

## Dietary fatty acids and CHD: from specific recommendations to dietary patterns<sup>‡</sup>

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### Abstract

Several countries have issued dietary recommendations about total and specific fatty acid (FA) intake for the prevention of CHD. For many years until today, controversies have existed especially about the deleterious effect or not of SFA, and the protective effect or not of *n*-3 PUFA, so that some authors have criticised these recommendations. There are many reasons for these controversies, including the different conclusions of prospective cohort studies compared with randomised clinical trials (RCT), and the contradictory conclusions of meta-analyses depending on the quality, number and type of studies included. The interrelationships between different FA in the diet make it difficult to analyse the specific effect of a particular class of FA on CHD. Furthermore, based on clinical practice and effectiveness of population-based prevention, it is very difficult at the individual level to assess in personal dietary intake the actual percentage and/or amount of SFA contained in each meal or consumed daily/weekly. In this critical narrative review, we try to answer the question of whether it would not be more relevant, in 2020, to promote dietary patterns, rather than FA intake recommendations. We critically analyse past and recent data on the association of FA with CHD, then propose that the Mediterranean diet and Japanese diet should be revitalised for Westerners and Asian populations, respectively. This does not exclude the usefulness of continuing research about effects of FA towards CHD, and accepting that what seems true today might be revised, at least partially tomorrow.

**Key words:** SFA: Unsaturated fatty acids: *n*-3 Fatty acids: Cardiovascular risk: Mediterranean diet: Japanese diet

(Received 10 August 2020; revised 19 December 2020; accepted 28 December 2020; accepted manuscript published online 7 January 2021)

### Historical background

Over recent years, new data from cohort studies, randomised clinical trials (RCT) and meta-analyses have changed the view regarding the association of fatty acids (FA) with CHD. This has not ended controversies about dietary fat guidelines in the USA and Europe<sup>(1,2)</sup>, and has even led to catchy headlines in some newspapers/magazines. Our objective, in this critical narrative review, is to try to answer this question: do recommendations about specific FA intake remain useful today for primary/secondary CHD prevention? This is not provocative and the response we propose aims to be pragmatic for clinical practice and/or prevention messages. We chose to analyse non-exhaustive past and recent data, which we hope will better help to understand the controversies and to answer that question.

Historically, three groups of studies/observations have been the source of interest regarding the association of intake of different types of FA with CHD risk: the Seven Countries Study led by Keys for both the potential deleterious role of SFA, and the protective role of the Mediterranean diet (MedDiet), Bang & Dyerberg's studies for the possible protective role of long-chain (LC) *n*-3 PUFA, and the epidemiological observation of a very

low rate of CHD in Japanese whose traditional diet (JapDiet) was very low in fat.

### Seven Countries Study

Ansel Keys and co-workers carried out the Seven Countries Study, which included, between 1958 and 1964, 12 763 healthy men aged 40–59 years. They were enrolled in one of the sixteen cohorts from seven countries. Eleven cohorts were formed from men living in rural areas of Finland, Greece, Italy, Yugoslavia and Japan, two from railroad employees living in USA and Italy, one from men working in a large cooperative in Serbia, one from university professors in Belgrade, and one from men living in a small market town in the Netherlands. After a 15-year follow-up, the rate of CHD was thirty-two times higher in East Finland than in Crete<sup>(3)</sup>. The Seven Countries Study was an ecological study that means that only the data from average populations, and not individual data, were used to establish the correlations. Differences in mean age, blood pressure, serum cholesterol and smoking habits explained 80 % of the variance of CHD deaths between cohorts. All death rates were positively related to total dietary energy percentage of SFA on the one hand, and

**Abbreviations:** ALA,  $\alpha$ -linolenic acid; CHO, carbohydrate; CV, cardiovascular; FA, fatty acid; HPFS, Health Professionals Follow-up Study; IMedDiet, Indo-Mediterranean diet; JapDiet, Japanese diet; LA, linoleic acid; LC, long-chain; MACE, major adverse cardiovascular event; MedDiet, Mediterranean diet; MI, myocardial infarction; NHS, Nurses' Health Study; OO, olive oil; RCT, randomised clinical trial; REDUCE-IT, Reduction of Cardiovascular Events with Icosapent Ethyl-Intervention Trial; RR, relative risk; STRENGTH, STatin Residual Risk Reduction With EpaNova in HiGh CV Risk PatientS With Hypertriglyceridemia; TEI, total energy intake; TFA, *trans*-fatty acids.

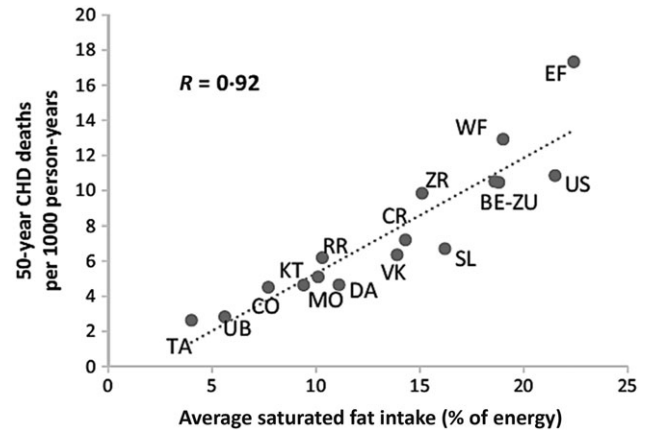
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<sup>‡</sup>This article was originally published with an omission in the author's affiliation. The affiliation has been corrected and the online PDF and HTML versions updated.

negatively to that of MUFA, oleic acid and the MUFA:SFA ratio on the other hand. No association was found with PUFA intake. All-cause and CHD deaths were low in cohorts with olive oil (OO) as the main dietary fat. Fifteen-year age-standardised CHD death rates per 10 000 was 2.3 times lower in rural Mediterranean areas (Dalmatia, Monte Giorgio, Crevalcore, Crete and Corfu) than in their rural counterparts in non-Mediterranean Europe (East and West Finland, Suwononia and Velika Krsna). After a 25-year follow-up, 47 % of men included had died<sup>(4)</sup>. Dietary samples had been taken at the beginning of the study and analysed 25 years later in 1987 from equivalent food composites. This analysis revealed a very huge difference in the amount of SFA consumed between cohorts at entry in the study (3.8 % of total energy intake (TEI) in Japan cohort *v.* 22.7 % in East Finland). The average intake of all major SFA as a whole was strongly positively associated with CHD deaths ( $r$  0.88;  $P < 0.001$ ) as well as individually lauric, myristic, palmitic and stearic acids ( $r$  0.81 to 0.86; all  $P < 0.001$ ). C18 : 1 *trans*-fatty acid (TFA) intake was also associated with CHD mortality ( $r$  0.78;  $P < 0.001$ ). The association with dietary cholesterol was weaker ( $r$  0.55;  $P < 0.05$ ). In multivariate analysis models, only SFA, antioxidants, flavonoids and smoking were independently associated with CHD mortality rates; SFA were the major determinant explaining 73 % of the total variance. Multivariate analysis selected butter, lard + margarine and meat as significant predictors of CHD mortality rate ( $r^2$  0.922)<sup>(5)</sup>. Lastly, the comparative (ecological) associations of SFA intake of the whole population from the sixteen cohorts at inclusion in the study and CHD mortality rate after 50 years of follow-up showed a very strong positive correlation ( $r$  0.92;  $P < 0.05$ ) (Fig. 1) with CHD mortality, while MedDiet adequacy index was negatively correlated ( $r = -0.91$ )<sup>(6)</sup>.

