Baseline fatty acids, food groups, a diet score and 50-year all-cause mortality rates. An ecological analysis of the Seven Countries Study

Menotti, Alessandro; Kromhout, Daan; Puddu, Paolo Emilio; Alberti-Fidanza, Adalberta; Hollman, Peter; Kafatos, Anthony; Tolonen, Hanna; Adachi, Hisashi; Jacobs, David R.

Published in:
Annals of medicine

DOI:
10.1080/07853890.2017.1372622

Annals of Medicine

ISSN: 0785-3890 (Print) 1365-2060 (Online) Journal homepage: http://www.tandfonline.com/loi/iann20

Baseline fatty acids, food groups, a diet score and 50-year all-cause mortality rates. An ecological analysis of the Seven Countries Study

Alessandro Menotti, Daan Kromhout, Paolo Emilio Puddu, Adalberta Alberti-Fidanza, Peter Hollman, Anthony Kafatos, Hanna Tolonen, Hisashi Adachi & David R. Jacobs Jr

To cite this article: Alessandro Menotti, Daan Kromhout, Paolo Emilio Puddu, Adalberta Alberti-Fidanza, Peter Hollman, Anthony Kafatos, Hanna Tolonen, Hisashi Adachi & David R. Jacobs Jr (2017) Baseline fatty acids, food groups, a diet score and 50-year all-cause mortality rates. An ecological analysis of the Seven Countries Study, Annals of Medicine, 49:8, 718-727, DOI: 10.1080/07853890.2017.1372622

To link to this article: http://dx.doi.org/10.1080/07853890.2017.1372622

ANNALS OF MEDICINE, 2017
VOL. 49, NO. 8, 718–727
https://doi.org/10.1080/07853890.2017.1372622

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Baseline fatty acids, food groups, a diet score and 50-year all-cause mortality rates. An ecological analysis of the Seven Countries Study

Alessandro Menotti[a], Daan Kromhout[b,c,d], Paolo Emilio Puddu[e], Adalberta Alberti-Fidanza[f], Peter Hollman[c], Anthony Kafatos[g], Hanna Tolonen[h], Hisashi Adachi[i] and David R. Jacobs Jr[d]

[a]Association for Cardiac Research, Rome, Italy; [b]Department of Epidemiology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands; [c]Division of Human Nutrition, Wageningen University, Wageningen, The Netherlands; [d]Division of Epidemiology and Community Health, School of Public Health, University of Minnesota, Minneapolis, MN, USA; [e]Department of Cardiovascular, Respiratory, Nephrological, Anesthesiological and Geriatric Sciences, Sapienza University of Rome, Rome, Italy; [f]Human Nutrition Section, Department of Neurosciences, Tor Vergata University of Rome, Rome, Italy; [g]Department of Social Medicine, Preventive Medicine and Nutrition Clinic, University of Crete, Heraklion, Crete, Greece; [h]Department of Public Health Solutions, National Institute for Health and Welfare, Helsinki, Finland; [i]Department of Internal Medicine, Division of Cardio-Vascular Medicine, Kurume University, School of Medicine, Kurume, Japan

ABSTRACT

Objectives: This analysis deals with the ecologic relationships of dietary fatty acids, food groups and the Mediterranean Adequacy Index (MAI, derived from 15 food groups) with 50-year all-cause mortality rates in 16 cohorts of the Seven Countries Study.

Material and methods: A dietary survey was conducted at baseline in cohorts subsamples including chemical analysis of food samples representing average consumptions. Ecologic correlations of dietary variables were computed across cohorts with 50-year all-cause mortality rates, where 97% of men had died.

Results: There was a 12-year average age at death population difference between extreme cohorts. In the 1960s the average population intake of saturated (S) and trans (T) fatty acids and hard fats was high in the northern European cohorts while monounsaturated (M), polyunsaturated (P) fatty acids and vegetable oils were high in the Mediterranean areas and total fat was low in Japan. The 50-year all-cause mortality rates correlated ($r = -0.51$ to -0.64) ecologically inversely with the ratios M/S, (M + P)/(S + T) and vegetable foods and the ratio hard fats/vegetable oils. Adjustment for high socio-economic status strengthened ($r = -0.62$ to -0.77) these associations including MAI diet score.

Conclusion: The protective fatty acids and vegetable oils are indicators of the low risk traditional Mediterranean style diets.

ARTICLE HISTORY
Received 21 June 2017
Revised 16 August 2017
Accepted 23 August 2017

KEYWORDS
50-year follow-up; all-cause mortality; fatty acids; Seven Countries Study; socio-economic status

KEY MESSAGES

- We aimed at studying the ecologic relationships of dietary fatty acids, food groups and the Mediterranean Adequacy Index (MAI, derived from 15 food groups) with 50-year all-cause mortality rates in the Seven Countries Study.
- The 50-year all-cause mortality rates correlated ($r = -0.51$ to -0.64) ecologically inversely with the ratios M/S [monounsaturated (M) + polyunsaturated (P)]/[saturated (S) + trans (T)] fatty acids and vegetable foods and the ratio hard fats/vegetable oils. After adjustment for high socio-economic status, associations with the ratios strengthened ($r = -0.62$ to -0.77) including also the MAI diet score.
- The protective fatty acids and vegetable oils are indicators of the low risk traditional Mediterranean style diets.

Introduction

The possible relationship of eating habits with health and disease have been studied systematically starting in the middle of the past century. A prototype of this approach was the Seven Countries Study (SCS) of cardiovascular diseases that had, among others, the purpose to establish whether various population groups suffer different rates of coronary heart disease as a function of contrasting diets. Details of the study were summarized in several monographs [1–3]. This led to the identification of the so-called Mediterranean

CONTACT Paolo Emilio Puddu paoloemilio.puddu@uniroma1.it Laboratory of Biotechnologies Applied to Cardiovascular Medicine, Department of Cardiovascular, Respiratory, Nephrological, Anesthesiological and Geriatric Sciences, Sapienza University of Rome, Viale del Policlinico, 155, Roma 00161

Supplemental data for this article can be accessed here.

