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The Mediterranean diet in relation to mortality and CVD: a Danish cohort study

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Abstract

The aim of the present study was to determine whether the Mediterranean Diet Score (MDS) is associated with reduced total mortality, cardiovascular incidence and mortality in a Danish population. Analyses were performed on 1849 men and women sampled during the 1982–83 Danish MONICA (MONItoring trends and determinants of Cardiovascular disease) population study, whose diet was assessed by means of a validated 7 d food record. The adherence to a Mediterranean dietary pattern was calculated by three different scores: one based on a classification excluding ingredients from mixed dishes and recipes (score 1); another based on a classification including ingredients (score 2); the last one based on a variant of the latter including wine instead of alcohol intake (score 3). The association between these scores and, respectively, total mortality, cardiovascular incidence and mortality was tested by a Cox proportional hazards model adjusted for several potential confounders of the association. Generally, all three scores were inversely associated with the end-points, although associations with score 1 did not reach statistical significance. Score 2 was inversely associated with total mortality (hazard ratio 0.94; 95% CI 0.88, 0.99). This association was confirmed for total cardiovascular as well as myocardial infarction (MI) incidence and mortality, but not for stroke. Score 3 was slightly more associated with the same outcomes. All associations were also resistant to adjustment for covariates related to potential CVD pathways, such as blood lipids, blood pressure and weight change after 11 years of follow-up. In a Danish cohort, the MDS was inversely associated with total mortality and with cardiovascular and MI incidence and mortality, but not with stroke incidence or mortality.

Key words: Mediterranean diet: Mortality: Cardiovascular incidence: Mortality

Lifestyle and eating habits are key modifiable factors that people can change to decrease chronic disease risk. Poor diet, in particular, has been convincingly linked to the incidence of CVD and some forms of cancer^(1,2), which are responsible for most of the disease burdens in the majority of developed countries⁽³⁾.

Dietary recommendations promoting healthy dietary patterns including whole-grain foods, legumes, vegetables and fruit, and suggesting that the intake of full-fat dairy products, sweets and red meat be limited, are an emerging approach for the prevention of several diseases, and the Mediterranean-style diet embodies many of these recommendations⁽⁴⁾. Indeed, in a recent meta-analysis, the Mediterranean eating pattern has turned out to be more effective than low-fat

diets in inducing important long-term changes in cardiovascular risk factors and inflammatory markers⁽⁵⁾.

Greater adherence to a Mediterranean-like dietary pattern, in particular, has been shown to be associated with improvement in health status^(6,7) as well as inversely associated with both premature⁽⁸⁾ and total mortality^(9,10). The latter effect has been observed in countries both inside⁽¹¹⁾ and outside^(12–14) the Mediterranean basin, for instance, in Nordic countries⁽¹²⁾, where we recently reported an inverse association with mortality both in elderly^(15,16) and in middle-aged adults living in Sweden⁽¹⁷⁾.

Dietary patterns in the Mediterranean countries are changing rapidly, with an increased consumption of animal products and saturated fats and a decline in the intake of vegetable-based foodstuffs^(18–20). On the other hand,

Abbreviations: FIL, food intake level; HR, hazard ratio; MDS, Mediterranean Diet Score; MI, myocardial infarction; PAL, physical activity level.

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Nordic populations have paradoxically increased their intakes of typical Mediterranean items such as fruit, vegetables and wine between 1970 and mid-2000^(21–23).

The aim of the present study was to determine whether the Mediterranean Diet Score (MDS) was inversely associated with all-cause mortality as well as cardiovascular incidence and mortality in the Danish MONICA (MONItoring trends and determinants of Cardiovascular disease) population study.

Subjects and methods

Subjects and measures

In 1982, a sample of 4807 subjects was randomly selected from the Central Person Register of citizens born in 1922, 1932, 1942 and 1952 living in eleven municipalities in the Copenhagen County⁽²⁴⁾. Of these, 226 were of non-Danish origin. Among the 4581 invited, 3608 participated in a subsequent health examination in 1982–3⁽²⁵⁾. The non-respondents have been described in detail elsewhere⁽²⁶⁾. The present study was part of the Danish MONICA project, an international study conducted under the auspices of the WHO to monitor CVD trends and determinants. One subject was excluded due to missing BMI information at baseline and three subjects for missing blood test results. The cohort described here included 1849 subjects (948 women) with complete information on diet intake and anthropometry and 1348 subjects (701 women) who also had results for blood tests and blood pressure.

The participants filled in a questionnaire describing their highest grade of education or highest year of regular schooling classified into 0–7, 8–11 and ≥ 12 years, their cigarette smoking habits and their leisure time activity. The latter was categorised into four classes: inactive; light (< 4 h/week); moderate (> 4 h/week); energetic (> 4 h/week).

Body weight was measured to the nearest 0.1 kg using a lever balance, with subjects dressed in light clothing or underwear. Height was measured without shoes to the nearest 1 cm. Weight change information after 11 years of follow-up was available for a subsample of 1348 subjects.

BMR (kcal/d) was calculated according to Schofield equations for subjects aged 30–59 years⁽²⁷⁾:

$$\text{BMR} = 11.5 \text{ weight (kg)} + 873 \text{ (men).}$$

$$\text{BMR} = 8.3 \text{ weight (kg)} + 846 \text{ (women).}$$

Food intake level (FIL) was then calculated based on the ratio of reported energy intake to BMR. Both systolic and diastolic blood pressures were measured with a London School of Hygiene sphygmomanometer, using one of the three different cuffs. Duplicate measurements were taken on the left arm after a minimum of 5 min of rest in a supine position. Means of duplicate measures were calculated and used in the analyses described below. Blood samples were drawn after a 12 h overnight fast. A commercial enzymatic method was used (Boehringer Mannheim) to measure the concentrations of total cholesterol, HDL-cholesterol and TAG (mmol/l) in the serum⁽²⁸⁾. The total cholesterol:HDL-cholesterol ratio was calculated, and a value greater than 4 was arbitrarily chosen as a threshold of a risky lipid profile.

