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ORIGINAL CONTRIBUTION

Association of a traditional Mediterranean diet and non-Mediterranean dietary scores with all-cause and cause-specific mortality: prospective findings from the Moli-sani Study

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Abstract

Purpose To evaluate in an Italian general population, the association with mortality of a traditional Mediterranean diet (MD) and non-Mediterranean dietary (non-MD) patterns, and their combined effect, and to test some biomarkers of cardiovascular (CVD) risk as potential mediators of such associations.

Methods Longitudinal analysis on 22,849 men and women aged ≥ 35 years, recruited in the Moli-sani Study (2005–2010), followed up for 8.2 years (median). The MD was assessed by the Mediterranean diet score (MDS). The Dietary Approaches to Stop Hypertension (DASH), the Palaeolithic diet, and the Nordic diet were chosen as reportedly healthy non-MD patterns. Hazard ratios (HR) with 95% confidence intervals (95% CI) were calculated by multivariable Cox regression.

Results Participants reaching higher MDS or DASH diet score experienced lower risk of both all-cause (HR 0.77; 95% CI 0.66–0.90 and 0.81; 0.69–0.96, respectively, highest vs lowest quartile) and CVD (0.77; 0.59–1.00 and 0.81; 0.69–0.96, respectively) death risk; risk reduction associated with the Palaeolithic diet was limited to total and other cause death, whereas the Nordic diet did not alter risk of mortality. Increasing adherence to MD was associated with higher survival in each stratum of non-MD diets. Biomarkers of glucose metabolism accounted for 7% and 21.6% of the association between either MDS or DASH diet, respectively, with total mortality risk.

Conclusions Both the traditional MD and DASH diet may reduce risk of all-cause mortality among Italians, as well as risk of dying from cardiovascular causes. The Palaeolithic diet did not appear to reduce cardiovascular risk, while the Nordic eating pattern was unlikely to be associated with any substantial health advantage.

Keywords Mediterranean diet · DASH diet · Palaeolithic diet · Nordic diet · Inflammation · CVD risk factors · Mortality

Moli-sani Study investigators are listed in the Acknowledgements.

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Introduction

The increasing globalization of diet and dietary habits resulting from the large availability and diversity of food poses new questions for the actual health benefits of different dietary models in populations not traditionally exposed to a certain dietary model.

Consuming a traditional Mediterranean diet (MD), the typical dietary pattern of the olive tree-growing areas of the Mediterranean basin characterised by high intake of plant foods, olive oil, high-to-moderate intakes of fish and seafood, moderate consumption of poultry and dairy products, low consumption of red meat, and moderate intake of

alcohol (mainly wine) during meals, has been found to be associated with lower mortality and disease risk in a number of observational epidemiological studies [1–3], meta-analyses [4, 5], and intervention trials, and in both primary [6] and secondary prevention trials [7]. This dietary pattern has been shown to be also effective in non-Mediterranean populations by reducing risk of cardiovascular (CVD) and cerebrovascular disease [8–10], cancer [11] or mortality [12–14]; in some cases, it performed even better than the typical dietary model of the studied population [15, 16]. All such studies have basically suggested that a shift to an MD would definitely provide health advantages also to non-MD populations, while, on the contrary, it is unclear whether healthful non-Mediterranean (non-MD) diets may reflect protective dietary patterns in Mediterranean populations, since few have addressed this topic so far [17].

A number of longitudinal studies have suggested that many healthy non-MD patterns, such as the Dietary Approaches to Stop Hypertension (DASH) diet, which attracted much attention due to its beneficial effects on blood pressure [18], the Palaeolithic dietary model, that describes the general diet *Homo sapiens* would have had prior to the development of agriculture [11], or a Nordic diet, reflecting the healthy diet consumed in Nordic countries [19, 20], may provide significant health advantages, being associated with less chronic disease [17–19], longer survival [13–16, 21], and favourable cardio-metabolic risk markers also in intervention studies [22–24].

All these non-MD dietary patterns are reflective of a healthy diet, and thus, it is reasonable to hypothesize that they may provide health advantages also in a Mediterranean population either by themselves or in combination with an MD.

Moreover, the biological mechanisms through which healthy dietary habits affect disease/mortality risk are still unclear, at least from a population-based perspective. It is well established that high-quality diets, such as the MD, are associated with more favourable levels of some markers of CVD risk, such as blood lipids or inflammatory markers; however, a few longitudinal studies to date have tested whether these markers could account for the beneficial association of an MD with health outcomes, at least as a primary purpose of the study [10, 12, 25–27].

As yet, little is known on the relationship between MD (and diet in general) with other established markers of CVD risk, such as cardiac risk markers or markers of renal function, which could be reasonably involved among the biological pathways through which diet exerts its effects on health.

The present study has three main purposes: first, to assess the individual association of a traditional MD and three widely used healthy non-MD dietary patterns with all-cause and cause-specific mortality in a Mediterranean population; second, to test the combination of MD with non-MD diets towards mortality risk; finally, to explore the biological mechanisms that could be on the pathway between diet quality and mortality.

Methods

Study population

We studied participants from the Moli-sani Study, a prospective cohort study established in 2005–2010 with an enrolment of 24,325 men and women (aged ≥ 35 years) randomly recruited from the general population of Molise, a Southern Mediterranean Italian region with the purpose of investigating genetic and environmental risk factors in the onset of cardiovascular, cerebrovascular, and tumour diseases. The study design and procedures have been previously described [28].

For the purpose of this study, we omitted subjects reporting implausible energy intakes (< 800 kcal/day in men and < 500 kcal/day in women or > 4000 kcal/day in men and > 3500 kcal/day in women; $n = 771$), unreliable medical/dietary questionnaires ($n = 235$ and $n = 955$, respectively), subjects lost to follow-up ($n = 23$) or with missing data on outcomes ($n = 68$), exposure ($n = 104$), and individuals with missing information on the main covariates of interest ($n = 65$). The final sample consisted of 22,849 subjects.

Dietary assessment

Food intake during the year before enrolment was assessed through the EPIC food-frequency questionnaire (FFQ) validated and adapted to the Italian population [29] for a total of 188 food items that were classified into 45 predefined food groups on the basis of similar nutrient characteristics or culinary usage (Supplemental Table 1). Components and food amounts for optimal scoring of each dietary index are summarized in Supplemental Table 2.

The traditional Mediterranean diet

Adherence to the traditional MD was defined through the Mediterranean Diet Score (MDS) developed by Trichopoulou et al. [1], which was obtained by assigning one point to healthy foods [fruits and nuts, vegetables, legumes, fish, cereals, and monounsaturated (MUFAs)-to-saturated fats (SFAs) ratio] whose consumption was above the sex-specific medians of intake of the Moli-sani Study population, free from CVD, cancer, and diabetes, and then applied to the whole population; foods presumed to be detrimental (meat and dairy products) were scored positively if their consumption was below the median. All other intakes received 0 points. For ethanol, men who consumed 10–50 g/

day and women who consumed 5–25 g/day received 1 point; otherwise, the score was 0. The MDS ranged from 0 to 9 (the latter reflecting maximal adherence).

The DASH diet

The DASH score was developed as indicated by Fung et al. [18] and is based on positive scoring of quintiles of fruits, vegetables, nuts and legumes, low-fat dairy products, whole grains (highest intake received five points; one point for the lowest); for food items presumed to be detrimental (dietary sodium, red and processed meats, and sweetened beverages), low intake was desired, therefore, the lowest quintile was given a score of five points and the highest quintile, one point. Men and women were classified into quintiles separately.

For soft drinks, we calculated approximate quintiles. In our cohort, data on whole-grain product intake were limited to whole-grain bread; for this reason, we used the cereal food group rather than whole grains. Low-fat dairy products, which are not commonly used in Italy, were limited to partially skimmed milk, skimmed milk yogurt, and ricotta cheese. The DASH diet score potentially ranged from 8 to 40.

The Palaeolithic diet

Adherence to a Palaeolithic diet was measured as proposed by Whalen et al. [11] and was obtained by scoring sex-specific quintiles of adherence to seven food items presumed to be healthful (fruits, vegetables, fruit and vegetables variety, legumes and nuts, fish, lean meat, and non-dairy calcium intake), while foods that should be consumed less were grains and starches, alcoholic beverages per week, dairy products, red and processed meat, baked products, dietary sodium, and sugar-sweetened beverages.

In our version, this score included also legumes in the nut food group, due to low nut consumption in our population. The final score could range from 14 to 70.

The Nordic diet

A Nordic diet index was calculated by scoring the following nine food items, as indicated by Galbete et al. [19]: whole-grain bread, apples and pears, berries (strawberries), fish, cabbage and cruciferous vegetables, root vegetables, milk and dairy products, potatoes, and vegetable fats (excluding olive oil). After categorizing each food component into sex-specific tertiles of intake, the participants received a score of 0–2 points according to the first, second, and third tertiles, respectively.

For whole-grain bread, the median intake was 0, as more than 80% of the cohort did not consume this food, and thus, one point was instead given to all participants with any intake of whole-grain bread (14.4%). The Nordic diet score potentially ranged from 0 to 17.

Combination of dietary scores

We undertook combined analyses stratified for low adherence (≤ 4, population median) and high (>4) adherence to the MD associated with each of non-MD dietary pattern (1 SD increase) with the purpose of testing whether low adherence to MD could be counterbalanced by improving adherence to non-MD dietary patterns, in relation to total and cause-specific mortality.

Similarly, we stratified analyses by degree of adherence (low/high) to non-MD dietary scores and tested mortality risk associated with 1 SD increase in the MDS.

To calculate the potential health advantages deriving from food items not included in the traditional MD, but present in non-MD scores, we alternately added food groups typical of non-MD patterns that were not part of the MDS. Each additional food group intake was calculated as sex-specific medians and was assigned a score 1 if the food was presumed healthy and of 0 if unhealthy. We then obtained the following three combined dietary scores: MDS + DASH food items (dietary sodium and soft drinks intake), MDS + Palaeolithic food items (non-dairy dietary calcium, fruit and vegetable variety, dietary sodium, soft drinks, and baked products), and MDS + Nordic food items (potatoes, vegetable fats excluding olive oil, and whole-grain bread). A fourth combined score (range 0–16) was also created and resulted by summing the MDS and the whole set of eight non-MD food items. Finally, a dietary score resulting from the difference between the combined score and the MDS was calculated to quantify the association of all non-MD food items with mortality risk.

Baseline covariate assessment

Demographics, including education attainment (highest qualification attained), household income, and smoking, were obtained by interviewer-administered questionnaires.

Leisure-time physical activity (PA) was expressed as daily energy expenditure in metabolic equivalent task-hours (MET-h/d) for sport, walking, and gardening, and then dichotomized as below/above the population median (2.27 MET-h/day). Height and weight were measured, and body mass index (BMI) was calculated as kg/m^2 and then grouped into three categories as normal (≤ 25), overweight (>25 to <30), or obese (≥ 30).

History of cardiovascular disease (angina, myocardial infarction, revascularization procedures, peripheral artery disease, and cerebrovascular events) was self-reported and confirmed by medical records and therapy. History of cancer

was self-reported and confirmed by medical records. Hypertension, hyperlipidaemia, and diabetes were defined by use of pharmacological treatment.