### Bang & Dyerberg studies in Inuits

The interest for the CHD protective effect of LC *n*-3 PUFA arose from the Bang & Dyerberg hypothesis in the 1970s that the lower prevalence of CHD among Inuits from Greenland compared with a Danish population might be explained by the high content in LC *n*-3 PUFA of their traditional food, leading, via the generation of A3 thromboxane and prostacyclin, to a lower platelet aggregability and vasodilatation. During the 1970s, the age-adjusted CHD mortality among Greenland Inuit males aged 45–65 years was 5.3 % as compared with 40.4 % in the USA. Bang & Dyerberg, plus a laboratory technician (Nielsen), made their first sled expedition in 1970 to Greenland. They found that among Inuit hunter-fishermen and their wives (aged 40 years and over) living in the Umanaq district of Igdlorsuit village, 500 km north of the Arctic Circle (latitude 71°N), all plasma lipoprotein concentrations were lower than those of Danes, except for HDL-cholesterol concentrations, which were higher<sup>(7)</sup>. In addition, plasma lipoprotein concentrations were higher in Inuit women who had emigrated and lived in Denmark than in those still living in Greenland<sup>(7)</sup>. This argued more in favour of a protective dietary environmental factor than in a genetic one. The *n*-6 PUFA plasma concentrations of Inuits were 50 to 30 % lower than those of Danes while LC *n*-3 PUFA concentrations were five to ten times higher. These differences were not observed between Inuits living in Denmark and Danes; hence,



**Fig. 1.** Relationship of average population saturated fat intake at baseline and 50-year CHD death rates (from Kromhout *et al.*<sup>(6)</sup>). US, US railroad; EF, East Finland; WF, West Finland; ZU, Zutphen, the Netherlands; CR, Crevalcore, Italy; MO, Montegiorgio, Italy; RR, Rome railroad, Italy; DA, Dalmatia, Croatia; SL, Slavonia, Croatia; VK, Velika Krsna, Serbia; ZR, Zrenjanin, Serbia; BE, Belgrade, Serbia; KT, Crete, Greece; CO, Corfu, Greece; TA, Tanushimaru, Japan; UB, Ushibuka, Japan.

the conclusion that a different content in FA of food consumed probably explained the differences<sup>(8)</sup>. Total LC *n*-3 PUFA intake accounted for 13.1 % of total FA among Inuit *v.* 0.8 % among Danes, 18 : *n*-6 accounted for 5 *v.* 10 %, and 18 : 3*n*-3, respectively: 0.6 *v.* 2 %<sup>(9)</sup>. The difference in FA composition was due to the high consumption of marine products (rich in LC *n*-3 PUFA) by Inuits *v.* a high consumption of dairy products by the Danes<sup>(10)</sup>. Another aspect highlighted by Bang & Dyerberg was the low platelet aggregation and long bleeding time in Inuits<sup>(11)</sup>. In their 1980 article<sup>(10)</sup>, the authors pointed out that the factors that could explain the lower incidence of CHD among Inuits, even though they were very heavy smokers, were: a protective lipid profile, lower platelet aggregation, low prevalence of obesity, hypertension and lack of diabetes. This perfectly reflected the knowledge of that time! The anti-thrombotic effects of LC *n*-3 PUFA – EPA increases the production of inactive thromboxane A3 (TBXA3), decreases that of thromboxane A2 (TBXA2), increases concentrations of anti-thrombin III, deformability of erythrocytes, and decreased blood viscosity – were subsequently confirmed by other studies<sup>(12)</sup>. These biochemical alterations led Dyerberg & Bang to hypothesise that the anti-thrombotic effect of LC *n*-3 PUFA probably explained the lower incidence of myocardial infarction (MI) in Greenland Inuits<sup>(13)</sup>. In a 1990 review<sup>(12)</sup>, Bang used the three following observations from scientific literature as arguments in favour of the potential protective role of LC *n*-3 PUFA towards MI: (a) Nelson's 1972 work<sup>(14)</sup> in eighty patients showing that 16–19 years following a CHD event, mortality was lower among those who consumed fish compared with those who never consumed it; (b) the observation during the Second World War of a significant decrease in thrombo-embolic pathologies in Norwegians whose intakes of EPA were of 4–5 g/d, due to a sharp increase in fish consumption at the expense of dairy products, eggs and meat whose availability was significantly decreased<sup>(15)</sup>; and (c) the confirmation of Bang & Dyerberg data in Japanese fishermen (consuming 250 g/d of fish, equivalent to 2.5 g/d EPA) compared with control



farmers (90 g/d of fish equivalent to 0.9 g/d EPA), showing higher EPA/DHA plasma concentrations in fishermen associated with lower platelet aggregation<sup>(16)</sup>.

### Very low incidence of CHD in Okinawan and Japanese populations

At the end of Second World War, individuals living in Okinawa prefecture (Ryukyu Islands) had the longest duration of life in the world. In 1995, death from CHD was 35 % lower in Okinawa than in Japan and 83 % lower than in the USA. This longevity, resulting mainly from lower incidence of CHD and cancers, has been attributed to their lifestyle and more specifically to their very specific diet resulting from multiple historic influences from Japan, continental China and countries of South-East Asia<sup>(17)</sup>. Dietary surveys from 1879<sup>(18)</sup>, 1919<sup>(19)</sup> and 1949<sup>(20,21)</sup> are available. The survey from 1879 showed that everyday diet was made of 93 % of sweet potato, the remaining being made of minor grains, wheat, millet, barnyard grains, sugar-beet and rice, which left not much place for fat. The survey from 1919 showed that 93 % of energy intake was from carbohydrates (CHO), 7.1 % from proteins and only 1.7 % from fat. The survey from 1949 showed that CHO accounted for 85 % of energy (79 % in other Japan areas), proteins for 9 % (13 % in other Japanese areas) and fat for 6 % only (8 % in other Japan areas). The composition of fat intake was 30.8 % SFA, 30 % MUFA and 40 % PUFA. Because of the Westernisation that began in the 1950s, the incidence of CHD has increased among the Okinawans, indirectly confirming the very likely protective role of their traditional diet and lifestyle in their low incidence of CHD and high longevity. In 2018, Japanese women had the longest (87.32 years) life span in the world and men had the third longest (81.25 years) life span in the world<sup>(22)</sup> with consistently low morbidity and mortality from CHD<sup>(23)</sup>. Japanese are not genetically protected from CHD risk factors. Indeed, the N-Hon-San study showed that Japanese immigrants living in Hawaii and San Francisco Bay Area and Honolulu had higher plasma cholesterol and CHD incidence than those living in Hiroshima and Nagasaki<sup>(24,25)</sup>. As underlined by Tada *et al.*<sup>(26)</sup> in their review, total cholesterol in Japanese is now reaching a level similar to that of Americans, but the incidence of CHD in Japanese remains approximately one-fifth to one-third of that in Americans. Altogether, this supports evidence that dietary habits, beyond a low-fat diet, and lifestyle influence CHD rates in Japanese and not genetic background.