Dietary assessment

Information on diet was obtained by means of a four-page (A3 format) weighed 7 d food record. The front page contained instructions for correct completion of the diet records together with the average household weights of nineteen frequently consumed foods, e.g. the white of an egg and a slice of bread. For butter and liver pâté, three examples were given of typical amounts put on a slice of bread. On the next pages, 100 foods were listed in the following nine subgroups: dairy products; bread and cereal grains; fats; cold cuts, etc.; vegetables; fruit; meat (including poultry and fish); drinks; miscellaneous items. The last page included space to record foods that did not fit into any of the nine groups, such as mixed dishes, home-made salads and cakes.

The participants were also given thorough verbal instructions on how to fill out the food record. It was emphasised that the diet record be completed during a typical 7 d period including a minimum of non-habitual social activities. Food quantities were expressed in grams, estimated as accurately as possible or preferably weighed. The same trained dietitian manually checked and coded all food records into dietary components.

Definitions of food groups and of the Mediterranean Diet Score

To assess the association of diet and lifestyle factors with all-cause mortality, a version of the modified MDS⁽²⁹⁾ adapted to the MONICA diet questionnaire was calculated. The score comprised eight positive or negative components: (1) ratio of (monounsaturated + polyunsaturated):saturated fats; (2) vegetables; (3) fruit; (4) cereal grains; (5) fish and fish products; (6) alcohol intake; (7) meat, meat products and eggs (negative); (8) dairy products (negative). As has been described previously^(9,29), the intake of each component was adjusted to the daily energy intakes of 10 467 kJ (2500 kcal) for men and 8 374 kJ (2000 kcal) for women, in order to obtain energy-adjusted associations in all the analyses. As sex-specific median intakes were taken as cut-off points, the final score must be considered as a measure of relative adherence to the Mediterranean dietary pattern. A value of 1 was assigned to subjects whose consumption was higher than the sex-specific median and 0 to the others, with the exception of meat and dairy products, where the reverse rule was applied. The final score was obtained by summing these values, and it varied from 0 (low adherence) to 8 (high adherence). Food groups were defined based on two different procedures, from which two different scores were produced. The first procedure included the identification of all the possible food items that could be classified according to the above-mentioned food categories (vegetables, fruit, etc.), but excluding mixed dishes (score 1). The second procedure included food items classified into food groups as in score 1, but now including ingredients extrapolated from mixed dishes or recipes (score 2). Finally, a variant of score 2 was created, now including wine instead of total alcohol intake (score 3).

Endpoints

Information on all-cause mortality as well as on CVD or myocardial infarction (MI) incidence and mortality was retrieved from the National Patient Registry of Hospital Discharges, the Cause of Death Register and the Central Person Register. The subjects were followed until 8 July 2007 for an average of 14 years. Survival time was parameterised in terms of lifetime until death or censoring event in years, with left truncation at the age of entrance into the study being equal to baseline examination. Total CVD cases were identified based on codes 390–458 (ICD8) and I00–I99 (ICD10) and MI cases were identified based on codes 410 (ICD8) and I21 (ICD10), while stroke cases were identified based on codes 430–434 + 436 (ICD8) and I60–I64 (ICD10). Analyses on CVD, MI and stroke incidence (both fatal and non-fatal cases) and mortality were performed given that there was no CVD, MI or stroke at baseline.

Statistical analyses

The associations of the three above-mentioned diet scores with mortality were determined by means of Cox proportional hazards models, either adjusted only by age or adjusted for potential confounders such as sex, age, obesity, smoking status (two dummy variables were included, describing pack years greater than 0 but less than 30 and pack years greater than 30, keeping the ‘never smoked’ category as the reference), education (the lowest level *v.* higher levels) and physical activity (see the above-mentioned categories). The latter model was then considered the main model. Additionally, in order to determine possible pathways of effects, we performed some confirmatory analyses adjusting for blood pressure (systolic and diastolic, included as continuous variables), blood lipids (TAG, included as a continuous variable, and total cholesterol:HDL-cholesterol ratio >4) and weight change (including two dummy variables describing weight change >3 kg or <–3 kg and keeping a weight change of 0 to ±3 kg as the reference category). All-cause mortality as well as cause-specific incidence and mortality, i.e. total CVD, MI and stroke, were studied in association with the MDS.

Effect modification of exposure (MDS) by each covariate was tested in the main model by including interaction terms. Stratified estimates were calculated in case a significant interaction was found. The association of single components of the MDS was also tested separately, by including as exposure a variable describing an intake over the sex-specific medians (fat ratio, vegetables, fruit, cereal grains, fish and alcohol) or below the median (meat and dairy products). To reduce bias for changes in diet or lifestyle factors due to subclinical disease, the main model was also run after excluding the first 2 years of follow-up. In analyses, different versions of the MDS were included, and these were excluded one at a time, and also single components of these scores were tested (while keeping all other components) in order to determine whether any associations could be explained by a single component.

With the aim of determining the additive effects of an increasing number of risk factors such as ever been a

smoker, having a low physical activity level (PAL) (<4 h/week) and obesity (BMI ≥30 kg/m²) and not consuming a Mediterranean-like diet (MDS ≤4), a new score was calculated, assigning an additional point for each of the above-mentioned risk factors. Different hazard ratios (HR) and 95% CI describing the association (adjusted by sex) of each level of the score with all-cause mortality were calculated and depicted in a histogram.

