*hearts*

MDPI

*Article*

# Dietary Fatty Acids Predicting Long Term Cardiovascular Mortality in a Cohort of Middle-Aged Men Followed-Up until Extinction

**Alessandro Menotti [1] and Paolo Emilio Puddu [1,2,*]**

[1] Association for Cardiac Research, Via Voghera, 31, 00182 Rome, Italy; amenotti2@gmail.com
[2] EA 4650, Signalisation, Électrophysiologie et Imagerie des Lésions d'Ischémie Reperfusion Myocardique, Université de Normandie, 14000 Caen, France
* Correspondence: puddu.pe@gmail.com; Tel.: +39-340-9323920

**Abstract:** Objectives: To report the relationships of major dietary fatty acids with major cardiovascular disease mortality groups in a cohort of middle-aged men followed up with until extinction. Material and Methods: In the early 1960s, a cohort of men aged 40 to 59 years was enrolled and examined within the Italian Rural Areas section of the Seven Countries Study including dietary history that allowed for the estimation of major fatty acid (FA) intake (saturated FAs: SAFAs; mono-unsaturated FAs: MUFAs; and poly-unsaturated FAs: PUFAs), their ratios, and the production of a dietary score derived from 18 food groups, the high levels of which corresponded to a Mediterranean diet profile. Results: During a follow-up of 61 years, the intake of SAFAs was directly while that of MUFAs was inversely and significantly associated with coronary heart disease (CHD) mortality (the hazard ratio for one standard deviation was 1.28 and 0.84, respectively) but not with other cases of Heart Disease of Uncertain Etiology (HDUE) and stroke mortality. The hazard ratio for SAFAs remained significant after factoring into the multivariate models the dietary score and other classical cardiovascular risk factors (age, smoking habits, cholesterol levels, and systolic blood pressure). The role of the dietary score was inverse and significant (hazard ratio of 0.73). Again, this was true for CHD but not for HDUE and stroke mortality. Conclusions: Both SAFAs and MUFAs predict long-term CHD mortality, together with a dietary score, but not HDUE and stroke, which represent different diseases also in relation to dietary habits.

**Keywords:** dietary fatty acids; coronary heart disease; cardiovascular mortality



**Citation:** Menotti, A.; Puddu, P.E. Dietary Fatty Acids Predicting Long Term Cardiovascular Mortality in a Cohort of Middle-Aged Men Followed-Up until Extinction. *Hearts* **2024**, *5*, 196–210. https://doi.org/10.3390/hearts5020013

Academic Editor: Matthias Thielmann

Received: 19 February 2024
Revised: 14 March 2024
Accepted: 21 March 2024
Published: 23 March 2024



## 1. Introduction

The purpose of this analysis was to show the relationship of three major dietary fatty acids with the occurrence of fatal cardiovascular diseases (CVDs) and coronary heart disease (CHD) in particular, in a cohort of middle-aged men followed-up for many years until the extinction of the cohort. In fact, in relatively recent analyses conducted on the same material, we showed the protective association with long-term mortality from CHD of a dietary profile corresponding to the Mediterranean diet, but not with HDUE or stroke mortality [1]. The availability of data on major dietary fatty acids prompted the idea to study their relationship with the same end-points, adding a comparison with the role of a dietary score.

We are aware that this contribution might be likely overwhelmed and swept away into the mess of the ongoing infinite debate on this issue where facts supporting the pros and the cons continuously contribute to a kind of never-ending study. The literature, mainly focused on the role of saturated fatty acids (SAFAs), offers three positions about this issue, and here, only a few references will be quoted as examples: (a) those asserting and/or demonstrating the actual role of dietary fatty acids and of SAFAs in particular in their association with major cardiovascular diseases and mainly CHD [2–5]; (b) those negating those facts [6–9];

(c) those suggesting that focusing on single nutrients might be a distorting way to look at the relationship between diet and disease due to the complexity of eating habits, thus proposing to place major attention on dietary profiles, such as the Mediterranean diet or other healthy diets [10,11]. A detailed comment of all quoted contributions can be found in the Discussion.

A balanced review of the problem can be found in several chapters of a book dedicated to food and nutrition science, based on the review of many studies and positions [12].

## 2. Material and Methods

### *2.1. Study Population and Measurements*

In early 1960s, two rural cohorts of middle-aged men (entry age, 40 to 59 years) were enrolled with a participation rate of 98.7% within the Italian section of the Seven Countries Study of Cardiovascular Diseases (SCS). The baseline examination included various personal characteristics such as social, behavioral, anthropometric, biophysical, biochemical, and clinical data [13].

In the early phases of the study, a dietary survey was performed on participants with the dietary history approach based on a questionnaire administered by experienced and supervised nutritionists [14]. The questionnaire included a long list of foods habitually consumed at each meal with an attempt to estimate weights and volumes. A proportion of about 9% of the participants were not interviewed and their data were estimated by imputation with a multivariate normal procedure based on the use of 30 other personal characteristics. Mean levels of the imputed levels were not substantially different from the original ones.

Major nutrients were estimated using local food tables [15]. For this analysis, we used the levels of saturated fatty acids (SAFAs), mono-unsaturated fatty acids (MUFAs) and poly-unsaturated fatty acids (PUFAs), plus three ratios, i.e., the P/S ratio (poly-unsaturated fats/saturated fats), M/S ratio (mono-unsaturated fat/saturated fats), and UN/SA ratio (unsaturated fats/saturated fats).

Moreover, 18 food groups were derived from the questionnaire, i.e., bread, cereals, potatoes, vegetables, legumes, fruit, oils, meat, fish, eggs, butter, margarine, milk, cheese, sugar, pastries, alcohol, and sugar beverages. After adjustment for 1000 calories, these 18 food groups were treated by a principal component analysis, which is a statistical reduction technique that produces one or more uncorrelated components representing many original variables, linearly combined after proper weighting, which explain the maximum amount of the variance of data. Using the coefficients of the resulting equation, a factor score can be produced for each individual, in this case, a dietary score.

The procedure is independent from the opinions of the investigators and relies on pre-defined mathematical procedures that exploit the association across the variables.

Scores of this type are called a posteriori scores in contrast with a priori scores, the structure of which is defined by the opinions of the investigators. The original factor score, the mean and median of which are reported in Table 1, was then divided into three tertiles classes and, comparing the prevalence of the various food groups in each of them, we arbitrarily assigned the target of Unhealthy Diet to the low tertile, Intermediate Diet to the intermediate tertile, and Healthy Diet to the high tertile. In fact, the profile of food groups in the Healthy Diet resembled that of the Mediterranean diet [1]. In other words, the higher the better. For analysis purposes, Unhealthy Diet was labelled with 1, the Intermediate Diet with 2, and the Healthy Diet with 3.

A few consolidated cardiovascular risk factors were used as possible confounding variables in the multivariate analysis. They were: (a) age, approximated to the nearest birthday (years); (b) smoking habits, derived from a questionnaire, classified as smokers (reference for multivariate analysis), ex-smokers, and current smokers (percentage); (c) systolic blood pressure, measured in supine position at the end of a medical examination, following the procedure described by the WHO Cardiovascular Survey Methods manual; the average of two consecutive measurements taken 1 min apart was used for

analysis (mmHg) [16]; (d) serum cholesterol, measured in casual blood samples following the technique of Anderson and Keys (mg/dL) [17].