In summary, the Seven Countries Study reported a very strong ecological association between SFA intake and CHD mortality in middle-aged men followed for 50 years. This was also the first study highlighting the possible/probable protective effect of a MedDiet pattern and CHD mortality (see the Seven Countries Study section). Bang & Dyerberg's pioneering works led to their hypothesis that the low incidence of MI in Inuits living in Greenland could be related to their high consumption of marine products rich in LC *n*-3 PUFA that favoured a less atherogenic lipid profile and lower platelet aggregation, without neglecting the role of their high physical activity during the 1970s. As to Okinawans and Japanese, although their traditional diet (JapDiet) is very low in total fat intake and high in LC *n*-3

PUFA, it is probably the whole diet, more than these amounts of specific FA, that explains their low incidence of CHD.

In the next sections, we critically analyse separately the data about the association of TFA, SFA, MUFA, plant PUFA and LC *n*-3 PUFA intake with CHD. The association is presented as relative risk (RR).

### Trans-fatty acids and CHD

Industrially produced TFA, formed during partial hydrogenation of vegetable oils, increase CHD risk<sup>(27–29)</sup>, which is now widely accepted, so that it does not deserve any more comment, except that they must be completely suppressed as soon as possible from global food supplies as proposed by the REPLACE action package released by the WHO<sup>(30)</sup>. In 2015, the Food & Drug Administration concluded that TFA were not generally recognised as safe and decided they must be removed from industrial products by 1 January 2021<sup>(31)</sup>. The main mechanisms advocated for their deleterious effects are an increase in LDL-cholesterol and decrease in HDL-cholesterol concentrations, an increase in inflammation and an induction of endothelial dysfunction<sup>(27)</sup>.

### SFA and CHD

#### Cohort studies

Zong *et al.*<sup>(32)</sup> included 81 557 women from the Nurses' Health Study (NHS) and 51 529 men from the Health Professionals Follow-up Study (HPFS) with 24–28 years of follow-up. Association with CHD was analysed according to quintiles of SFA intake. The sum of LC SFA (12 : 0 to 18 : 0; major SFA of the diet) was positively related to CHD for each cohort as well as for the pooled cohorts (RR 1.18; 95 % CI 1.09, 1.28;  $P_{\text{trend}} < 0.001$ ). RR was 1.04 (95 % CI 0.96, 1.12; NS) for short-medium-chain SFA. RR for each of LC SFA were all significant (1.07 for 12 : 0, 1.13 for 14 : 0, 1.18 for 16 : 0 and 1.18 for 18 : 0) (Table 1). It is noteworthy that a recent meta-analysis of sixteen studies<sup>(33)</sup> shows that coconut oil (rich in short-medium-chain SFA) increases LDL-cholesterol by 10.47 mg/dl (0.27 mmol/l) and HDL-cholesterol by 4.00 mg/dl (0.10 mmol/l) as compared with non-tropical vegetable oils, leading the authors to conclude that it is not a healthy oil. The study of association of SFA with CHD by comparing their foods sources (by example meats *v.* dairy or different dairy sources between them) is confounded by the other constituents of these foods as recently reviewed<sup>(34)</sup>, which precludes a clear conclusion about a direct association of their content in SFA with CHD. The Prospective Urban Rural Epidemiology (PURE) cohort<sup>(35)</sup> included 135 335 participants, aged 35–70 years from eighteen countries (three high-income, eleven middle-income and four low-income); the USA and European countries except Sweden were not included. After 7.4 years of follow-up, the higher quintile of SFA intake was not associated with MI or CHD mortality. The National Institutes of Health-American Association of Retired Persons (AARP) Diet and Health Study cohort<sup>(36)</sup> included 521 120 participants aged 50–71 years (16 years of follow up). The highest quintile of SFA intake was associated with CHD mortality (RR 1.29; 95 % CI 1.25, 1.33;  $P_{\text{trend}} < 0.0001$ ).

**Table 1.** Relative risks of intake of individual SFA and risk of CHD in the Nurses' Health Study (NHS; 1984–2010) and Health Professionals Follow-up Study (HPFS; 1986–2010) (adapted from Zong *et al.*<sup>(32)</sup>) (Relative risks (RR) and 95 % confidence intervals)

	Quintile categories of SFA as a percentage of total energy intake									<i>P</i> <sub>trend</sub>
	1 (reference)	2		3		4		5		
	RR	RR	95 % CI	RR	95 % CI	RR	95 % CI	RR	95 % CI	
4 : 0–10 : 0										
NHS + HPFS*	1	1.03	0.95, 1.11	1.01	0.93, 1.09	1.04	0.96, 1.12	1.04	0.96, 1.12	0.30
12 : 0										
NHS + HPFS*	1	1.00	0.93, 1.08	1.03	0.95, 1.11	1.01	0.94, 1.09	1.07	0.99, 1.15	0.05
14 : 0										
NHS + HPFS*	1	1.02	0.94, 1.10	1.06	0.98, 1.15	1.09	1.01, 1.17	1.13	1.05, 1.22	<0.001
16 : 0										
NHS + HPFS*	1	1.06	0.98, 1.15	1.09	1.01, 1.18	1.15	1.06, 1.24	1.18	1.09, 1.27	<0.001
18 : 0										
NHS + HPFS*	1	1.08	1.00, 1.17	1.11	1.02, 1.20	1.20	1.11, 1.30	1.18	1.09, 1.28	<0.001
12 : 0–18 : 0										
NHS + HPFS*	1	1.06	0.98, 1.15	1.08	1.00, 1.17	1.17	1.08, 1.26	1.18	1.09, 1.28	<0.001

\* Multivariable model (for more details, see Nelson<sup>(14)</sup>).

### Meta-analyses

Skeaff & Miller<sup>(37)</sup>, in 2009, found no association of SFA intake with CHD death (eight cohorts) (RR 1.14; 95 % CI 0.82, 1.60) or CHD events (five cohorts) (RR 0.93; 95 % CI 0.83, 1.05). Siri-Tarino *et al.*<sup>(38)</sup>, in 2010, included twenty-one prospective cohorts (347 747 subjects; 5–23 years of follow-up; 11 006 CHD events or stroke). SFA intake was not associated with CHD (sixteen cohorts; RR 1.07; 95 % CI 0.96, 1.19). There was heterogeneity among studies, not explained by age, sex, sample size, duration of follow-up, medical record review and study quality score. Chowdhury *et al.*<sup>(39)</sup> performed, in 2014, a meta-analysis of thirty-two observational studies (512 420 subjects, 5–23 years of follow-up, 15 945 CHD events). They found no association of SFA with CHD, comparing the highest tertile of intake with the lowest (RR 1.03; 95 % CI 0.98, 1.07). TFA were significantly associated with a higher risk (RR 1.16; 95 % CI 1.06, 1.27) and LC *n*-3 PUFA with a lower risk (RR 0.87; 95 % CI 0.78, 0.97). The conclusion of Chowdhury *et al.*<sup>(39)</sup> was: 'Current evidence does not clearly support cardiovascular guidelines that encourage high consumption of PUFA and low consumption of total saturated fats'. That conclusion led Mark Bittman to write an article in *The New York Times* entitled 'Butter is back'<sup>(40)</sup> and, 3 months later, Brian Walsh to cover 'Eat Butter. Scientists labelled fat the enemy. Why they were wrong' on the front page of *Time* magazine<sup>(41)</sup>. Zhu *et al.*<sup>(29)</sup> performed, in 2019, a meta-analysis including fifty-six cohorts. Highest *v.* lowest SFA intake was not associated with CHD (RR 0.97; CI 0.93, 1.02). There was a significant heterogeneity, poorly explained by all covariates explored in meta-regression.