A sensitivity analysis was performed, aiming to confirm the main results in a subsample of subjects who could be considered ‘adequate reporters’. The ratio of FIL:PAL was calculated. Adequate reporters were defined according to three different definitions based on increasingly stringent criteria, i.e. exclusion of the lowest 30% (less stringent criterion) and of the lowest 50% and of the lowest 65% of the FIL:PAL ratio (most stringent criteria).

Statistical analyses were performed with SAS software (version 9.0; SAS Institute). All tests were considered significant if *P*<0.05.

Bioethics

The present study was conducted according to the guidelines laid down in the Declaration of Helsinki (1989) of the World Medical Association, and all procedures involving human subjects were approved by the Ethics Committee of the Medicine Faculty of the Copenhagen County. All subjects were informed of the aims and procedures of the study, and they gave their written consent.

Results

Cohort description

The population consisted of 948 women (51.3%) and 901 men (48.7%). Only a small number were obese (*n* 138, 7.5%).

Table 1. Intake value of each food group included in the Mediterranean Diet Score, adjusted to 8.374 MJ (2000 kcal) in women and 10.467 MJ (2500 kcal) in men*

(Medians with their 5th–95th percentiles)

	Median	5th–95th percentile
(Monounsaturated + polyunsaturated): saturated fats	1.1	0.8–1.6
Vegetables (g/d)	192.1	79.0–418.5
Fruit (g/d)	109.8	0–401.5
Cereal grains (g/d)	179.9	94.3–297.0
Fish and shellfish (g/d)	25.2	0–87.5
Alcohol among users (g/d)	15.5	2.0–62.6
Wine among users (g/d)	2.5	0.8–13.0
Dairy products (g/d)	297.4	55.0–750.6
Meat products (g/d)	182.3	95.6–310.5
Energy intake (kJ/d) in men	10571.7	6514.7–15993.6
Energy intake (kJ/d) in women	7460.9	4450.6–11852.8
FIL (energy intake:BMR)	1.4	0.8–2.1
Mediterranean Diet Score 1	5	2–7
Mediterranean Diet Score 2	4	2–6
Mediterranean Diet Score 3	4	2–6

FIL, food intake level.

* Energy intake and the ratio of energy intake:BMR are also reported.

Table 2. Survival analyses on the association between an increase in one unit of the three different versions of the Mediterranean Diet Score (scores 1, 2 and 3) and all-cause mortality as well as cardiovascular mortality and incidence (Hazard ratios (HR) and 95 % confidence intervals)

	n	All-cause mortality (n 553)						CVD						Myocardial infarction						Stroke									
		HR		95% CI		Incidence† (n 755)		Mortality (n 223)		Incidence† (n 161)		Mortality (n 64)		Incidence† (n 167)		Mortality (n 40)		HR		95% CI		Incidence† (n 167)		Mortality (n 40)		HR		95% CI	
Score 1 (main model)‡	1849	0.95	0.91, 1.00	0.97	0.93, 1.02	0.96	0.89, 1.05	1.00	0.91, 1.01	0.88	0.76, 1.02	0.96	0.88, 1.06	1.06	0.88, 1.29	0.96	0.88, 1.06	1.06	0.88, 1.29	0.96	0.88, 1.06	1.06	0.88, 1.29	1.06	0.88, 1.29	1.06	0.88, 1.29		
Age-only-adjusted model	1849	0.97	0.92, 1.02	0.97	0.93, 1.02	0.98	0.91, 1.06	0.99	0.90, 1.08	0.90	0.78, 1.05	0.96	0.87, 1.06	1.06	0.88, 1.29	0.96	0.87, 1.06	1.06	0.88, 1.29	0.96	0.87, 1.06	1.06	0.88, 1.29	1.06	0.88, 1.29	1.06	0.88, 1.29		
Score 2 (main model)‡	1849	0.94*	0.88, 0.99	0.94*	0.89, 0.99	0.90*	0.82, 0.99	0.89*	0.80, 1.00	0.80*	0.67, 0.96	0.96	0.86, 1.07	1.03	0.82, 1.30	0.96	0.86, 1.07	1.03	0.82, 1.30	0.96	0.86, 1.07	1.03	0.82, 1.30	1.03	0.82, 1.30	1.03	0.82, 1.30		
Age-only-adjusted model	1849	0.95	0.89, 1.00	0.94*	0.89, 0.99	0.92	0.84, 1.00	0.91	0.82, 1.02	0.84*	0.70, 0.99	0.95	0.86, 1.06	1.01	0.81, 1.25	0.95	0.86, 1.06	1.01	0.81, 1.25	0.95	0.86, 1.06	1.01	0.81, 1.25	1.01	0.81, 1.25	1.01	0.81, 1.25		
Score 3 (main model)‡	1849	0.93*	0.87, 0.98	0.92***	0.87, 0.97	0.87**	0.79, 0.95	0.89*	0.80, 1.00	0.79**	0.66, 0.94	0.93	0.84, 1.04	0.97	0.77, 1.21	0.93	0.84, 1.04	0.97	0.77, 1.21	0.93	0.84, 1.04	0.97	0.77, 1.21	0.97	0.77, 1.21	0.97	0.77, 1.21		
Age-only-adjusted model	1849	0.93*	0.88, 0.99	0.91***	0.87, 0.96	0.88**	0.80, 0.96	0.91	0.82, 1.01	0.80**	0.67, 0.95	0.92	0.83, 1.02	0.94	0.76, 1.17	0.92	0.83, 1.02	0.94	0.76, 1.17	0.92	0.83, 1.02	0.94	0.76, 1.17	0.94	0.76, 1.17	0.94	0.76, 1.17		

Values were significantly different: * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

† Fatal and non-fatal cases.

‡ Adjusted for sex, BMI, education, physical activity and cigarette smoking.

