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RESEARCH PAPER

Dietary atherogenicity and thrombogenicity indexes predicting cardiovascular mortality: 50-year follow-up of the Seven Countries Study

Alessandro Menotti [a], Paolo Emilio Puddu [a,b,*], Johanna M. Geleijnse [c], Anthony Kafatos [d], Hanna Tolonen [e]

[a] *Association for Cardiac Research, Rome, Italy*
[b] *EA 4650, Signalisation, Électrophysiologie et Imagerie des Lésions d'ischémie Reperfusion Myocardique, Université de Normandie, Caen, France*
[c] *Division of Human Nutrition and Health, Wageningen University, Wageningen, the Netherlands*
[d] *Department of Social Medicine, Preventive Medicine and Nutrition Clinic, University of Crete, Heraklion, Greece*
[e] *Finnish Institute for Health and Welfare, Helsinki, Finland*

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Population cohorts;
50-Year follow-up

Abstract *Background and aim:* To study the relationships of an Atherogenicity Index (ATI) and a Thrombogenicity Index (THI), with 50-year mortality from coronary heart disease (CHD), other heart diseases of uncertain etiology (HDUE) and cerebrovascular disease or stroke (STR), in 16 international cohorts of middle-aged men.
Methods and results: Foods from a dietary survey in subsamples of men in each cohort of the Seven Countries Study (SCS) were chemically analyzed for several types of fatty acids that were converted into ATI and THI identifying each of 16 cohorts. Ecological correlations of the ATI and THI were calculated with the three fatal CVD conditions and with all-cause mortality at 25 and 50 years. Correlation coefficients (Rs) were positive and highly significant between ATI and THI versus CHD mortality, with levels ranging from 0.79 to 0.97, depending on the duration of follow-up and the choice of 10 or of 16 cohorts. This was not the case for HDUE and STR mortality for which Rs were variable and not significant. A strong direct association was also found with all-causes deaths at 25 and 50-years. ATI and THI were also directly related with dietary saturated fat and cholesterol levels and inversely with the Mediterranean Adequacy Index (a score identifying the Mediterranean diet).
Conclusion: These findings indicate that CHD has a different relationship with dietary lipids intake than HDUE and STR. This suggests that HDUE and STR have different underlying pathways or are different diseases.

* Corresponding author. Via Voghera, 31, Rome 00182, Italy.
E-mail addresses: amenotti2@gmail.com (A. Menotti), puddu.pe@gmail.com (P.E. Puddu), marianne.geleijnse@wur.nl (J.M. Geleijnse), kafatos@med.uoc.gr (A. Kafatos), hanna.tolonen@thl.fi (H. Tolonen).

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1. Introduction

The relationship between diet and coronary heart disease (CHD) has been studied systematically in the Seven Countries Study of Cardiovascular Diseases (SCS) since the middle of the last century for more than 50 years along a number of steps, as follows: a) the relationship of saturated fat, the PS ratio (poly-unsaturated fatty acid/saturated fatty acids) and serum cholesterol with 5 and 10 year incidence of CHD [1,2]; b) the same nutrients plus the monounsaturated fatty acids/saturated fatty acids ratio (M/S) as a marker of the Mediterranean Diet in the 15 year mortality from CHD and all causes [3]; c) the definition of a number of food groups and their relationship with 25 year follow-up mortality from CHD [4,5]; d) the creation of the MAI dietary index (Mediterranean Adequacy Index) based on the diet of an Italian rural village examined in the feasibility study of the SCS [6]; e) the systematic analysis of food groups and major nutrients versus the 50-year mortality from all causes and CHD [7,8] and the use of the same variables as determinants of inflammation and 50-year events [9]; the measurement of several dietary fatty acids in the diet and their relationship with 25-year CHD fatalities [10]. All these procedures were based on "ecological" comparison of cohorts, where the 16 cohorts of the study represented the statistical units. Major findings were the positive association of average population intake of saturated fats (as a whole and as sub-types) and CHD mortality among the 16 cohorts of the study and the association of food groups and of a dietary score corresponding to the Mediterranean diet with both CHD and all-cause mortality.

