het verband tussen de Mediterrane voedingsscore voor verschillende HVZ er uit ziet (**hoofdstuk 2**). Dat deze score geassocieerd is met een lager risico op HVZ is in meerdere prospectieve cohort studies aangetoond. Echter, HVZ is een verzamelnaam van ziektebeelden met verschillende oorzaken (etiologie). Elke toename van twee punten op de score ging gepaard met een 22% lager risico op sterfte aan HVZ en een 5% lager risico op totaal (ziekte + sterfte) HVZ. Voor

de specifieke HVZ, was elke toename van twee punten op de score gerelateerd aan een 14% lager risico op een hartinfarct, een 12% lager risico op beroerte en een 26% lager risico op longembolie. Angina pectoris, TIA, en perifeer arterieel vaatlijden lieten geen verband zien met de Mediterrane voedingsscore. Dit is mogelijk een gevolg van het feit dat deze aandoeningen minder makkelijk vast zijn te stellen dan die van hartinfarct, beroerte en longembolie.

In de **Doetinchem Cohort Studie** is de associatie tussen de Mediterrane voedingsscore en HVZ op basis van één meting van de Mediterrane voedingsscore aan het begin van de studie vergeleken met dezelfde associatie op basis van het gemiddelde van drie metingen over een periode van 10 jaar (**hoofdstuk 3**). Herhaalde metingen kunnen de meetfout verkleinen aangezien een gemiddelde blootstelling van meerdere metingen een kleinere variatie binnen personen geeft. Daarnaast kan door het gebruik van herhaalde metingen rekening worden gehouden met mogelijke veranderingen in eetgewoonten in de tijd. Zoals verwacht is de associatie van HVZ met de eenmalige meting van het Mediterrane voedingspatroon minder sterk dan die met het gemiddelde van drie metingen. In welke mate dit komt door het verminderen van de meetfout of door mogelijke veranderingen in eetgewoonten is niet duidelijk. Onze resultaten laten zien dat de sterkte van de associatie tussen de Mediterrane voedingsscore en HVZ mogelijk onderschat wordt in onderzoeken met slechts één meting van deze score.

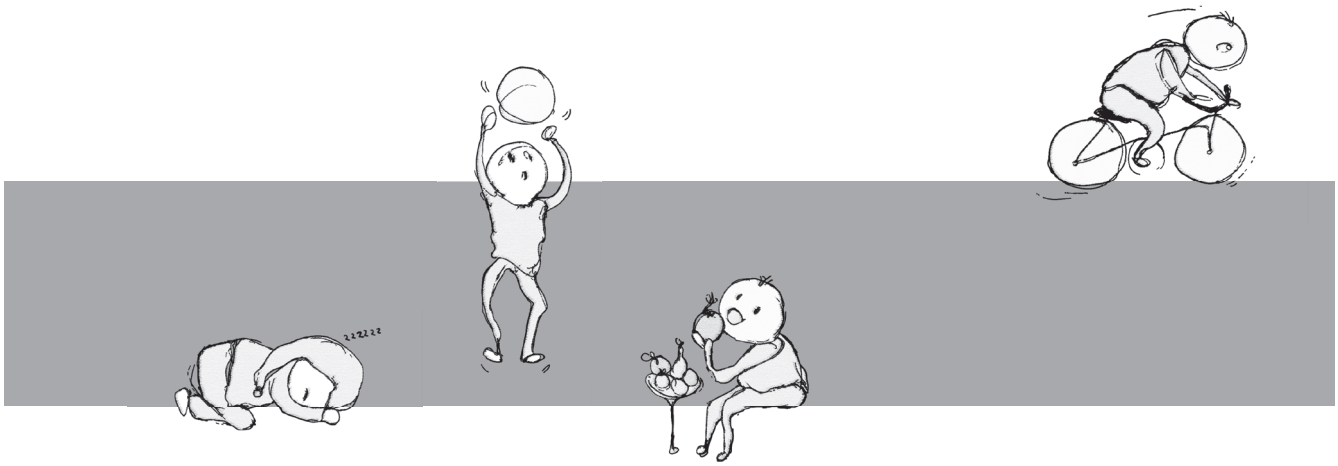
In het **MORGEN cohort** is de associatie tussen specifieke vormen van lichaamsbeweging en HVZ onderzocht (**hoofdstuk 4**). Fietsen (wel versus niet) en sporten (wel versus niet) zijn geassocieerd met respectievelijk een 18 en 26% lager risico op HVZ. In onze studie zijn wandelen en tuinieren niet aan HVZ gerelateerd. Wandelen en tuinieren zijn activiteiten met over het algemeen een relatief lage intensiteit. Er was geen informatie over de intensiteit van wandelen en tuinieren. Daarom was het niet mogelijk om wandelen en tuinieren met een lage en matige intensiteit te scheiden. Dit heeft gezorgd voor misclassificatie en dus voor het mogelijk missen van een associatie. Een andere verklaring voor het feit dat er geen associatie voor wandelen en tuinieren was, is dat de MORGEN-populatie uit vrij jonge en actieve mensen bestaat. Het zou kunnen dat wandelen niet veel meer toevoegt als men al activiteiten met een hogere intensiteit uitvoert. Deze hypothese wordt ondersteund door het feit dat in andere – veelal Amerikaanse – onderzoeken, met minder actieve deelnemers, vaak wel een associatie tussen wandelen en HVZ gevonden wordt.