**Table 1.** Descriptive statistics of characteristics of the study population.

| Dietary Variables | Mean | SD | Median | 25 Perc | 75 Perc | % of Energy |
|---|---|---|---|---|---|---|
| SAFAs g/day | 28.7 | 11.7 | 28.7 | 20.4 | 34.5 | 8.8 |
| MUFAs g/day | 49.3 | 16.3 | 48.2 | 38.3 | 58.0 | 15.1 |
| PUFAs g/day | 11.5 | 6.8 | 9.6 | 7.1 | 12.8 | 3.5 |
| Fat g/day | 89.4 | 28.1 | 88.0 | 69.7 | 104.2 | 27.4 |
| P/S ratio | 0.43 | 0.25 | 0.36 | 0.28 | 0.47 | --- |
| M/S ratio | 1.86 | 0.64 | 1.74 | 1.40 | 2.18 | --- |
| UN/S ratio | 2.29 | 0.72 | 2.16 | 1.80 | 2.65 | --- |
| Dietary score | −0.012 | 0.924 | −0.014 | −0.667 | 0.690 | --- |
| **Confounding risk factors** | | | | | | |
| Age years | 49.1 | 5.1 | 49.0 | 45.0 | 53.0 | --- |
| Never smoker % (*) | 25.4 | 1.1 | --- | --- | --- | --- |
| Ex-smoker % (*) | 13.6 | 0.8 | --- | --- | --- | --- |
| Smoker % (*) | 61.1 | 1.2 | --- | --- | --- | --- |
| Systolic blood pressure mmHg | 143.6 | 21.0 | 140.0 | 130.0 | 155.0 | --- |
| Serum cholesterol mg/dL | 201.6 | 40.8 | 198.0 | 175.0 | 224.0 | --- |
| **Other variables** | | | | | | |
| Body mass index kg/m$^2$ | 25.2 | 3.7 | 24.8 | 22.6 | 27.4 | --- |
| Protein g/day | 76.5 | 24.5 | 75.6 | 60.1 | 89.1 | --- |
| Oligosaccharides g/day | 57.9 | 64.5 | 55.8 | 30.7 | 78.7 | --- |
| Polysaccharides g/day | 254.7 | 90.8 | 247.0 | 197.2 | 298.5 | --- |
| Energy kcal | 2941 | 642 | 2935 | 2584 | 3232 | --- |
| **61-year mortality** | **N** | **Rate per 1000** | | | | |
| All causes rate | 1708 | 998 | --- | --- | --- | --- |
| Coronary heart disease | 281 | 164 | --- | --- | --- | --- |
| Heart disease uncertain etiology | 216 | 124 | --- | --- | --- | --- |
| Stroke | 230 | 134 | --- | --- | --- | --- |

(SD = standard deviation), (*) proportion and standard error, perc: percentile.

We coded mortality data using official causes of death with the addition of other information from various sources, like field examinations, hospital clinical records, other medical reports, and especially interviews with hospital and family doctors and relatives of the deceased. All these details were obtained before the era of the Helsinki declaration, and consent was implied in participation. All details were anonymously coded without any possibility to connect the coded information to the actual name of the participant, whether deceased or still alive. The procedure was similar to what years later would be called "verbal autopsy" by WHO standards.

Causes of death were assigned using the 8th Revision of the WHO International Classification of Diseases (ICD-8) [18] following the strict rules of the study protocol. The analysis used only 3 wide groups of cardiovascular disease mortality, that was: (a) CHD only if typical major ischemic syndromes, including sudden coronary death, could be identified; (b) stroke of all types; and (c) HDUE including major heart diseases usually manifested with heart failure, or chronic arrhythmia and blocks in the absence of a clear etiology. All other types of cardiovascular diseases were excluded and covered few cases with other defined etiologies or that were very rare. Details and reasons for these choices are provided elsewhere [1,13,19].

In 61 years of follow-up, in the original 1712 men, there were 1708 deaths, 1 lost to follow-up, and 3 survivors. Cardiovascular disease mortality covered 45.7% of all causes, while those belonging to the 3 major groups described above were 705, covering the 92.4% of all cardiovascular events.

### *2.2. Statistical Analysis*

Variables used in the analysis were treated for descriptive statistics and tabulated. A correlation matrix involving some dietary variables was computed. Mean levels of the 18 food groups were tabulated and compared across the levels of dietary score. The three major fatty acids and the three ratios were used as covariates in a series of Cox proportional hazard models with CHD, HDUE, and stroke as dependent variables, separately. Each model was followed by another model with the addition of the dietary score in order to test the possible improvement of prediction as evaluated by the informativeness test of Peto (prediction ability), which is directly proportional to the $chi^2$ (twice the change of the model likelihoods). This series of models did not include possible confounders that could have distorted this preliminary evaluation of the analysis.

Finally, three Cox models, separately conducted for CHD, HDUE, and stroke were computed including the three fatty acids, the dietary score, and the classical risk factors as confounding variables to obtain a comprehensive and adjusted predictive picture.

## 3. Results

The baseline descriptive statistics of the variables used in the analysis (Table 1) showed that the study population had a high energy intake (due to heavy physical activity at work), a moderate intake of total fat, a relatively low intake of SAFAs, and a relatively high intake of MUFAs. Among the risk factors used as confounding variables, high levels of blood pressure and cigarette smoking were somewhat counterbalanced by low levels of serum cholesterol. Another small group of general characteristics of the study population was also reported.

The death rates for three groups of major cardiovascular diseases described the outcome linked to the extinction of the population with CHD to be somewhat more common than those of HDUE and stroke.

A description of the mean levels of the food groups in the three dietary score classes (Table 2) suggested several significant differences across classes confirmed by the *p* value of the ANOVA. In particular, the class defined as Healthy Diet had a significantly higher intake (compared with the other two classes) of bread, potatoes, vegetables, fish, eggs, margarine, and alcohol, and a lower intake of fruits, meat, butter, milk, cheese, sugar, pastries, and sugar beverages.

A correlation matrix involving the fatty acids, their ratios, and the dietary score (not reported as being too bulky) indicated the existence of a high association among most of the variables, suggesting the possibility of difficult handling and interpretation of further analyses. In particular: (a) the highest R (correlation coefficient) for SAFAs was with MUFAs (+0.63), and the same was the case for MUFAs with SAFAs; (b) the highest R for PUFAs was with the P/S ratio (+0.69); (c) the highest R for the M/S ratio was with the UN/SA ratio (+0.94) as well as that of the UN/SA with MS; (d) the highest R for P/S was with UN/SA (+0.47); and (e) the highest R for the dietary score was with M/S (+0.30). Overall, out of 21 Rs, there were 6 $\geq$ 0.32, a level that explained at least 10% of the variance between two variables, and 12 Rs $\leq$ 0.23, a level that explained at least 5% of the variance. In another version of the correlation matrix, we added serum cholesterol, of which the relationship with the other dietary variables was limited, ranging from 0.04 with SAFAs and MUFAs to $-0.07$ with the dietary score.

**Table 2.** Food group quantities adjusted for 1000 Kcal in three categories of the dietary score. Means and standard deviation in parentheses.