Venous blood samples were obtained from participants who had fasted overnight and had refrained from smoking for at least 6 h.

Serum lipids (total cholesterol, HDL-cholesterol, and triglycerides) and blood glucose were assayed by enzymatic reaction methods using an automatic analyzer [ILab 350, Instrumentation Laboratory (IL), Milan, Italy].

Quality control for lipids and glucose was obtained by a commercial standard (Ser 1 and Ser 2) provided by the IL and an in-house serum standard pool. The coefficients of variability (CV) were, respectively, 4.9%, 5.2%, and 4% for blood cholesterol; 3.2%, 3%, and 4.5% for HDL-cholesterol; 5.2%, 5.3%, and 5% for triglycerides; 4.7%, 4.1%, and 3.9% for blood glucose.

High-sensitivity C-reactive protein (CRP) was measured in fresh serum samples by a particle-enhanced immunoturbidimetric assay (ILab 350, IL, Milan, Italy). Quality control for CRP was maintained using in-house serum pool and internal laboratory standard at 1.5 mg/L; inter-day coefficients of variability for CRP were 5.5% and 4.2%, respectively.

Hemocromocytometric analysis was performed by cell count (Coulter HMX, Beckman Coulter, IL Milan, Italy) within 3 h from blood collection.

Quality control was performed using three different levels of standards Abn I, Abn II, and Normal (Coulter HMX, Beckman Coulter). Coefficient of variability for white blood cells (WBC) was 6.2%, 3.3%, and 3.0% for Abn I, Abn II, and Normal, respectively.

Blood pressure (BP) was measured by an automatic device (OMRON-HEM-705CP) three times on the non-dominant arm and the last two values were taken as the BP. Measurements were made in a quiet room with comfortable temperature with the participants lying down for at least 5 min.

N-terminal pro B-type natriuretic peptide (NTproBNP), high-sensitivity assayed Troponin I (hsTnI), apolipoprotein A1 (ApoA), apolipoprotein B100 (ApoB100), lipoprotein a [Lp(a)], markers of renal function (cystatin C, creatinine), insulin, C-peptide, and serum vitamin D were measured in the framework of the collaborative BiomarCaRE project [30].

Statistical analysis

Characteristics of the study population by levels of adherence to MD, DASH, Palaeolithic, and Nordic diets were presented as numbers and percentages, or mean values and standard deviation (SD) for continuous variables. Differences in the distribution of baseline covariates according to degree of adherence to each dietary score were calculated using the analysis of variance adjusted for age and sex (GENMOD procedure for categorical variables and GLM procedure for continuous variables in SAS software; Table 1). Correlation between dietary scores was calculated with a Spearman correlation coefficient.

Risk estimates for all-cause and cause-specific deaths were expressed as hazard ratios (HRs) with 95% confidence interval (95% CI) and calculated using Cox regression models with time-on-study on the time scale and competing risk of dying for other causes (PHREG procedure in SAS software).

Multivariable-adjusted HRs were calculated across quartiles of dietary pattern scores, as well as considering the dietary patterns as continuous variables [by 1 SD].

Selection of potential confounders was made through directed acyclic graphs (DAG) using DAGitty (http://dagitty.net/), a browser-based environment for analysing causal models through diagrams to minimize bias (Supplemental Figure 1).

Two multivariable models were fitted: the first (model 1) was adjusted for age (continuous), sex, and energy intake (kcal/day; continuous); the second (model 2) as in model 1 further controlled for educational level, household income, smoking, leisure-time PA, BMI, diabetes, hypertension, hyperlipidaemia, history of CVD, and cancer at baseline.

Several panels of biomarkers were tested as possibly mediating the association of dietary scores with mortality risk. In addition to cardiac troponin (hsTnI) and NTproBNP, we tested biomarkers of renal function (cystatin C, creatinine), glucose metabolism (blood glucose, insulin, C-peptide), lipid metabolism [total blood cholesterol, HDL-cholesterol, triglycerides, Lp(a), apoA1, and apoB100] serum vitamin D, inflammatory markers (CRP and WBC), and a panel including systolic and diastolic blood pressure (BP).

The multivariable model 2 served as the reference for the mediation analysis used to quantify the contribution of each set of potential mediators, which were alternately included into model 2. For the mediation analysis, we used the %MEDIATE macro in SAS [31] which calculates the point and interval estimates of the percent of exposure effect (PTE) explained by one or more intermediate variables, with 95% confidence interval and *P* values. Biomarkers were entered into the mediation analysis as ordered quintiles.

We performed sensitivity analyses for all-cause mortality to assess potential effect modification by various risk factors: age (35–65 and ≥ 65 years), sex, socioeconomic strata (education and household income), lifestyles (smoking status, leisure-time PA), health conditions at baseline (CVD, cancer, diabetes, hypertension, and hyperlipidaemia), and within a healthy sample (without CVD nor cancer at baseline). Appropriate multiplicative terms for testing interaction

Table 1 Baseline characteristics of the Moli-sani Study population across categories (quartiles) of adherence to a priori-defined dietary scores

	Total population	Mediterranean diet (MDS)		DASH diet		Palaeolithic diet		Nordic diet	
		Q1 (0–3)	Q4 (6–9)	Q1 (10–21)	Q4 (27–39)	Q1 (21–39)	Q4 (49–65)	Q1 (0–6)	Q4 (11–17)
Subjects (*n*)	22,849	6961	5833	6368	6013	5982	5146	6588	4751
Age (years)	55 (12)	54 (12)	56 (11)	53 (11)	58 (12)	53 (12)	57 (11)	57 (12)	54 (11)
Men (%)	47.7	43.7	52.8	49.8	45.0	52.2	43.5	46.2	47.6
Postsecondary education (%)	12.9	12.4	14.0	12.0	14.5	12.7	13.4	11.4	14.9
High income (> 40,000 Euros/year), (%)	12.2	11.1	14.2	11.1	15.0	12.1	12.7	10.7	13.3
Leisure-time PA (MET-h/day)[a] (%)	49.4	43.1	55.7	42.6	56.5	41.9	56.9	42.9	56.8
Current smokers (%)	23.0	23.1	22.4	27.2	19.4	27.7	19.7	25.9	19.4
Obesity (body mass index ≥ 30 kg/m^2) (%)	29.6	29.4	29.5	29.1	29.3	27.1	31.3	30.6	28.8
History of CVD (%)	5.2	4.8	5.6	3.8	6.5	4.0	6.6	5.4	4.9
History of cancer (%)	3.2	3.2	3.1	2.6	3.6	2.7	3.5	3.4	2.9
Diabetes (%)	4.8	5.1	4.7	3.7	5.6	3.3	6.2	5.0	4.2
Hyperlipidaemia (%)	7.7	6.4	9.7	5.3	10.3	5.1	10.5	8.3	7.0
Hypertension (%)	27.3	25.8	29.6	22.5	31.9	22.5	31.2	29.4	24.8
hsTnI (pg/mL)[b]	2.24 (2.22–2.27)	2.19 (2.15–2.22)	2.34 (2.30–2.38)*	2.16 (2.12–2.20)	2.34 (2.29–2.38)*	2.19 (2.15–2.23)	2.27 (2.23–2.32)*	2.27 (2.23–2.31)	2.27 (2.23–2.32)
NTproBNP (pg/mL)[b]	50.8 (50.1–51.5)	51.5 (50.4–52.6)	48.2 (47.1–49.3)*	49.3 (48.2–50.4)	48.5 (47.4–49.5)	48.6 (47.6–49.7)	49.1 (48.0–50.3)	51.0 (50.0–52.1)	49.8 (48.5–51.0)
Markers of renal function									
Cystatin C (mg/dL)[b]	0.96 (0.96–0.96)	0.96 (0.96–0.97)	0.96 (0.95–0.96)*	0.96 (0.95–0.96)	0.96 (0.96–0.96)	0.96 (0.95–0.96)	0.95 (0.95–0.96)	0.97 (0.97–0.98)	0.96 (0.95–0.96)*
Creatinine (mg/dL)[b]	0.80 (0.80–0.80)	0.80 (0.79–0.80)	0.81 (0.81–0.81)*	0.79 (0.79–0.80)	0.81 (0.81–0.82)*	0.80 (0.79–0.80)	0.81 (0.81–0.81)*	0.81 (0.80–0.81)	0.80 (0.80–0.81)
Markers of glucose metabolism									
Blood glucose (mg/dL)[b]	99 (97–101)	100 (99–100)	99 (98–99)	100 (99–100)	99 (98–99)*	99 (98–99)	100 (99–100)*	99 (98–99)	99 (99–100)
Insulin (pmol/L)[b]	51.9 (51.6–52.3)	52.9 (52.3–53.6)	50.4 (49.7–51.1)*	54.2 (53.5–54.9)	49.6 (48.9–50.2)*	51.9 (51.2–52.6)	51.7 (51.0–52.4)	52.0 (51.4–52.7)	51.6 (50.8–52.3)
C-peptide (ng/mL)[b]	1.58 (1.57–1.59)	1.63 (1.61–1.65)	1.51 (1.49–1.52)*	1.66 (1.64–1.68)	1.48 (1.46–1.49)*	1.59 (1.57–1.61)	1.55 (1.54–1.57)*	1.60 (1.58–1.62)	1.56 (1.54–1.58)*
Markers of lipid metabolism									
Blood cholesterol (mg/dL)	213 (42)	212 (42)	214 (41)*	214 (42)	212 (41)*	214 (41)	212 (42)*	212 (42)	213 (41)
HDL-cholesterol (mg/dL)	57 (15)	57 (15)	57 (15)	57 (15)	57 (15)	58 (15)	57 (15)*	57 (15)	58 (15)
Triglycerides (mg/dL)[b]	112 (106–119)	111 (110–113)	115 (113–116)*	113 (112–115)	112 (110–113)	113 (112–114)	112 (110–113)	115 (114–116)	110 (108–111)*
ApoB100 (g/L)	0.98 (0.24)	0.96 (0.23)	1.01 (0.25)*	0.97 (0.24)	0.99 (0.25)*	0.98 (0.24)	0.99 (0.25)*	0.98 (0.25)	0.98 (0.25)
ApoA (g/L)	1.56 (0.32)	1.52 (0.31)	1.59 (0.33)*	1.52 (0.31)	1.59 (0.34)*	1.55 (0.31)	1.56 (0.33)	1.55 (0.33)	1.56 (0.32)
Lp(a) (mg/dL)	18.1 (18.9)	17.4 (18.3)	18.6 (19.2)*	17.7 (18.7)	18.8 (19.4)*	17.8 (18.7)	18.4 (19.0)	17.7 (18.8)	18.1 (18.8)
Serum vitamin D (ng/mL)	18.9 (9.3)	19.1 (9.3)	19.0 (9.3)	18.8 (9.2)	19.1 (9.3)	18.9 (9.5)	19.3 (9.1)*	18.8 (9.2)	19.1 (9.4)*
Inflammatory markers[b]									
C-reactive protein (mg/L)	1.51 (1.49–1.53)	1.54 (1.51–1.58)	1.47 (1.43–1.51)*	1.59 (1.55–1.63)	1.39 (1.35–1.42)*	1.54 (1.50–1.58)	1.46 (1.42–1.50)*	1.53 (1.50–1.57)	1.47 (1.43–1.52)*
Leukocyte count ($\times 10^9$/L)	6.0 (6.0–6.0)	6.1 (6.0–6.1)	6.0 (5.9–6.0)*	6.1 (6.1–6.2)	5.9 (5.9–5.9)*	6.1 (6.0–6.1)	6.0 (6.0–6.0)*	6.1 (6.1–6.2)	6.0 (5.9–6.0)*
Systolic BP (mmHg)	141 (21)	140 (20)	141 (20)*	140 (20)	141 (21)	140 (20)	140 (21)	141 (21)	141 (20)