To better clarify the nature of the association of SFA with CHD, it is quite useful to look at the effect of their partial replacement by other FA or different types of CHO.

### Substitution/reduction studies of SFA and CHD

Several studies from the same group<sup>(28,42,43)</sup> assessed the effects of the different types of FA in 83 349 women from the NHS and 42 884 men from the HPFS. Comparisons were made by quintiles of fat intake. The percentage of energy intakes from total fat or

specific FA intake was calculated as cumulative average up to the start of each 2- or 4-year follow-up interval (Table 2). For multivariable analyses, isoenergetic substitution models were built, which simultaneously included energy intake, percentages of energy derived from protein and specific types of fat and other potentially confounding variables. Thus, as underlined by the authors, the coefficients from these models can be interpreted as the estimated effect of substituting a certain percentage of energy from fat for equivalent energy from CHO. By using these models, TFA were associated with a 20 % increase in cardiovascular (CV) mortality (*P*<sub>trend</sub> < 0.001); SFA intake, when replaced by total CHO, was not related to CV mortality. PUFA and MUFA intakes were inversely associated with CV mortality (both *P*<sub>trend</sub> < 0.001). The replacement of 5 % of SFA by CHO from whole grains was associated with a 9 % lower risk of CHD (*P* = 0.01), whereas it was not if replaced with refined CHO (*P* > 0.10) (Table 3). When 5 % of SFA intake was replaced by MUFA or PUFA, the risk of CHD decreased by 15 % (*P* = 0.02) and 25 % (*P* = 0.0001), respectively. The Women's Health Initiative Dietary Modification Trial<sup>(44)</sup>, a RCT (48 835 US women, 50–79 years, follow-up 8.3 years), showed that a low-fat-diet (20 % TEI) decreased CHD by 30 % in primary prevention (RR 0.70; 95 % CI 0.56, 0.87; *P* = 0.003), but increased it by 47 % in secondary prevention (RR 1.47; 95 % CI 1.12, 1.93). The authors indicated that secondary prevention outcomes were skewed by post-randomisation use of cholesterol-lowering drugs. Mozaffarian *et al.*<sup>(45)</sup>, in 2010, performed a meta-analysis of eight RCT (13 614 participants; 1042 CHD events; follow-up > 1 year). Average PUFA consumption was 14.9 % TEI in intervention groups *v.* 5.0 % in controls. Overall, CHD risk reduction was 19 % (RR 0.81; 95 % CI 0.70, 0.95; *P* = 0.008), corresponding to 10 % reduced risk (RR 0.90; 95 % CI 0.83, 0.97) for each 5 % energy increase from PUFA. Hooper *et al.*<sup>(46)</sup>, in 2015, performed a meta-analysis of fifteen RCT (59 000 participants). Reducing SFA intake reduced CHD events by 17 % (RR 0.83; 95 % CI 0.72, 0.96). Harcombe *et al.*<sup>(2)</sup>, in 2016, performed a meta-analysis of ten RCT (62 421 participants; 1218 CHD deaths) and found no effect of reduced/modified fat intake towards CHD mortality (RR 0.98; 95 % CI 0.88, 1.08).

**Table 2.** Hazard ratios of CHD by intake of fatty acids as percentages of total energy (adapted from Li *et al.*<sup>(42)</sup>) (Hazard ratios (HR) and 95 % confidence intervals)

	Quintile categories of fat intake as a percentage of total energy intake									
	1 (reference)	2		3		4		5		<i>P</i> <sub>trend</sub>
	HR	HR	95 % CI	HR	95 % CI	HR	95 % CI	HR	95 % CI	
Total fatty acids										
NHS + HPFS*	1	0.94	0.87, 1.01	0.91	0.85, 0.99	0.97	0.89, 1.04	0.88	0.8, 0.96	0.02
SFA										
NHS + HPFS*	1	0.96	0.89, 1.04	0.96	0.87, 1.05	0.95	0.85, 1.05	0.93	0.82, 1.05	0.46
TFA										
NHS + HPFS*	1	1.13	1.05, 1.22	1.17	1.08, 1.27	1.18	1.08, 1.30	1.20	1.09, 1.32	0.002
PUFA										
NHS + HPFS*	1	0.96	0.89, 1.03	0.85	0.78, 0.92	0.90	0.84, 0.9	0.80	0.73, 0.88	<0.0001
MUFA										
NHS + HPFS*	1	0.99	0.91, 1.08	1.03	0.93, 1.13	0.99	0.89, 1.11	1.00	0.87, 1.13	0.86

NHS, Nurses' Health Study (1984–2010); HPFS, Health Professionals Follow-up Study (1986–2010); TFA, *trans*-fatty acids.

\* Multivariable model (for more details, see Division of Health, Labor and Welfare, Okinawa Prefectural Government<sup>(21)</sup>).

**Table 3.** Multivariable hazard ratios of CHD with isoenergetic (percentage of energy) substitutions of one dietary component for another in the Nurses' Health Study (NHS; 1984–2010) and the Health Professionals Follow-up Study (HPFS) (1986–2010) (adapted from Li *et al.*<sup>(42)</sup>) (Hazard ratios (HR) and 95 % confidence intervals)

Isoenergetic substitutions of SFA by energy from	NHS + HPFS		<i>P</i>
	HR	95 % CI	
<i>Trans</i> -fatty acids (2 % of energy)	1.05	0.93, 1.19	0.41
MUFA (5 % of energy)	0.85	0.74, 0.97	0.02
PUFA (5 % of energy)	0.75	0.67, 0.84	< 0.0001
Carbohydrate from refined starches/sugars (5 % of energy)	1.01	0.95, 1.06	0.81
Carbohydrate from whole grains (5 % of energy)	0.91	0.85, 0.98	0.01

In summary, during many years, especially since the Seven Countries Study<sup>(3)</sup>, it has been advocated that CHD was related to SFA intake and most if not all of international dietary recommendations counselled that their intake should be less than 10 % of TEI. Metabolic studies have shown that diets high in SFA and low in PUFA increased plasma cholesterol concentrations<sup>(47)</sup>. It was generally advocated that SFA increased CHD risk by elevating plasma cholesterol on the one hand, and that different types of SFA had a different effect on plasma lipoproteins, so that they could not be similarly atherogenic<sup>(48)</sup> on the other hand. Meta-analysis failed to demonstrate a link between SFA intake and CHD events and/or CHD mortality at odds with individual cohorts (except the Prospective Urban Rural Epidemiology (PURE) cohort, which found no association). As very often in science and life, the truth is probably intermediate between these extremes. SFA are probably not as deleterious as previously thought, but the lack of association observed in most meta-analyses between their intake and CHD does not prove their total safety. Indeed, the substitution of a part of SFA for PUFA, MUFA or CHO from whole grains reduces CHD risk, which pleads against complete safety of SFA toward CHD. Thus, their consumption *ad libitum* ('eat butter') is certainly not a good way to prevent CHD. Altogether, this could explain controversies about adequacy of dietary SFA guidelines for CHD risk<sup>(1,2)</sup>. Among the reasons of controversy about the deleterious effect or not of SFA, the following can be cited: the CV effect of

decreasing SFA depends on the nutrients and foods that are substituted, the effect of SFA on plasma LDL-cholesterol concentrations can vary significantly among individuals, individual SFA have differing biological effects, the food matrix can modify the CV risk in response to SFA, and overall nutrient distribution should be considered, which is also true for other types of FA, except industrially formed TFA which are clearly always deleterious.