A total of 476 (25.7%) had never smoked cigarettes, while 303 (16.4%) smoked more than thirty pack years. Finally, 241 subjects (13%) abstained from any alcohol intake. Among the cardiovascular risk factors, 450 subjects (24.3%) had high blood pressure (either systolic ≥ 140 mmHg or diastolic ≥ 90 mmHg), almost half of the population had either a total cholesterol:HDL-cholesterol ratio >4 (n 955, 51.6%), while about a third had gained more than 3 kg at follow-up (n 666, 36.0%).

Energy-adjusted median values of each score component (Table 1) showed that the ratio of unsaturated:saturated fats was 1.1 (5th–95th percentiles: 0.8–1.6), indicating a similar level of consumption of each type of fat. Vegetable intake was higher than fruit intake, while the median intake for dairy products was almost 300 g/d. Median alcohol intake among users was about one standard unit and a half (15.5 g/d), while median wine intake was 2.5 g/d. The median reported daily energy intakes were 10 571.7 MJ (2525 kcal) in men and 7460.9 MJ (1782 kcal) in women. The ratio of energy intake:BMR was 1.4 (5th–95th percentiles: 0.8–2.1), indicating a good estimation of energy intake compared with the metabolic rate. The differences among the median values of the three scores were very small.

Main analyses and effect modification

The association between the three different versions of the MDS (scores 1, 2 and 3) and all-cause mortality as well as CVD incidence and mortality was assessed (Table 2), adjusting for only age or for different potential confounders such as sex, age, BMI, education, physical activity and cigarette smoking (main model). Since no statistical interaction ($P > 0.05$) with sex was found in relation to almost all outcomes, all analyses were conducted on men and women together, adjusting for sex.

Results were, in general, in the direction of an inverse association between the three scores and all the outcomes, with the exception that the association between the MDS calculated excluding mixed dishes and recipes (score 1) did not reach statistical significance in either the main model or in the age-only-adjusted model. In contrast, the second version of the MDS, including the ingredients from mixed dishes and recipes (score 2), was inversely associated with all outcomes except with stroke incidence (HR 0.96; 95% CI 0.86, 1.07) and mortality (HR 1.03; 95% CI 0.82, 1.30). In the age-only-adjusted model, score 2 was significantly associated only with CVD incidence and MI mortality. Finally, score 2, recalculated to include wine instead of total alcohol intake (score 3), was significantly associated with all outcomes, but, again, not with stroke.

No effect modification was found when testing the interaction with potential confounders, with the exception of the association between the MDS and MI mortality, which was significant only at low PAL (moderate activity <4 h/week) (score 3: HR 0.61; 95% CI 0.43, 0.86; P for interaction: 0.04).

In order to determine possible pathways of effects in relation to the cardiovascular risk, the main model was further adjusted for blood lipids and blood pressure as well as for

the latter plus weight change (Table 3). With the exception of MI incidence, which did not resist the adjustment for weight change (HR 0.91; 95% CI 0.79, 1.05), the results obtained after these additional adjustments were in line with the previous ones (main model, Table 2) or slightly stronger. Finally, the exclusion of early deaths (i.e. subjects who died during the first 2 years of follow-up) did not materially change the results, with the exception, again, of MI incidence, where association with the score was only of borderline significance (HR 0.91; 95% CI 0.81, 1.02).

Supplementary analyses

Table 4 summarises the results of a supplementary analysis, where the association of all endpoints with different versions of score 3 was determined. The alternative versions were created by excluding, one at a time, each single score component to check if any of these could explain alone the association of the score. The results show that the association was robust, and in the few cases where the association was not significant, a borderline association was found. The association of single MDS components (high or low intake according to median values) with all the endpoints investigated revealed only a few significant results (Table 5). The most robust one was found with high fruit intake, which was inversely associated with all-cause mortality (HR 0.74; 95% CI 0.62, 0.88), CVD incidence (HR 0.86; 95% CI 0.75, 1.00) and mortality (HR 0.72; 95% CI 0.55, 0.94). Also, vegetables showed some significant associations with all-cause mortality (HR 0.81; 95% CI 0.68, 0.96) as well as with MI incidence (HR 0.73; 95% CI 0.54, 1.00) and mortality (HR 0.58; 95% CI 0.35, 0.96).

The results of the sensitivity analyses, based on three different definitions of adequate diet reporters (>30% FIL:PAL, >50% and >65%), showed that although score 3 was always inversely associated with both incidence and mortality, not all these analyses were statistically significant due to a highly reduced number of cases, especially when the most stringent criterion was applied (>65% FIL:PAL). No large variations of these estimates were observed comparing these three analyses. Both all-cause mortality and CVD mortality were shown to be the strongest associations, since they were statistically significant no matter how adequate diet reporters were defined.

Mediterranean Diet Score and other lifestyle factors

Diet and lifestyle are strongly associated, and the level of the MDS was associated with smoking status, obesity and physical activity. We determined the cumulative effect of diet and lifestyle-related risk factors on mortality (not to be confused with a dose response to the number of levels of the MDS) by means of an additional score (Fig. 1). With increased number of risk factors (i.e. ever been a smoker, having a low physical activity level, being obese and not consuming a Mediterranean-like diet) the mortality risk increased linearly. In the highest risk category (three or four risk factors) the sex-adjusted association will all-cause