Table 1 (continued)

	Total population	Mediterranean diet (MDS)		DASH diet		Palaeolithic diet		Nordic diet	
		Q1 (0–3)	Q4 (6–9)	Q1 (10–21)	Q4 (27–39)	Q1 (21–39)	Q4 (49–65)	Q1 (0–6)	Q4 (11–17)
Diastolic BP (mmHg)	82 (10)	82 (9)	83 (10)*	82 (9)	82 (9)	82 (9)	82 (9)	82 (10)	82 (10)

Values are mean ± standard deviation (SD) unless otherwise indicated. The range of scores are shown in parentheses for Q1 and Q4 for each diet. Differences were calculated using the analysis of variance. Biomarkers are reported as age and sex-adjusted means. Differences were calculated using the analysis of variance adjusted for age and sex

CVD cardiovascular disease, *NTproBNP* N-terminal pro B-type natriuretic peptide, *hsTnI* high-sensitivity assayed Troponin I, *ApoA* apolipoprotein A1, *ApoB100* apolipoprotein B100, *Lp*(*a*) Lipoprotein a

*Significance across quartiles ($P < 0.05$)

[a]Leisure-time physical activity (PA) above the population median (2.27 MET-h/day)

[b]Geometric means with corresponding 95% confidence intervals are reported for log-transformed variables

were included in the multivariable models to test for a difference of effect of the dietary scores across subgroups.

Sensitivity analyses were run by excluding missing categories.

Potential dose–response relationship between dietary patterns and all-cause mortality risk was tested via a restricted cubic spline regression. The used RCS_Reg SAS macro [32] created a restrictive cubic spline function of dietary patterns with three knots, located at the 5th, 50th, and 95th percentile, and displays the dose–response association (with its 95% confidence interval) between the cubic spline dietary scores and all-cause mortality, tested via a multivariable Cox model.

Dummy variables for missing values (30.9% household income; 0.9% hyperlipidaemia; 0.7% hypertension; 1.2% diabetes; 1.6% CVD; 0.4% cancer, and up to 7.7% of biomarkers of CVD risk) were created. Positively skewed variables were log transformed before analysis.

The data analysis was generated using SAS/STAT software, Version 9.4 of the SAS System for Windows©2009.

Results

Closer adherence (highest quartile) to all 'a priori'-defined dietary scores was associated with a healthier lifestyle, being positively associated with more leisure-time PA and lower prevalence of smokers, and with higher socioeconomic status (Table 1), although with higher prevalence of subjects with hyperlipidaemia or hypertension (with the exception of the Nordic diet). Blood cholesterol levels were higher in subjects adhering most to MD and lower among those with greater adherence to the DASH or Palaeolithic diet (Table 1); biomarkers of inflammation were reduced in each high adherence group (Table 1); generally, differences in biomarkers distribution were more apparent across MD levels as compared to other dietary scores (Table 1). Mean daily intake of main food groups across levels of adherence to each dietary pattern is reported in Supplemental Table 3.

Spearman correlation coefficients of MDS with DASH, Palaeolithic and Nordic diets were $r = 0.56$, $r = 0.49$, $r = 0.28$, respectively ($P < 0.0001$).

Distribution of study participants across 16-level combination of adherence to four different a priori dietary patterns is shown in Supplemental Table 4, showing that about 22.7% of subjects reported low adherence to all four dietary scores, while about 16% was likely to be positioned in the higher adherence group to each dietary score.

Over a median follow-up of 8.2 years (interquartile ranges 7.3–9.3 years; 187,899 person years), a total of 1237 deaths were ascertained (CVD = 444, IHD/cerebrovascular = 258, cancer = 483, and 310 from other causes).

In the multivariable model, high adherence to MD was associated with 23% lower risk of death as compared to the lowest quartile (95% CI 10–34; Table 2, model 2); mortality risks from CVD, IHD/cerebrovascular, and other causes were lowered by 23%, 31%, and 34%, respectively, while a non-statistically significant trend towards protection was found for cancer death.

Among non-MD patterns, the DASH and the Palaeolithic diets were associated with lower risk of total mortality (HR 0.81; 0.69–0.96 and HR 0.84; 0.71–0.99 for Q4 vs Q1, respectively) and higher adherence to a DASH diet was inversely associated with CVD (HR 0.80; 0.62–1.04 for Q4 vs Q1) and IHD/cerebrovascular mortality risk (HR 0.73; 0.52–1.03 for Q4 vs Q1; Table 2, model 2). All three dietary scores were associated with lower risk of death from the other causes (Table 3, model 2).

No consistent association between mortality risk and the Nordic diet was found. The risk prediction of dietary scores over time is illustrated in Figs. 1 and 2, in which mortality rates are well separated during follow-up for MD (1A) and to a less extent for DASH diet (1B), but not for Palaeolithic (2A) or Nordic diets (2B).

Table 2 Association of a priori-defined dietary patterns with risk of all-cause, CVD and IHD/cerebrovascular death in the Moli-sani Study cohort ($n = 22{,}849$)

	Subjects, *n* (%)	All-cause mortality			CVD mortality			IHD/cerebrovascular mortality		
		Deaths, *n* (%)	Model 1 HR (95% CI)	Model 2 HR (95% CI)	Deaths, *n* (%)	Model 1 HR (95% CI)	Model 2 HR (95% CI)	Deaths, *n* (%)	Model 1 HR (95%CI)	Model 2 HR (95% CI)
Quartiles										
Mediterranean diet										
Q1 (0–3)	6961 (30.5)	390 (5.6)	–1–	–1–	144 (2.1)	–1–	–1–	91 (1.3)	–1–	–1–
Q2 (4)	5129 (22.4)	278 (5.4)	0.83 (0.71–0.97)	0.89 (0.76–1.03)	98 (1.9)	0.79 (0.61–1.03)	0.88 (0.68–1.14)	51 (1.0)	0.66 (0.47–0.93)	0.73 (0.52–1.03)
Q3 (5)	4926 (21.6)	271 (5.5)	0.84 (0.72–0.98)	0.88 (0.75–1.03)	97 (2.0)	0.83 (0.64–1.08)	0.85 (0.65–1.11)	57 (1.2)	0.79 (0.57–1.11)	0.80 (0.57–1.12)
Q4 (6–9)	5833 (25.5)	298 (5.1)	0.71 (0.61–0.84)	0.77 (0.66–0.90)	105 (1.8)	0.72 (0.55–0.93)	0.77 (0.59–1.00)	59 (1.0)	0.67 (0.47–0.94)	0.69 (0.49–0.97)
DASH diet										
Q1 (10–21)	6368 (27.9)	289 (4.5)	–1–	–1–	105 (1.6)	–1–	–1–	66 (1.0)	–1–	–1–
Q2 (22–23)	4226 (18.5)	232 (18.5)	0.89 (0.75–1.06)	0.90 (0.76–1.07)	76 (1.8)	0.77 (0.58–1.04)	0.81 (0.60–1.09)	45 (1.1)	0.74 (0.50–1.08)	0.77 (0.53–1.13)
Q3 (24–26)	6242 (27.3)	364 (5.8)	0.92 (0.79–1.07)	0.94 (0.81–1.10)	132 (2.1)	0.90 (0.70–1.17)	0.96 (0.74–1.24)	72 (1.1)	0.79 (0.56–1.10)	0.84 (0.60–1.18)
Q4 (27–39)	6013 (26.3)	352 (5.8)	0.75 (0.64–0.88)	0.81 (0.69–0.96)	131 (2.2)	0.75 (0.58–0.97)	0.80 (0.62–1.04)	75 (1.2)	0.70 (0.50–0.99)	0.73 (0.52–1.03)
Palaeolithic diet										
Q1 (21–39)	5982 (26.2)	281 (4.7)	–1–	–1–	95 (1.6)	–1–	–1–	52 (0.9)	–1–	–1–
Q2 (40–43)	5308 (23.2)	283 (5.3)	0.88 (0.74–1.03)	0.89 (0.75–1.05)	110 (2.1)	0.97 (0.74–1.28)	0.98 (0.74–1.22)	65 (1.2)	1.03 (0.71–1.49)	1.04 (0.72–1.51)
Q3 (44–48)	6414 (28.1)	393 (6.1)	0.92 (0.79–1.07)	0.97 (0.83–1.13)	131 (2.0)	0.86 (0.66–1.12)	0.90 (0.68–1.17)	80 (1.2)	0.95 (0.66–1.34)	0.99 (0.69–1.41)
Q4 (49–65)	5146 (22.5)	280 (5.4)	0.79 (0.67–0.94)	0.84 (0.71–0.99)	108 (2.1)	0.87 (0.66–1.15)	0.92 (0.70–1.22)	61 (1.2)	0.89 (0.61–1.29)	0.92 (0.63–1.34)
Nordic diet										
Q1 (0–6)	6588 (28.8)	441 (6.7)	–1–	–1–	155 (2.3)	–1–	–1–	94 (1.4)	–1–	–1–
Q2 (7–8)	5818 (25.5)	289 (5.0)	0.83 (0.72–0.97)	0.82 (0.71–0.96)	115 (2.0)	0.98 (0.77–1.25)	0.95 (0.75–1.22)	68 (1.2)	0.97 (0.71–1.34)	0.93 (0.68–1.28)
Q3 (9–10)	5692 (24.9)	293 (5.1)	0.95 (0.82–1.11)	0.97 (0.84–1.14)	95 (1.7)	0.95 (0.73–1.24)	0.92 (0.71–1.21)	57 (1.0)	0.97 (0.69–1.37)	0.91 (0.64–1.28)
Q4 (11–17)	4751 (20.8)	214 (4.5)	0.91 (0.77–1.09)	0.93 (0.78–1.11)	79 (1.7)	1.05 (0.78–1.41)	1.03 (0.76–1.38)	39 (0.8)	0.91 (0.61–1.36)	0.84 (0.56–1.26)

The range of scores is shown in parentheses for quartiles for each diet. Hazard ratio (HR) with 95% confidence interval (95% CI). Model 1 adjusted for age, sex and energy intake (Kcal/d). Model 2 as in model 1 further adjusted for education (up to lower secondary school, upper secondary school, postgraduate), household income (< 10,000; 10,000–25,000; 25,000–40,000; > 40,000 Euros/year), leisure-time physical activity (MET-h/day; continuous), smoking status (non-smokers, current smokers, former smokers), BMI (kg/m^2; < 25; 25–30; > 30), diabetes, hypertension, hyperlipidaemia, history of CVD, and history of cancer at baseline

Table 3 Association of a priori-defined dietary patterns with risk of cancer and other cause death in the Moli-sani Study cohort ($n = 22,849$)