Diet, whose characteristics were later defined in detail by other investigators [4–6]. Subsequently, many prospective studies, including trials, conducted independently in different countries identified subgroups of populations with dietary habits that could be defined as "Mediterranean", "prudent" or "healthy" that were associated with a lower risk of coronary heart disease, other cardiovascular diseases, different types of cancer and all-cause mortality [7–31].

The SCS investigated the associations of different aspects of diet with long-term all-cause mortality at the ecological level because of the large variation in dietary patterns at entry and all-cause mortality rates during 10 and 15 years of follow-up among the cohorts [32–34]. After 25-years of follow-up the all-cause mortality rates of originally middle-aged men varied from about 30% in the cohorts of university professors from Belgrade (Serbia) and farmers on the island of Crete (Greece) and about 60% in the cohorts of farmers in East Finland and Slavonia (Croatia) [35]. The early analysis on diet and all-cause mortality rates showed that after 10 years of follow-up the ratio of mono (M) and polyunsaturated (P) to saturated (S) fatty acids was significantly inversely related to all-cause mortality rates [32]. Five years later Keys et al. showed that the M/S ratio was also inversely related to 15-year all-cause mortality rates [33]. These results suggest that at the ecological level the fatty acid composition of the diet is an important predictor of all-cause mortality rates.

Recently, the 50-year mortality follow-up of the SCS was completed. The purpose of the current analysis is not only to study the average population daily intake of dietary fatty acids at base-line with long-term all-cause mortality rates but also for the first time the associations of food groups and the Mediterranean Adequacy Index (MAI), a diet score, with all-cause mortality rates during 50 years of follow-up.

Materials and methods

Populations

Sixteen cohorts of middle-aged men (age 40 to 59 years) were enrolled in late 1950s and early 1960s. Eleven of the cohorts were rural: East Finland and West Finland (Finland), Crevalcore and Montegiorgio (Italy), Dalmatia and Slavonia (Croatia, former Yugoslavia), Velika Krsna (Serbia, former Yugoslavia), Crete and Corfu (Greece) and Tanushimaru and Ushibuka (Japan). The remaining five cohorts were the US and Rome railroad, men from the town of Zutphen, The Netherlands and two cohorts from Serbia, one consisting of workers in a large agricultural cooperative in Zrenjanin and another of Belgrade university professors. A total of 12,763 men aged 40–59 were first examined in the late 1950s and early 1960s [1–3].

Dietary data

Dietary surveys were carried out at baseline, spread across all seasons of the year, in subsamples of 13 cohorts from 1959 to 1964, including food records with precise weighing and chemical analysis for macronutrients [36]. Only food records were collected in the US railroad in 1960–1962, in the Rome railroad in 1969 and in Ushibuka, Japan in 1971[1,36]; the estimated average diet in Rome and Ushibuka was assumed to be similar to the average diets approximately 10 years earlier. The cohort subsamples for diet survey across cohorts numbered 498 men with a total of 3,282 days of food consumption surveyed. The number of men surveyed in each cohort are reported in Supplementary Table 1. These were random subsamples of all participants in each cohort, with replacements for the few who refused the diet survey.

Foods and food groups

Individual food items and their weighed amounts eaten were recorded for each participant. All foods eaten by the participants in the subsamples at entry were coded in a standardized way as the edible part of raw products [34] and classified into 15 homogenous food groups and a group of foods not otherwise classified. Those 15 groups were combined into five larger groups i.e. vegetable foods (bread, cereals, potatoes, vegetables, legumes, fruit, vegetable oils), animal foods (meat, milk (solid fraction), hard fats (butter, lard, hard margarine) cheese, eggs), fish, sweet products (added sugars and pastries) and alcohol. Although included into the five larger groups, vegetable oils and hard fats were also independently considered for analysis.

Dietary pattern

The MAI is an a priori dietary pattern score computed according to Fidanza et al. [37], based on 15 food groups at the entry survey [34]. The numerator of the score included foods typical of the traditional Mediterranean diet (bread, cereals, legumes, potatoes, vegetables, fresh and dry fruit, fish, vegetable oils (mainly olive) and wine), while the denominator includes foods not typical of the Mediterranean diet (milk and milk products, cheese, meat, eggs, hard fatsand sweet products), all expressed as g per 1000 kcal. Highest scores for the MAI represented the "traditional" Mediterranean diet. In this analysis, all alcoholic beverages were replaced by alcohol due to the wide variety of drinking patterns (wine, beer and hard liquor) in the different countries.

Chemically analyzed nutrients

In 1987–1988, as close as possible to identical foods in the 1960s were purchased from local markets in each cohort and shipped for chemical analysis in the laboratory of the Division of Human Nutrition of Wageningen University, The Netherlands. The foods were cleaned and food composites were prepared according to the average consumption pattern of each cohort. The food samples were analyzed for total proteins, fats and carbohydrates and total energy was calculated from these macronutrients. Total lipids were isolated using the Soxhlet method [38] and individual fatty acids were determined by gas chromatography [39] and infrared spectrometry [40], with cis and trans fatty acids identified using different columns [41,42]. The values of the macronutrients were expressed as percent (%) of total energy.

Risk factors

Self-reported information was collected about socio-economic-status (SES) based on occupation of all men at entry who were classified as high SES or others (coded as 1 and 0 respectively). High SES included professionals, businessmen, public administrators, foremen and high rank clerical workers. Self-reported information on the prevalence of current smokers, derived from a standard questionnaire administered at entry, expressed as percent of each cohort. Body mass index (BMI) derived from height and weight, was measured at entry, following the rules of the WHO Cardiovascular Survey Methods manual [43]. A BMI equal or greater than 30 was the cut-off for the prevalence of obesity.

Baseline data were collected before the era of the Helsinki Declaration, with consent implied by participation in the examinations, while verbal or written consent was obtained to collect follow-up data.