Table 3. Survival analyses on the association between an increase in one unit of Mediterranean Diet Score 3 and all-cause mortality as well as cardiovascular (CV) mortality and incidence (Hazard ratios (HR) and 95% confidence intervals)

	n	CVD						Myocardial infarction						Stroke								
		All-cause mortality (n 553)			Incident (n 755)			Mortality (n 223)			Incident (n 161)			Mortality (n 64)			Incident (n 167)			Mortality (n 40)		
		HR	95% CI		HR	95% CI		HR	95% CI		HR	95% CI		HR	95% CI		HR	95% CI		HR	95% CI	
Covariates related to CV risk†§	1849	0.93**	0.87, 0.98	0.93**	0.88, 0.97	0.86**	0.79, 0.95	0.89*	0.80, 1.00	0.79**	0.66, 0.94	0.93	0.84, 1.04	0.97	0.78, 1.22							
Weight change¶	1348	0.90*	0.83, 0.98	0.91**	0.85, 0.97	0.81**	0.71, 0.93	0.91	0.79, 1.05	0.73*	0.56, 0.96	0.89	0.78, 1.02	0.98	0.72, 1.32							
Excluding early death‡	1822	0.93*	0.88, 0.99	0.90***	0.85, 0.95	0.84***	0.77, 0.93	0.91	0.81, 1.02	0.80*	0.67, 0.96	0.93	0.84, 1.04	0.96	0.76, 1.20							

Values were significantly different: * P≤0.05, ** P≤0.01, *** P≤0.001.

† Fatal and non-fatal cases.

‡ Adjusted for sex, BMI, education, physical activity and cigarette smoking.

§ Also adjusted for covariates potentially related to possible pathways of CV risk (i.e. blood pressure, TAG and total cholesterol:HDL-cholesterol ratio).

|| Also adjusted for both covariates potentially related to possible pathways of CV risk and weight change.

Table 4. Sensitivity analyses excluding, one at a time, each level of Mediterranean Diet Score (MDS) 3† (Hazard ratios (HR) and 95 % confidence intervals)

	MDS recalculated excluding													
	All-cause mortality (n 553)		CVD				Myocardial infarction				Stroke			
	HR	95% CI	Incidence‡ (n 755)		Mortality (n 223)		Incidence‡ (n 161)		Mortality (n 64)		Incidence‡ (n 167)		Mortality (n 40)	
			HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI
Fat ratio	0.92*	0.86, 0.98	0.91**	0.86, 0.96	0.85**	0.76, 0.94	0.91	0.80, 1.03	0.79*	0.65, 0.96	0.93	0.82, 1.05	0.92	0.71, 1.18
Vegetables	0.94	0.89, 1.01	0.92**	0.87, 0.97	0.88**	0.79, 0.97	0.92	0.82, 1.03	0.82*	0.68, 0.99	0.93	0.83, 1.04	0.98	0.77, 1.24
Fruit	0.96	0.90, 1.02	0.92**	0.87, 0.97	0.89*	0.80, 0.98	0.88*	0.78, 0.99	0.77**	0.64, 0.93	0.94	0.84, 1.05	1.04	0.82, 1.32
Cereal grains	0.92*	0.86, 0.98	0.92**	0.87, 0.97	0.88*	0.80, 0.97	0.89*	0.79, 1.00	0.80*	0.66, 0.96	0.95	0.84, 1.06	0.96	0.75, 1.22
Fish and shellfish	0.91**	0.86, 0.97	0.92**	0.87, 0.97	0.84***	0.76, 0.93	0.88*	0.78, 0.99	0.75**	0.62, 0.91	0.92	0.82, 1.03	0.92	0.72, 1.17
Wine	0.92**	0.87, 0.98	0.92**	0.87, 0.97	0.87**	0.79, 0.96	0.89*	0.79, 1.00	0.76**	0.63, 0.92	0.92	0.82, 1.03	0.98	0.78, 1.24
Dairy products	0.93*	0.87, 0.98	0.91***	0.87, 0.96	0.88**	0.80, 0.97	0.90	0.80, 1.01	0.85	0.71, 1.02	0.93	0.83, 1.04	0.96	0.76, 1.20
Meat products	0.92*	0.87, 0.98	0.92**	0.87, 0.97	0.87**	0.79, 0.96	0.90	0.80, 1.01	0.76**	0.63, 0.92	0.94	0.84, 1.05	0.99	0.78, 1.25

Values were significantly different: * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

† All results were obtained from a Cox proportional hazards model, adjusted for sex, BMI, education, physical activity and cigarette smoking.

‡ Fatal and non-fatal cases.

Table 5. Survival analyses on the association between the intake of single food groups above or below the median and several outcomes related to all-cause mortality and cardiovascular incidence and mortality†

(Hazard ratios (HR) and 95 % confidence intervals)

Single MDS component	MDS recalculated excluding													
	All-cause mortality (n 553)		CVD				Myocardial infarction				Stroke			
	HR	95% CI	Incidence‡ (n 755)		Mortality (n 223)		Incidence‡ (n 161)		Mortality (n 64)		Incidence‡ (n 167)		Mortality (n 40)	
			HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI	HR	95% CI
Intake above the median														
Fat ratio	0.93	0.79, 1.10	0.92	0.80, 1.06	0.92	0.71, 1.20	0.76	0.55, 1.03	0.68	0.41, 1.11	0.90	0.67, 1.22	1.32	0.70, 2.51
Vegetables	0.81*	0.68, 0.96	0.88	0.76, 1.02	0.81	0.62, 1.06	0.73*	0.54, 1.00	0.58*	0.35, 0.96	0.94	0.69, 1.27	0.90	0.48, 1.68
Fruit	0.74***	0.62, 0.88	0.86*	0.75, 1.00	0.72*	0.55, 0.94	1.01	0.73, 1.38	0.85	0.52, 1.42	0.87	0.64, 1.18	0.59	0.31, 1.12
Cereal grains	0.97	0.82, 1.15	0.90	0.78, 1.04	0.76*	0.58, 1.00	0.90	0.66, 1.24	0.69	0.41, 1.16	0.82	0.60, 1.11	1.00	0.53, 1.89
Fish and shellfish	1.05	0.88, 1.24	0.90	0.78, 1.04	1.12	0.86, 1.46	1.02	0.75, 1.39	1.12	0.68, 1.85	1.01	0.74, 1.37	1.38	0.72, 2.63
Total alcohol	1.09	0.92, 1.29	1.14	0.98, 1.31	1.24	0.95, 1.62	0.98	0.72, 1.35	1.32	0.80, 2.20	1.33	0.98, 1.81	1.48	0.78, 2.81
Wine	1.00	0.84, 1.18	0.93	0.80, 1.07	0.91	0.70, 1.19	0.96	0.70, 1.32	1.03	0.63, 1.70	1.05	0.77, 1.43	0.89	0.47, 1.68
Intake below the median														
Dairy products	1.00	0.84, 1.18	1.02	0.89, 1.18	0.85	0.65, 1.11	0.90	0.65, 1.22	0.49**	0.30, 0.82	0.97	0.72, 1.33	1.06	0.56, 2.02
Meat products	0.96	0.81, 1.14	0.91	0.79, 1.06	0.87	0.67, 1.15	0.89	0.65, 1.23	1.06	0.64, 1.74	0.89	0.65, 1.22	0.82	0.43, 1.58