The next step is represented by this analysis that exploited the availability of several dietary fatty acids measurements and consisting in combining them into the so-called Indexes of Atherogenicity and Thrombogenicity. Recently, a review [11] described 10 different types of these indexes, suggesting that the best ones are those proposed by Ulbricht and Southgate in 1991 [12] called index of Atherogenicity (ATI) and index of Thrombogenicity (THI). This review prompted the idea to apply those indexes to the cohorts of the SCS, and to relate them to long-term cardiovascular mortality. Since the literature is extremely poor of analyses of this type, the only possibility to discuss the issue was to tackle the problem debated during the last 20 year or so, about the role of dietary saturated and polyunsaturated fats as determinants of atherosclerosis, CHD, other major cardiovascular conditions and all-cause mortality. In fact, there are three opposite positions, i.e (a) the one supporting and/or demonstrating the role of dietary fatty acids and of SAFAs in particular in their association with major cardiovascular diseases and mainly CHD [3,8,13,14]; and (b) that negating those facts [15–18]; c) that suggesting that focusing on single nutrients might be a wrong way to look at the relationship between diet and disease due to the complexity of eating habits, proposing instead to give major attention to dietary profiles, such as the Mediterranean diet or other healthy diets [19,20]. A detailed comment of all the quoted contributions will be given in Discussion.

2. Methods

2.1. Populations

Of the 16 cohorts in the SCS, 11 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 other Serbian cohorts, namely workers in a large agricultural cooperative in Zrenjanin and Belgrade university professors. A total of 12,763 men aged 40–59 were initially examined in the late 1950s and early 1960s with an average participation rate of 90.4 % [21].

2.2. Dietary data

Dietary habits were measured in subsamples of each cohort through complex procedures described elsewhere, where few major nutrients were chemically analyzed [22] and a number of food groups were defined [4].

Years later after the entry surveys, foods identical to those consumed in the 1950s and 60s were purchased from SCS local markets in each community of the 16 cohorts and shipped for chemical analysis for nutrients and individual fatty acids to the laboratory of the Division of Human Nutrition and Health of Wageningen University, The Netherlands [10]. The availability of fatty acid measurements allowed the estimate of dietary ATI and THI following the formulas proposed in 1991 by Ulbricht and Southgate [12]. This choice was based on the fact that the list of fatty acids used by those authors was the same available to the SCS. The correlation coefficients of the single fatty acids with the 25-year CHD mortality rates were already published together with the analytical procedures for the measurement of fatty acids [10].

For this analysis, we used the following variables: a) mean cohort levels of serum cholesterol measured at entry examination, determined by the Anderson and Keys procedure [23]; b) the Mediterranean Adequacy Index (MAI), an a-priori dietary score based on dietary data collected in a rural community of Southern Italy during the feasibility study of the SCS; it is based on the ratio of vegetable foods plus fish to animal foods plus sugar and pastry (its high levels correspond to what is intended as the Mediterranean diet); for analytical purposes MAI is transformed into its natural log (ln(MAI) [6]; c) the food groups consumed by each cohort as described in previous analyses [4,5,7,8]; and d) the cohort levels of dietary saturated (SAFA), monounsaturated (MUFA), poly-unsaturated (PUFA) and trans (TRANS) fatty acids [10].

2.3. Mortality data collection and coding

Mortality data were systematically collected during 50 years in 10 cohorts, 45 years in three cohorts from Serbia, former Yugoslavia and 25 years in 1 cohort in Italy and 2 in Croatia, former Yugoslavia. Out of 12,763 men enrolled at entry examination, 67 were lost to follow-up (5 per 1000) and their data censored at defined times.

Causes of death were adjudicated by a single reviewer following pre-defined criteria and exploiting also other information from interim examinations, review of hospital and other clinical records, or interviewing family and hospital doctors and the relatives of the deceased. In the presence of multiple causes of death or serious doubts about principal cause, it was assigned hierarchically as violence, cancer, CHD, stroke and other causes, in that order.

Cardiovascular mortality end-points were chosen as follows using the 8th Revision of the WHO-ICD8 [24]: a) CHD including cases of myocardial infarction, acute ischemic attacks, and sudden coronary death, in the last case after the exclusion of other possible causes (ICD-8 codes 410, 411, 412, 795); cases with only mention or evidence of chronic or other types of CHD (part of code 412 and code 414) were not included in this group for reasons given elsewhere [18] while healed myocardial infarction was retained; b) heart diseases of uncertain etiology (HDUE) includes a pool of symptomatic heart diseases (ICD-8 code 427) (heart failure, arrhythmia, blocks, covering 64% of all cases), ill-defined hypertensive heart disease (usually in the absence of documented left ventricular hypertrophy, covering 8% of cases) (ICD-8 codes 402–404), and cases quoted as chronic or other types of coronary heart disease, without any indication of typical coronary syndromes (ICD-8 part of codes 412, and 414, covering 28% of cases). The reason for keeping separated CHD versus HDUE is that these two conditions contrast in several characteristics [25]: age at death is higher for HDUE than in (typical) CHD, and serum cholesterol predicts CHD but not HDUE; c) cerebrovascular diseases (i.e. stroke; STR) included any type of cerebrovascular disease (ICD-8 codes 430–438); d) in the analysis we used also the sum of STR + HDUE (STHD) as a counterpart of CHD; e) ALL cause mortality including all ICD-8 codes.