Mortality data

The vital status of the men was checked at regular intervals. The collection of mortality data included dates and causes of death and was carried out systematically over 50 years in 10 cohorts, 45 years in three cohorts and 25 years in three other cohorts. During 25 years of follow-up 13 men from the two Croatian cohorts and after 45 years 6 men from two of the three Serbian cohorts were lost to follow-up. Of the other 11 cohorts, 20 men from the US, 2 from Zutphen, 1 from Crete and 25 from the two Japanese cohorts were also lost to follow-up. In the remaining 7 cohorts no one was lost to follow-up. Those lost to follow-up were censored at the date of their last examination.

Statistical analysis

Because of the large variation in average population energy intake macronutrients were expressed in percent of energy and foods in g/1000 kcal. For 10 cohorts 50-year mortality rates were computed and for six cohorts (one from Italy, two from Croatia and three from Serbia) and 50-year mortality data were estimated using regression equations derived from the 10 cohorts with complete mortality data. The dependent variable was the 50-year findings and the independent variables were either 25- or 45-year findings (Supplementary Table 2). All-cause mortality was expressed in rates per 1000 person-years and adjusted for the mean age of the 16 cohorts. For reference and comparison with all-cause mortality rates, age at death was computed in years or in age at 50 years of follow-up after adjustment for the mean age of the cohorts.

Only ecologic analysis could be carried out on these dietary data because individual level data at baseline were collected only in subsamples of the 16 cohorts, each represented by its average. Pearson linear correlation coefficients were computed between the dietary variables and 50-year all-cause mortality rates. Correlations coefficients $\geq$0.50 for 16 cohorts were statistically significant (two-sided p values $< .05$). For analytical purposes, the MAI diet score was transformed by the natural log, which improved the fit. Each correlation coefficient of a dietary variable with all-cause mortality rates was separately adjusted for high SES or prevalence of obesity or prevalence of smoking in multivariable regression equations and was represented by partial correlation coefficients.

Results

At the baseline survey risk factors were collected between 1958 and 1964 (Supplementary Table 2). The prevalence of high SES varied in the 11 rural cohorts from 3 to 14% and in four other cohorts (US and Rome railroad, Zrenjanin and Zutphen) from 13 to 38%. The 16th cohort of Belgrade university professors

Table 1. Average population intake of fatty acids and energy intake in the 1960s and age-adjusted 50-year all-cause mortality rates in 16 cohorts of the Seven Countries Study.

	% of energy					50-year all-cause
Area[a]	SAFA	MUFA	PUFA	Trans	Energy (kcal)	mortality rates 1000 p-y[b]
US railroad	21.5	18.8	7.9	2.3	2326	37.3
East Finland	22.4	13.7	3.3	2.3	3577	44.0
West Finland	19.0	12.9	3.2	2.0	3440	40.4
Zutphen NL	18.8	15.3	5.7	8.0	2922	38.8
Crevalcore IT	14.3	19.0	5.3	0.5	3432	39.1
Montegiorgio IT	10.1	16.2	4.7	0.4	2791	36.2
Rome railroad IT	10.3	19.9	3.6	0.6	2455	34.4[c]
Dalmatia HR	11.1	21.9	6.2	0.6	3201	36.3[c]
Slavonia HR	16.2	19.1	4.9	0.5	3816	45.3[c]
Velika Krsna SR	13.9	11.3	4.1	1.0	3388	39.9[d]
Zrenjanin SR	15.1	18.0	6.1	0.4	3256	43.1[d]
Belgrade SR	18.6	17.5	8.3	1.0	2780	31.1[d]
Crete GR	9.4	27.9	4.2	0.2	2712	31.1
Corfu GR	7.7	21.9	5.2	0.1	2594	35.9
Tanushimaru JP	4.0	4.0	4.8	0.2	2243	35.7
Ushibuka JP	5.6	6.8	5.2	0.2	2267	38.6
Mean	13.6	16.5	5.2	1.3	2950	37.9
± SD	5.6	5.9	1.5	2.0	503	4.1

[a]Symbols of countries: NL: the Netherlands; IT: Italy; HR: Croatia, former Yugoslavia; SR: Serbia, former Yugoslavia; GR: Greece; JP: Japan.
[b]Person-years.
[c]Projected from 25-year data using regression equations of 10 cohorts with 50-year follow-up.
[d]Projected from 45-year data using regression equations of 10 cohorts with 50-year follow-up.
SAFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; TRANS: trans fatty acid.

scored 100%. The lowest prevalence of obesity (<1%) was observed in Japan and the highest (approximately 15%) in the Italian cohorts of Crevalcore and Rome railroad. The Belgrade professors had the lowest prevalence of smoking (44%) while the highest prevalence (>70%) was found in the rural Japanese cohorts and the Dutch cohort of Zutphen. The age-adjusted death rates per 1000 person-years ranged from 31.1 in Crete and Belgrade up to 44.0 in East Finland and 45.3 in Slavonia. The all-cause mortality rates highly correlated ($r = 0.96$) with age at death and the extreme experiences of the death rates corresponded to an average difference of 12 years.

There was a 5.6-fold range in average population intake of saturated fatty acids (Table 1). The average population intake of trans fatty acids was low in 15 cohorts and varied from 0.1 to 2.3% of energy. The Zutphen cohort was an outlier with an average intake of 8.0 energy %. There was a 7.0-fold range in average intake of monounsaturated fatty acids and a 2.5-fold range in polyunsaturated fatty acids. The average energy intake ranged from approximately 2250 kcal/day in the Japanese cohorts to about 3500 kcal/day or more in the Finnish cohorts and in Slavonia. The consumption of vegetable oils was highest in Greek cohorts and not consumed in the northern European cohorts (Table 2). The reverse was true for the consumption of hard fats. The consumption of vegetable foods was also highest in Greek cohorts and lowest in the cohorts East Finland and Slavonia. The highest consumption of animal foods was found in the US and the lowest in Japan. The MAI was high in Italy, Greece, Dalmatia and Japan and low in the northern and central European cohorts.