| Food Groups | Unhealthy Diet | Intermediate Diet | Healthy Diet | *p* of ANOVA |
|---|---|---|---|---|
| Bread g | 93.5 (41.4) | 122.2 (58.3) | 146.7 (65.5) | <0.0001 |
| Cereals g | 40.8 (20.4) | 41.2 (19.0) | 42.8 (19.7) | 0.1773 |
| Potatoes g | 7.2 (7.7) | 8.3 (8.7) | 9.8 (90.1) | <0.0001 |
| Vegetables g | 15.6 (15.5) | 17.1 (16.1) | 24.5 (19.6) | <0.0001 |
| Legumes g | 1.7 (6.5) | 1.5 (2.9) | 1.2 (2.6) | 0.1404 |
| Fruit g | 93.5 (59.4) | 68.8 (49.9) | 32.8 (38.2) | <0.0001 |
| Oils g | 13.4 (6.9) | 14.1 (7.0) | 13.5 (6.4) | 0.1261 |
| Meat g | 50.7 (26.6) | 43.8 (23.9) | 27.8 (18.2) | <0.0001 |
| Fish g | 6.7 (6.4) | 7.0 (6.5) | 9.2 (8.4) | <0.0001 |
| Eggs g | 5.1 (6.0) | 5.9 (7.9) | 6.9 (7.9) | 0.0003 |
| Butter g | 7.0 (6.2) | 4.6 (5.0) | 0.7 (2.0) | <0.0001 |
| Margarine g | 0.6 (1.7) | 4.1 (4.8) | 0.7 (1.9) | <0.0001 |
| Milk g of solid part | 7.7 (7.1) | 4.1 (4.8) | 0.7 (1.9) | <0.0001 |
| Cheese g | 7.0 (7.8) | 4.8 (8.0) | 3.2 (5.9) | <0.0001 |
| Sugar g | 7.4 (5.2) | 4.7 (3.5) | 1.8 (2.7) | <0.0001 |
| Pastries g | 7.8 (12.2) | 4.0 (4.8) | 1.5 (2.8) | <0.0001 |
| Alcohol g | 23.1 (15.4) | 28.7 (18.3) | 29.0 (20.7 | <0.0001 |
| Sugar beverages g of sugar | 0.4 (1.8) | 0.1 (0.7) | 0.04 (0.2) | <0.0001 |

Tables 3–5 should be inspected in comparison, since they deal, separately, with the three CVD end-points. In general, models dealing with CHD as an end-point (Table 3) showed the direct, significant association of SAFAs with events, and the indirect significant role of MUFAs and two ratios. The addition of the dietary score improved the prediction as documented by the significant $chi^2$ of the informativeness test, and the same score was inversely related to events, suggesting a protective effect of its high levels.

The case of the HDUE model was entirely different, since single fatty acids and their ratios never produced significant coefficients in any direction. The addition of the dietary score was never associated with an improvement in the models and by itself never produced significant coefficients. Also, in the case of stroke, the coefficients of the three fatty acids and their ratios were not statistically significant. The addition of the dietary score was associated with a significant improvement in informativeness in the model dealing with the M/S ratio and close to significance in the other cases, while the dietary score was simply close to significance.

The three Cox models including the three fatty acids, the dietary score, and the selected confounding cardiovascular risk factors tended to replicate the findings obtained in the preliminary analysis where the confounding variables were not considered (Table 6). The model for CHD confirmed the positive and significant coefficients for SAFAs and the negative and significant coefficient for MUFAs and for the dietary score, but, at the same time, it showed the traditional expected direct relationship with the CHD events of age, systolic blood pressure, and serum cholesterol and the protective effect of never-smokers. In particular, one standard deviation excess of MUFAs (16 g) did correspond to a reduced CHD risk of 15%, and the same applied to one excess deviation for the dietary score (roughly less than one-third of the distribution), while one extra standard deviation of SAFAs (12 g) was associated with a 20% extra risk that could be roughly transformed into a reduced risk of the same amount if the SAFAs were reduced of the same percentage.

**Table 3.** Basic analyses for CHD versus dietary fatty acids and dietary score.

| | Coefficient | *p* Value | Delta | HR | lcl | hcl | |
|---|---|---|---|---|---|---|---|
| **Model 3 fatty acids** | | | | | | | |
| **SAFAs** | 0.0209 | 0.0014 | 12 | 1.28 | 1.10 | 1.50 | |
| **MUFAs** | −0.0109 | 0.0244 | 16 | 0.84 | 0.72 | 0.98 | |
| **PUFAs** | 0.0019 | 0.8305 | 7 | 1.01 | 0.90 | 1.15 | |
| **Model 3 fatty acids + dietary score** | | | | | | | |
| **SAFAs** | 0.0164 | 0.0153 | 12 | 1.22 | 1.04 | 1.43 | *p* of informativeness <0.0001 |
| **MUFAs** | −0.0088 | 0.0677 | 16 | 0.87 | 0.75 | 1.01 | |
| **PUFAs** | 0.0021 | 0.8148 | 7 | 1.01 | 0.90 | 1.15 | |
| **Dietary score** | −0.3810 | <0.0001 | 0.82 | 0.73 | 0.65 | 0.83 | |
| **Model P/S ratio** | | | | | | | |
| **P/S ratio** | 0.0178 | 0.9372 | 0.25 | 1.00 | 0.90 | 1.12 | |
| **Model P/S ratio + dietary score** | | | | | | | |
| **P/S ratio** | 0.0786 | 0.7236 | 0.25 | 1.02 | 0.91 | 1.14 | *p* of informativeness <0.0001 |
| **Dietary score** | −0.4093 | <0.0001 | 0.82 | 0.71 | 0.63 | 0.81 | |
| **Model M/S ratio** | | | | | | | |
| **M/S ratio** | 0.3191 | 0.0029 | 0.65 | 0.81 | 0.71 | 0.93 | |
| **Model M/S ratio + dietary score** | | | | | | | |
| **M/S ratio** | −0.2271 | 0.0324 | 0.65 | 0.86 | 0.75 | 0.99 | *p* of informativeness <0.0001 |
| **Dietary score** | −0.3812 | <0.0001 | 0.82 | 0.73 | 0.65 | 0.83 | |
| **Model UN/SA ratio** | | | | | | | |
| **UN/SA ratio** | −0.2429 | 0.0090 | 0.72 | 0.78 | 0.65 | 0.94 | |
| **Model UN/SA ratio + dietary score** | | | | | | | |
| **UN/SA ratio** | −0.1634 | 0.0770 | 0.72 | 0.85 | 0.71 | 1.02 | *p* of informativeness <0.0001 |
| **Dietary score** | −0.1634 | <0.0001 | 0.82 | 0.68 | 0.59 | 0.79 | |

HR = hazard ratio; delta = changes for HR computation; lcl = low 95% confidence limit; hcl = high 95% confidence limit.

In the model for the HDUE, the three fatty acids and the dietary score produced no significant coefficients, as well as serum cholesterol as largely documented in previous analyses [1], while the predictive roles of age, systolic blood pressure, and never smoking were retained. The model for stroke had an intermediate result since the three fatty acids had no significant coefficients, but there was the adverse effect of serum cholesterol and systolic blood pressure, while never-smokers had a negative and significant (protective) role.

Separately, we produced replicas of the above three Cox models where the levels of the SAFAs, MUFAs, and PUFAs were expressed after adjustment for 1000 calories considering the large spread of energy intake of this population (not reported in detail). The outcome, in terms of multivariate coefficients and their *p* values, was practically the same as the original ones. Another Cox model was computed using as an end-point the combination of HDUE with stroke (not reported in detail), representing a solid counterpart to CHD, and the findings confirmed the picture described above.

We attempted to produce parallel Cox models forcing the three ratios (P/S, M/S, and UN/SA) as covariates instead of the three fatty acids. The consequence was that, due to multicollinearity problems, the computer program automatically excluded one of the three ratios in each model. In particular, in the model for CHD, the P/S ratio was excluded and the other two ratios were not significant, while the dietary score retained its significance. In the model for HDUE, the M/S ratio was excluded, and the other variables were not significant. The same happened for the model of stroke where the excluded ratio was that of UN/SA. The major findings of this analysis were the strong predictive power of dietary SAFAs (direct) and partly that of MUFAs (inverse) on the occurrence of long-term

CHD mortality. This was not true for the other two end-points (HDUE and stroke), the relationship of which was absolutely neutral. These conclusions should be considered in the light of a number of details that characterize this population sample of originally middle-aged men.