	Subjects, *n* (%)	Cancer mortality			Other cause mortality		
		Deaths, *n* (%)	Model 1 HR (95% CI)	Model 2 HR (95% CI)	Deaths, *n* (%)	Model 1 HR (95% CI)	Model 2 HR (95% CI)
Quartiles							
Mediterranean diet							
Q1 (0–3)	6961 (30.5)	139 (2.0)	–1–	–1–	107 (1.5)	–1–	–1–
Q2 (4)	5129 (22.4)	107 (2.1)	0.91 (0.71–1.17)	0.94 (0.73–1.21)	73 (1.4)	0.79 (0.59–1.07)	0.84 (0.62–1.13)
Q3 (5)	4926 (21.6)	112 (2.3)	0.96 (0.75–1.24)	1.00 (0.78–1.29)	62 (1.3)	0.71 (0.52–0.97)	0.76 (0.55–1.04)
Q4 (6–9)	5833 (25.5)	125 (2.1)	0.82 (0.64–1.05)	0.88 (0.68–1.12)	68 (1.2)	0.61 (0.44–0.83)	0.66 (0.48–0.91)
DASH diet							
Q1 (10–21)	6368 (27.9)	106 (1.7)	–1–	–1–	78 (1.2)	–1–	–1–
Q2 (22–23)	4226 (18.5)	91 (2.1)	1.01 (0.76–1.33)	1.01 (0.76–1.33)	65 (1.5)	0.91 (0.66–1.27)	0.93 (0.67–1.29)
Q3 (24–26)	6242 (27.3)	145 (2.3)	1.04 (0.81–1.34)	1.06 (0.82–1.37)	87 (1.4)	0.81 (0.59–1.10)	0.83 (0.61–1.13)
Q4 (27–39)	6013 (26.3)	141 (2.3)	0.88 (0.68–1.14)	0.95 (0.74–1.23)	80 (1.3)	0.63 (0.46–0.87)	0.71 (0.51–0.97)
Palaeolithic diet							
Q1 (21–39)	5982 (26.2)	109 (1.8)	–1–	–1–	77 (1.3)	–1–	–1–
Q2 (40–43)	5308 (23.2)	105 (2.0)	0.89 (0.68–1.17)	0.90 (0.69–1.18)	68 (1.3)	0.75 (0.54–1.03)	0.77 (0.56–1.08)
Q3 (44–48)	6414 (28.1)	160 (2.5)	1.05 (0.82–1.34)	1.09 (0.85–1.40)	102 (1.6)	0.84 (0.62–1.13)	0.89 (0.66–1.20)
Q4 (49–65)	5146 (22.5)	109 (2.1)	0.86 (0.66–1.12)	0.90 (0.68–1.18)	63 (1.2)	0.63 (0.45–0.88)	0.69 (0.49–0.97)
Nordic diet							
Q1 (0–6)	6588 (28.8)	160 (2.4)	–1–	–1–	126 (1.9)	–1–	–1–
Q2 (7–8)	5818 (25.5)	107 (1.8)	0.81 (0.64–1.04)	0.81 (0.63–1.03)	67 (1.1)	0.69 (0.51–0.93)	0.69 (0.51–0.93)
Q3 (9–10)	5692 (24.9)	134 (2.3)	1.11 (0.87–1.41)	1.17 (0.92–1.49)	64 (1.1)	0.75 (0.55–1.02)	0.77 (0.56–1.06)
Q4 (11–17)	4751 (20.8)	82 (1.7)	0.86 (0.65–1.15)	0.89 (0.67–1.19)	53 (1.1)	0.83 (0.59–1.18)	0.89 (0.63–1.27)

The range of scores is shown in parentheses for quartiles for each diet. Hazard ratio (HR) with 95% confidence interval (95% CI). Model 1 adjusted for age, sex and energy intake (kcal/day). Model 2 as in model 1 further adjusted for education (categorical), income (categorical), leisure-time physical activity (MET-h/d, continuous), smoking status (categorical), BMI (kg/m^2, categorical), diabetes, hypertension, hyperlipidaemia, history of CVD, and history of cancer at baseline

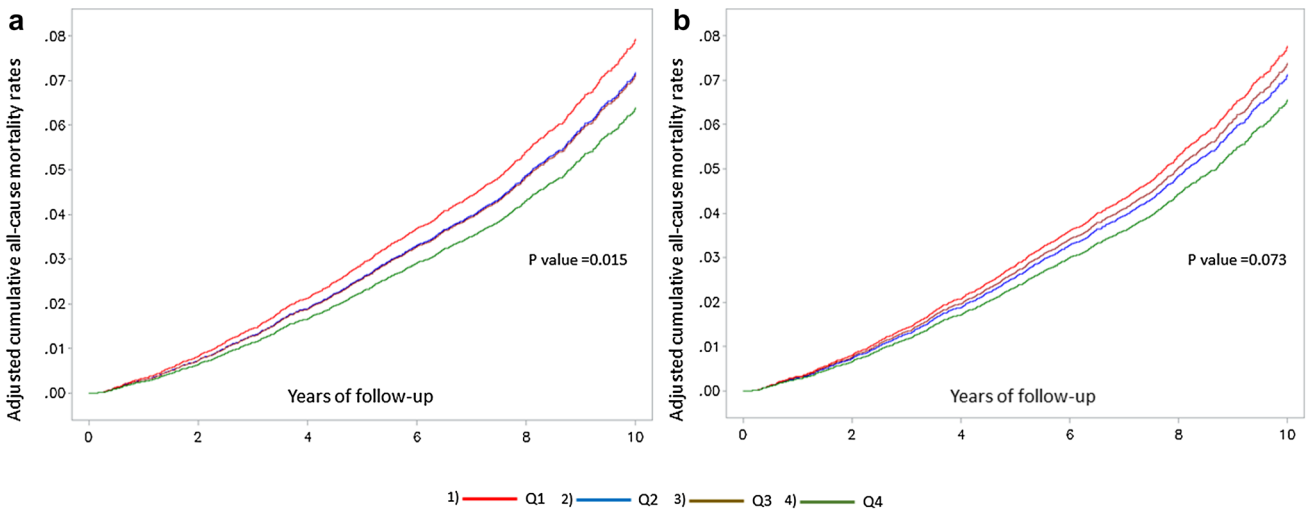

Fig. 1 Cumulative all-cause mortality rates according to four-level adherence to Mediterranean diet (**a**) and DASH diet (**b**). The mortality rates are obtained from a multivariable model adjusted for age, sex, energy intake, education (categorical), income (categorical), leisure-time physical activity (MET-h/day; continuous), smoking status (categorical), BMI (kg/m^2; categorical), diabetes, hypertension, hyperlipidemia, history of CVD, and history of cancer at baseline

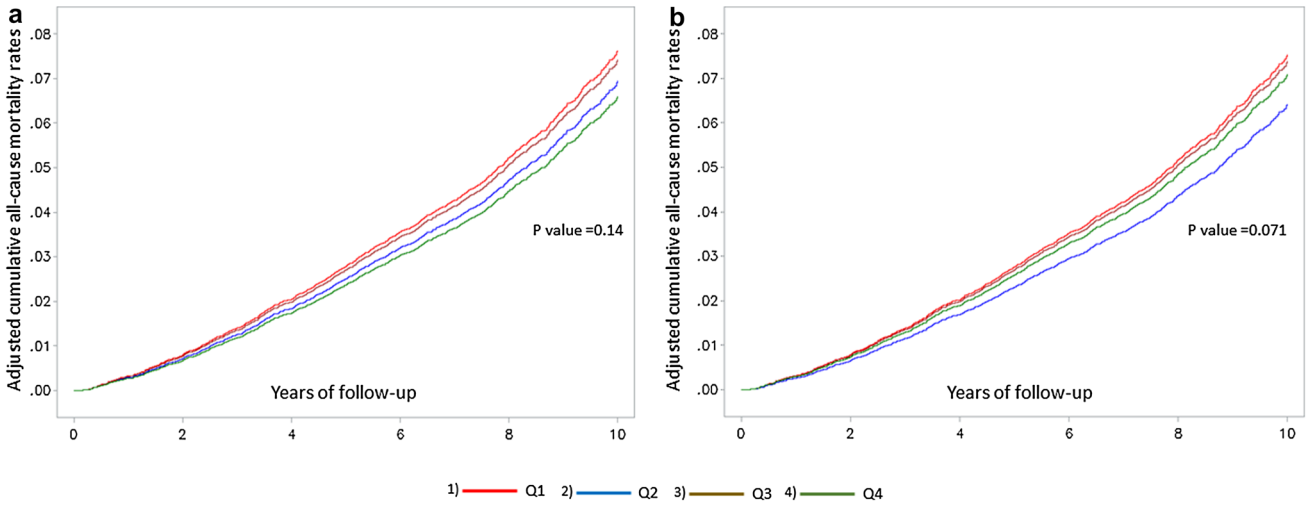

Fig. 2 Cumulative all-cause mortality rates according to four-level adherence to Palaeolithic diet (**a**) and Nordic diet (**b**). The mortality rates are obtained from a multivariable model adjusted for age, sex, energy intake, education (categorical), income (categorical), leisure-time physical activity (MET-h/day; continuous), smoking status (categorical), BMI (kg/m^2; categorical), diabetes, hypertension, hyperlipidemia, history of CVD, and history of cancer at baseline

Supplemental Figures 2 and 3 report the adjusted dose–response association between dietary scores and risk of all-cause death showing an inverse linear dose–response relationship limited to MDS (*P* for overall association = 0.0029; *P* for non-linear association = 0.77).

Among individual food groups, higher consumption of MUFAs over SFAs, cabbages and cruciferous vegetables, and fish and grains was likely associated with lower risk of total and CVD death, while for total, cancer, and other cause mortality, moderate alcohol intake also played a major role. For IHD/cerebrovascular risk, there was also a healthful role of low-fat dairy products and berries, while cancer risk was likely to be altered by higher consumption of fruits and nuts/legumes (Supplemental Table 5).

Mediation analysis

Each panel of explanatory factors was tested independently and in a multivariable model including all factors simultaneously. Overall, biomarkers of glucose metabolism (i.e., blood glucose, insulin, and C-peptide) explained the largest, although modest, proportion of the association of 1 SD increment in MDS and DASH diet score with total mortality (7.4 and 21.6%, respectively; Table 4).

All explanatory factors explained from 13.4 to 21.2% of the relation of diet with the risk of dying from non-CVD/non-cancer causes. Biomarkers of lipid metabolism (e.g., total blood cholesterol and triglycerides) were on the pathway between MDS, DASH, and Palaeolithic diets and risk of other cause mortality, while markers of renal function likely attenuated the association between the Nordic diet and health outcomes (Table 4).

Combination of MDS with non-MD dietary scores

We stratified main analyses across low and high levels of adherence to the MDS to test whether an increase in adherence to each non-MD dietary score would modulate mortality risk (Table 5).

Among the group with the lowest MDS (≤ 4), increased adherence to non-MD dietary patterns was not associated with improved survival, while CVD mortality risk was likely to be lowered at increased adherence to the Nordic diet. No additional health benefits were found among subjects with high MDS (Table 5).

On the other hand, analyses stratified for non-MD dietary patterns revealed higher survival associated with 1 SD increment in the MDS independently from the baseline degree of adherence to each non-MD score. Also, downward trends for cause-specific death risks were found (Table 6).