In het **MORGEN cohort** is onderzocht of slaapkwaliteit, naast slaapduur, van belang is voor de preventie van HVZ (**hoofdstuk 5**). Andere onderzoeken hebben aangetoond dat zowel een korte slaapduur als een lange slaapduur het risico op HVZ verhoogt. Wij vonden bij mensen die kort slapen (≤ 6 uur per 24-uurs periode) ten opzichte van mensen met een normale slaapduur (7 uur) een 15% hoger risico op totaal HVZ en een 23% hoger risico op coronaire hartziekten. Er zijn echter mensen die kort slapen maar 's ochtends wel fris en uitgerust opstaan. Waarschijnlijk combineren deze mensen een korte slaap met een goede slaapkwaliteit. Kort slapen en een slechte slaapkwaliteit was geassocieerd met een 65% hoger risico op totaal HVZ en een 85%

hoger risico op coronaire hartziekten ten opzichte van een normale slaapduur en een goede slaapkwaliteit. Bij alléén een korte slaapduur (maar goede slaapkwaliteit) of alléén een slechte slaapkwaliteit (maar normale slaapduur) was het risico op totaal HVZ en coronaire hartziekten wel verhoogd, maar niet statistisch significant. Wij zagen geen verhoogd risico op totaal HVZ en coronaire hartziekten bij mensen met lange slaapduur (≥ 9 uur per 24-uurs periode), in tegenstelling tot andere cohortstudies. Onze conclusie was dat een korte slaapduur het sterkst geassocieerd was met HVZ in combinatie met een slechte slaapkwaliteit. Wij bevelen daarom aan om in toekomstig onderzoek niet alleen slaapduur, maar ook slaapkwaliteit mee te nemen.

Vervolgens is in het **MORGEN cohort** onderzocht of voldoende slapen iets toevoegt aan de verlaging van het risico op HVZ door traditionele gezonde leefstijlfactoren – een gezond voedingspatroon, voldoende lichaamsbeweging, matig alcoholgebruik en niet-roken (**hoofdstuk 6**). Mensen die gezond eten, voldoende bewegen, matig alcohol gebruiken en niet-roken hadden een 57% lager risico op totaal HVZ en een 67% lager risico op sterfte aan HVZ ten opzichte van de mensen met hoogstens één van deze gezonde leefstijlfactoren. Als de mensen met een gezonde leefstijl ook nog voldoende sliepen, hadden ze zelfs een 65% lager risico op totaal HVZ en een 83% lager risico op sterfte aan HVZ. Onze conclusie was dat voldoende slaap een extra bijdrage levert aan de verlaging van het risico op HVZ door de vier traditionele leefstijlfactoren.

In **hoofdstuk 7** worden de belangrijkste bevindingen van dit proefschrift, enkele methodologische aspecten, en het effect van de leefstijlfactoren op biologische risicofactoren voor HVZ bediscussieerd. Dit hoofdstuk wordt afgesloten met een discussie over de implicaties van onze resultaten voor de volksgezondheid met betrekking tot HVZ. Ongeveer één op drie gevallen van HVZ zou voorkomen of uitgesteld kunnen worden door een gezonde leefstijl. De resultaten in dit proefschrift onderschrijven het belang van een gezonde leefstijl voor preventie van HVZ.



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Nu dit proefschrift bijna afgerond is, is het tijd om terug te denken aan het traject en alle fijne mensen die er op mijn pad zijn gekomen. Er zijn zoveel mensen om dankbaar te zijn, dat ik bang ben dat ik ze niet allemaal kan noemen. Toch ga ik een dappere poging doen...

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About the author

CURRICULUM VITAE

Marieke Hoevenaar-Blom was born on March 5th, 1983 in Brummen, the Netherlands. In 2001 she completed secondary school (VWO) at the "Isendoorn College" in Warnsveld. In 2005 she received her bachelor's degree in "Nutrition and Dietetics" at HAN University of Applied Sciences, followed by her masters degree in "Nutritional Epidemiology" at Wageningen University in 2007.