The pool of the selected CVD end-points (CHD, HDUE and STR) covered 92% of all CVD deaths in 50 years while other rare or etiologically defined cardiovascular diseases were not included in this analysis.

2.4. Statistical analysis

The 25 and 50-year mortality rates were expressed per 1000 person-years and adjusted for mean cohort age using a linear regression modeling. For six cohorts, Rome Railroad, in Italy, Dalmatia and Slavonia in Croatia, (former Yugoslavia), Velika Krsna, Zrenjanin and Belgrade in Serbia (former Yugoslavia), 50-year mortality data were estimated using regression equations derived from the 10 cohorts having complete mortality data. The dependent variable were the 50-year findings, the independent variables were either 25- or 45-year findings. The regression equations developed for this purpose are reported in Appendix Table 1.

The dietary indexes were computed using the formulas proposed by Ulbricht and Southgate in 1991 [12] but were modified by the addition in the numerators of TRANS (fatty acids) that were available to us but were not used in the original formulas, as follows.

A) ATI = (C12:0 + C14:0*4 + C16:0+TRANS)/(n6PUFA + n3PUFA + C18:1+othMUFA);
B) THI= (C14:0 + C16:0 + C18:0+TRANS)/(C18.1 * 0.5+othMUFA*0.5 + n6PUFA*0.5 + n3PUFA* 3+(n3PUFA/n6PUFA))

where: C12:0 = lauric fatty acid; C14:0 = myristic fatty acid; C16:0 = palmitic fatty acid; C18:0 = stearic fatty acid; C18:1 = oleic acid; othMUFA = other monounsaturated fatty acids; n6PUFA = poly-unsaturated fatty acid type n6; n3PUFA = polyunsaturated fatty acid type 3n; TRANS = trans fatty acids; MUFA = mono unsaturated fatty acids; PUFA = poly-unsaturated fatty acids.

For comparison purposes we computed two other simpler indexes found in the paper presenting the review of the literature [11] as follows: 1) Health-promoting index (HPI) [26]: (MUFA + PUFA)/(C12:0 + C14:0*4 + C16:0); 2) Hypocholesterolemic/hypercholesterolemic ratio (HH) [27]: (C18:1+PUFA)/(C12:0 + C14:0 + C16:0) where the meanings of symbols are as above.

The amounts of fatty acids used in the above computations consumed in each cohort have been tabulated in detail.

An ecological analysis, using the 16 cohorts as statistical units, was run computing the Pearson linear correlation coefficients of the modified ATI and THI versus CHD, HDUE, STR, STHD and ALL-cause mortality at 25 and 50 years of follow-up. A similar procedure was performed using only the 10 cohorts with full 50-year mortality data.

Correlation coefficients were computed and tabulated between the two main indexes and the food groups consumed (all adjusted per 1000 Kcal), in the 16 cohorts and separately in 10 cohorts. The selected food groups were those published in 1989 [4] and then already used in other papers to be related with events occurred in 25 and 50 years of follow-up [5,7–9]. Linear correlation coefficients were also computed and tabulated for 50-year mortality of 5 end-points versus 4 indexes of atherogenicity (ATI, TSI, HPI and HH), 4 major fatty acid groups, the dietary score MAI and the means of serum cholesterol from the 16 cohorts.

3. Results

Death rates from the three selected CVD groups and for all-causes are reported for 25 and 50 years of follow-up, together with the estimated (modified) ATI and THI of the 16 cohorts (Table 1). Large cohort differences in CVD death rates were already presented elsewhere [7–9].

Table 1 Mortality in 25 and 50 years from 3 CVD groups and all-causes in rates per 1000 person/years and levels of Indexes of Atherogenicity (ATI) and Thrombogenicity (THI) (modified from Ref. [12].