### MUFA intake and CHD

The most common MUFA of the human diet is oleic acid. The specific interest for their potential protective CV effect was born with the Seven Countries Study<sup>(3)</sup>, showing a lower CHD risk in populations consuming the MedDiet known to be rich in OO. We will separate studies aiming to assess the association of total MUFA whatever their dietary sources (animal and plant) from those studying the effect of OO, knowing that its content is not exclusively oleic acid but also polyphenols (especially in extra-virgin OO).

### Cohorts

Hu *et al.*<sup>(49)</sup> prospectively studied 80 082 women aged 34–59 years who had no history of CHD events, followed up for 14 years. Dietary intake was assessed by a FFQ. The analysis of

the association of FA intake with CHD risk was performed comparing quintiles of FA intake and by analysis of the effect of substitution of a dietary component by another one. There was no association between MUFA intake and CHD risk along the five quintiles of intake (from 11 to 19.3 % of energy) (RR 0.95; 95 % CI 0.64, 1.39). As compared with CHO, an increase in 5 % of energy from MUFA was associated with a 29 % decreased risk (RR 0.81; 95 % CI 0.65, 1.00;  $P=0.05$ ). In an extension of follow-up to 20 years of the NHS, the authors<sup>(50)</sup> found no association across the quintiles of MUFA intake and CHD, while they found a significant inverse association for PUFA and a significant positive association for TFA. In the PREvencion con Dieta MEDiterranea (PREDIMED) study (7038 subjects at high CV risk followed up for 6 years)<sup>(51)</sup>, the highest quintile of MUFA intake (compared with the lowest) was inversely associated with CHD (RR 0.50; 95 % CI 0.31, 0.81). In substitution analysis, replacing 5 % of energy from SFA by MUFA decreased CHD by 37 % (RR 0.63; 95 % CI 0.43, 0.94)<sup>(24)</sup>. In that study, MUFA were mainly from extra-virgin OO, which also contains polyphenols.

### Meta-analyses

Jakobsen *et al.*<sup>(52)</sup>, in 2009, performed a meta-analysis of eleven cohort studies (344 696 participants; 4–10 years of follow-up; 5249 CHD events; 2155 CHD deaths). There was tendency to a positive association between MUFA intake and CHD events (RR 1.19; 95 % CI 1.00, 1.42) when substituted for SFA. Chowdhury *et al.*<sup>(39)</sup>, in 2014, included nine prospective cohorts (144 219 participants; 6031 events); they found no association of MUFA intake with CHD (RR 1.00; 95 % CI 0.91, 1.10). Schwingshackl & Hoffmann, in 2014, performed two meta-analyses<sup>(53,54)</sup>, one including thirty-two cohorts (841 211 participants; primary prevention)<sup>(53)</sup>, the other, twelve RCT (7150 participants; follow-up 1–6 years; secondary prevention)<sup>(54)</sup>. Regarding cohorts, MUFA intake was inversely associated with CHD mortality (RR 0.88; 95 % CI 0.80, 0.96;  $P=0.004$ ) and CHD events (RR 0.91; 95 % CI 0.86, 0.96;  $P=0.001$ ). The association was only significant with OO for CHD events. Regarding meta-analysis of RCT, comparing a reduced/modified fat intake (fat < 30 % of TEI, MUFA: 8–26 % TEI) with a control diet, MUFA intake was not associated with CHD mortality, CHD events and MI. Zhu *et al.*<sup>(29)</sup>, in 2019, performed a meta-analysis of forty-three cohort studies and found no association of MUFA with CHD events. Martínez-González *et al.*<sup>(55)</sup> performed a meta-analysis of case-control, cohort and intervention studies to assess the association of OO consumption with CHD and/or stroke. Nine studies, all conducted in Mediterranean countries, were included (101 460 participants for CHD and 38 673 for stroke; follow-up 4.8 to 10.6 years). Combining all studies, with CHD and stroke as end points, showed an inverse association of OO consumption with CHD (RR 0.82; 95 % CI 0.70, 0.96;  $P=0.01$ ). Secondary analysis showed no association of OO with CHD (ten studies) (for an increase in 25 g/d: RR 0.87; 95 % CI 0.78, 1.18;  $P=0.14$ ).

In summary, discrepancies clearly exist; there is reasonable evidence of an inverse association of MUFA intake with CHD when substituted for SFA. For OO, there is no proof of a protective effect *per se* towards CHD, but possibly towards stroke.

Some reasons for the controversy are that MUFA are tightly associated with SFA in many foodstuffs, such as red meat, and that OO is not only rich in oleic acid but also contains SFA and polyphenols (extra-virgin OO).