Cumulative mortality rates for joint analysis of MDS (low/high) with non-MD diets (low/high) showed higher survival for those adopting an MD rather than non-MD dietary patterns (Supplemental Figure 4).

The inclusion of non-MD food groups did not provide any additional risk reduction to the MDS, with the exception of a modest improvement in CVD mortality risk when DASH food items were included into the MDS (Supplemental Table 7). A global score of MDS supplemented with eight non-MD food items was not associated with lower mortality, nor was the score including non-MD foods only.

Table 4 Mediation analysis for the association of 1-SD increment in the dietary scores with all-cause and cause-specific mortality risks in the Moli-sani Study cohort ($n=22,849$)

	All-cause mortality		CVD mortality		IHD/cerebrovascular mortality		Cancer mortality		Other causes mortality	
	HR (95% CI)	PTE-% (95% CI); *P* value	HR (95% CI)	PTE-% (95% CI); *P* value	HR (95% CI)	PTE-% (95% CI); *P* value	HR (95% CI)	PTE-% (95% CI); *P* value	HR (95% CI)	PTE-% (95% CI); *P* value
Mediterranean diet										
Model 2	0.90 (0.85–0.96)	–	0.92 (0.83–1.01)	–	0.86 (0.76–0.99)	–	0.95 (0.86–1.04)	–	0.82 (0.74–0.92)	–
Model 2 + cardiac troponin	0.90 (0.85–0.95)	Null	0.90 (0.82–1.00)	Null	0.85 (0.75–0.97)	Null	0.95 (0.86–1.04)	1.2 (0.0–88.0); 0.37	0.82 (0.74–0.92)	–
Model 2 + natriuretic peptide	0.90 (0.85–0.96)	Null	0.91 (0.82–1.01)	Null	0.86 (0.75–0.98)	Null	0.95 (0.87–1.04)	4.9 (0.5–32.5); 0.073	0.82 (0.73–0.91)	Null
Model 2 + renal function	0.90 (0.85–0.95)	Null	0.91 (0.83–1.01)	Null	0.86 (0.75–0.98)	Null	0.95 (0.87–1.04)	4.3 (0.2–50.8); 0.21	0.82 (0.73–0.92)	Null
Model 2 + glucose metabolism	0.91 (0.86–0.96)	7.4 (2.8–18.1); 0.0033	0.92 (0.83–1.02)	5.1 (0.4–44.2); 0.19	0.86 (0.75–0.98)	Null	0.96 (0.87–1.05)	17.0 (1.7–70.5); 0.013	0.83 (0.74–0.93)	5.7 (1.6–18.1); 0.023
Model 2 + lipid metabolism	0.91 (0.85–0.96)	3.0 (0.2–34.6); 0.23	0.91 (0.82–1.01)	Null	0.84 (0.74–0.96)	Null	0.94 (0.86–1.04)	Null	0.85 (0.75–0.95)	14.3 (5.6–32.0); 0.0011
Model 2 + serum vitamin D	0.90 (0.85–0.95)	Null	0.91 (0.83–1.01)	Null	0.86 (0.76–0.99)	Null	0.95 (0.86–1.04)	Null	0.82 (0.73–0.92)	Null
Model 2 + inflammation	0.90 (0.85–0.96)	Null	0.92 (0.83–1.01)	Null	0.87 (0.76–0.99)	1.7 (0.0–48.4); 0.31	0.95 (0.86–1.04)	Null	0.82 (0.73–0.92)	Null
Model 2 + BPs	0.90 (0.85–0.95)	Null	0.91 (0.83–1.01)	Null	0.86 (0.75–0.98)	Null	0.95 (0.86–1.04)	Null	0.83 (0.74–0.93)	2.0 (0.2–18.5); 0.19
Model 2 + all factors	0.91 (0.86–0.96)	6.4 (0.6–41.4); 0.18	0.91 (0.83–1.01)	Null	0.85 (0.74–0.97)	Null	0.95 (0.86–1.04)	1.4 (0.0–*100.0*); 0.47	0.85 (0.75–0.95)	13.4 (4.0–36.5); 0.019
DASH diet										
Model 2	0.95 (0.89–1.01)	–	0.97 (0.87–1.07)	–	0.95 (0.83–1.08)	–	0.98 (0.89–1.08)	–	0.90 (0.80–1.02)	–
Model 2 + cardiac troponin	0.94 (0.88–1.009)	Null	0.94 (0.85–1.04)	Null	0.92 (0.80–1.05)	Null	0.98 (0.89–1.08)	Null	0.89 (0.79–1.01)	Null
Model 2 + natriuretic peptide	0.94 (0.89–1.00)	Null	0.95 (0.86–1.05)	Null	0.93 (0.81–1.07)	Null	0.98 (0.89–1.08)	4.0 (0.0–98.4); 0.32	0.90 (0.80–1.01)	Null

Table 4 (continued)

	All-cause mortality		CVD mortality		IHD/cerebrovascular mortality		Cancer mortality		Other causes mortality	
	HR (95% CI)	PTE-% (95% CI); *P* value	HR (95% CI)	PTE-% (95% CI); *P* value	HR (95% CI)	PTE-% (95% CI); *P* value	HR (95% CI)	PTE-% (95% CI); *P* value	HR (95% CI)	PTE-% (95% CI); *P* value
Model 2 + renal function	0.94 (0.89–1.00)	Null	0.96 (0.86–1.06)	Null	0.94 (0.82–1.07)	Null	0.98 (0.89–1.08)	Null	0.89 (0.79–1.01)	Null
Model 2 + glucose metabolism	0.96 (0.90–1.02)	21.6 (5.0–59.2); 0.0003	0.98 (0.88–1.09)	39.7 (0.3–99.2); 0.016	0.95 (0.83–1.09)	16.5 (0.6–86.5); 0.11	0.99 (0.90–1.09)	55.9 (0.0–100.0); 0.012	0.92 (0.81–1.04)	14.5 (2.8–50.0); 0.011
Model 2 + lipid metabolism	0.95 (0.89–1.01)	Null	0.96 (0.86–1.06)	Null	0.93 (0.81–1.06)	Null	0.98 (0.88–1.08)	Null	0.92 (0.81–1.04)	16.7 (3.3–54.6); 0.011
Model 2 + serum vitamin D	0.95 (0.89–1.01)	1.4 (0.0–94.9); 0.39	0.97 (0.88–1.07)	10.4 (0.2–89.7); 0.23	0.95 (0.83–1.09)	8.3 (0.3–74.5); 0.17	0.98 (0.89–1.08)	2.1 (0.0–100.0); 0.43	0.90 (0.80–1.02)	1.0 (0.0–36.7); 0.31
Model 2 + inflammation	0.95 (0.90–1.01)	6.7 (0.8–37.5); 0.11	0.97 (0.88–1.08)	11.3 (0.2–90.6); 0.22	0.95 (0.83–1.09)	9.3 (0.4–74.5); 0.16	0.98 (0.89–1.09)	13.5 (0.0–99.4); 0.25	0.90 (0.80–1.02)	1.0 (0.0–97.7); 0.40
Model 2 + BPs	0.95 (0.90–1.01	3.2 (0.3–27.2); 0.17	0.97 (0.87–1.07)	Null	0.94 (0.82–1.08)	Null	0.98 (0.89–1.08)	2.1 (0.0–100); 0.42	0.91 (0.80–1.03)	5.6 (0.9–27.6); 0.054
Model 2 + all factors	0.96 (0.90–1.02)	17.9 (2.4–65.9); 0.087	0.96 (0.87–1.07)	Null	0.94 (0.82–1.07)	Null	0.99 (0.89–1.09)	30.7 (0.0–99.9); 0.24	0.92 (0.82–1.04)	21.2 (3.3–67.8); 0.044
Palaeolithic diet										
Model 2	0.95 (0.89–1.00)	–	0.97 (0.87–1.08)	–	0.98 (0.85–1.12)	–	0.97 (0.88–1.06)	–	0.89 (0.79–1.00)	–
Model 2 + cardiac troponin	0.94 (0.89–1.00)	Null	0.95 (0.86–1.06)	Null	0.96 (0.84–1.10)	Null	0.97 (0.88–1.06)	Null	0.88 (0.79–0.99)	Null
Model 2 + natriuretic peptide	0.95 (0.89–1.00)	Null	0.96 (0.87–1.07)	Null	0.97 (0.84–1.11)	Null	0.97 (0.88–1.07)	4.8 (0.1–73.000); 0.18	0.89 (0.79–1.00)	Null
Model 2 + renal function	0.94 (0.89–1.00)	Null	0.96 (0.87–1.07)	Null	0.97 (0.85–1.11)	Null	0.97 (0.89–1.07)	4.1 (0.0–82.1); 0.29	0.89 (0.79–1.00)	Null
Model 2 + glucose metabolism	0.95 (0.89–1.01)	2.1 (0.0–62.1); 0.32	0.97 (0.88–1.08)	10.1 (0.1–94.7); 0.25	0.98 (0.85–1.12)	4.7 (0.0–99.9); 0.41	0.97 (0.89–1.07)	6.9 (0.1–85.3); 0.23	0.89 (0.79–1.01)	1.7 (0.0–64.5); 0.33
Model 2 + lipid metabolism	0.95 (0.89–1.01)	Null	0.96 (0.87–1.07)	Null	0.96 (0.84–1.10)	Null	0.96 (0.88–1.06)	Null	0.91 (0.81–1.02)	17.0 (4.3–48.4); 0.0039

Table 4 (continued)