AREA	CHD 25	CHD 50	HDUE 25	HDUE 50	STR 25	STR 50	ALL 25	ALL 50	ATI	THI
US	8.35	10.86	2.07	4.02	1.82	3.31	23.53	37.32	55.3	49.8
EF	14.24	17.34	1.13	2.63	2.53	4.31	31.80	43.98	101.0	76.6
WF	9.34	12.93	0.71	2.39	2.48	4.42	26.55	40.36	82.5	64.7
ZU	8.67	10.47	1.29	2.56	1.84	3.22	24.15	38.77	64.6	53.4
CR	5.02	7.22	1.79	3.85	2.66	4.18	25.29	39.09	48.7	49.7
MO	2.85	5.11	2.44	5.53	3.60	6.35	21.80	36.18	24.5	29.7
RR	4.07	6.21	1.82	3.98	2.07	3.92	17.86	34.39	27.8	26.1
DA	2.66	4.66	1.47	3.34	4.06	5.92	21.93	36.27	34.0	36.9
SL	4.54	6.72	3.44	6.79	6.57	8.52	34.99	45.26	57.6	66.3
VK	2.02	6.37	4.43	8.82	4.63	7.60	25.73	39.94	53.4	46.6
ZR	5.77	9.85	2.88	5.89	5.97	9.50	29.14	43.06	43.7	52.8
BE	4.99	10.52	0.52	2.56	1.89	4.23	13.50	31.09	54.5	51.8
KT	1.11	4.65	0.98	3.85	3.20	4.83	13.86	31.06	25.3	25.0
CO	2.20	4.52	2.11	4.80	3.25	6.48	19.05	35.87	20.3	19.8
TA	1.51	2.64	1.23	1.33	5.19	6.48	21.43	35.72	9.8	9.3
UB	2.51	2.84	1.00	2.19	5.52	7.18	26.31	38.58	13.4	13.2

US=US Raiload; EF = East Finland; WF=West Finland; ZU = Zutphen, the Netherlands; CR= Crevalcore, Italy; MO=Montegiorgio, Italy; RR= Rome Railroad, Italy; DA = Dalmatia, Croatia, former Yugoslavia; SL= Slavonia, Croatia, former Yugoslavia; VK= Velika Krsna, Serbia, former Yugoslavia; ZR=Zrenjanin, Serbia former Yugoslavia; BE=Belgrade, Serbia, former Yugoslavia; KT=Crete, Greece; CO=Corfu, Greece; Tanushimaru, Japan; UB= Ushibuka, Japan.
Mortality.
CHD25 = Coronary heart disease, 25 years; CHD50 = Coronary heart disease, 50 years; HDUE25 = Heart diseases of uncertain etiology, 25 years; HDUE50 = Heart diseases of uncertain etiology, 50 years; STR25 = stroke, 25 years; STR50 = stroke, 50 years; ALL25 = all causes, 25 years; ALL50 = all causes, 50 years.

The amounts of single fatty acids used for the computation of the various indexes (Table 2) showed an excess of SAFA subgroups in Northern European countries and in the USA, while levels of MUFA were high in the Mediterranean areas, as expected from previous simpler data and from the structure of consumed food groups.

The two indexes, ATI and THI, showed large variations, considering that their standard deviations are around half of the mean and that differences between the two extreme values are 10-fold for ATI and more than 8-fold for THI. The two indexes are highly correlated one each other, with an R of 0.95 for the group of 16 cohorts and 0.98 for the group of 10 cohorts. In the majority of cases, values for the ATI are larger than those for THI but this probably depends upon the different use and combinations of fatty acids in the respective formulas. In general, values for ATI are higher in the North American and Northern European cohorts (range of 55–101) while all the others are definitely lower (that is smaller than 55 except a value of 57 in Slavonia, part of former Yugoslavia). Something similar is shown for the THI since in the 5 North American and Northern European cohorts the values are 49 or more, while in the other countries they are less than 49 except the value of 66 for Slavonia (former Yugoslavia) and those of two of the Serbian cohorts. The two indexes were also significantly correlated with cohort average serum cholesterol with R of 0.73 for ATI and 0.60 for THI suggesting the possible role of cholesterol as mediator

Table 2 Levels of fatty acids used to compute ATI and THI indexes in the 16 cohorts (g/day).