## $\alpha$ -Linolenic acid and CHD

### Cohorts

The Multiple Risk Factor Intervention Trial<sup>(56)</sup> was a US primary prevention study in men at high CHD risk (usual-care group 6250 participants; 10.5 years of follow-up); the highest quintile of  $\alpha$ -linolenic acid (ALA) intake expressed as a percentage of TEI was inversely associated with CHD deaths (RR 0.58;  $P<0.05$ ). When expressed as quintiles of g/d of intake, the association was not significant (RR 0.66; NS). It is noteworthy that mean LC *n*-3 PUFA intake was low (175 mg/d). The HPFS<sup>(57)</sup> included 43 757 participants, followed for 16 years. Intake of ALA (as % TEI) was inversely associated with MI (RR 0.41 for a 1 % increase in energy;  $P_{\text{trend}} < 0.01$ ), but not with fatal CHD. The association was not significant for a range of ALA intake of 0.9–1.5 g/d. In an update of the HPFS, Mozaffarian *et al.*<sup>(58)</sup> reported that after 14 years of follow-up, for each 1 g/d intake of ALA, there was lower risk of non-fatal MI (RR 0.42; 95 % CI 0.23, 0.75;  $P=0.003$ ) and total CHD (RR 0.53; 95 % CI 0.34, 0.83;  $P=0.006$ ), but only when LC *n*-3 PUFA intake was < 100 mg/d. The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study was a Finnish study in male smokers ( $n$  21 930; 6.1 years of follow-up)<sup>(59)</sup>. No association was found between ALA intake and CHD death over a range of intake of 0.9 to 2.5 g/d. The Singapore Chinese Health Study<sup>(60)</sup> included 63 257 participants, aged 45–74 years, with a follow-up of 13 to 18 years. ALA intake was inversely associated with CHD mortality when comparing the highest (0.80 g/d) with lowest (0.40 g/d) quartiles (RR 0.82; 95 % CI 0.71, 0.93;  $P_{\text{trend}} = 0.001$ ). The association was significant only in individuals without prior history of CHD. The NHS<sup>(61)</sup> recruited 76 283 US women without prior CHD, followed for 10 years. In multivariate analysis, fatal CHD was inversely associated with the highest quintile of intake of ALA (1.36 g/d) as compared with the lowest (0.71 g/d) (RR 0.55; 95 % CI 0.32, 0.94;  $P_{\text{trend}} = 0.01$ ). Non-fatal CHD was not significantly associated (RR 0.85; 95 % CI 0.61, 1.19;  $P=0.50$ ). An updated analysis of the NHS (76 763 women; follow-up 18 years)<sup>(62)</sup> showed an inverse association with the fourth (0.60 % TEI) and fifth (0.75 % TEI) quintiles of ALA intake as compared with the lowest (0.37 % TEI) for sudden cardiac death (RR 0.62 (95 % CI 0.39, 0.98) and RR 0.60 (95 % CI 0.37, 0.96), respectively;  $P_{\text{trend}} = 0.02$ ) but not for other fatal CHD or non-fatal CHD. A Netherlands cohort<sup>(63)</sup> included 20 069 healthy men and women, aged 20–65 years with a follow-up of 8–13 years. No association was observed between the highest quintile of ALA intake (> 1.9 g/d) *v.* the lowest (< 0.9 g/d) (RR 1.12; 95 % CI 0.72, 1.75). The Pooling Project of Cohort Studies on Diet and Coronary Disease<sup>(64)</sup> gathered eight cohorts (four from the USA, two from Finland and one from Sweden) including 148 675 women and 80 368 men with a follow-up of 4–10 years. For each additional 1 g of ALA consumed, a 15 % lower risk of CHD events (hazard ratio 0.85; 95 % CI 0.72, 1.01; NS) and a 23 % lower risk of CHD deaths (hazard ratio 0.77, 95 % CI 0.58, 1.01; NS) were observed. No consistent



association was observed among women. No effect of the modification by the intake of LC *n*-3 PUFA was observed. Other studies found no association<sup>(65–68)</sup>.

### Adipose tissue $\alpha$ -linolenic acid and CHD

Three studies assessed the association between adipose tissue content of ALA with CHD. The Diet, Cancer and Health Study<sup>(69)</sup> included 3500 Danish participants, aged 50–64 years, with a 17-year follow-up. Multivariate analysis showed that ALA dietary intake (range < 1.67 g/d to  $\geq$  2.54 g/d) was not associated with MI (RR 0.90 (95 % CI 0.76, 1.07) for men; RR 1.10 (95 % CI 0.83, 1.45) for women). ALA concentrations in adipose tissue were also not associated with MI (RR 1.27 (95 % CI 0.94, 1.72) for men, and RR 1.13 (95 % CI 0.75, 1.71) for women). In a case–control study from Costa-Rica<sup>(70)</sup> including 1819 cases with a first MI and 1819 controls, the levels of ALA intake ranged from 0.42 to 0.86 % of TEI. Greater ALA (in adipose tissue or as dietary intake) was associated with a lower risk of MI (RR 0.41 (95 % CI, 0.25, 0.67) for adipose tissue ALA, and 0.61 (95 % CI 0.42, 0.88) for dietary ALA). The relationship between ALA and MI was non-linear; risk did not decrease with intakes > 0.65 % of energy (1.79 g/d). The EUROpean multicenter case–control study on Antioxidants, Myocardial Infarction and breast Cancer study (EURAMIC)<sup>(71)</sup> is a case–control study (639 cases and 700 controls) conducted in eight European countries and Israel. After adjustment for classical CHD risk factors, comparing the highest quintile with the lowest concentrations of ALA in adipose tissue, there was no significant association with MI (RR 0.68; 95 % CI 0.31, 1.49).

### Meta-analyses

Pan *et al.*<sup>(72)</sup>, in 2012, included nineteen prospective cohorts and eight retrospective case–control studies (251 049 individuals, 15 327 CVD events, follow-up 5–30.7 years). The overall pooled RR was 0.86 (95 % CI 0.77, 0.97). In subgroups, the association was significant only for fatal CHD (RR 0.80; 95 % CI 0.65, 0.98). Chowdhury *et al.*<sup>(39)</sup>, in 2014, included seven prospective cohorts (157 258 participants) and four RCT (9444 participants). No association was found. Del Gobbo *et al.*<sup>(73)</sup>, in 2016, pooled nineteen cohort studies. Biomarkers of ALA were inversely associated with fatal CHD (RR 0.91; 95 % CI 0.84, 0.98) but not total CHD (RR 1.00; 95 % CI 0.95, 1.05). Balk & Lichtenstein<sup>(74)</sup> have summarised the 2016 update of the 2004 Agency of Healthcare Research and Quality evidence review of *n*-3 FA and CVD. By meta-analysis, no association was found. Wei *et al.*<sup>(75)</sup>, in 2018, carried out a meta-analysis of fourteen cohorts. Higher ALA intake was associated with modest reduced CHD (RR 0.91; 95 % CI 0.85, 0.97) and fatal CHD (RR 0.85; 95 % CI 0.75, 0.96). Abdelhamid *et al.*<sup>(76)</sup>, in 2020, performed a meta-analysis of RCT and found no effect of ALA on CHD mortality or events.

In summary, results are contradictory. Most cohorts do not find association of ALA intake or its biomarkers with CHD. Meta-analyses generally conclude that there is a lack of or a weak significant association. Whether ALA has a protective effect towards CHD, it might exist in individuals with a very low intake of LC *n*-3 PUFA (< 100 or < 175 mg/d) as suggested by two studies<sup>(56,58)</sup>. Among reasons explaining the controversial effect of

ALA, one should consider that it may not be protective *per se*, but only when LC *n*-3 PUFA intake is low so that it could participate in restoring by elongation and desaturation to a higher, even if modest, increase in LC *n*-3 PUFA concentrations in membrane phospholipids, or when given as part of a MedDiet.

### *n*-6 PUFA and CHD

The main *n*-6 PUFA in the diet is linoleic acid (LA). Because maize or safflower oil is used as the main source of PUFA in RCT, the LA effect *per se* is included in this section.