**Table 4.** Basic analyses for HDUE versus dietary fatty acids and dietary score.

| | Coefficient | *p* Value | Delta | HR | lcl | hcl | |
|---|---|---|---|---|---|---|---|
| **Model 3 fatty acids** | | | | | | | |
| **SAFAs** | 0.0040 | 0.6243 | 12 | 1.05 | 0.86 | 1.27 | |
| **MUFAs** | 0.0041 | 0.4513 | 16 | 1.07 | 0.90 | 1.26 | |
| **PUFAs** | −0.0094 | 0.4192 | 7 | 0.94 | 0.80 | 1.10 | |
| **Model 3 fatty acids + dietary score** | | | | | | | |
| **SAFAs** | 0.0034 | 0.6864 | 12 | 1.04 | 0.86 | 1.27 | *p* of informativeness 0.4386 |
| **MUFAs** | 0.0043 | 0.4265 | 16 | 1.07 | 0.90 | 1.27 | |
| **PUFAs** | −0.0093 | 0.4225 | 7 | 0.94 | 0.80 | 1.10 | |
| **Dietary score** | −0.0685 | 0.4328 | 0.82 | 0.95 | 0.82 | 1.09 | |
| **Model P/S ratio** | | | | | | | |
| **P/S ratio** | −0.4588 | 0.1178 | 0.25 | 0.89 | 0.77 | 1.03 | |
| **Model P/S ratio + dietary score** | | | | | | | |
| **P/S ratio** | −0.4471 | 0.1270 | 0.25 | 0.89 | 0.77 | 1.03 | *p* of informativeness 0.5271 |
| **Dietary score** | −0.0662 | 0.4474 | 0.82 | 0.95 | 0.82. | 1.09 | |
| **Model M/S Ratio** | | | | | | | |
| **M/S ratio** | 0.0590 | 0.5935 | 0.65 | 1.04 | 0.90 | 1.20 | |
| **Model M/S ratio + dietary score** | | | | | | | |
| **M/S ratio** | 0.0712 | 0.5208 | 0.65 | 1.05 | 0.91 | 1.21 | *p* of informativeness 0.3711 |
| **Dietary score** | −0.0801 | 0.3604 | 0.82 | 0.94 | 0.81 | 1.08 | |
| **Model UN/SA ratio** | | | | | | | |
| **UN/SA ratio** | −0.0141 | 0.8882 | 0.72 | 0.99 | 0.86 | 1.14 | |
| **Model UN/SA ratio + dietary score** | | | | | | | |
| **UN/SA ratio** | −0.0034 | 0.9728 | 0.72 | 1.00 | 0.87 | 1.15 | *p* of informativeness 0.2732 |
| **Dietary score** | −0.0730 | 0.4053 | 0.82 | 0.94 | 0.82 | 1.08 | |

HR = hazard ratio; delta = changes for HR computation; lcl = low 95% confidence limit; hcl = high 95% confidence limit.

The dietary components used here referred to a population group comprising, for more than two-thirds, heavy workers due to their farming activity typical in the middle of the last century. The energy intake was high on average, with almost 3000 calories/day. The levels of the three major fatty acid intakes were relatively low for SAFAs and PUFAs and relatively high for MUFAs, depicting a situation rather similar to that of the ideal Mediterranean diet. The high levels of MUFAs were partly justified by the common use of olive oil that covered about 10% of total calories. Moreover, the about 3000 calories/day were about 20% covered by alcohol intake in the form of wine (other sources only accounted for 3% of the total). This was the reason why we recomputed the percent contribution of the three major fatty acids after having excluded alcohol from the total energy. The outcome was that SAFAs moved from 8.8% to 11.9%, MUFAs from 15.1% to 18.8%, and PUFAs from 3.5% to 4.4%, which were relatively small increases justifying the negligible changes observed in the replicas of the final Cox models.

**Table 5.** Basic analyses for stroke versus dietary fatty acids and dietary score.

| | Coefficient | *p* Value | Delta | HR | lcl | hcl | |
|---|---|---|---|---|---|---|---|
| **Model 3 fatty acids** | | | | | | | |
| **SAFAs** | 0.0017 | 0.8310 | 12 | 1.02 | 0.90 | 1.16 | |
| **MUFAs** | 0.0018 | 0.7337 | 16 | 0.92 | 0.65 | 1.29 | |
| **PUFAs** | −0.0054 | 0.6202 | 7 | 1.01 | 0.91 | 1.13 | |
| **Model 3 fatty acids + dietary score** | | | | | | | |
| **SAFAs** | −0.0003 | 0.9707 | 12 | 1.00 | 0.82 | 1.20 | *p* of informativeness 0.0736 |
| **MUFAs** | 0.0026 | 0.6258 | 16 | 1.04 | 0.88 | 1.23 | |
| **PUFAs** | −0.0052 | 0.6325 | 7 | 0.96 | 0.83 | 1.12 | |
| **Dietary score** | −0.1506 | 0.0724 | 0.82 | 0.88 | 0.77 | 1.01 | |
| **Model P/S ratio** | | | | | | | |
| **P/S ratio** | −0.1788 | 0.4973 | 0.25 | 0.96 | 0.84 | 1.09 | |
| **Model P/S ratio + dietary score** | | | | | | | |
| **P/S ratio** | −0.1550 | 0.5541 | 0.25 | 0.96 | 0.85 | 1.09 | *p* of informativeness 0.0833 |
| **Dietary score** | −0.1467 | 0.0776 | 0.82 | 0.89 | 0.78 | 1.01 | |
| **Model M/S Ratio** | | | | | | | |
| **M/S ratio** | 0.0674 | 0.5206 | 0.65 | 1.04 | 0.91 | 1.19 | |
| **Model M/S ratio + dietary score** | | | | | | | |
| **M/S ratio** | 0.0969 | 0.3553 | 0.65 | 1.07 | 0.93 | 1.22 | *p* of informativeness 0.0278 |
| **Dietary score** | −0.1605 | 0.0556 | 0.82 | 0.88 | 0.77 | 1.00 | |
| **Model UN/SA ratio** | | | | | | | |
| **UN/SA ratio** | 0.0291 | 0.7574 | 0.72 | 1.02 | 0.89 | 1.17 | |
| **Model UN/SA ratio + dietary score** | | | | | | | |
| **UN/SA ratio** | 0.0556 | 0.5554 | 0.72 | 1.04 | 0.91 | 1.19 | *p* of informativeness 0.0652 |
| **Dietary score** | −0.1566 | 0.0620 | 0.82 | 0.88 | 0.77 | 1.01 | |

HR = hazard ratio; delta = changes for HR computation; lcl = low 95% confidence limit; hcl = high 95% confidence limit.

Altogether, the low levels of PUFAs and the high levels of MUFAs allowed us to understand why MUFAs and the M/S ratio were so relevant in the outcome of the analysis. Incidentally, the M/S ratio was probably used for the first time in a Seven Countries contribution dealing with the first 15 years of the study, using it as a kind of marker of the Mediterranean diet [2]. Another feature of the diet was the relatively high consumption of carbohydrates that covered 43% of the total calories and 53% of the calories after subtracting the alcohol contribution.

The dietary score showed to be a strong predictor, at least for CHD mortality, confirming its role already documented in previous analyses conducted on the same material and being characterized, in its higher levels, by a food group profile resembling the Mediterranean diet. It should be recalled that this was not an intentional arbitrary choice of the investigators but the outcome of the a posteriori process of a principal component analysis that was originally used for the construction of the score, exploiting data from 18 food groups including alcohol intake and adjusting the food group intake for 1000 calories. When the dietary score was added in the same model to the three fatty acids, its role was always significantly predictive, adding value to the power of the model according to the informativeness test. However, this was again true only for the end-point made by CHD group of cardiovascular diseases. The fact that fatty acids and the dietary score could survive with significant coefficients in the same predictive models probably tells us that although the score incorporates, still indirectly, information on fatty acids from the existing food groups, it offers some extra hidden property that allows this situation. In fact, considering three equi-numerical classes (tertiles of the dietary score), we found an excess

of 16% of SAFAs in class 1 (the most exposed to CHD risk) versus class 3, a 17% excess in the level of the M/S ratio, a 15% excess in the level of the UN/SA ratio, and an 11% excess in the level of the P/S ratio versus class 1 (M/S and UN/SA being the least exposed to CHD risk), confirming the indirect connection of the dietary score with the three fatty acid intakes.