AREA	Lauric	Myristic	Palmitic	Stearic	Oleic	Other MUFA	PUFA N6	PUFA N3	TRANS	MUFA	PUFA
US	1.8	5.8	30.2	13.7	37.1	11.6	18.4	2.7	6.0	48.7	20.3
EF	4.3	14.0	40.6	21.8	42.0	13.0	10.9	4.4	9.0	55.0	13.1
WF	3.4	11.0	35.0	18.5	37.8	11.2	10.5	3.0	7.6	49.0	12.1
ZU	2.2	8.0	30.0	14.8	29.5	20.0	17.2	2.9	26.0	49.5	18.4
CR	1.1	4.2	30.8	14.7	61.6	11.3	18.4	2.5	1.8	72.9	20.2
MO	0.3	1.3	19.0	9.4	42.1	7.8	13.2	1.6	1.1	49.9	14.6
RR	0.7	2.4	17.5	6.2	46.9	6.9	8.9	1.2	1.6	53.8	9.8
DA	0.5	2.5	23.9	10.5	67.0	10.6	19.4	4.6	2.3	77.6	22.0
SL	0.6	3.7	42.2	20.4	64.4	16.4	17.6	4.0	2.1	80.8	20.6
VK	1.5	5.8	28.6	12.1	33.1	9.3	13.6	2.2	3.8	42.4	15.6
ZR	0.4	2.6	32.9	17.3	52.4	12.7	20.1	2.6	1.4	65.1	22.1
BE	1.3	5.6	30.8	15.3	43.1	10.7	22.5	3.3	3.0	53.8	25.7
KT	0.5	1.8	17.6	5.6	77.2	6.9	11.7	1.7	0.6	84.1	12.7
CO	0.3	1.1	15.6	3.1	55.8	7.5	13.3	2.1	0.3	63.3	15.1
TA	0.1	0.8	6.5	2.0	6.9	2.9	8.0	3.8	0.4	9.8	11.9
UB	0.2	1.0	9.2	3.0	13.3	4.0	8.2	4.8	0.4	17.3	13.2

between diet and the atherosclerosis process. Moreover, ATI and THI were highly correlated with lnMAI (Rs of −0.93 and −0.90, respectively).

The correlation coefficients between the indexes and CVD mortality for the analysis with 16 cohorts are highly significant for both indexes when the end-points are CHD, both at 25 and 50 years of follow-up, but also for all-cause mortality (Table 3). For all the other CVD end-points, Rs are negative, small, and not significant. For CHD, ATI is larger than THI while the reverse is true for all-cause mortality, but the differences are not significant.

The picture offered by the correlation coefficients computed only on 10 cohorts (those with complete 50-year mortality data) is similar but with some specifics. In general, Rs are larger than for the 16 cohorts and again highly significant at 25 and 50 years for both CHD and all-cause mortality. Moreover, all Rs are negative and not significant for HDUE, while for STR and STHD they are again negative but also larger and significant.

The associations between ATI and THI and food groups consumed in the 16 cohorts (Table 3) suggest the substantial correctness of the fatty acid measurement involved in the indexes. In fact, systematically, the correlation is direct with butter, milk, and sugar products plus pastries and inverse with cereals, vegetables, and legumes. The negative role of fish is evident only in the analysis on 16 cohorts, while the positive role of cheese is so in the analysis with 10 cohorts. Correlation coefficients from olive oil are negative but do not reach significant levels.

Table 4 using only the 50-year mortality data of the 5 end-points (Table 5) CHD relationships with the ATI and THI are shown and they appear to be coherent with other similar indexes, the major classes of fatty acids, the lnMAI and mean serum cholesterol. For CHD, there is clear coherence with the magnitude of four indexes as ATI, THI, HPI, HH and the levels of SAFA intake and serum cholesterol levels, and the inverse correlation with lnMAI. This also happens for all-cause mortality, while for the other CVD end-points the relationship with this chain of findings does not exist and becomes negative and significant for STR and STHD.

Table 3 Linear correlation coefficients between mortality from 4 CVD end-points and all-cause mortality in 25 and 50 years using 16 cohorts (all) and separately 10 cohorts (those with full 50 years data). ATI and THI (modified from Ref. [12].