	All-cause mortality		CVD mortality		IHD/cerebrovascular mortality		Cancer mortality		Other causes mortality	
	HR (95% CI)	PTE-% (95% CI); *P* value	HR (95% CI)	PTE-% (95% CI); *P* value	HR (95% CI)	PTE-% (95% CI); *P* value	HR (95% CI)	PTE-% (95% CI); *P* value	HR (95% CI)	PTE-% (95% CI); *P* value
Model 2 + serum vitamin D	0.95 (0.89–1.01)	1.3 (0.0–93.1); 0.39	0.97 (0.88–1.08)	11.0 (0.1–94.2); 0.24	0.98 (0.86–1.12)	18.2 (0.0–99.6); 0.17	0.97 (0.88–1.06)	2.0 (0.0–98.0); 0.39	0.89 (0.79–1.00)	Null
Model 2 + inflammation	0.95 (0.89–1.00)	Null	0.97 (0.87–1.08)	Null	0.98 (0.85–1.12)	10.5 (0.0–99.4); 0.32	0.97 (0.88–1.06)	Null	0.89 (0.79–1.00)	Null
Model 2 + BPs	0.94 (0.89–1.00)	Null	0.97 (0.87–1.08)	Null	0.97 (0.84–1.11)	Null	0.97 (0.88–1.06)	Null	0.89 (0.79–1.01)	2.1 (0.1–33.3); 0.24
Model 2 + all factors	0.95 (0.89–1.01)	1.4 (0.0–100); 0.45	0.96 (0.87–1.07)	Null	0.96 (0.84–1.10)	Null	0.97 (0.88–1.06)	Null	0.91 (0.81–1.02)	17.8 (3.6–55.9); 0.035
Nordic diet										
Model 2	0.96 (0.90–1.02)	–	0.97 (0.88–1.08)	–	0.93 (0.81–1.07)	–	1.00 (0.91–1.10)	–	0.89 (0.78–1.01)	–
Model 2 + cardiac troponin	0.96 (0.90–1.02)	Null	0.97 (0.88–1.08)	Null	0.93 (0.81–1.06)	Null	1.00 (0.91–1.10)	Null	0.89 (0.78–1.01)	Null
Model 2 + natriuretic peptide	0.96 (0.90–1.02)	Null	0.97 (0.87–1.08)	Null	0.92 (0.80–1.06)	Null	1.00 (0.91–1.10)	21.8 (0.0–100.0); 0.39	0.89 (0.78–1.00)	Null
Model 2 + renal function	0.97 (0.91–1.03)	14.0 (2.1–55.2); 0.017	0.98 (0.88–1.09)	26.4 (0.1–99.0); 0.081	0.93 (0.81–1.07)	3.1 (0.0–85.2); 0.34	1.01 (0.92–1.11)	Null	0.89 (0.79–1.01)	6.2 (1.2–26.3); 0.047
Model 2 + glucose metabolism	0.96 (0.90–1.02)	Null	0.97 (0.87–1.07)	Null	0.92 (0.80–1.05)	Null	1.00 (0.91–1.10)	15.7 (0.0–100.0); 0.45	0.88 (0.78–1.00)	Null
Model 2 + lipid metabolism	0.96 (0.90–1.02)	Null	0.97 (0.88–1.08)	Null	0.92 (0.80–1.06)	Null	1.00 (0.91–1.10)	Null	0.89 (0.79–1.01)	4.1 (0.2–54.8); 0.26
Model 2 + serum vitamin D	0.96 (0.90–1.02)	1.5 (0.0–99.2); 0.41	0.97 (0.88–1.08)	Null	0.92 (0.81–1.06)	Null	1.00 (0.91–1.10)	Null	0.89 (0.78–1.01)	1.1 (0.0–35.2); 0.30
Model 2 + inflammation	0.96 (0.90–1.02)	Null	0.97 (0.87–1.08)	Null	0.93 (0.81–1.06)	Null	1.00 (0.91–1.10)	11.1 (0.0–100.0); 0.47	0.89 (0.78–1.01)	Null
Model 2 + BPs	0.96 (0.90–1.02)	Null	0.97 (0.88–1.08)	Null	0.93 (0.81–1.07)	Null	1.00 (0.91–1.10)	38.8 (0.0–100.0); 0.33	0.89 (0.78–1.00)	Null
Model 2 + all factors	0.96 (0.90–1.02)	Null	0.97 (0.87–1.07)	Null	0.92 (0.80–1.05)	Null	1.00 (0.91–1.10)	60.4 (0.0–100.0); 0.41	0.89 (0.78–1.01)	Null

Table 4 (continued)

Hazard ratio (HR) with 95% confidence interval (95% CI) for all-cause and cause-specific mortality risk associated with 1 standard deviation (SD) increase in each dietary score (Mediterranean diet = 1.6; Dash diet = 4.0; Palaeolithic diet = 6.3; Nordic diet = 2.6). Model 2 is adjusted for sex, age (years, continuous), energy intake (kcal/day, continuous), education (categorical), income (categorical), leisure-time physical activity (MET-h/day, continuous), smoking status (categorical), BMI (kg/m^2, categorical), diabetes, hypertension, hyperlipidaemia, history of CVD, and history of cancer at baseline. Cardiac troponin = high-sensitivity assayed Troponin I (hsTnI; pg/mL; logarithm). Natriuretic peptide = N-terminal pro B-type natriuretic peptide (NTproBNP; pg/mL; logarithm). Biomarkers of renal function include Cystatin C and Creatinine (mg/L; logarithm). Biomarkers of glucose metabolism include blood glucose (mg/dL; logarithm), insulin (pmol/L; logarithm), and C-peptide (ng/mL; logarithm). Biomarkers of lipid metabolism = blood cholesterol (mg/dL), HDL-cholesterol (mg/dL), triglycerides (mg/dL; logarithm), apolipoprotein A1 (ApoA; g/L), apolipoprotein B100 (ApoB100, g/L), and Lipoprotein a [Lp(a); mg/dL]. Serum vitamin D expressed as ng/mL. Inflammatory biomarkers include C-reactive protein (mg/dL; logarithm) and white blood cell count (× 10^9/L; logarithm)

BP systolic and diastolic blood pressure (mm Hg), *BMI* body mass index (normal weight, overweight, obese), *PTE* percent of exposure effect explained by intermediate variables, with 95% confidence interval and *P* values, *Null* not mediating the effect

Sensitivity analyses

The main results on dietary scores and all-cause mortality risk were confirmed by sensitivity analysis ran for age, sex, and different baseline health conditions, lifestyles, and socioeconomic status. We found that age was an effect modifier of the association between the DASH diet and total death, with risk reduction limited to youngest people (*P* for interaction = 0.019); also, history of cancer at baseline modified the risk of dying from any cause associated with the Palaeolithic diet (*P* for interaction = 0.0046; Supplemental Table 7).

Discussion

Findings from a large population-based cohort of adult Italians showed that adherence to the traditional Mediterranean diet is associated with lower risk of all-cause, CVD, and other cause mortality.

Improved survival was also found with higher scores on DASH and Palaeolithic diets, while no reduction in total mortality risk was found with the Nordic diet.

The strength of the association between the DASH diet pattern with CVD and IHD/cerebrovascular mortality was weaker than that observed with MD. All dietary scores showed a non-significant downtrend with cancer death risk, although the magnitude of the association of MDS (and Nordic diet) was greater than the other diets, and seemed in line with risk estimates provided by prior investigations [33]. Mortality from the other causes was also lower for subjects with higher adherence to all dietary patterns, with the exception of the Nordic diet.

To the best of our knowledge, this is the first study to test the ability of non-MD scores to shape mortality risk among a Mediterranean population. In a prior investigation conducted within the Italian segment of the EPIC study [17], along with the MD (as measured by the Italian Mediterranean Index), the DASH diet and the Healthy eating index (HEI) were associated with lower risk of ischemic stroke, thus documenting health advantages associated with non-MD diets in a Mediterranean population. However, no evidence is available to date with respect to mortality.

Although the four dietary patterns here analysed are all proxies of healthy eating models, some differences still persist. It is worth noting that the traditional MD is the only to include MUFAs-to-SFAs ratio, and this may likely account for the observed differences among the four dietary patterns in relation to CVD death risk; indeed, the high scoring of MUFAs over SFAs was consistently associated with lower death risk, especially form cardiovascular causes as also discussed in previous epidemiological observations [34].

Table 5 All-cause and cause-specific mortality risk associated with 1 standard deviation increase in the DASH, Palaeolithic and Nordic diet scores across low and high levels of adherence to the Mediterranean diet (MDS) in the Moli-sani Study cohort ($n = 22,849$)

	Deaths, *n*/subjects, *n*	DASH diet	Palaeolithic diet	Nordic diet
All-cause mortality				
Low MDS	668/12,090	0.98 (0.89–1.08)	0.97 (0.87–1.07)	0.94 (0.86–1.03)
High MDS	569/10,759	0.98 (0.89–1.08)	0.97 (0.88–1.07)	1.02 (0.93–1.11)
P interaction		0.80	0.98	0.083
CVD mortality				
Low MDS	242/12,090	1.08 (0.93–1.26)	0.97 (0.82–1.14)	0.89 (0.76–1.03)
High MDS	202/10,759	0.92 (0.77–1.09)	1.05 (0.89–1.23)	1.12 (0.96–1.30)
P interaction		0.29	0.36	0.017
IHD/cerebrovascular mortality				
Low MDS	142/12,090	1.11 (0.91–1.37)	1.07 (0.86–1.33)	0.92 (0.76–1.12)
High MDS	116/10,759	0.86 (0.69–1.08)	0.96 (0.78–1.19)	0.99 (0.81–1.21)
P interaction		0.12	0.27	0.45
Cancer mortality				
Low MDS	246/12,090	0.98 (0.84–1.14)	1.04 (0.89–1.22)	1.04 (0.90–1.20)
High MDS	237/10,759	1.01 (0.87–1.18)	0.91 (0.79–1.06)	0.99 (0.86–1.14)
P interaction		0.81	0.25	0.65
Other cause mortality				
Low MDS	180/12,090	0.89 (0.41–1.06)	0.90 (0.75–1.09)	0.87 (0.74–1.04)
High MDS	130/10,759	1.04 (0.84–1.28)	0.96 (0.78–1.18)	0.94 (0.78–1.14)
P interaction		0.17	0.71	0.28

Hazard ratio (HR) with 95% confidence interval (95% CI) for all-cause and cause-specific mortality risk associated with 1 standard deviation (SD) increase in each dietary score (Dash diet = 4.0; Palaeolithic diet = 6.3; Nordic diet = 2.8). Multivariable models are adjusted for sex, age (years, continuous), energy intake (kcal/day, continuous), education (categorical), income (categorical), leisure-time physical activity (MET-h/day, continuous), smoking status (categorical), BMI (kg/m^2, categorical), diabetes, hypertension, hyperlipidaemia, history of CVD, and history of cancer at baseline. Low and high categories of adherence to the Mediterranean diet defined as MDS ≤ 4 or > 4 (population median)

Table 6 All-cause and cause-specific mortality risk associated with 1 standard deviation increase in the Mediterranean diet score across low and high levels of adherence to the DASH, Palaeolithic, and Nordic diets in the Moli-sani Study cohort ($n = 22,849$)

	Subjects, *n*	All-cause mortality	CVD mortality	IHD/cerebrovascular mortality	Cancer mortality	Other cause mortality
DASH diet						
Low	12,817	0.91 (0.83–0.997)	0.93 (0.80–1.09)	0.89 (0.73–1.10)	0.95 (0.82–1.10)	0.83 (0.69–0.99)
High	10,032	0.90 (0.82– 0.99)	0.92 (0.79–1.07)	0.87 (0.70–1.07)	0.93 (0.81–1.08)	0.83 (0.69–1.005)
P interaction		0.70	0.74	0.95	0.86	0.71
Palaeolithic diet						
Low	12,672	0.93 (0.84–1.01)	0.90 (0.77–1.05)	0.88 (0.71–1.09)	1.00 (0.87–1.13)	0.86 (0.72–1.03)
High	10,177	0.87 (0.79–0.96)	0.93 (0.79–1.09)	0.81 (0.66–0.99)	0.87 (0.75–1.01)	0.80 (0.66–0.97)
P interaction		0.87	0.36	0.94	0.34	0.74
Nordic diet						
Low	12,406	0.88 (0.81–0.96)	0.84 (0.73–0.98)	0.85 (0.70–1.02)	0.96 (0.83–1.11)	0.84 (0.71–1.00)
High	10,443	0.92 (0.85–0.997)	0.99 (0.86–1.14)	0.90 (0.75–1.09)	0.93 (0.82–1.05)	0.82 (0.69–0.97)
P interaction		0.43	0.084	0.47	0.69	0.93

Hazard ratio (HR) with 95% confidence interval (95% CI) for all-cause and cause-specific mortality risk associated with 1 standard deviation (SD) increase in the Mediterranean diet score (1 SD = 1.6). Multivariable models are adjusted for sex, age (years, continuous), energy intake (kcal/day, continuous), education (categorical), income (categorical), leisure-time physical activity (MET-h/d, continuous), smoking status (categorical), BMI (kg/m^2, categorical), diabetes, hypertension, hyperlipidaemia, history of CVD, and history of cancer at baseline. Low and high categories of adherence were defined as dietary score $\leq$ or $>$ population median (24, 44, and 8 for the DASH, Palaeolithic, and the Nordic diets, respectively)

Moreover, the different impact on health outcomes is potentially linked to the use of a different methodology to classify each score (e.g., population cut-offs and quintiles).