**Table 6.** Multivariate final analysis with Cox models for CHD, HDUE, and stroke, including some CVD risk factors as possible confounders.

| | Coefficient | *p* Value | Delta | HR | Lcl | hcl |
|---|---|---|---|---|---|---|
| **Model CHD** | | | | | | |
| **Age** | 0.0711 | <0.0001 | **5** | **1.43** | **1.25** | **1.63** |
| **Smoker** | Reference | --- | --- | --- | --- | --- |
| **Ex-smoker** | −0.2328 | 0.2009 | 1 | 0.79 | 0.55 | 1.13 |
| **Never-smoker** | −0.3095 | 0.0294 | 1 | **0.73** | **0.56** | **0.97** |
| **Systolic blood pressure** | 0.0154 | <0.0001 | 20 | **1.36** | **1.20** | **1.54** |
| **Serum cholesterol** | 0.0063 | <0.0001 | 40 | **1.29** | **1.15** | **1.44** |
| **SAFAs** | 0.0166 | 0.0164 | **12** | **1.22** | **1.04** | **1.44** |
| **MUFAs** | −0.0098 | 0.0383 | **16** | **0.85** | **0.74** | **0.99** |
| **PUFAss** | 0.0011 | 0.9072 | 7 | 1.01 | 0.89 | 1.14 |
| **Dietary score** | −0.1895 | 0.0182 | **0.82** | **0.86** | **0.75** | **0.97** |
| **Model HDUE** | | | | | | |
| **Age** | 0.1808 | <0.0001 | **5** | **2.47** | **2.09** | **2.91** |
| **Smoker** | Reference | --- | --- | --- | --- | --- |
| **Ex-smoker** | −0.1975 | 0.3157 | 1 | 0.82 | 0.56 | 1.21 |
| **Never-smoker** | −0.5696 | 0.0006 | 1 | **0.57** | **0.41** | **0.78** |
| **Systolic blood pressure** | 0.0143 | 0.0039 | 20 | **1.33** | **1.14** | **1.55** |
| **Serum cholesterol** | 0.0007 | 0.3989 | 40 | 1.03 | 0.90 | 1.18 |
| **SAFAs** | 0.0046 | 0.5821 | 12 | 1.06 | 0.87 | 1.28 |
| **MUFAs** | 0.0037 | 0.4877 | 16 | 1.06 | 0.90 | 1.26 |
| **PUFAs** | −0.0142 | 0.2072 | 7 | 0.91 | 0.78 | 1.06 |
| **Dietary score** | 0.1636 | 0.0756 | 0.82 | 1.14 | 0.99 | 1.33 |
| **Model Stroke** | | | | | | |
| **Age** | 0.1182 | <0.0001 | 5 | **1.81** | **1.56** | **2.10** |
| **Smoker** | Reference | --- | --- | --- | --- | --- |
| **Ex-smoker** | 0.1357 | 0.4448 | 1 | 1.15 | 0.81 | 1.62 |
| **Never-smoker** | −0.4333 | 0.0080 | 1 | **0.65** | **0.47** | **0.89** |
| **Systolic blood pressure** | 0.0161 | <0.0001 | 20 | **1.38** | **1.20** | **1.58** |
| **Serum cholesterol** | 0.0038 | 0.0182 | 40 | **1.17** | **1.03** | **1.33** |
| **SAFAs** | 0.0037 | 0.6557 | 12 | 1.05 | 0.86 | 1.27 |
| **MUFAs** | 0.00002 | 0.9971 | 16 | 1.00 | 0.85 | 1.18 |
| **PUFAs** | −0.0075 | 0.4895 | 7 | 0.95 | 0.82 | 1.10 |
| **Dietary score** | 0.1720 | 0.0493 | 0.82 | **1.15** | **1.00** | **1.33** |

HR = hazard ratio; delta = changes for HR computation; lcl = low 95% confidence limit; hcl = high 95% confidence limit.

Moreover, the crude death rate in three tertile classes of the dietary score showed a smaller rate of 29% for CHD in class 3 versus class 1, versus a larger rate of +49% for HDUE and of +30% for stroke, suggesting that, even in the absence of adjustments for other variables, the tertile roughly corresponding to the Mediterranean diet had a favorable effect on CHD and an adverse effect on the other two end-points. These findings, beyond the

corrective role of the confounding variables, suggested the possible existence of competing risks across the three cardiovascular groups.

All the main findings are reported in Tables 3–6, and the many details described above tend to suggest that the three CVD groups were different diseases, with likely different etiologies, risk factors, and relationships with some characteristics of the diet. This was somewhat expected on the basis of a series of analyses carried out in the same epidemiological material where we found important differences across the three CVD conditions. All the abovementioned small details that could not be tabulated without a waste of space were presented for the need to focus the attention on the substantial differences that we found between CHD and the other two CVD end-points.

## 4. Discussion

The hypothesis was that dietary habits and serum cholesterol may trigger the process of gross atherosclerosis in the large coronary vessels, which may later reach the stage of typical minor or major coronary events, yet still supported by other risk factors. This should not be the case for HDUE, where the clinical manifestations are different and mainly oriented towards heart failure and chronic arrhythmia. The situation could be somewhat intermediate for stroke also because a limit of this study was the inability, in the majority of cases, to segregate thrombotic from hemorrhagic strokes. The relationship of serum cholesterol with the occurrence of stroke was null during the first 40 years of follow-up [20], suggesting the presence of a majority of hemorrhagic strokes also supported by the high fatality rate of the first event that was around 40% or more. The relationship with serum cholesterol became marginally significant after 50 years of follow-up, suggesting an increase in the unknown quota of the thrombotic type of stroke. Probably, this was not enough to detect the possible relationship between the dietary score and the fatty acid intake.

Attention to this problem is not common in the literature, and one of the best, although indirect, explanatory contributions in the differences between the two major heart disease groups came from an old pathology report [21] showing that the myocardial scars had a bimodal distribution, where the large ones corresponded to the presence of gross atherosclerosis of large coronary vessels and frequent myocardial infarction, while the small scars were multiple and diffuse and not more common in the cases carrying large scars. The small scars were associated with diffuse fibrosis, likely promoting heart failure, but the authors, in those times, could not provide evidence for their etiology and made only hypotheses.

Since a major interest of our studies is to show the epidemiological difference across three large and relatively homogenous groups of cardiovascular disease mortalities, we briefly summarized, in Table 7, the differences found in previous analyses and those added by the present paper, all of them obtained from the same population group followed-up until extinction. Most documentation for old analyses can be found in a single report [13].

Some limitations of this study were the small size of the cohort, only partially compensated by the extremely long follow-up, the absence of subjects of the female sex, and the use of only three large classes of dietary fatty acids. The last point is bound to the starting time of the Seven Countries Study, i.e., the late 1950s–early 1960s, when knowledge and measurement techniques of subcategories or of single fatty acids were not developed enough. Actually, the measurements of single fatty acids in replicas of the food eaten at baseline could be performed several years later, but only on small subsamples of the 16 original cohorts in the Seven Counties Study.