	16 cohorts		10 cohorts	
	ATI	**THI**	**ATI**	**THI**
CHD25	**0.88***	**0.79***	**0.97***	**0.94***
CHD50	**0.93***	**0.86***	**0.99***	**0.97***
HDUE25	−0.03	0.13	−0.28	−0.17
HDUE50	−0.02	0.16	−0.24	−0.12
STR25	−0.36	−0.19	**−0.73***	**−0.79***
STR50	−0.34	−0.15	**−0.75***	**−0.80***
STHD25	−0.26	−0.07	**−0.84***	**−0.85***
STHD50	−0.20	0.0006	**−0.70***	**−0.66***
ALL25	**0.50***	**0.57***	**0.68***	**0.65***
ALL50	**0.55***	**0.62***	**0.76***	**0.73***

Levels of Rs => 0.50 have p=< 0.05 for the analysis on 16 cohorts.
Levels of Rs => 0.64 have p=< 0.05 for the analysis on 10 cohorts.
(*) Significant p (in bold).
Mortality.
CHD25 = Coronary heart disease, 25 years; CHD50 = Coronary heart disease, 50 years; HDUE25 = Heart disease of uncertain etiology, 25 years; HDUE50 = Heart disease of uncertain etiology, 50 years; STR25 = stroke, 25 years; STR50 = stroke, 59 years; STHD25 = STROKE + HDUE, 25 years; STHD50 = STROKE + HDUE, 50 years; ALL25 = all causes, 25 years; ALL50 = all causes, 50 years.

Table 4 Linear correlation coefficients between ATI and of THI versus food groups consumed by the 16 cohorts and 10 cohorts.

	16 cohorts		10 cohorts	
	ATI	THI	ATI	THI
Bread	0.17	0.23	0.18	0.23
Cereals	**−0.56***	**−0.62***	−0.59	**−0.65***
Potatoes	0.44	0.32	0.59	0.50
Vegetables	**−0.72***	**−0.70***	**−0.78***	**−0.79***
Legumes	**−0.63***	**−0.68***	**−0.72***	**−0.80***
Fruit	−0.31	−0.33	−0.32	−0.29
Meat	0.27	0.36	0.41	0.49
Butter	**0.84***	**0.67***	**0.95***	**0.90***
Milk	**0.87***	**0.73***	**0.94***	**0.89***
Cheese	0.19	0.17	0.59	**0.65***
Eggs	−0.17	−0.09	−0.22	−0.13
Margarine	0.34	0.25	0.37	0.35
Lard	−0.02	0.24	−0.21	−0.11
Fish	**−0.52***	**−0.60***	−0.53	−0.61
Olive oil	−0.46	−0.46	−0.41	−0.38
N6polyoils	013	0.23	0.13	0.25
Sugar products plus pastries	**0.71***	0.47	**0.71***	**0.70***
Alcohol	**−0.52***	−0.45	−0.54	−0.42

Levels of Rs => 0.50 have p=< 0.05 for the analysis on 16 cohorts.
Levels of Rs => 0.64 have p=< 0.05 for the analysis on 10 cohorts.
(*) Significant p (in bold).

4. Discussion

The purpose of this analysis was to study the relationships of these two dietary indexes, ATI and THI, in three groups of CVD mortality that previously showed different characteristics in their relationships with some risk factors and their natural history. Due to the ecological nature of the analysis, these findings could not be proposed and used for predictive purposes at individual level. Our main interest was to look at the possible coherence of this approach with previous findings based on different analytical choices and procedures. Findings showed that the indexes were strongly associated with CHD and partly with all-cause mortality, but not with STR and HDUE mortality. This is a unique study in this field since only a single other one comparable to our own could be found in the literature. In any case, whatever other study is difficult to compare since we dealt with “ecological“ analyses while almost all other studies use single individuals as statistical units. A possible limitation of our investigation is the limited number of statistical units (N = 16) that anyhow represented a noticeable accomplishment considering the starting time of the study located around the mid of last century.

Evidence from this analysis points to important, possibly debatable issues. Measurements of major dietary lipid fractions (SAFA, MUFA, PUFA) were made in food groups of the 16 cohorts at entry examination and then

repeated years later. They were highly correlated with each other despite some possible changes in food composition, leaving their ranks substantially unchanged [10,22] and becoming a warranty for the measurement of the single fatty acids made on the second occasion. Relationships of individual fatty acids with 25-year CHD mortality were published in 1995 [10] but at the time no such complex indexes were computed. On that occasion, a strong positive and significant association was found between trans fatty acids and 25-year mortality from CHD and similar associations were seen between SAFA subgroups, such as lauric, myristic and palmitic fatty acids.

The addition of trans fatty acids to the original indexes was evaluated by comparing the difference in the performance of indexes without versus those with trans fatty acids in the analysis using all 16 cohorts. In the majority of cases (13 out of 24 comparisons) we found a better performance by adding trans fatty acids. However, this was more common for ATI than for THI, for CHD mortality than for other end-points and, in any case, none of the differences reached statistical significance likely due to the small number of statistical units. A number of PUFA subgroups fatty acids did not enter into the indexes formulas but this was probably due to the knowledge and technical procedures existing at the time when the indexes were proposed [11,12].