As yet, we acknowledge the possibility that the intake of some of non-typical Mediterranean foods (e.g., cabbages, root vegetables, and whole-grain bread) may not be commonly consumed in our Mediterranean population to detect beneficial effects.

Finally, the MDS was originally created based on a Mediterranean population and this sort of "internal bias" may also be responsible for the more favourable results observed with MDS which may be a better index to describe or summarize diet quality in a Mediterranean country than the other dietary indices. However, we should recall that an MDS, or its modified versions, were found effective in reducing disease/death risk also in non-Mediterranean countries [9–12] and sometimes resulted as the best performing dietary model as compared to other healthy dietary patterns [13, 15, 16].

On the other side, the health advantages of healthy non-MD diets in non-Mediterranean populations have been widely demonstrated and frequently resulted of the same magnitude of the MDS [35, 36].

Combination of dietary scores

In the present work, we also aimed to test whether increasing adherence to healthy non-MD dietary patterns would represent a healthful option in the absence of an optimal adherence to a traditional MD and vice versa.

We then calculated mortality risk associated with non-MD diets across levels of adherence to the traditional MD and found that increasing conformity to DASH, Palaeolithic, or Nordic diets did not provide any additional advantage for survival, independently from baseline adherence to the MD. However, we found lower CVD mortality risk associated with increased adherence to the Nordic diet among subjects with poor adherence to MD, which is likely due to its high fish content, thoroughly associated with CVD benefits [37].

On the contrary, increasing adherence to a traditional MD was linked to improved survival in each stratum of non-MD scores, and health advantages were found also for CVD death risk and other cause mortality.

Our results on the combination of dietary scores are in agreement with the apparently only study of this type available to date from the Swedish Mammography Cohort [15] which classified nearly 40,000 women in joint exposure strata reflecting the combined adherence to the MD and the Healthy Nordic Food Index (HNFI); findings showed that a higher adherence to the MD was associated with lower mortality in each stratum of the HNFI, whereas results for HNFI were not independent of MD.

In our study, we also aimed to test whether inclusion of healthful non-MD food items into the traditional MDS could improve risk prediction. However, we found no additional health benefits associated with an MDS supplemented with non-MD food items, such as lower intake of baked foods or soft drinks. This finding may indicate that MDS itself is able to almost completely discriminate between healthy and non-healthy diets, at least in our Mediterranean population.

Possible pathways linking diet quality to mortality

All dietary models under study may reduce risk of chronic diseases that lead to premature mortality through several biological mechanisms. Plenty of studies have shown that high adherence to each of these diets is associated with a more favourable CVD profile as measured by established markers of CVD risk, such as lipids or inflammatory markers.

Among the merits of the present study, we acknowledge the investigation on different mediating mechanisms possibly linking diet quality to mortality.

So far, only a few studies have tested some mediating pathways possibly linking diet to health end-points as a primary purpose; moreover, some were mainly focussed on pre-existing health conditions rather than on biological mechanisms [38], while five prospective cohort investigations [10, 12, 25–27] analysed the role of traditional (i.e., blood lipids and BMI) and inflammatory CVD risk factors. Some biomarkers have been recently shown to improve CVD risk prediction [39] beyond more traditional risk factors (i.e., high blood pressure, lipids, smoking, etc.), but there have been few reported studies exploring the association between diet and, for example, markers of renal function or markers of myocardial injury, with a few exceptions showing that some of these markers are favourably associated with a Mediterranean diet [40, 41].

In general, we were more likely to observe differences in biomarkers distribution between those who adhered to MD or the DASH diet (i.e., being in the highest quartile) and those who did not adhere (i.e., being in the lowest quartile) as compared to individuals with different degree of adherence to the Palaeolithic and the Nordic diets. Differences in baseline markers of glucose metabolism appear to mediate the associations of the traditional MD and the DASH diet with total mortality by 7% and 17%, respectively; markers of lipid metabolism were on the pathway between diet quality and other cause mortality, while a weak role was found for inflammatory markers.

This is apparently in contrast with the previous studies, showing that MD favourably modulates inflammation both in intervention [42] and observational settings [43], and with numerous studies linking inflammation to increased disease/mortality risk [44, 45].

Our findings may indicate that a favourable modulation of markers of glucose metabolism, renal function, and lipid

metabolism may be among the main pathways through which diet exerts its beneficial effects on health. Yet, we acknowledge that the use of only two markers to assess the inflammatory status could lead to an underestimation of the role of inflammation as a likely mediating mechanism linking diet quality to death risk.

Strengths and limitations

The strengths of our study include the large sample size, its prospective population-based design, and the inclusion of detailed information on diet and other lifestyle factors to accommodate possible confounding by these variables. Moreover, this is one of the few studies aimed to assess a number of biological mechanisms as possibly mediating the relation between diet quality and mortality.

Yet, our findings should be interpreted in light of several limitations. First, the observational nature of the study cannot fully rule out residual or unmeasured confounding. Second, dietary data were based on self-reported information and, therefore, may be susceptible to error and bias, and further to difficulties in assessing portion sizes and inadequacies in food composition tables. However, these problems are partially mitigated by exclusion of participants with implausible energy intakes and by energy adjustment [46, 47].

Third, our data were gathered from an adult cohort from a small Southern Italian region, which might limit the generalizability of our findings, although our cohort is representative of the whole Italian population.

Finally, subjects' information (e.g., dietary and biological data) was collected at baseline only, thus life-course changes possibly occurred during the follow-up may have influenced the strength of the findings and led to an underestimation of true effect size. However, the large study size likely compensates for random misclassification.

Conclusions

A traditional Mediterranean diet, a DASH diet, and a Palaeolithic diet were all favourably associated with longer survival. Lower cardiovascular mortality risk was only associated with the MD and the DASH diets, while the Nordic diet was unlikely to provide any substantial health advantage.

Additionally, increasing adherence to MD was associated with higher survival in each stratum of all three non-MD diets.

Part of the health benefits associated with higher adherence to the MD, the DASH, or to the Palaeolithic diets was accounted by a modulation of established biomarkers of CVD risk, such as markers of glucose or lipid metabolism and renal function.

From a public health perspective, our findings provide further incentive and support to preserve a traditional Mediterranean diet to prolong survival among Mediterranean populations, and to reduce CVD mortality risk.

Reportedly healthy non-Mediterranean dietary patterns, although associated with some health benefits also in our Mediterranean population, are unlikely to provide additional health benefits over those offered by a traditional MD; rather, high adherence to the traditional MD may implement the weak effects on CVD mortality risk associated with non-MD diets, as the Palaeolithic and the Nordic diets.

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Moli-sani Study Investigators: The enrolment phase of the Moli-sani Study was conducted at the Research Laboratories of the Catholic University in Campobasso (Italy), and the follow-up of the Moli-sani cohort is being conducted at the Department of Epidemiology and Prevention of the IRCCS Neuromed, Pozzilli, Italy. *Steering Committee:* Licia Iacoviello*°(Chairperson), Giovanni de Gaetano* and Maria Benedetta Donati*. *Scientific secretariat:* Licia Iacoviello*° (Coordinator), Marialaura Bonaccio*, Americo Bonanni*, Chiara Cerletti*, Simona Costanzo*, Amalia De Curtis*, Giovanni de Gaetano*, Augusto Di Castelnuovo§, Maria Benedetta Donati*, Francesco Gianfagna*°, Mariarosaria Persichillo*, Teresa Di Prospero* (Secretary). *Safety and Ethical Committee:* Jos Vermylen (Catholic Univesity, Leuven, Belgio) (Chairperson), Ignacio De Paula Carrasco (Accademia Pontificia Pro Vita, Roma, Italy), Simona Giampaoli (Istituto Superiore di Sanità, Roma, Italy), Antonio Spagnuolo (Catholic University, Roma, Italy). *External Event adjudicating Committee:* Deodato Assanelli (Brescia, Italy), Vincenzo Centritto (Campobasso, Italy). *Baseline and Follow-up data management*: Simona Costanzo* (Coordinator), Marco Olivieri (Università del Molise, Campobasso, Italy). *Informatics:* Marco Olivieri (Università del Molise, Campobasso, Italy). *Data Analysis:* Augusto Di Castelnuovo§ (Coordinator), Marialaura Bonaccio*, Simona Costanzo*, Alessandro Gialluisi*, Francesco Gianfagna*°, Emilia Ruggiero*. *Biobank and biomedical analyses:* Amalia De Curtis* (Coordinator), Sara Magnacca*. *Genetic analyses:* Benedetta Izzi* (Coordinator), Francesco Gianfagna*°, Annalisa Marotta*, Fabrizia Noro*. *Communication and Press Office:* Americo Bonanni* (Coordinator), Francesca De Lucia (Associazione Cuore Sano, Campobasso, Italy). *Recruitment staff*: Mariarosaria Persichillo* (Coordinator), Francesca Bracone*, Francesca De Lucia (Associazione Cuore Sano, Campobasso, Italy), Salvatore Dudiez*, Livia Rago*. *Follow-up Event adjudication:* Livia Rago* (Coordinator), Simona Costanzo*, Amalia De Curtis*, Licia Iacoviello*°, Teresa Panzera*, Mariarosaria Persichillo*. *Regional Health Institutions:* Direzione Generale per la Salute - Regione Molise; Azienda Sanitaria Regionale del Molise (ASReM, Italy); Molise Dati Spa (Campobasso, Italy); Offices of vital statistics of the Molise region. *Hospitals:* Presidi Ospedalieri ASReM: Ospedale A. Cardarelli—Campobasso, Ospedale F. Veneziale – Isernia, Ospedale San Timoteo - Termoli (CB), Ospedale Ss. Rosario - Venafro (IS), Ospedale Vietri—Larino (CB), Ospedale San Francesco Caracciolo - Agnone (IS); Casa di Cura Villa Maria - Campobasso; Fondazione di Ricerca e Cura Giovanni Paolo II - Campobasso; IRCCS Neuromed - Pozzilli (IS). * Department of Epidemiology and Prevention, IRCCS

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Compliance with ethical standards

Conflict of interest None of the authors has conflicts of interest to disclose.