**Table 7.** Major differences across three CVD mortality groups documented in the past and added to in the present analysis on the same population study.

| | **Previously Documented Differences** | | |
|---|---|---|---|
| | **CHD** | **HDUE** | **Stroke** |
| Relationship with serum cholesterol | Direct | Null or inverse | Marginally significant |
| Age at occurrence | Younger | Older | Intermediate |
| Age at death | Younger | Older | Intermediate |
| Relationship with healthy diet | Inverse | Null | Null |
| Relationship with physical activity | Inverse | Null | Marginal |
| Relationship with smoking habits | Direct | Direct | Direct |
| | **Present Additional Evidence of Differences** | | |
| Relationship with SAFAs | Direct | Null | Null |
| Relationship with MUFAs | Inverse | Null | Null |
| Relationship with PUFAs | Null | Null | Null |
| Relationship with P/S ratio | Null | Null | Null |
| Relationship with M/S ratio | Inverse | Null | Null |
| Relationship with UN/SA ratio | Inverse | Null | Null |

A comparison of our findings with other contributions is too difficult considering the enormous and contrasting number of papers available in the literature. We may only attempt to construct a short story of this problem quoting only a few papers as examples. In the first half of last century, before the start of cardiovascular epidemiology, some reports usually called "geographical pathology" suggested the idea of a possible connection between dietary habits and CHD. Two relevant contributions were those of Cornelis De Langen and Isidore Snapper, two Dutch internists teaching medicine in Indonesia and China, respectively, who attributed the low serum cholesterol levels and the rarity of coronary syndromes in the native populations to their dietary habits based on plant food [22,23]. The identification of the relationships between serum cholesterol and the occurrence of CHD syndromes in the early 1960s prompted the classical metabolic studies investigating the effect on serum cholesterol levels of changing types and the amount of dietary fatty acids, mainly SAFAs [24,25]. However, a close relationship between dietary habits and serum cholesterol at individual levels was not found. The Seven Countries Study of Cardiovascular Disease provided evidence, at an ecological level that compared different and contrasting cohorts used as statistical units, of a strong association of average saturated fat intake and average serum cholesterol with incidence and mortality from CHD [2,3,26]. Although these findings were only observational and valid only at an ecological level, they met at least six out the nine Bradford–Hill criteria of causality. Two studies, conducted in parallel and investigating in population groups the relationship between dietary habits and serum cholesterol, were carried out in a Finnish community where the diet was changed toward the Mediterranean style, while in the Italian counterpart the Mediterranean diet was shifted toward high saturated fat intake, typical of the usual Finnish diet [27,28]. The outcome was a substantial decrease of serum cholesterol in Finland and a significant increase in Italy.

A population-level experimental approach in terms of prevention was that of the North Karelia Project, in Finland, where two large areas were subjected to intervention and control, respectively [29]. The preventive action included an anti-smoking campaign, the start of community control for hypertension, changing dietary habits with the substitution of whole milk with skimmed milk, the substitution of butter with soft margarine, and an increase in plant food intake. This approach, which later spread all over the country, was accompanied by a drastic reduction in incidence and mortality from CHD in a country that once was probably the one with the highest rates of the disease and now is classified as a low-risk country [30].

The observational studies at individual levels were frequently summarized by a large meta-analysis dealing with the relationship of saturated fat with CHD events, the conclusions of which suggested the absence of a clear correlation that, in any case, should be taken with some caution due to the possible uncertainties bound to this statistical procedure [6]. Some years later, again at the individual level, the PURE project conducted in cohorts of 18 countries, mainly belonging to the so-called developing nations, did not find an association of saturated fat intake with CHD mortality, suggesting instead the adverse role of carbohydrates [7]. In this case, it was difficult to interpret the findings since the overall saturated fat intake was low (close to ideal suggestions of dietary guidelines), the intake of carbohydrates was not segregated into poly- and oligosaccharides, and many cohorts had a diet close to that causing malnutrition.

A preventive trial ending in success was the PREDIMED, conducted in Spain, where the increased intake of two food groups typical of the Mediterranean diet (olive oil and nuts) in the treatment group was followed by a reduction in LDL-cholesterol, an increase in HDL-cholesterol, and a significant decrease (around 30%) in major coronary and cerebrovascular events [31]. From this point of view, we should not forget some other characteristics of the Mediterranean diet that were considered in this analysis and largely summarized in a recent review [32]. The adverse effects of SAFAs and the beneficial effects of MUFAs and of the dietary score, the high levels of which resemble the Mediterranean diet, were confirmed in this analysis in support of its general role in health.

The many observational studies and preventive trials can be followed only through reviews and meta-analysis. A meta-analysis published in 2009 on 11 observational studies estimated that lowering SAFA intake by 5% of one's energy and replacing them with PUFAs in the same proportion corresponded to a reduction in CHD risk by 13% and in CHD death by 26% [33]. The last figure roughly coincided with an estimate made on the predictive power of SAFAs in our study. In a review of many randomized preventive trials, SAFAs were partly substituted by PUFAs, resulting in PUFAs covering 14.9% of the energy among the treated and 5% among the controls. This corresponded to a decline of 10% in CHD risk, and studies of longer durations showed more benefits [34]. The same authors in a subsequent commentary paper expressed the idea that a need exists to develop further investigations to clarify an issue of which recent findings were not as satisfactory as expected [35]. A more recent review of 15 randomized controlled trials concluded that the substitution of SAFAs with PUFAs or carbohydrates for at least two years may produce important effects on combined cardiovascular events, but not on smaller subgroups with a greater reduction in larger decreases in SAFA intake [36]. Finally, a review of the literature, observational studies, randomized preventive trials, and their meta-analyses concluded that the relationship of SAFAs with CVD and CHD in particular has not been demonstrated by the present research evidence [37].

The more recent literature on the problem provides an excess of articles dedicated to the role of omega-3 fatty acids or to single fatty acids such as linoleic acid, but rare comparisons can be made, in these cases, with findings offered by the present analysis. However, several interesting papers could be identified, still presenting the contrasting differences quoted in the Introduction.

In a large population study, the end-point was CHD incidence, while the role of fatty acids was played by their circulating levels [4]. In this US study, SAFAs and MUFAs were directly related to an excess risk of, while PUFAs had an inverse association with CHD incidence. The positive association between SAFAs and MUFAs was partly attributed to their correlation with serum triglycerides. Two analyses conducted on the NHNES in the USA were related specifically to the role of larger fatty acid categories and separately to various types of PUFAs [5,38]. Major findings in the first analysis were the positive association of SAFA and MUFA intake with CHD mortality and their inverse association with PUFAs. These findings could not be confirmed in subjects with previous myocardial infarctions. In the second analysis, the various subtypes of PUFAs were inversely and significantly associated with mortality from CHD, heart failure, and stroke.

In a peculiar review of the problem, the authors were convinced that there was no evidence that reducing SAFAs is beneficial to the incidence of cardiovascular diseases and total mortality, but a benefit was obtained for the incidence of stroke. Therefore, their suggestion was that no limitations should be placed on the use of whole-fat dairy, unprocessed meat, and dark chocolate [9].

An interesting study was conducted in the Netherlands where dairy SAFAs were substituted with meat SAFAs, of which the consequence was an increase in CHD risk on the basis of a modeling procedure [39]. A meta-analysis dedicated to the intake of MUFAs alone reached the conclusion that their higher levels were associated with a lower all-cause mortality, while no benefits were seen for CVD and cancer mortality, which seems contradictory considering that usually these two conditions account for, everywhere, around three-quarters of all-cause mortality [40]. Another review article indicated that the effect of the substitution of SAFAs heavily depended on the replacement fat [41]. The best way to do it would be to increase the amount of PUFAs, removing the same amount of SAFAs. This also indicates that a supplement of fish oil still seems uncertain in terms of the benefit.

In the famous US Nurses and Health Professionals Studies, the intake of at least 7 g/day of olive oil was associated with a lower all-cause mortality (−19%), cardiovascular mortality (−19%), and cancer mortality (−17%) [42]. The replacement of 10 g/day of butter, margarine, or dairy fat with 10 g of olive oil was associated with lower risks of various conditions ranging from 8 to 34%. In this case, the favorable contribution of MUFAs and PUFAs was probably complemented by the micronutrients typically contained in olive oil.