### Randomised clinical trials

Several RCT have been designed over the years. Rose *et al.*<sup>(77)</sup>, in 1965, performed a secondary prevention RCT (eighty London patients included but analysis from forty-three after 2 years). Patients were divided into three groups: no advice (controls), reduction of animal fats plus OO (80 g/d), and reduction of animal fats plus maize oil (80 g/d). After 2 years of follow-up, 75 % of controls remained free of new CHD, whereas only 57 and 52 % in the OO and maize oil groups, respectively (0.1 >  $P$ >0.05). The conclusion of the authors was that under the circumstances of their study, maize oil could not be recommended in the treatment of CHD. The main limitation of the study is the small number of patients remaining included at 2 years. The Oslo Diet-Heart Study<sup>(78)</sup>, initiated in the 1950s, is a secondary prevention RCT (412 men; aged 30–64 years; followed for 11 years). Patients were randomly allocated to the experimental diet group (39 % of TEI from fat including 8.5 % SFA, 20.7 % PUFA and 10.1 % MUFA) or to the control diet group. The detailed clinical follow-up including dietary advice was undertaken only during the first 5 years of the 11-year follow-up. We only report the results of the 11-year follow-up. The experimental diet analysed in seventeen participants contained 39 % of TEI from fat including 8.5 % SFA, 20.7 % PUFA and 10.1 % MUFA. The composition of the control diet is not reported but probably was the usual Norwegian diet at that time. After 5 years of follow-up the mean cholesterol reduction in the experimental diet group was 17.6 % *v.* 3.7 % in the control group, and combined CHD major relapses (MI and new angina pectoris) were reduced by 24.7 % ( $P=0.05$ ), but not MI or CV deaths. After 11 years of follow-up fatal MI was 43.9 % lower in the experimental diet group ( $P=0.004$ ). The Finnish Mental Hospital Study<sup>(79)</sup>, initiated in the 1960s, aimed to study the association of an experimental diet rich in PUFA with CHD mortality in patients from two mental hospitals regardless of pre-existing CHD, with a 12-year follow-up. A cross-over design was used (6 years  $\times$  6 years). The PUFA:SFA ratio of the usual diet was 0.22–0.29 *v.* 1.42–1.78 for the experimental one. Mean plasma cholesterol was 12–18 % lower with the experimental diet. The experimental diet decreased age-adjusted CHD deaths: 6.61 *v.* 14.8 per 1000 person-years for males ( $P<0.002$ ) and 5.21 *v.* 7.90 for females (NS). The Minnesota Coronary Survey<sup>(80,81)</sup> was a secondary prevention RCT conducted from 1968 in six Minnesota mental state hospitals and one nursing home (4393 men and 4664 women; follow-up 4.5 years). The trial compared a 39 % fat control diet (18 % SFA; 5 % PUFA; 16 % MUFA; 446 mg cholesterol per d) with a 38 % fat



treatment diet (9 % SFA; 15 % PUFA; 14 % MUFA; 166 mg cholesterol per d) on plasma cholesterol levels and the incidence of MI and sudden deaths. No difference was observed between the two groups for those end points. The Sydney Diet Heart Study<sup>(82)</sup> is a secondary prevention RCT (men aged 30–59 years followed from 1966 to 1973). The intervention group ( $n$  221) increased PUFA intake (from safflower) to about 15 % of TEI, and reduced SFA to less than 10 % of TEI and dietary cholesterol to 300 mg/d. The control group ( $n$  237) received no instructions. CHD mortality was higher in the intervention group (16.3 *v.* 10.1 %; RR 1.74; 95 % CI 1.04, 2.92;  $P=0.036$ ). It is noteworthy that the *n*-6 PUFA intervention was delivered in a margarine that probably had a high content of TFA, which may have contributed to the higher CHD mortality. The other studies modelling, in cohorts such as the NHS, HPHS and others, the replacement of percentage of energy (generally 5 %) from SFA with PUFA have been discussed earlier (Substitution/reduction studies of SFA and CHD section). They generally showed a decrease in CHD events and/or mortality.

In summary, all these studies increased the PUFA:SFA ratio to decrease plasma cholesterol, so that the specific effect of *n*-6 PUFA towards CHD is not proved.

### Meta-analyses

Ramsden *et al.*<sup>(83)</sup>, in 2010, performed a meta-analysis including seven RCT responding to the following criteria: dietary PUFA were increased in replacement of SFA and/or TFA, and end points were non-fatal MI and CHD deaths. A separate analysis of whole datasets, of RCT with specific *n*-6 PUFA diet, and of RCT with mixed *n*-6/*n*-3 PUFA diets was made. For non-fatal MI + CHD death, the pooled risk reduction for mixed *n*-3/*n*-6 PUFA diets was 22 % (RR 0.78; 95 % CI 0.65, 0.93) compared with an increased risk of 13 % for *n*-6 specific PUFA diets (RR 1.13; 95 % CI 0.84, 1.53). Risk of non-fatal MI + CHD death was higher in *n*-6 specific PUFA diets compared with mixed *n*-3/*n*-6 PUFA diets ( $P=0.02$ ). It was concluded that substitution of SFA with *n*-6 PUFA was not useful and even deleterious, whereas substituting with mixed *n*-6/*n*-3 PUFA was protective. Ramsden *et al.*<sup>(81)</sup>, in 2016, have included the results of their recovered data from the Minnesota Coronary Experiment in an updated meta-analysis of LA intervention trials. There was a tendency towards an increased risk of death from CHD (RR 1.33; 95 % CI 0.99, 1.79;  $P=0.06$ ). Farvid *et al.*<sup>(84)</sup>, in 2014, included thirteen cohort studies (310 602 individuals; 12 470 CHD events including 5882 CHD deaths). Comparing the highest *v.* the lowest category of intake, LA was associated with a lower total CHD risk (RR 0.85; 95 % CI 0.78, 0.92) and CHD deaths (RR 0.79; 95 % CI 0.71, 0.89). A 5 % energy increment in LA intake replacing the equivalent energy from SFA was associated with a 9 % lower risk of events (RR 0.91; 95 % CI 0.86, 0.96) and a 13 % lower risk of CHD deaths (RR 0.85; 95 % CI 0.82, 0.94). Hamley<sup>(85)</sup>, in 2017, performed a meta-analysis of nineteen RCT aiming to analyse the replacement of SFA with mostly *n*-6 PUFA. Five of them were adequately controlled. No association of replacement of SFA with *n*-6 PUFA was found with major CHD events (RR 1.06; 95 % CI 0.86, 1.31), total CHD events (RR 1.02; 95 % CI 0.84, 1.23) or CHD mortality (RR 1.13; 95 % CI 0.91, 1.40). The pooled results from all nineteen trials suggested

that replacing SFA with mostly *n*-6 PUFA significantly reduced total CHD events (RR 0.80; 95 % CI 0.65, 0.98,  $P=0.03$ ), but neither major CHD events nor CHD mortality. Hooper *et al.*<sup>(86)</sup>, in 2018, performed a meta-analysis of twelve trials. *n*-6 PUFA generally replaced SFA or MUFA. They found no association with CHD events (RR 0.88; 95 % CI 0.66, 1.17; seven trials) or MI (RR 0.88; 95 % CI 0.76, 1.02; seven trials). Marklund *et al.*<sup>(87)</sup>, in 2019, performed an individual-level pooled analysis of thirty cohort studies aiming to assess the association between biomarkers of dietary *n*-6 FA intake (in plasma and erythrocyte phospholipids, total plasma or cholesteryl esters, adipose tissue) and incident CVD and mortality. Mean age was 49–77 years, median follow-up was 2.5–31.9 years. Higher levels of LA were significantly associated with lower risks of total CVD and CV mortality, with RR per interquintile range of 0.93 (95 % CI 0.88, 0.99) and 0.78 (95 % CI 0.70, 0.85), respectively. By comparing extreme quintiles, higher levels of LA were associated with a significantly lower risk of CVD mortality (RR 0.77; 95 % CI 0.69, 0.86) but not of total CVD or CHD. Li *et al.*<sup>(88)</sup>, in 2020, performed a meta-analysis of the association of dietary intake and biomarkers of LA with mortality including CVD mortality. They included forty-four prospective cohorts (eighteen reporting association of LA intake and twenty-two of LA biomarkers with mortality; 811 069 participants with dietary intake assessment (50 786 CVD deaths) and 65 411 participants with biomarker measurements (6492 CVD deaths)). The pooled RR comparing extreme categories of dietary LA intake was 0.87 (95 % CI 0.82, 0.92) for CVD mortality. The pooled RR for each 1 sd increment in LA concentrations in adipose tissue/blood compartments was 0.89 (95 % CI 0.85, 0.94) for CVD mortality. The different causes of CVD mortality are not reported.