Linear correlation coefficients were computed for the average population intake of energy, macronutrients, food groups and the MAI with the 50-year all-cause mortality rates (Table 3). Significant correlation coefficients were observed for energy ($r = 0.68$) and for the nutrients M/S ratio (monounsaturated/saturated fatty acids); $r = 0.53$) and (M + P)/(S + T) ratio ((monounsaturated + polyunsaturated fatty acids)/(saturated + trans fatty acids); $r = 0.56$). The parallel analysis on food groups found similar results as for fatty acids since vegetables foods (including vegetable oils), vegetable oils alone and the vegetable oils/hard fat ratio were all significantly associated with 50-year all-cause mortality rates with correlation coefficients ranging from −0.51 to −0.64. The Belgrade cohort of professors was an outlier and after exclusion of the cohort the correlation coefficients generally increased (Table 3). For all 16 cohorts, partial correlation coefficients were adjusted for high SES. The strength of the partial correlation coefficients was 0.66 for saturated fatty acids and 0.72 for hard fat and varied from −0.62 to −0.77 for the vegetable oils/hard fat ratio, the M/S ratio, the (M + P)/(S + T) ratio and the MAI showing similar associations for fatty acids and food groups.

Table 2. Average population consumption of selected food groups and the dietary pattern MAI (Mediterranean Adequacy Index) in the 1960s in the 16 cohorts of the Seven Countries Study.

	g/day per 1000 calories				
Area[a]	Vegetable oils	Hard fat	Vegetable foods	Animal foods	lnMAI[b]
US railroad	1.3	12.9	281.6	166.1	−0.09
East Finland	0.0	27.1	244.1	101.5	−0.53
West Finland	0.0	21.2	261.3	102.6	−0.51
Zutphen NL	0.0	27.0	284.7	110.9	−0.03
Crevalcore IT	11.4	5.8	263.1	85.5	0.94
Montegiorgio IT	8.6	14.3	305.3	62.3	1.96
Rome railroad IT	17.5	2.4	346.2	121.6	1.07
Dalmatia HR	22.5	5.3	311.8	67.7	1.64
Slavonia HR	2.1	16.3	245.3	91.0	0.65
Velika Krsna SR	1.5	7.1	293.4	104.8	0.52
Zrenjanin SR	3.7	13.5	338.1	95.6	0.73
Belgrade SR	10.1	9.7	267.6	114.0	0.13
Crete GR	35.0	0.0	508.9	36.5	1.49
Corfu GR	28.9	0.0	540.9	23.8	2.37
Tanushimaru JP	1.3	0.0	402.6	13.4	2.45
Ushibuka JP	3.1	0.0	371.9	21.9	2.12
Mean	9.2	10.2	329.2	82.3	0.93
± SD	11.2	9.3	88.7	42.0	0.99

[a]Symbols of countries: NL: the Netherlands; IT: Italy; HR: Croatia, former Yugoslavia; SR: Serbia, former Yugoslavia; GR: Greece; JP: Japan.
[b]Natural log of Mediterranean Adequacy Index.

Table 3. Linear correlation coefficients of energy, macronutrients and food groups at baseline vs. 50-year age-adjusted death rates in the 16 cohorts of the Seven Countries Study.

Energy Nutrients Food groups	Linear correlation vs. Death rates 16 cohorts	Linear correlation vs. Death rates 15 cohorts Belgrade excluded	Linear partial correlation vs. Death rates Adjusted for SES 16 cohorts
Energy and nutrients			
Energy	0.68*	0.72*	0.67*
Protein	−0.26	−0.33	−0.30
Carbohydrates	0.02	−0.09	−0.16
Fat	0.05	0.17	0.24
Saturated fatty acids	0.41	0.59*	0.66*
Monounsaturated fatty acids	−0.29	−0.30	−0.30
Polyunsaturatred fatty acids	−0.30	−0.05	−0.03
Trans-fatty acids	0.17	0.17	0.31
M/S ratio[a]	−0.53*	−0.68*	−0.74*
(M + P)/(S + T) ratio[b]	−0.56*	−0.70*	−0.77*
Food groups			
Vegetable foods[c]	−0.51*	−0.67*	−0.71*
Animal foods[d]	0.22	0.35	0.45
Fish	−0.07	−0.13	−0.17
Sweet products	0.08	0.14	0.26
Alcohol	−0.28	−0.43	−0.44
Vegetable oils	−0.64*	−0.70*	−0.75*
Hard fats	0.58*	0.64*	0.72*
Vegetable foods/Animal foods	−0.32	−0.45	0.48
Vegetable oils/Hard fats	−0.51*	−0.63*	−0.68*
lnMAI	−0.39	−0.56*	−0.62*

*$p < .05$.
[a]Monounsaturated fatty acids/Saturated fatty acids.
[b](Monounsaturated + Polyunsaturated fatty acids)/(Saturated + Trans fatty acids).
[c]Including vegetable oils.
[d]Including hard fats.

The MAI was also related to the M/S ratio ($r = 0.63$) and the (M + P)/(S + T) ratio ($r = 0.83$). Carbohydrates and sweet products were not significantly related to all-cause mortality, also not after excluding the Belgrade cohort or after adjustment for SES.

All-cause mortality rates were not related to the prevalence of obesity ($r = 0.09$) and the prevalence of smokers ($r = 0.29$) and the partial correlation coefficients of dietary variables with all-cause mortality rates did not change after adjustment for obesity or smoking. Figures 1–3 presents the relationships between observed vs. predicted all-cause mortality rates derived from the regression equations of the (M + P)/(S + T) ratio and SES ($r = 0.82$), the ratio of vegetable oils/hard fat and SES ($r = 0.75$) and the MAI and SES ($r = 0.71$).