MDS, Mediterranean Diet Score.

Values were significantly different: * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

† All results were obtained from a Cox proportional hazards model, adjusted for sex, BMI, education, physical activity and cigarette smoking.

‡ Fatal and non-fatal cases.

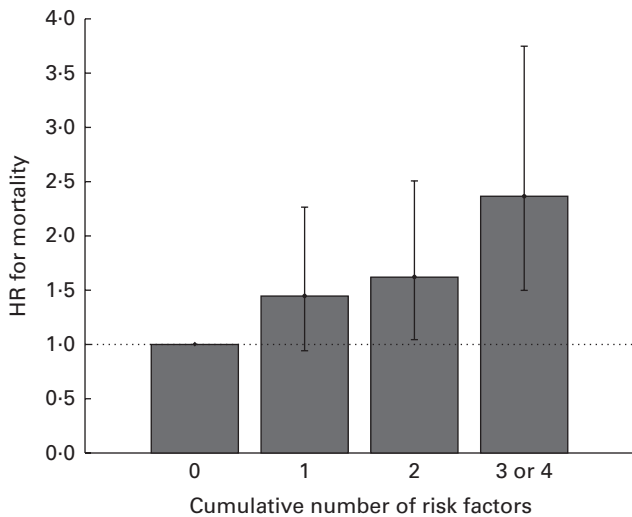


Fig. 1. Association between a cumulative number of risk factors related to both diet and lifestyle (including a modified Mediterranean Diet Score ≤ 4 , ever being a smoker, low levels of physical activity and being obese) and all-cause mortality (adjusted by age). Values are hazard ratios (HR), with 95% CI represented by vertical bars. *P* for trend < 0.0001 . Cumulative number of risk factors: 0 – *n* 144 (HR 1); 1 – *n* 606 (HR 1.45, 95% CI 0.93, 2.26); 2 – *n* 787 (HR 1.62, 95% CI 1.05, 2.51); 3 or 4 – *n* 312 (HR 2.37, 95% CI 1.50, 3.75).

mortality was strong and significant (HR 2.37; 95% CI 1.50, 3.75; *P* for trend < 0.0001).

Discussion

The present study shows that the MDS was inversely associated both with all-cause mortality and with cardiovascular incidence and mortality, but not with stroke incidence and mortality. All associations proved to be robust to the adjustment for several potential confounders (i.e. sex, BMI, education, physical activity and smoking status) as well as covariates potentially related to possible pathways of CV risk, such as blood pressure, blood lipids and, notably, weight change after 11 years of follow-up. Increasing the number of unhealthy dietary or lifestyle-related factors additively increased the mortality risk by more than two times compared with those with no risk factors, as suggested by previous studies^(29,30). Fruit and vegetables were particularly relevant, as they attenuated the association of the diet score with some outcomes when excluded from score calculations.

Notably, only the MDS including ingredients from mixed dishes and recipes produced statistically significant results. It is not clear how ingredients have been treated in previous studies^(9,29,31,32). The present results imply that they should be included in the calculations of this score, because they allow for a more accurate depiction of a Mediterranean-like dietary pattern.

CVD prevention is of major public health importance, because of the negative impact of CVD on the quality of life⁽³³⁾ and the expected increase in the number of deaths⁽³⁴⁾. Lifestyle changes and particularly dietary interventions are cost-effective means for the prevention of CVD and the reduction of the social and economic burden of this condition⁽³⁵⁾.

Several studies^(13,29,32) have shown associations between the adoption of a Mediterranean dietary pattern and the lower risk of overall mortality and death because of IHD and CVD in general. Particularly, the MDS has been shown to be inversely associated with mortality in Swedish elderly⁽¹⁵⁾ and in middle-aged Swedes where an inverse association with cardiovascular mortality (excluding stroke) was found in women and an association with cancer mortality was found in men⁽¹⁷⁾. In the latter study, fruit intake was inversely associated with all-cause mortality in women. The results of the present study again suggest that fruit and vegetable intakes can have particular importance in the reduction of cardiovascular risk. A few years ago, a meta-analysis of prospective studies had calculated a CHD risk reduction of 4% for each additional portion of fruit and vegetables consumed per day⁽³⁶⁾. However, the exact mechanisms by which these food items reduce CV risk are still unclear⁽³⁷⁾.

We do not have a clear explanation as to why the MDS and its single components were not at all associated with stroke mortality, also when separately analysing haemorrhagic *v.* non-haemorrhagic cases as well as ischaemic *v.* non-ischaemic cases. However, a tendency towards an inverse association was found for stroke incidence. Some epidemiological studies have also examined the association between a MDS and the risk of stroke. Among these, some^(31,38,39) found an inverse association, while others did not^(17,40). Moreover, a recent paper has found an inverse association between adherence to a Mediterranean-like diet and total cerebrovascular disease⁽⁴¹⁾. Stroke mortality, in particular, could be more related to factors such as the use of anticoagulant therapy and the course of related infections, although the influence of the latter on stroke outcome is still debated⁽⁴²⁾.