References

1. Trichopoulou A, Costacou T, Bamia C, Trichopoulos D (2003) Adherence to a Mediterranean diet and survival in a Greek population. N Engl J Med 348:2599–2608
2. Carlos S, De La Fuente-Arrillaga C, Bes-Rastrollo M, Razquin C, Rico-Campà A, Martínez-González MA, Ruiz-Canela M (2018) Mediterranean diet and health outcomes in the SUN cohort. Nutrients. https://doi.org/10.3390/nu10040439
3. Bonaccio M, Di Castelnuovo A, Costanzo S, Persichillo M, De Curtis A, Donati MB, de Gaetano G, Iacoviello L, MOLI-SANI study Investigators (2016) Adherence to the traditional Mediterranean diet and mortality in subjects with diabetes. Prospective results from the MOLI-SANI study. Eur J Prev Cardiol 23:400–407
4. Dinu M, Pagliai G, Casini A, Sofi F (2018) Mediterranean diet and multiple health outcomes: an umbrella review of meta-analyses of observational studies and randomised trials. Eur J Clin Nutr 72:30–43
5. Schwingshackl L, Schwedhelm C, Galbete C, Hoffmann G (2017) Adherence to Mediterranean diet and risk of cancer: an updated systematic review and meta-analysis. Nutrients. https://doi.org/10.3390/nu9101063
6. Estruch R, Ros E, Salas-Salvadó J et al (2018) Primary prevention of cardiovascular disease with a Mediterranean diet supplemented with extra-virgin olive oil or nuts. N Engl J Med 378:e34
7. de Lorgeril M, Salen P, Martin JL, Monjaud I, Boucher P, Mamelle N (1998) Mediterranean dietary pattern in a randomized trial: prolonged survival and possible reduced cancer rate. Arch Intern Med 158:1181–1187
8. Tsivgoulis G, Psaltopoulou T, Wadley VG, Alexandrov AV, Howard G, Unverzagt FW, Moy C, Howard VJ, Kissela B, Judd SE (2015) Adherence to a Mediterranean diet and prediction of incident stroke. Stroke 46:780–785
9. Tektonidis TG, Åkesson A, Gigante B, Wolk A, Larsson SC (2015) A Mediterranean diet and risk of myocardial infarction, heart failure and stroke: a population-based cohort study. Atherosclerosis 243:93–98
10. Tong TY, Wareham NJ, Khaw KT, Imamura F, Forouhi NG (2016) Prospective association of the Mediterranean diet with cardiovascular disease incidence and mortality and its population impact in a non-Mediterranean population: the EPIC-Norfolk study. BMC Med 14:135
11. Whalen KA, McCullough M, Flanders WD, Hartman TJ, Judd S, Bostick RM (2014) Palaeolithic and Mediterranean diet pattern scores and risk of incident, sporadic colorectal adenomas. Am J Epidemiol 180:1088–1097
12. Stefler D, Malyutina S, Kubinova R, Pajak A, Peasey A, Pikhart H, Brunner EJ, Bobak M (2017) Mediterranean diet score and total and cardiovascular mortality in Eastern Europe: the HAPIEE study. Eur J Nutr 56:421–429
13. Whalen KA, Judd S, McCullough ML, Flanders WD, Hartman TJ, Bostick RM (2017) Palaeolithic and Mediterranean diet pattern scores are inversely associated with all-cause and cause-specific mortality in adults. J Nutr 147:612–620
14. Neelakantan N, Koh WP, Yuan JM, van Dam RM (2018) Diet-quality indexes are associated with a lower risk of cardiovascular, respiratory, and all-cause mortality among Chinese adults. J Nutr 148:1323–1332
15. Warensjö Lemming E, Byberg L, Wolk A, Michaëlsson K (2018) A comparison between two healthy diet scores, the modified Mediterranean diet score and the Healthy Nordic Food Index, in relation to all-cause and cause-specific mortality. Br J Nutr 119:836–846
16. Ratjen I, Schafmayer C, di Giuseppe R, Waniek S, Plachta-Danielzik S, Koch M, Nöthlings U, Hampe J, Schlesinger S, Lieb W (2017) Postdiagnostic Mediterranean and healthy Nordic dietary patterns are inversely associated with all-cause mortality in long-term colorectal cancer survivors. J Nutr 147:636–644
17. 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
18. Fung TT, Chiuve SE, McCullough ML, Rexrode KM, Logroscino G, Hu FB (2008) Adherence to a DASH-style diet and risk of coronary heart disease and stroke in women. Arch Intern Med 168:713–720
19. Galbete C, Kröger J, Jannasch F, Iqbal K, Schwingshackl L, Schwedhelm C, Weikert C, Boeing H, Schulze MB (2018) Nordic diet, Mediterranean diet, and the risk of chronic diseases: the EPIC-Potsdam study. BMC Med 16(1):99
20. Sanches Machado d'Almeida K, Ronchi Spillere S, Zuchinali P, Corrêa Souza G (2018) Mediterranean diet and other dietary patterns in primary prevention of heart failure and changes in cardiac function markers: a systematic review. Nutrients. https://doi.org/10.3390/nu10010058
21. Lassale C, Gunter MJ, Romaguera D et al (2016) Diet quality scores and prediction of all-cause, cardiovascular and cancer mortality in a pan-European cohort study. PLoS ONE 11:e0159025
22. Hikmat F, Appel LJ (2014) Effects of the DASH diet on blood pressure in patients with and without metabolic syndrome: results from the DASH trial. J Hum Hypertens 28:170–175
23. Genoni A, Lyons-Wall P, Lo J, Devine A (2016) Cardiovascular, metabolic effects and dietary composition of ad-libitum Palaeolithic vs. Australian guide to healthy eating diets: a 4-week randomised trial. Nutrients. https://doi.org/10.3390/nu8050314
24. Adamsson V, Reumark A, Fredriksson IB, Hammarström E, Vessby B, Johansson G, Risérus U (2011) Effects of a healthy Nordic diet on cardiovascular risk factors in hypercholesterolaemic subjects: a randomized controlled trial (NORDIET). J Intern Med 269:150–159
25. Sjögren P, Becker W, Warensjö E, Olsson E, Byberg L, Gustafsson IB, Karlström B, Cederholm T (2010) Mediterranean and

carbohydrate-restricted diets and mortality among elderly men: a cohort study in Sweden. Am J Clin Nutr 92:967–974
26. Atkins JL, Whincup PH, Morris RW, Lennon LT, Papacosta O, Wannamethee SG (2014) High diet quality is associated with a lower risk of cardiovascular disease and all-cause mortality in older men. J Nutr 144:673–680
27. Reedy J, Krebs-Smith SM, Miller PE, Liese AD, Kahle LL, Park Y, Subar AF (2014) Higher diet quality is associated with decreased risk of all-cause, cardiovascular disease, and cancer mortality among older adults. J Nutr 144:881–889
28. Di Castelnuovo A, Costanzo S, Persichillo M, Olivieri M, De Curtis A, Zito F, Donati MB, de Gaetano G, Iacoviello L, MOLI-SANI Project Investigators (2012) Distribution of short and lifetime risks for cardiovascular disease in Italians. Eur J Prev Cardiol 19:723–730
29. Pisani P, Faggiano F, Krogh V, Palli D, Vineis P, Berrino F (1997) Relative validity and reproducibility of a food frequency dietary questionnaire for use in the Italian EPIC centres. Int J Epidemiol 26:S152–S160
30. Zeller T, Hughes M, Tuovinen T et al (2014) BiomarCaRE: rationale and design of the European BiomarCaRE project including 300,000 participants from 13 European countries. Eur J Epidemiol 29:777–790
31. Hertzmark E, Pazaris M, Spiegelman D (2012) The SAS MEDIATE Macro. Boston: Harvard T.H. Chan School of Public Health. http://www.hsph.harvard.edu/donna-spiegelman/software/mediate/. Accessed Sept 2019
32. Desquilbet L, Mariotti F (2010) Dose-response analyses using restricted cubic spline functions in public health research. Stat Med 29:1037–1057. Accessed Sept 2019
33. Schwingshackl L, Hoffmann G (2015) Adherence to Mediterranean diet and risk of cancer: an updated systematic review and meta-analysis of observational studies. Cancer Med 4:1933–1947
34. Guasch-Ferré M, Babio N, Martínez-González MA et al (2015) Dietary fat intake and risk of cardiovascular disease and all-cause mortality in a population at high risk of cardiovascular disease. Am J Clin Nutr 102:1563–1573
35. Hashemian M, Farvid M, Poustchi H et al (2019) The application of six dietary scores to a Middle Eastern population: a comparative analysis of mortality in a prospective study. Eur J Epidemiol 34:371–382
36. Harmon BE, Boushey CJ, Shvetsov YB et al (2015) Associations of key diet-quality indexes with mortality in the Multiethnic Cohort: the Dietary Patterns Methods Project. Am J Clin Nutr 101:587–597
37. Bonaccio M, Ruggiero E, Di Castelnuovo A et al (2017) Fish intake is associated with lower cardiovascular risk in a Mediterranean population: prospective results from the Moli-sani study. Nutr Metab Cardiovasc Dis 27:865–873
38. Gardener H, Wright CB, Gu Y, Demmer RT, Boden-Albala B, Elkind MS, Sacco RL, Scarmeas N (2011) Mediterranean-style diet and risk of ischemic stroke, myocardial infarction, and vascular death: the Northern Manhattan Study. Am J Clin Nutr 94:1458–1464
39. Folsom AR (2013) Classical and novel biomarkers for cardiovascular risk prediction in the United States. J Epidemiol 23:158–162
40. Fitó M, Estruch R, Salas-Salvadó J et al (2014) Effect of the Mediterranean diet on heart failure biomarkers: a randomized sample from the PREDIMED trial. Eur J Heart Fail 16:543–550
41. Foscolou A, Georgousopoulou E, Magriplis E et al (2018) The mediating role of Mediterranean diet on the association between Lp(a) levels and cardiovascular disease risk: a 10-year follow-up of the ATTICA study. Clin Biochem. https://doi.org/10.1016/j.clinbiochem.2018.07.011
42. Ros E, Martínez-González MA, Estruch R, Salas-Salvadó J, Fitó M, Martínez JA, Corella D (2014) Mediterranean diet and cardiovascular health: teachings of the PREDIMED study. Adv Nutr 5:330S–336S
43. Bonaccio M, Pounis G, Cerletti C, Donati MB, Iacoviello L, de Gaetano G, MOLI-SANI Study Investigators (2017) Mediterranean diet, dietary polyphenols and low grade inflammation: results from the MOLI-SANI study. Br J Clin Pharmacol 83:107–113
44. Bonaccio M, Di Castelnuovo A, Pounis G, De Curtis A, Costanzo S, Persichillo M, Cerletti C, Donati MB, de Gaetano G, Iacoviello L, Moli-sani Study Investigators (2016) A score of low-grade inflammation and risk of mortality: prospective findings from the Moli-sani study. Haematologica 101:1434–1441
45. Parrinello CM, Lutsey PL, Ballantyne CM, Folsom AR, Pankow JS, Selvin E (2015) Six-year change in high-sensitivity C-reactive protein and risk of diabetes, cardiovascular disease, and mortality. Am Heart J 170:380–389
46. Kipnis V, Subar AF, Midthune D, Freedman LS, Ballard-Barbash R, Troiano RP, Bingham S, Schoeller DA, Schatzkin A, Carroll RJ (2003) Structure of dietary measurement error: results of the OPEN biomarker study. Am J Epidemiol 158:14–21
47. Rhee JJ, Sampson L, Cho E, Hughes MD, Hu FB, Willett WC (2015) Comparison of methods to account for implausible reporting of energy intake in epidemiologic studies. Am J Epidemiol 181:225–233