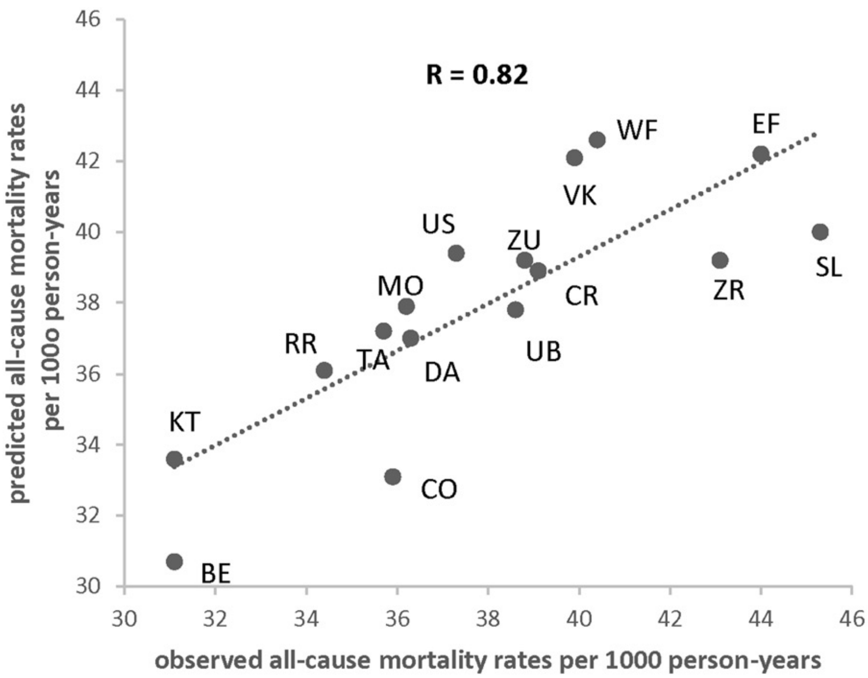

Figure 1. Observed and predicted death rates as a function of (M + P)/(S + T) ratio adjusted for higher SES for the 16 cohorts in the Seven Countries Study. Observed rates in 50 years were estimated by regression equations for six cohorts with shorter follow-up (see: Supplementary Table 2). Regression equation of this figure was computed as:

$$\text{Predicted Death rates (Y} - \text{axis)} = 46.0 - 3.51 \times (M + P)/(S + T) - 0.11 \times \text{SES.}$$

$$(M + P)/(S + T) = (\text{mono} + \text{polyunsaturated}) \text{ to } (\text{saturated} + \text{trans fatty acids}) \text{ ratio.}$$

SES: socio-economic status; US: US railroad; EF: East Finland; WF: West Finland, ZU: Zutphen, the Netherlands; CR: Crevalcore, Italy; MO: Montegiorgio, Italy; RR: Rome railroad, Italy; DA:Dalmatia, Croatia; SL: Slavonia, Croatia; VK: Velika Krsna, Serbia; ZR: Zrenjanin, Serbia; BE: Belgrade, Serbia; KT: Crete, Greece; CO: Corfu, Greece; TA: Tanushimaru, Japan; UB: Ushibuka, Japan

Discussion

After 50 years of follow-up the lowest all-cause mortality rates were observed in the rural cohort of Crete and the Belgrade cohort of professors and the highest rates in the rural cohorts East Finland and Slavonia. The average population difference in age at death was 12 years for the extreme cohorts. After adjustment for high SES the ratios of fatty acids, vegetable oils, vegetable foods and the MAI were inversely related to all-cause mortality rates. This suggests that the protective mono- and polyunsaturated fatty acids, vegetable oils and vegetable foods are indicators of healthful diets as operationalized in the MAI diet score.

The M/S ratio was used for the first time in a SCS paper of 1986 [33] and was strongly inversely related to 15-year all-cause mortality rates. Subsequently, our data showed that the M/S ratio is a proxy for olive oil consumption ($r = 0.97$). We also computed the MP/ST ratio, an extension of the M/S ratio, that was also positively correlated with vegetable oils ($r = 0.87$). Animal foods were not associated with all-cause mortality rates but vegetable foods and the MAI were inversely related to all-cause mortality rates. This suggests a beneficial role of "traditional Mediterranean style diets", in which vegetables dominated over animal foods that are rich in fiber and/or vitamins and bioactive compounds such as flavonoids. The beneficial role of flavonoids was already documented in the Seven Countries Study, in relation to coronary heart disease [44]. In our data, the correlation coefficient ($r = -0.69$) between vegetable foods and animal foods suggested that cohorts with a high consumption of vegetable foods had a low consumption of animal foods. Carbohydrates and sweet products were not significantly related to all-cause mortality. Actually, the debate on this issue is largely confined to coronary heart disease and in the 25-year follow-up of the Seven Countries Study the consumption of added sugars and pastries was positively related to coronary heart disease mortality, although to a lesser extent compared with saturated fatty acids and food groups rich in saturated fatty acids [41,45].

A sensitivity analysis was done after excluding the men lost to follow-up and of those who died from infectious diseases or violence. The outcome was similar to the basic analysis and therefore, to be conservative, we retained all men. Also caution should be