Comparing the methodology used in the present study with the one that we had applied previously to elderly Swedes (based on a refined version of the MDS⁽¹⁵⁾), here a further score refinement was possible by adding the components of mixed dishes and recipes, which enabled us to identify the healthiest dietary pattern in relation to the health effects of a Mediterranean-like dietary pattern. Considering the alcohol component, in particular, one point should be noted. Based on the known U-shaped relationship between alcohol intake and CHD, previous studies using the MDS have given one point for moderate alcohol intakes (< 50 g/d in men and 25 g/d in women) instead of negative scoring for high intakes^(43,44). However, since in our cohort only about 10% of the subjects exceeded these thresholds, we decided to categorise alcohol intakes according to medians, as for the other food items.

The strengths of the present study include the adjustment of the analyses for several potential confounders as well as for covariates potentially related to possible pathways of cardiovascular risk such as blood pressure and lipids, our ability to examine effects on both MI and stroke, and the availability of a detailed food database based on a validated dietary assessment method that provided details of ingredients from mixed dishes and recipes. Additionally, there is almost 100% follow-up for endpoints in the Danish registries as well as 100% coverage of the Danish population⁽⁴⁵⁾, which means a

negligible loss to follow-up. However, some limitations must also be acknowledged, such as possible residual confounding due to physical activity, as well as the likelihood that diets could have been misreported. However, random misreporting would have resulted in attenuation and not inflation of the hazards, suggesting that the observed significant association is likely to be true.

In conclusion, the present Danish population study demonstrates that within a Nordic setting, a high MDS is associated with longer survival and with a reduction in cardiovascular mortality, particularly, MI. Fruit and vegetable intakes seem to be of particular importance in influencing the strength of this association. Thus, the study supports evidence that the Mediterranean diet may be beneficial to health even among populations outside the Mediterranean basin. This is of importance considering that in Europe this pattern is not so common any more among the young generation⁽⁴⁶⁾.

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References

1. WHO/FAO (2003) *Diet, Nutrition and the Prevention of Chronic Diseases. Joint WHO/FAO Expert Consultation. WHO Technical Report Series* no. 916. Geneva: WHO/FAO.
2. AICR (2007) *Food, Nutrition, Physical Activity and the Prevention of Cancer: A Global Perspective*. Washington DC: AICR.
3. Allender S, Peto V, Scarborough P, *et al.* (2008) Coronary Heart Disease Statistics. London: British Heart Foundation. <http://www.heartstats.org>
4. Willett WC, Sacks F, Trichopoulou A, *et al.* (1995) Mediterranean diet pyramid: a cultural model for healthy eating. *Am J Clin Nutr* **61**, Suppl. 6, 1402S–1406S.
5. Alain JN, Katja S-Z, Heiner CB, *et al.* (2011) Meta-analysis comparing Mediterranean to low-fat diets for modification of cardiovascular risk factors. *Am J Med* **124**, 841–851.
6. Sofi F, Abbate R, Gensini GF, *et al.* (2010) Accruing evidence on benefits of adherence to the Mediterranean diet on health: an updated systematic review and meta-analysis. *Am J Clin Nutr* **92**, 1189–1196.
7. Sofi F, Cesari F, Abbate R, *et al.* (2008) Adherence to Mediterranean diet and health status: meta-analysis. *BMJ* **337**, a1344.
8. Martinez-Gonzalez MA, Guillen-Grima F, De Irala J, *et al.* (2012) The Mediterranean diet is associated with a reduction in premature mortality among middle-aged adults. *J Nutr* **142**, 1672–1678.
9. Trichopoulou A, Kouris-Blazos A, Wahlqvist ML, *et al.* (1995) Diet and overall survival in elderly people. *BMJ* **311**, 1457–1460.
10. McNaughton SA, Bates CJ & Mishra GD (2012) Diet quality is associated with all-cause mortality in adults aged 65 years and older. *J Nutr* **142**, 320–325.
11. Buckland G, Agudo A, Travier N, *et al.* (2011) Adherence to the Mediterranean diet reduces mortality in the Spanish cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC-Spain). *Br J Nutr* **106**, 1581–1591.
12. Lagiou P, Trichopoulos D, Sandin S, *et al.* (2006) Mediterranean dietary pattern and mortality among young women: a cohort study in Sweden. *Br J Nutr* **96**, 384–392.
13. Mitrou P, Kipnis V, Thiebaut A, *et al.* (2007) Mediterranean dietary pattern and prediction of all-cause mortality in a US population: results from the NIH-AARP Diet and Health Study. *Arch Intern Med* **167**, 2461–2468.
14. Osler M & Schroll M (1997) Diet and mortality in a cohort of elderly people in a north European community. *Int J Epidemiol* **26**, 155–159.
15. Tognon G, Rothenberg E, Eiben G, *et al.* (2011) Does the Mediterranean diet predict longevity in the elderly? A Swedish perspective. *Age (Dordr)* **33**, 439–450.
16. Sjogren P, Becker W, Warensjo E, *et al.* (2010) Mediterranean and carbohydrate-restricted diets and mortality among elderly men: a cohort study in Sweden. *Am J Clin Nutr* **92**, 967–974.
17. Tognon G, Nilsson LM, Lissner L, *et al.* (2012) The Mediterranean diet score and mortality are inversely associated in adults living in the subarctic region. *J Nutr* **142**, 1547–1553.
18. Martinez E, Lull R, Del Mar Bibiloni M, *et al.* (2010) Adherence to the Mediterranean dietary pattern among Balearic Islands adolescents. *Br J Nutr* **103**, 1657–1664.
19. di Giuseppe R, Bonanni A, Olivieri M, *et al.* (2008) Adherence to Mediterranean diet and anthropometric and metabolic parameters in an observational study in the 'Alto Molise' region: the MOLI-SAL project. *Nutr Metab Cardiovasc Dis* **18**, 415–421.
20. Garcia-Closas R, Berenguer A & Gonzalez CA (2006) Changes in food supply in Mediterranean countries from 1961 to 2001. *Public Health Nutr* **9**, 53–60.
21. Forebyggelseskommissionen (2009) Vi kan leve længere og sundere – Forebyggelseskommissionens anbefalinger til en styrket forebyggende indsats (We can live longer and healthier – Prevention Commission's recommendations for enhanced prevention efforts). <http://www.forebyggelseskommissionen.dk>
22. Eiben G, Andersson CS, Rothenberg E, *et al.* (2007) Secular trends in diet among elderly Swedes – cohort comparisons over three decades. *Public Health Nutr* **7**, 637–644.
23. Osler M, Heitmann BL & Schroll M (1997) Ten year trends in the dietary habits of Danish men and women. Cohort and cross-sectional data. *Eur J Clin Nutr* **51**, 535–541.
24. Hartz AJ, Rupley DC & Rimm AA (1984) The association of girth measurements with disease in 32,856 women. *Am J Epidemiol* **119**, 71–80.