Reviewing a large number of observational studies from PubMed, CINAHL, and the Cochrane library, it can be seen that several of them were able to subdivide SAFAs into subgroups with short, medium, and long chains and their association with CVD [43]. In particular long-chain SAFAs increased the CVD risk, while medium- and short-chain SAFAs were neutral or perhaps beneficial. This finding reflected the use of long-chain SAFAs in the numerator of the Atherogenicity Index proposed in 1991 by Ulbricht and Southgate, while PUFAs and some MUFAs were located in the denominator, and the other SAFAs were disregarded [44].

Overall, the contributions to the literature are full of discrepancies and uncertainties. To be sure, concentrating on a single nutrient may distort the meaning of findings because people eat foods and not selected nutrients. The outcome of the PREDIMED trial was instructive since simple changes of two components typical of the Mediterranean diet produced substantial benefits in terms of CVD incidence, yet still preceded by a reduction in LDL-cholesterol and increase in HDL-cholesterol. It is also clear that results obtained 60 years ago in controlled metabolic studies [24,25] are almost impossible to obtain in randomized controlled trials. Probably, the role of dietary fatty acids cannot be negated, but beyond clarifying several uncertainties, it will be wiser to concentrate on food groups and dietary profiles and their role in disease, health, and prevention.

## 5. Conclusions

This investigation supported the idea that dietary SAFAs (adverse) and MUFAs (favorable) play a role in the prediction and perhaps etiology of CHD, but not of HDUE or stroke. The confusion about the problem probably derives from the complexity of diet in general, and in focusing on a single nutrient or food group while forgetting that probably a dietary profile, or even more than one profile, are more important for prediction and prevention. This was another finding of this analysis where the role of a dietary score was strongly predictive even in its presence in the models of single fatty acids or their ratios.

**Author Contributions:** Conceptualization, A.M. and P.E.P.; methodology and analysis, A.M.; writing—original draft, A.M. and P.E.P. All authors have read and agreed to the published version of the manuscript.

**Funding:** Analysis and writing of this paper received no external funding.

**Institutional Review Board Statement:** The board of directors of the institution involved in data collections were de facto playing the role of the ethical committee, approving the execution of the study on the basis of the local existing legislation by the date this investigation started. The study was conducted in accordance with the Declaration of Helsinki, and since it was started before this Declaration, it was not formally approved by an Institutional Review Board (or Ethics Committee).

**Informed Consent Statement:** Baseline measurements were taken before the era of the Helsinki Declaration and approval was implied in participation. All details were anonymously coded without any possibility to connect the coded information to the actual name of the participant, either deceased or still alive.

**Data Availability Statement:** The original data are not publicly available. However, research projects are centrally evaluated by an ad hoc committee.

**Acknowledgments:** The authors acknowledge the contribution of Giovina Catasta for help obtaining recent follow-up data on the participants in the Italian areas.

**Conflicts of Interest:** The authors declare no conflicts of interest.

## References

1. Menotti, A.; Puddu, P.E.; Catasta, G. Lifestyle behaviors predicting major cardiovascular diseases mortality in a practically extinct cohort of middle-aged men followed-up for 61 years. *Acta Cardiol.* **2023**, *78*, 578–585. [CrossRef] [PubMed]
2. Keys, A.; Menotti, A.; Karvonen, M.J.; Aravanis, C.; Blackburn, H.; Buzina, R.; Djordjevic, B.S.; Dontas, A.S.; Fidanza, F.; Keys, M.H.; et al. The diet and 15-year death rate in the Seven Countries Study. *Am. J. Epidemiol.* **1986**, *124*, 903–915, Reprinted as Historic Article in *Am. J. Epidemiol.* **2017**, *185*, 1130–1142. [CrossRef] [PubMed]
3. Kromhout, D.; Menotti, A.; Alberti-Fidanza, A.; Puddu, P.E.; Hollman, P.; Kafatos, A.; Tolonen, H.; Adachi, H.; Jacobs, D.R., Jr. Comparative ecologic relationships of saturated fat, sucrose, food groups and a Mediterranean food pattern score to 50-year coronary heart disease mortality rates among 16 cohorts of the Seven Countries Study. *Eur. J. Clin. Nutr.* **2018**, *72*, 1102–1110. [CrossRef] [PubMed]
4. Jin, D.; Tricha, F.; Islam, N.; Lewington, S.; Lacey, B. Associations of circulating fatty acids with incident coronary heart disease: A prospective study of 89,242 individuals in UK Biobank. *BMC Cardiovasc. Dis.* **2023**, *23*, 365. [CrossRef]
5. Wang, Y.; Fang, Y.; Witting, P.K.; Charchar, F.J.; Sobey, C.G.; Drummond, G.R.; Golledge, J. Dietary fatty acids and mortality risk from heart disease in US adults: An analysis based on NHANES. *Sci. Rep.* **2023**, *13*, 1614. [CrossRef] [PubMed]
6. Siri-Tarino, P.W.; San, Q.; Hu, F.B.; Krauss, R.M. Meta-analysis of prospective cohort studies evaluating the association of saturated fat with cardiovascular disease. *Am. J. Clin. Nutr.* **2010**, *91*, 535–546. [CrossRef] [PubMed]
7. Dehghan, M.; Mente, A.; Zhang, X.; Swaminathan, S.; Li, W.; Mohan, V.; Iqbal, R.; Kumar, R.; Wentzel-Viljoen, E.; Rosengren, A.; et al. Associations of fats and carbohydrates intake with cardiovascular disease and mortality in 18 countries from five continents (PURE): A prospective cohort study. *Lancet* **2017**, *390*, 2050–2062. [CrossRef]
8. Valk, R.; Hammill, J.; Grip, J. Saturated fat: Villain and bogeyman in the development of cardiovascular disease? *Eur. J. Prev. Cardiol.* **2022**, *29*, 2312–2321. [CrossRef]
9. Astrup, A.; Magkos, F.; Bier, D.M.; Brenna, J.T.; de Olivera Otto, M.C.; Hill, J.O.; King, J.C.; Mente, A.; Ordovas, J.M.; Volek, J.S.; et al. Saturated fats and health: A reassessment and proposal for food-based recommendations: JACC State-of-the-Art Review. *J. Am. Coll. Cardiol.* **2020**, *76*, 844–857. [CrossRef]
10. Chareonrungrueangchai, K.; Wongkawinwoot, K.; Anothaisintawee, T.; Reutrakul, S. Dietary factors and risks of cardiovascular diseases: An umbrella review. *Nutrients* **2020**, *12*, 1088. [CrossRef]
11. Delarue, J. Dietary fatty acids and CHD: From specific recommendations to dietary patterns. *Nutr. Res. Rev.* **2021**, *34*, 240–258. [CrossRef] [PubMed]
12. Katz, D.L. *The Truth about Food 2018*; Dystel & Goderich: New York, NY, USA, 2018; pp. 1–752.
13. Menotti, A.; Puddu, P.E. How the Seven Countries Study contributed to the launch and development of cardiovascular epidemiology in Italy. A historical perspective. *Nutr. Metab. Cardiovasc. Dis.* **2020**, *30*, 368–383. [CrossRef]
14. Alberti Fidanza, A.; Seccareccia, F.; Torsello, S.; Fidanza, F. Diet of two rural population groups of middle-aged men in Italy. *Intern. J. Vit. Nutr. Res.* **1988**, *58*, 442–451.
15. Fidanza, F.; Versiglioni, N. *Tabelle di Composizione Degli Alimenti*; Food Composition Tables; Idelson: Napoli, Italy, 1981.
16. Rose, G.; Blackburn, H. *Cardiovascular Survey Methods*; World Health Organization (WHO): Geneva, Switzerland, 1968; pp. 1–188.
17. Anderson, J.T.; Keys, A. Cholesterol in serum and lipoprotein fractions: Its measurement and stability. *Clin. Chem.* **1956**, *2*, 145–159. [CrossRef] [PubMed]
18. World Health Organization. *International Classification of Diseases and Causes of Death*; 8th revision; ICD-8; World Health Organization: Geneva, Switzerland, 1965; pp. 1–671.
19. Puddu, P.E.; Menotti, A. Heart disease of uncertain etiology: A new definition of heart failure for epidemiological studies. *J. Cardiov Dev. Dis.* **2023**, *10*, 132. [CrossRef]