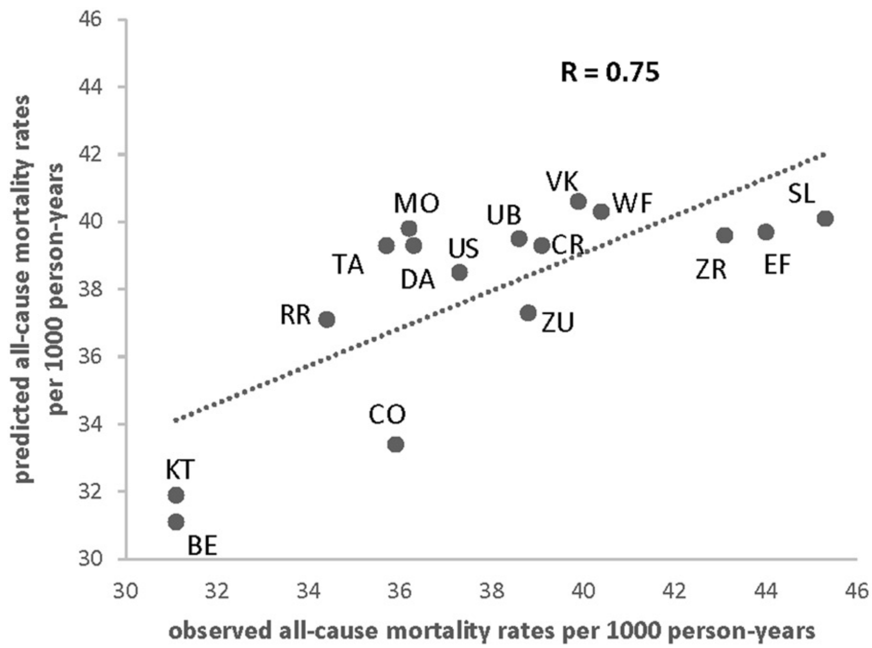

Figure 2. Observed and predicted death rates as a function of vegetable oils/hard fat ratio adjusted for higher SES for the 16 cohorts in the Seven Countries Study. Observed rates in 50 years were estimated by regression equations for six cohorts with shorter follow-up (see: Supplementary Table 2). Regression equation of this figure was computed as:

$$\text{Predicted Death rates (Y} - \text{axis)} = 40.91 - 0.24 \times \text{Vegetable oils/hard fats} - 0.10 * \text{SES.}$$

SES: socio-economic status; US: US railroad; EF: East Finland; WF: West Finland, ZU: Zutphen, the Netherlands; CR: Crevalcore, Italy; MO: Montegiorgio, Italy; RR: Rome railroad, Italy; DA:Dalmatia, Croatia; SL: Slavonia, Croatia; VK: Velika Krsna, Serbia; ZR: Zrenjanin, Serbia; BE: Belgrade, Serbia; KT: Crete, Greece; CO: Corfu, Greece; TA: Tanushimaru, Japan; UB: Ushibuka, Japan

taken in interpreting the reported findings of the cohorts whose 50-year mortality rates were projected from shorter follow-up periods. This is particularly true for the Slavonia cohort that was not only characterized by high death rates, but was also hit by the war with Serbia in the early 1990s, several years after the end of the documented 25-year mortality follow-up. Other limitations were that the SCS was confined to men, a small age range and only 16 statistical units, with inherent problems in interpretation of the findings. Moreover, systematic information was not available about changes of dietary habits during follow-up. Advantages of the study are the accuracy of the chemical analysis of the food, detailed fatty acid information at entry as well as complete and near lifetime mortality follow-up.

Parallel evidence from similar ecological studies is not available. However, the results of a worldwide meta-analysis were based on sparse data derived from heterogenous sources [46–48]. In the most recent report [48], 79 risk factors collected in 188 countries were related to all-cause mortality and DALYs disability-adjusted life-years (DALYs) in the period 1990–2013. The most important risk factor of that study was "bad dietary habits" characterized by low fruit, vegetables, whole grain, nuts and seeds consumption and high sodium intake, confirming the role of diet as determinant of all-cause mortality and quality of life. The characteristic of those "bad habits" did roughly correspond to the opposite of Mediterranean or East Asian diets that are protective also in the present study.

The findings of the current ecological analysis cannot be directly transferred to the experience of individuals because they describe population sample means only and not personalized health problems. A large number of prospective cohort studies showed that healthful diets are associated with a lower risk of all-cause mortality and differ in sex, age, length of follow-up, countries and cultures [7–31] including contributions of some SCS cohorts [8,14,22,30]. This is in accord with the results of a meta-analysis of prospective cohort studies on Mediterranean style diets and all-cause mortality risk [49]. A large review investigating the association between intake of different food groups and chronic diseases from 266 worldwide surveys reached similar conclusions [50].

Randomized controlled trials in which saturated fatty acids were replaced by polyunsaturated fatty acids [51] and the PRimary prEvention by DIey

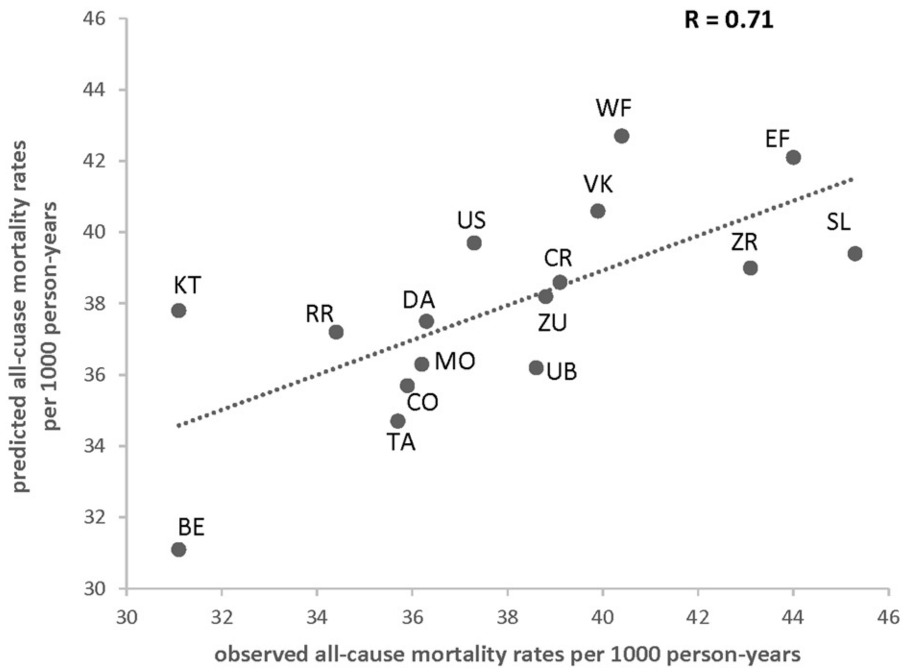

Figure 3. Observed and predicted death rates as a function of the lnMAI adjusted for prevalence of higher SES. Observed rates in 50 years are estimated by regression equations for six cohorts with shorter follow-up. Observed rates in 50 years were estimated by regression equations for six cohorts with shorter follow-up (see: Supplementary Table 2). Regression equation of this figure was computed as:

$$\text{Predicted Death rates (Y} - \text{axis)} = 42.17 - 2.46 \times \text{lnMAI} - 0.11 * \text{SES}.$$

lnMAI: natural log of the Mediterranean Adequacy Index; SES: socio-economic status; US: US railroad; EF: East Finland; WF: West Finland, ZU: Zutphen, the Netherlands; CR: Crevalcore, Italy; MO: Montegiorgio, Italy; RR: Rome railroad, Italy; DA:Dalmatia, Croatia; SL: Slavonia, Croatia; VK: Velika Krsna, Serbia; ZR: Zrenjanin, Serbia; BE: Belgrade, Serbia; KT: Crete, Greece; CO: Corfu, Greece; TA: Tanushimaru, Japan; UB: Ushibuka, Japan