ATI and THI derived from chemical measurements made on food groups of the 16 international cohorts of middle-aged men are directly and strongly associated with long-term mortality from CHD and partly from all-cause mortality but not with mortality attributable to STR and HDUE. The latter group of major, frequent CVD conditions contrasts with CHD in several characteristics. This suggests that processes of large vessel atherosclerosis and thrombosis should not influence the occurrence of the two other diseases. This statement is not fully documented since in this study we were unable, in the majority of cases, to segregate thrombotic from hemorrhagic events of STR.

Indications provided by the ATI are more powerful than those offered by the THI but they are highly intercorrelated and their association with CVD end-points are not significantly different. When inspecting the outcome using 10 cohorts only, all values of both indexes are definitely larger than in the analysis on 16 cohorts, but the differences are not significant while those higher values of Rs in the 10-cohort analysis allow to reach statistical significance. The correlation of the two indexes with food groups reflect the expected composition of the various foods in terms of fatty acids and the significant associations do correspond (at least for milk and butter) to those reported by the paper proposing the two indexes [12].

The inverse correlations of the two indexes with STR and HDUE mortality will surely raise questions and doubts, mainly because average serum cholesterol was also inversely related with those two end-points. There are at least two precedent cases in the history of the SCS. In 1997, our research group published a paper showing that blood pressure is highly correlated with STR mortality at the individual level while the correlation is negative in the ecological analysis dealing with the 16 cohorts [28]. Probably, the two types of analysis should be read and interpreted in different ways. A similar problem was found in a more recent paper where in 10 cohorts with a follow-up of 60 years and the extinction of the cohorts it was found that serum cholesterol was not predictive of STR and HDUE mortality in both individual and ecological analyses [29]. For STR, somewhat similar findings were reported by a large metanalysis published in 2007 [30]. In fact, differences of CHD mortality across populations are large but opposite differences were found for STR and HDUE. Moreover, populations (and individuals) with baseline low serum cholesterol levels have a lower risk of CHD mortality, and a definitely higher risk of STR and HDUE mortality, but the advantage of higher age at death for those two CVD conditions. The role of serum cholesterol is indirect and can explain why the ecological relationships are null or inverse with HDUE and STR mortality. The populations with higher serum cholesterol were shown to have non-healthy, non-Mediterranean diets and higher levels of ATI and THI. We conclude that these two indexes confirm observations made beforehand in relation with CHD, using different approaches including food groups, specific nutrients, average population serum cholesterol, and now the two indexes studied here.

This analysis, as others with an ecological approach, may arouse criticism from several points of view. However, they suggest the existence of mass phenomena that need attention and should be further tackled by mass strategies. We like to recall that already several decades ago the association of dietary SAFA with incidence-mortality from CHD in the SCS was coherent with at least 6 of the 9 Bradford Hill criteria for causality [31].

The literature is poor on this issue since the majority of contributions deal with the effects of feeding food animals with special foods that may reduce both ATI and THI, including the two extra indexes HPI and HH [26,27]. Many more strictly medical papers exhibit those concepts in their titles but almost none used indexes of this type to study their association with CVD or other conditions. As an alternative they use single fatty acids and call them “indexes” or focus on specific molecules such as lipoprotein (a).

Remarkably, the original Ulbricht and Southgate paper [12] was quoted more than 3700 times, according to Pubmed (accessed in November 2023) but apparently no one replicated their proposals or applied them successfully to human populations. The only sure exception deals with the Caerphilly Prospective Study in UK [32] where in a sample of about 500 middle-aged men the various fatty acids were estimated from foods intake recorded during seven days intake allowing the computation of both ATI and THI. After a follow-up of 5 years there were direct but not significant relationships between the indexes and the occurrence of CHD events, an outcome likely explainable by the low power of the study enrolling so few individuals.

The consequence of this apparently abnormal situation is that the comparison with present knowledge on the issue must be downsized to the infinite ongoing debate

about the role of fatty acids, and mainly of SAFA in the causality of atherosclerosis and its organ complications, mainly CHD. Examples of data in favor of the role of SAFA are those of the SCS [3,8] summarized in the Introduction to which we may add, as examples, two more recent large studies run in the UK and in the USA [13,14].