In September 2007 she started her PhD project of which the results are described in this thesis. This project was conducted at the Centre for Prevention and Health Services of the National Institute of Public Health and the Environment (RIVM) in Bilthoven in collaboration with the Division of Human Nutrition of Wageningen University. In 2011, she won the 'Young Investigator Award – Clinical Sciences' at the annual meeting of the European Society of Cardiology on Preventive Cardiology (EuroPRevent) in Geneva for her research paper on sleep and cardiovascular diseases (chapter 5 of this thesis).

PUBLICATIONS

Hoevenaar-Blom MP, Spijkerman AM, Kromhout D, Van den Berg JF,Verschuren WM. Sleep duration and sleep quality in relation to 12-year cardiovascular disease incidence: The MORGEN Study. *Sleep*. 2011;34(11):1487-1492.

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Hoevenaar-Blom MP, Nooyens ACJ, Kromhout D, Spijkerman AMW, Beulens JWJ, van der Schouw YT, Bueno-de-Mesquita HB, Verschuren WMM. Mediterranean Style Diet and 12-year Incidence of Cardiovascular Diseases: The EPIC-NL Cohort Study. *PLoS ONE*. 2012;7(9): e45458. doi:10.1371/journal.pone.0045458.

Hoevenaar-Blom MP, Spijkerman AMW, Boshuizen HC, Boer JMA, Kromhout D, Verschuren WMM. Effect of using repeated measurements of a Mediterranean style diet on the strength of the association with 12-year cardiovascular disease incidence. The Doetinchem Cohort Study. Submitted.

Hoevenaar-Blom MP, Spijkerman AMW, Kromhout D, Verschuren WMM. Sufficient sleep duration contributes to lower cardiovascular disease risk in addition to four traditional lifestyle factors. The MORGEN Study. Submitted.

Berentzen NE, Beulens JWJ, **Hoevenaar-Blom MP**, Kampman E, Bueno-de-Mesquita HB, Romaguera D, Peeters P, May AM. Adherence to the WHO's healthy diet indicator and overall cancer risk in the EPIC-NL cohort. Submitted.

OVERVIEW OF COMPLETED TRAINING ACTIVITIES

Discipline specific activities

- Principles of epidemiologic data analysis (1), NIHES, Rotterdam, 2008
- Regression analysis, NIHES, Roterdam, 2008
- Survival analysis, NIHES, Rotterdam, 2008
- Principles of epidemiologic data analysis (2), NIHES, Lunteren, 2008
- "Voedings- en gezondheidscongres" (Conference on nutrition and health), Amsterdam, 2008
- Annual meeting of the Netherlands Epidemiology Society (WEON), Amsterdam, 2009
- Annual meeting of the European Society of Cardiology on Preventive Cardiology (EuroPRevent), Paris, 2008 and Geneva, 2011
- Annual meeting of the European Society of Cardiology, Barcelona, 2009
- Missing data analysis, RIVM, Bilthoven, 2009
- EPIC-NL symposium, UMCU, Utrecht, 2008 and 2010
- EPIC-NL symposium, RIVM, Bilthoven, 2009 and 2011
- Symposium "Lifestyle and chronic disorders", VU, Amsterdam, 2010
- Symposium "Sodium, potassium and cardiovascular health", WUR, Wageningen, 2010
- Longitudinal data analyses, EpidM, Amsterdam, 2011
- Cardiovascular epidemiology, UMCU, Utrecht, 2011
- Analysis using R, VLAG, Wageningen, 2012

General courses

- PhD retreat, WUR, Wageningen, 2007
- PhD competence assessment, VLAG, Wageningen, 2007
- "Tutorentraining" (course on guiding students), WUR, Wageningen, 2007
- Making attractive poster presentations, RIVM/ Proneri, Bilthoven, 2008
- Scientific writing, WUR, Wageningen, 2009
- Use of software, RIVM/ Proneri, Bilhoven, 2010
- Mini symposium: "How to write a world-class paper", WUR/ Elsevier, Wageningen, 2010

- PhD skills, RIVM/ Proneri, Bilthoven, 2010
- Workshop "zakelijk flirten" (professional interaction), RIVM, Bilthoven, 2010
- Career perspectives, VLAG, Wageningen, 2012
- Philosophy and ethics of food sciences and technology, VLAG, Wageningen, 2012
- Writing grant proposals, WUR, Wageningen, 2011
- How to write a competitive proposal for Framework 7, RIVM, Bilthoven, 2011

Optional courses and activities

- Lectures of the Centre for Nutrition and Health, RIVM, Bilthoven, 2007–2011
- Lunch lectures sector Public Health and Health Services, RIVM, 2007–2012
- Methodology meetings, RIVM, Bilthoven, 2007–2012
- Epi research meetings, WUR, Wageningen, 2007–2009
- Monday morning lectures, RIVM, Bilthoven, 2008–2012
- Doetinchem Cohort Study research meetings, RIVM, Bilthoven, 2009–2012

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