MEDiterranean (PREDIMED) study [6] in which additional amounts of extra virgin olive oil and mixed nuts did not significantly reduce all-cause mortality risk possibly due to lack of power and short-term follow-up. More and larger trials and a longer follow-up are needed to definitively show that healthful diets reduce all-cause mortality rates.

In conclusion, our ecological findings suggest that low all-cause mortality rates are associated with a high intake of vegetable oils and vegetable foods and a low intake of hard fats and animal foods. The results of our study correspond also to high levels of the fatty acid ratios M/S and (M + P)/(S + T) and a dietary pattern describing traditional Mediterranean style diets. The characteristics of the protective dietary habits are summarized in food patterns such as the MAI and others, whose levels were not only particularly favorable in the rural cohort Crete (Greece), but also in the rural cohorts Corfu (Greece), Montegiorgio (Italy), Dalmatia (Croatia) and Tanushimaru (Japan) although, in the last cohort on the basis of different types of food. These dietary characteristics explain only a part of population differences in all-cause mortality rates that depend also upon a large number of other determinants e.g. lifestyle, health care and SES variables, that greatly vary with time and location.

Disclosure statement

The authors declare that they have no conflict of interest to disclose.

ORCID

Paolo Emilio Puddu http://orcid.org/0000-0002-6191-7838

References

[1] Keys A, Blackburn H, Menotti A, et al. Coronary heart disease in seven countries. Circulation. 1970;41(suppl 1):1–211.

[2] Keys A, Aravanis C, Blackburn H, et al. Seven Countries Study. A multivariate analysis of death and coronary heart disease. Cambridge (MA): Harvard University Press; 1980.

[3] Kromhout D, Menotti A, Blackburn H. Prevention of Coronary Heart Disease. Diet, Lifestyle and Risk Factors in the Seven Countries Study. Norwell Massachusetts, USA and Dordrecht, NL: Kluwer Publishers; 2002.

[4] Trichopoulou A, Kouris-Blazos A, Wahlquist ML, et al. Diet and overall survival in elderly people. BMJ. 1995;311:1457–1460.
[5] Willett W. Mediterranean diet pyramid: a cultural model for healthy eating. Am J Clin Nutr. 1995;61: 1402S–1406S.
[6] Estruch R, Ros E, Salas-Salvadó J, et al. Primary prevention of cardiovascular disease with a Mediterranean diet. N Engl J Med. 2013;368: 1279–1290.
[7] Trichopoulou A, Costacou T, Bamia C, et al. Adherence to a Mediterranean diet and survival in a Greek population. N Engl J Med. 2003;348:2599–2608.
[8] Huijbregts P, Feskens E, Rasanen L, et al. Dietary pattern and 20 year mortality in elderly men in Finland, Italy and the Netherlands: longitudinal cohort study. Brit Med J. 1997;315:13–17.
[9] Trichopoulou A, Bamia C, Norat T, et al. Modified Mediterranean diet and survival: EPIC-elderly prospective cohort study. BMJ. 2005;330:991.
[10] Lagiou P, Trichopoulos D, Sandin S, et al. Mediterranean dietary pattern and mortality among young women: a cohort study in Sweden. Br J Nutr. 2006;96:384–392.
[11] Mitrou PN, Kipnis V, Thiébaut AC, et al. 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. 2007;167:2461–2468.
[12] Trichopoulou A, Bamia C, Trichopoulos D. Anatomy of health effects of Mediterranean diet: Greek EPIC prospective cohort study. BMJ. 2009;338:b2337.
[13] Buckland G, Agudo A, Travier N, et al. 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. 2011;106:1581–1591.
[14] Menotti A, Alberti-Fidanza A, Fidanza F, et al. Factor analysis in the identification of dietary patterns and their predictive role in morbid and fatal events. Public Health Nutr. 2012;15:1232–1239.
[15] Hoevenaar-Blom MP, Nooyens AC, Kromhout D, et al. Mediterranean style diet and 12-year incidence of cardiovascular diseases: the EPIC-NL cohort study. PLoS One. 2012;7:e45458.
[16] Martinez-Gonzalez MA, Guillén-Grima F, De Irala J, et al. The Mediterranean diet is associated with a reduction in premature mortality among middle-aged adults. J Nutr. 2012;142:1672–1678.
[17] McNaughton SA, Bates CJ, Mishra GD. Diet quality is associated with all-cause mortality in adults aged 65 years and older. J Nutr. 2012;142:320–325.
[18] Tognon G, Lissner L, Saebye D, et al. The Mediterranean diet in relation to mortality and CVD: a Danish cohort study. Br J Nutr. 2014;111:151–159.
[19] Davis MA, Neuhaus JM, Moritz DJ, et al. Health behaviors and survival among middle-aged and older men and women in the NHANES I epidemiologic follow-up study. Prev Med. 1994;23:369–376.
[20] Ruigómez A, Alonso J, Antó JM. Relationship of health behaviours to five-year mortality in an elderly cohort . Age Ageing. 1995;24:113–119.
[21] Haveman-Nies A, de Groot L, Burema J, et al. Dietary quality and lifestyle factors in relation to 10-year mortality in older Europeans: the SENECA study. Am J Epidemiol. 2002;156:962–968.
[22] Knoops KTB, de Groot LCPGM, Kromhout D, et al. Mediterranean diet, lifestyle factors and 10-year mortality in elderly European men and women: the HALE project. JAMA. 2004;292:1433–1439.
[23] van Dam RM, Li T, Spiegelman D, et al. Combined impact of lifestyle factors on mortality: prospective cohort study in US women. BMJ. 2008;337:a1440.
[24] Kvaavik E, Batty GD, Ursin G, et al. Influence of individual and combined health behaviors on total and cause-specific mortality in men and women: the United Kingdom health and lifestyle survey. Arch Intern Med. 2010;170:711–718.
[25] Nechuta SJ, Shu XO, Li HL, et al. Combined impact of lifestyle-related factors on total and cause-specific mortality among Chinese women: prospective cohort study. PLoS Med. 2010;7:e1000339.
[26] Odegaard AO, Koh WP, Gross MD, et al. Combined lifestyle factors and cardiovascular disease mortality in Chinese men and women: the Singapore Chinese health study. Circulation. 2011;124:2847–2854.
[27] Carlsson AC, Wändell PE, Gigante B, et al. Seven modifiable lifestyle factors predictand all-cause mortality regardless of body mass index: a cohort study. Int J Cardiol. 2013;168:946–952.
[28] Yun JE, Won S, Kimm H, et al. Effects of a combined lifestyle score on 10-year mortality in Korean men and women: a prospective cohort study. BMC Public Health. 2012;12:673.
[29] Behrens G, Fischer B, Kohler S, et al. Healthy lifestyle behaviors and decreased risk of mortality in a large prospective study of U.S. women and men. Eur J Epidemiol. 2013;28:361–372.
[30] Menotti A, Puddu PE, Lanti M, et al. Lifestyle habits and mortality from all and specific causes of death: 40-year follow-up in the Italian rural areas of the Seven Countries Study. J Nutr Health Aging. 2014;18:314–321.
[31] Prinelli F, Yannakoulia M, Anastasiou CA, et al. Mediterranean Diet and other lifestyle factors in relation to 20-year all-cause mortality: a cohort study in an Italian population. Br J Nutr. 2015;113:1003–1011.
[32] Keys A, Aravanis C, Van Buchem F, et al. The diet and all causes death rate in the Seven Countries Study. Lancet. 1981;2:58–61.
[33] Keys A, Menotti A, Karvonen MJ, et al. The diet and 15-year death rate in the Seven Countries Study. Am J Epidemiol. 1986;124:903–915.
[34] Kromhout D, Keys A, Aravanis C, et al. Food consumption patterns in the 1960s in seven countries. Am J Clin Nutr. 1989;49:889–894.
[35] Menotti A, Blackburn H, Kromhout D, et al. Cardiovascular risk factors as determinants of 25-year all-cause mortality in the seven countries study. Eur J Epidemiol. 2001;17:337–346.
[36] Den Hartog C, Buzina R, Fidanza F, et al. Dietary studies and epidemiology of heart diseases [Internet]. The Hague, The Netherlands: Voeding; 1968. 1–157; [cited 2017 Apr] Available from: http://edepot.wur.nl/380320.