Among the few selected findings that negate the role of SAFA [15–18], a large metanalysis did not find any connection between SAFA intake and CHD but this conclusion should be taken with much caution since frequently the metanalytic process may distort the truth due to important methodological problems [15]. On the other hand, the PURE project [16] run in population samples of 18 mainly developing countries, attributed to carbohydrates a major causal role in etiology of CHD. However, in this case carbohydrates were not distinguished between simple and complex ones [8,33], the SAFA intake was in general of a magnitude suggested for preventive purposes and in many areas the overall diets were close to those producing malnutrition. Other contributions were simply narrative and expressed opinions more than producing facts. Probably, the wisest position is that of the "third party" that suggests to not insist on single nutrients and prefers to concentrate on dietary patterns such as the Mediterranean Diet and other healthy diets [19,20]. There are suggestions that considering the null role of SAFA, the whole causality should be made by PUFA, but this idea is contradicted by our data where PUFA alone are not significantly associated with any of the considered end-points as shown in Table 5.

Beyond the uncertainties of the present knowledge and literature, we like to briefly report the facts, as seen from two different research projects and different approaches. The Global Burden of Disease Study 2017 [34] provided the so-called dietary risks in 195 countries worldwide and defined a list of 15 exposures dangerous for health. Some aspects reflect the need to adhere to some characteristics of the Mediterranean Diet, but since nowadays it is fashion, not a single word is mentioned about SAFA while a low PUFA intake is classified as dangerous for health as well a generic high consumption of meat.

On the other hand, rare mention in the literature is made of the North Karelia Project [35] where two large regions of Finland were chosen as community treatment and control, respectively. The preventive action included anti-smoking campaign, community control of hypertension and dietary advice, the last one including substitution of butter with soft margarine, of whole milk with skimmed milk and an increased intake of plant foods. The effects were a substantial reduction of population serum cholesterol and a drastic decrease of incidence and mortality from CHD. These attitudes later spread to the whole country and presently Finland in classified as a low-risk country after having been the country with the highest rate of CHD worldwide.

Table 5 Linear correlation coefficients between mortality from 4 CVD end-points and all-cause mortality in 50 years using 16 cohorts versus 4 indexes of atherogenicity and thrombogenicity, mean cohort intake of SAFA, MUFA, PUFA and TRANS, lnMAI and serum cholesterol.

	CHD50	HDUE50	STR50	STHD50	ALL50
ATI	**0.93***	−0.02	−0.34	−0.20	**0.55***
THI	**0.86***	0.16	−0.15	0.0006	**0.62***
HPI	**−0.73***	0.05	0.24	0.17	**−0.53***
HH	**−0.71***	0.003	0.16	0.09	**−0.59***
SAFA	**0.89***	0.11	−0.22	−0.06	**0.60***
MUFA	0.14	0.33	−0.01	0.18	0.05
PUFA	0.17	0.16	0.11	0.16	0.07
TRANS	**0.53***	−0.25	−0.49	−0.41	0.22
lnMAI	**−0.91***	0.01	0.42	0.25	−0.39
CHOL	**0.78***	−0.39	**−0.73***	**−0.62***	0.11

CHD50 = Coronary heart disease, 50 years; HDUE50 = Heart disease of uncertain etiology, 50 years; STR50 = stroke, 50 years; STHD50 = STROKE + HDUE, 50 years; ALL50 = all causes, 50 years.
ATI =Atherogencity index.
THI= Thrombogenicity index.
HPI=Health promoting index.
HH = hypocholesterolemic/hypercholesterolemic ratio.
SAFA = saturated fatty acids.
MUFA = mono-unsaturated fatty acids.
PUFA = poly-unsaturated fatty acids.
lnMAI = natural log of Mediterranean Adequacy Index.
CHOL = serum cholesterol.
(*) Significant p (in bold).

5. Conclusion

The ATI and THI proposed in 1991 and slightly modified here, were highly associated at ecological level with long-term CHD mortality in the 16 cohorts of the SCS, confirming previous findings dealing with lipid intake and metabolism. This association was not found for mortality from STR and HDUE, suggesting that CHD is a substantially different disease than STR or HDUE. Findings contradict the idea that saturated fat are irrelevant in the etiology of atherosclerosis and cardiovascular disease, mainly CHD, but the counterproof would be the replication of this study using single individuals as statistical units instead of entire cohorts.

Informed consent

Baseline measurements were taken before the era of the Helsinki Declaration, and approval was implied in participation, while verbal or written consent was obtained for the collection of follow-up data.

Certifications

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.numecd.2024.05.010.

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