25. Jensen KH & Jorgensen T (1991) Incidence of gallstones in a Danish population. *Gastroenterology* **100**, 790–794.
26. Heitmann BL (1991) Body fat in the adult Danish population aged 35–65 years: an epidemiological study. *Int J Obes* **15**, 535–545.
27. Schofield WN (1985) Predicting basal metabolic rate, new standards and review of previous work. *Hum Nutr Clin Nutr* **39**, Suppl. 1, 5–41.
28. Heitmann B (1992) The effects of gender and age on associations between blood lipid levels and obesity in Danish men and women aged 35–65 years. *J Clin Epidemiol* **45**, 693–702.
29. Knuops KT, de Groot LC, Kromhout D, *et al.* (2004) Mediterranean diet, lifestyle factors, and 10-year mortality in elderly European men and women: the HALE project. *JAMA* **292**, 1433–1439.
30. van den Brandt PA (2011) The impact of a Mediterranean diet and healthy lifestyle on premature mortality in men and women. *Am J Clin Nutr* **94**, 913–920.
31. Fung T, Rexrode K, Mantzoros C, *et al.* (2009) Mediterranean diet and incidence of and mortality from coronary heart disease and stroke in women. *Circulation* **119**, e379.
32. Trichopoulos A, Costacou T, Bamia C, *et al.* (2003) Adherence to a Mediterranean diet and survival in a Greek population. *N Engl J Med* **348**, 2599–2608.
33. Kahn R, Robertson RM, Smith J, *et al.* (2008) The impact of prevention on reducing the burden of cardiovascular disease. *Diabetes care* **118**, 576–585.
34. WHO (2012) World health statistics 2012. http://www.who.int/gho/publications/world_health_statistics/EN_WHS2012_Full.pdf
35. Nishida C, Uauy R & Kumanyika S (2004) The joint WHO/FAO expert consultation on diet, nutrition and the prevention of chronic diseases: process, product and policy implications. *Public Health Nutr* **7**, 245–250.
36. Dauchet L, Amouyel P, Hercberg S, *et al.* (2006) Fruit and vegetable consumption and risk of coronary heart disease: a meta-analysis of cohort studies. *J Nutr* **136**, 2588–2593.
37. Dauchet L, Amouyel P & Dallongeville J (2009) Fruits, vegetables and coronary heart disease. *Nat Rev Cardiol* **6**, 599–608.
38. Agnoli C, Krogh V, Grioni S, *et al.* (2011) *A priori*-defined dietary patterns are associated with reduced risk of stroke in a large Italian cohort. *J Nutr* **141**, 1552–1558.
39. Kastorini C-M, Milionis H, Kantas D, *et al.* (2011) Adherence to the Mediterranean diet in relation to ischemic stroke nonfatal events in nonhypercholesterolemic and hypercholesterolemic participants: results of a case/case–control study. *Angiology* **63**, 509–515.
40. Gardener H, Wright C, Gu Y, *et al.* (2011) Mediterranean-style diet and risk of ischemic stroke, myocardial infarction, and vascular death: the Northern Manhattan Study. *Am J Clin Nutr* **94**, 1458–1522.
41. Misirli G, Benetou V, Lagiou P, *et al.* (2012) Relation of the traditional Mediterranean diet to cerebrovascular disease in a Mediterranean population. *Am J Epidemiol* **176**, 1185–1192.
42. Roquer J, Cuadrado-Godia E, Giralte-Steinhauer E, *et al.* (2012) Previous infection and stroke: a prospective study. *Cerebrovasc Dis* **33**, 310–315.
43. Milaneschi Y, Bandinelli S, Corsi AM, *et al.* (2011) Mediterranean diet and mobility decline in older persons. *Exp Gerontol* **46**, 303–308.
44. Romaguera D, Guevara M, Norat T, *et al.* (2011) Mediterranean diet and type 2 diabetes risk in the European Prospective Investigation into Cancer and Nutrition (EPIC) study: the Inter-Act project. *Diabetes Care* **34**, 1913–1918.
45. Abildstrom SZ, Torp-Pedersen C & Madsen M (2011) Register-based studies of cardiovascular disease. *Scand J Public Health* **39**, Suppl. 7, 165–169.
46. Tognon G, Hebestreit A, Lanfer A, *et al.* (2013) Mediterranean diet, overweight and body composition in children from eight European countries: cross-sectional and prospective results from the IDEFICS study. *Nutr Metab Card Dis* (In Press).