[37] Alberti-Fidanza A, Fidanza F. Mediterranean Adequacy Index of Italian diets. Public Health Nutr. 2004;7: 937–941.

[38] Osborne DR, Voogt P. The Analysis of Nutrients and Foods. Academic Press Inc. London/New York: Academic Press Inc.; 1978.

[39] Metcalfe LD, Schmitz A, Pekka JR. Rapid preparation of fatty acid esters from lipids for gas chromatographic analyses. Anal Chem. 1966;18:514–515.

[40] Sleeter RT, Matlock MG. Automated quantitative analysis of isolated (nonconjugated) trans isomers using Fourier transform infrared spectroscopy incorporating improvements of procedures. J Am Oil Chem. 1989;66:121–127.

[41] Kromhout D, Menotti A, Bloemberg B, et al. Dietary saturated and trans fatty acids and cholesterol and 25-year mortality from coronary heart disease: the Seven Countries Study. Prev Med. 1995;24:308–315.

[42] De Vries JHM, Jansen A, Kromhout D. The fatty acid and sterol content of food composites of middle-aged men in seven countries. J Food Comp Anal. 1997;10:115–141.

[43] Rose G, Blackburn H. Cardiovascular survey methods. Geneva: World Health Organization; 1968.

[44] Hertog MGL, Kromhout D, Aravanis C, et al. Flavonoid intake and long-term risk of coronary heart disease and cancer in the Seven Countries Study. Arch Intern Med. 1995;155:381–386.

[45] Menotti A, Kromhout D, Blackburn H, et al. Food intake patterns and 25-year mortality from coronary heart disease: cross-cultural correlations in the Seven Countries Study. Eur J Epidemiol. 1999;15:507–515.

[46] The World Health Report 2002. Reducing risks - promoting healthy life. Geneva: World Health Organization; 2002.

[47] Ezzati M, Van der Hoorn S, Lopez AD, et al. Comparative quantification of mortality and burden of disease attributable to selected risk factors. In: Lopez AD, Mathers CD, Ezzati M, et al., editors. Global burden of disease and risk factors. Washington (DC): World Bank; 2006.

[48] GBD 2013 Risk Factors Collaborators. Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks in 188 countries, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet. 2015;386:2287–2323.

[49] Sofi F, Macchi C, Abbatte R, et al. Mediterranean diet and health status: an updated meta-analysis and a proposal for a literature-based adherence score. Public Health Nutr. 2013;17:2769–2782.

[50] Micha R, Khatibzadeh S, Shi P, et al. Global, regional and national consumption of major food groups in 1990 and 2010: a systematic analysis including 266 country-specific nutrition surveys worldwide. BMJ Open. 2015;5:e008705.

[51] Mozaffarian D, Micha R, Wallace S. Effects on coronary heart disease of increasing polyunsaturated fat in place of saturated fat: systematic review and meta-analysis of randomized controlled trials. PLOS Med. 2010;7:e1000252.