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

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

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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.

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, \((M + P)/(S + T)\) 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
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
fats and 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 socioeconomic-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 ≥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
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 (>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 %.

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>
<thead>
<tr>
<th>Area</th>
<th>% of energy</th>
<th>50-year all-cause mortality rates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SAFA</td>
<td>MUFA</td>
</tr>
<tr>
<td>US railroad</td>
<td>21.5</td>
<td>18.8</td>
</tr>
<tr>
<td>East Finland</td>
<td>22.4</td>
<td>13.7</td>
</tr>
<tr>
<td>West Finland</td>
<td>19.0</td>
<td>12.9</td>
</tr>
<tr>
<td>Zutphen</td>
<td>18.8</td>
<td>15.3</td>
</tr>
<tr>
<td>Crevalcore IT</td>
<td>14.3</td>
<td>19.0</td>
</tr>
<tr>
<td>Montegiorgio IT</td>
<td>10.1</td>
<td>16.2</td>
</tr>
<tr>
<td>Rome railroad IT</td>
<td>10.3</td>
<td>19.9</td>
</tr>
<tr>
<td>Dalmatia HR</td>
<td>11.1</td>
<td>21.9</td>
</tr>
<tr>
<td>Slavonia HR</td>
<td>16.2</td>
<td>19.1</td>
</tr>
<tr>
<td>Velika Krsna SR</td>
<td>13.9</td>
<td>11.3</td>
</tr>
<tr>
<td>Zrenjanin SR</td>
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<tr>
<td>Corfu GR</td>
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</tr>
<tr>
<td>Tanushimarou JP</td>
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<td>4.0</td>
</tr>
<tr>
<td>Ushibuka JP</td>
<td>5.6</td>
<td>6.8</td>
</tr>
<tr>
<td>Mean</td>
<td>13.6</td>
<td>16.5</td>
</tr>
<tr>
<td>± SD</td>
<td>5.6</td>
<td>5.9</td>
</tr>
</tbody>
</table>

(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 acids.
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 \( (r = 0.71) \).
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
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 Dley

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

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 Crsna, 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 Tanushimar (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.

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References