20. Menotti, A.; Lanti, M.; Maiani, G.; Kromhout, D. Forty-year mortality from cardiovascular diseases and their risk factors in men of the Italian rural areas of the Seven Countries Study. *Acta Cardiol.* **2005**, *60*, 521–531. [CrossRef]
21. Schwartz, C.J.; Mitchell, J.R. The relation between myocardial lesions and coronary artery disease. I. An unselected necropsy study. *Br. Heart J.* **1962**, *24*, 761–786. [CrossRef] [PubMed]
22. De Langen, C.D. Cholesterol metabolism and racial pathology. *Geneeskd. Tijdschr. Voor Ned. -Indie* **1916**, *56*, 1–34. (In Dutch)
23. Snapper, I. *Chinese Lessons to Western Medicine*; Interscience: New York, NY, USA, 1941; Grune & Stratton: New York, NY, USA.
24. Keys, A.; Anderson, J.T.; Grande, F. Serum cholesterol response to changes in diet. IV. Particular saturated fatty acids in the diet. *Metabolism* **1965**, *14*, 776–787. [CrossRef] [PubMed]
25. Hegsted, D.M.; McGandy, R.B.; Myers, M.L.; Stare, F.J. Quantitative effects of dietary fat on serum cholesterol on man. *Am. J. Clin. Nutr.* **1965**, *17*, 281–295. [CrossRef] [PubMed]
26. Menotti, A.; Kromhout, D.; Blackburn, H.; Fidanza, F.; Buzina, R.; Nissinen, A. 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. [CrossRef]
27. Ehnholm, C.; Huttunen, J.K.; Pietinen, P.; Leino, U.; Mutanen, M.; Kostiainen, E.; Pikkarainen, J.; Dougherty, R.; Iacono, J.; Puska, P. Effect of diet on serum lipoproteins in a population with a high risk of coronary heart disease. *N. Engl. J. Med.* **1982**, *307*, 850–855. [CrossRef] [PubMed]
28. Ferro-Luzzi, A.; Strazzullo, P.; Scaccini, C.; Siani, A.; Sette, S.; Mariani, M.A.; Mastranzo, P.; Dougherty, R.M.; Iacono, J.M.; Mancini, M. Changing the Mediterranean diet: Effects on blood lipids. *Am. J. Clin. Nutr.* **1984**, *40*, 1027–1037. [CrossRef] [PubMed]
29. Puska, P.; Tuomilehto, J.; Nissinen, A.; Vartiainen, E. (Eds.) *The North Karelia Project. 20 Years Results and Experiences*; National Public Health Institute: Helsinki, Finland, 1995; pp. 1–363.
30. Jousilahti, P.; Laatikainen, T.; Salomaa, V.; Pietila, A.; Vartiainen, E.; Pusk, P. 40-Year CHD mortality trends and the role of risk factors in mortality decline: The North Karelia Project Experience. *Glob. Heart* **2016**, *11*, 207–221. [CrossRef] [PubMed]
31. Estruch, R.; Ros, R.; Sala-Salvado, J.; Covas, M.I.; Corella, D.; Aros, F.; Gómez-Gracia, E.; Ruiz-Gutiérrez, V.; Fiol, M.; Lapetra, J.; et al. Primary prevention of cardiovascular disease with a Mediterranean diet supplemented with extra-virgin olive oil or nuts. *N. Engl. J. Med.* **2018**, *378*, e34. [CrossRef] [PubMed]
32. Delarue, J. Mediterranean diet and cardiovascular health: An historical perspective. *Br. J. Nutr.* **2022**, *128*, 1335–1348. [CrossRef]
33. Jakobsen, M.U.; O'Reilly, E.J.; Heitmann, B.L.; Pereira, M.A.; Bälter, K.; Fraser, G.E.; Goldbourt, U.; Hallmans, G.; Knekt, P.; Liu, S.; et al. Major types of dietary fat and risk of coronary heart disease: A pooled analysis of 11 cohort studies. *Am. J. Clin. Nutr.* **2009**, *89*, 1425–1432. [CrossRef]
34. Mozaffarian, D.; Micha, R.; Wallace, S. Effects on coronary heart disease of increasing polyunsaturated fat in place of saturated fat: A systematic review and meta-analysis of randomized controlled trials. *PLoS Med.* **2010**, *7*, e1000252. [CrossRef]
35. Micha, R.; Moraffarian, D. Saturated fat and cardiometabolic risk factors, coronary heart disease, stroke, and diabetes: A fresh look at the evidence. *Lipids* **2010**, *45*, 893–905. [CrossRef] [PubMed]
36. Hooper, L.; Martin, N.; Jimoh, O.F.; Kirk, C.; Foster, E.; Abdelhamd, A.S. Reduction in saturated fat intake for cardiovascular disease. *Cochrane Database Syst. Rev.* **2020**, *8*, CD011737. [CrossRef] [PubMed]
37. Astrup, A.; Dyerberg, J.; Elwood, P.; Hermansen, K.; Hu, F.B.; Jakobsen, M.U. The role of reducing intakes of saturated fat in the prevention of cardiovascular disease: Where does the evidence stand in 2010? *Am. J. Clin. Nutr.* **2011**, *94*, 684–688. [CrossRef]
38. Zhong, N.A.; Han, P.; Wand, Y.; Wheng, C. Associations of polyunsaturated fatty acids with cardiovascular disease and mortality: A study of NHANES database in 2003–2018. *BMC Endocr. Disord.* **2023**, *23*, 185. [CrossRef]
39. Vissers, L.E.T.; Rijksen, J.; Boer, J.M.A.; Verschuren, W.M.M.; van der Schouw, Y.T.; Sluijs, I. Fatty acids from dairy and meat and their association with risk of coronary heart disease. *Eur. J. Nutr.* **2019**, *58*, 2639–2647. [CrossRef] [PubMed]
40. Lotfi, K.; Slari-Moghaddam AYousfinia, M.; Larijani, B.; Esmaillzadeh, A. Dietary intakes of monounsaturated fatty acids and risk of mortality from all causes, cardiovascular disease and cancer: A systematic review and dose-response meta-analysis of prospective cohort studies. *Ageing Res. Rev.* **2021**, *72*, 101467. [CrossRef] [PubMed]
41. Wang, D.D.; Hu, F.H. Dietary fat and risk of cardiovascular disease: Recent controversies and advances. *Annu. Rev. Nutr.* **2017**, *37*, 423–446. [CrossRef] [PubMed]
42. Guasch-Ferré, M.; Li, Y.; Willett, W.C.; Sun, Q.; Sampson, L.; Salas-Salvadó, J.; Martínez-González, M.A.; Stampfer, M.J.; Hu, F.B. Consumption of olive oil and risk of total and cause-specific mortality among U.S. adults. *Am. J. Coll. Cardiol.* **2022**, *79*, 101–112. [CrossRef] [PubMed]
43. Perna, M.; Hewlings, S. Saturated fatty acid chain length and risk of cardiovascular disease: A systematic review. *Nutrients* **2022**, *15*, 30. [CrossRef] [PubMed]
44. Ulbricht, T.L.V.; Southgate, D.A.T. Coronary heart disease: Seven dietary factors. *Lancet* **1991**, *338*, 985–992. [CrossRef]