In summary, *n*-6 PUFA are not deleterious for CV risk as suggested before, but are probably not cardioprotective *per se*. They contribute, in association with ALA, to decrease the CHD risk when replacing SFA. There is not much controversy about the fact that *n*-6 PUFA have been studied in substitution of SFA to decrease plasma cholesterol or associated with ALA as a component of plant PUFA, which can explain that their protective effect *per se* cannot be studied, on difference with ALA or LC *n*-3 PUFA (EPA/DHA) which can be added as a dietary supplementation and not as substitutes of other FA.

### Long-chain *n*-3 PUFA and CHD

The CV protective effect of LC *n*-3 PUFA has become over time, as well as for SFA, a subject of hard controversy due mainly to an extreme heterogeneity of designs of RCT, further complicated by the multiplication of contradictory meta-analyses. They sometimes include studies of a very different nature, with very different populations, different doses given, different forms (fish oils, TAG, ethyl esters, others), EPA + DHA or EPA alone, sometimes associated with CV treatments (statin, aspirin), and different placebos.

### Primary prevention

In the NHS, the highest quintile of LC *n*-3 PUFA intake was inversely associated with CHD events (RR 0.69; 95 % CI 0.57, 0.84;  $P<0.001$ )<sup>(89)</sup>. As to non-fatal MI, RR were 0.23 (95 % CI



0.09, 0.55;  $P=0.001$ ) for plasma EPA and 0.46 (95 % CI 0.18, 1.16;  $P_{\text{trend}}=0.07$ ) for DHA<sup>(90)</sup>. Still in the NHS (30-year follow-up)<sup>(91)</sup>, sudden cardiac death was reduced by 37 % for the highest quintile of intake (RR 0.63; 95 % CI 0.44, 0.90;  $P_{\text{trend}}=0.03$ ). In the Physicians' Health Study, Albert *et al.*<sup>(92)</sup> (22 071 men; aged 40–80 years; 17 years of follow-up) found an inverse association of plasma LC *n*-3 PUFA with sudden cardiac death for the highest quartile of intake (RR 0.19; 95 % CI 0.05, 0.71;  $P_{\text{trend}}=0.001$ ). Lemaitre *et al.*<sup>(93)</sup> carried out a case–control study from the Cardiovascular Health Study. In those who died from MI, plasma phospholipid concentrations of EPA + DHA were lower than those of controls (RR 0.23; 95 % CI 0.12, 0.76;  $P=0.01$ ), but no association was observed in those victims of a non-fatal MI. In the Ludwigshafen Risk and Cardiovascular Health Study<sup>(94)</sup> (3316 patients hospitalised for coronary angiography; follow-up 9.9 years), the highest tertile of omega-3 index (sum of EPA + DHA/total FA in phospholipids of erythrocyte membranes)<sup>(95)</sup> was associated with a lower CHD mortality (RR 0.78; 95 % CI 0.64, 0.95;  $P=0.015$ ). The Framingham Heart Study Offspring cohort<sup>(96)</sup> (2500 participants; mean age 66 years; follow-up of 7.3 years) found that an omega-3 index > 6.8 % was inversely associated with total CVD events (RR 0.61; 95 % CI 0.37, 0.99;  $P=0.008$ ). The NIH-AARP-HS (617 119 participants aged 50–71 years; 16 years of follow-up)<sup>(96)</sup> found that the highest quintile of intake was associated with a modest lower CHD mortality (RR 0.98; 95 % CI 0.97, 0.99). The Japan Public Health Centre-based study<sup>(97)</sup> (116 896 participants, aged 40–69 years, 14–17 years of follow-up) showed that the highest quartile of plasma concentrations of LC *n*-3 PUFA (divided into quartiles) was inversely associated with sudden cardiac death (RR 0.08; 95 % CI 0.01, 0.88;  $P_{\text{trend}}=0.04$ ) and CHD mortality (RR 0.12; 95 % CI 0.02, 0.75;  $P_{\text{trend}}=0.03$ ). ASCEND (A Study of Cardiovascular Events in Diabetes)<sup>(98)</sup> is a primary prevention study involving 15 480 diabetics followed for 7.4 years. Four groups were compared. Two groups received 460 mg EPA plus 380 mg DHA/d with ( $n$  3870) or without ( $n$  3870) concomitant 100 mg/d aspirin. There was a significant decrease in CHD mortality (RR 0.81; 95 % CI 0.67, 0.99). VITAL (Vitamin D and Omega-3 Trial)<sup>(99,100)</sup> is a primary prevention study aiming to evaluate the effect of daily vitamin D<sub>3</sub> (2000 IU) or fish oil (460 mg EPA + 380 mg DHA) on primary prevention of cancer and CHD (in patients at high CHD risk; 25 871 US men aged ≥ 50 years and women aged ≥ 55 years, including 5106 African Americans). After a 5.3-year follow-up, LC *n*-3 PUFA supplementation had no effect on the composite end point (MI, stroke, CHD mortality), but was associated with significant reductions in total MI (RR 0.72; 95 % CI 0.59, 0.90), percutaneous coronary intervention (RR 0.78; 95 % CI 0.63, 0.95) and fatal MI (RR 0.50; 95 % CI 0.26, 0.97). In African Americans, MI was reduced by 77 % (RR 0.23; 95 % CI 0.11, 0.47). The UK Biobank study<sup>(101)</sup> (427 648 men and women aged 40–69 years from the general population living in England, Scotland and Wales; follow-up 8–12 years) assessed the association of habitual fish oil supplementation with CVD and mortality. RR associated with fish oil use was 0.84 (95 % CI 0.78, 0.91) for CVD mortality, 0.80 (95 % CI 0.70, 0.91) for MI mortality, 0.93 (95 % CI 0.90, 0.96) for CVD, and 0.92 (95 % CI 0.88, 0.96) for MI (all  $P<0.05$ ). The association was stronger among participants with hypertension ( $P=0.005$ ).

### Secondary CHD prevention

The Diet and Reinfarction Trial<sup>(102)</sup> (2033 patients; 2-year follow-up) showed that the consumption of 200–400 g/week of fatty fishes was inversely associated with CHD mortality (RR 0.71; 95 % CI 0.54, 0.93;  $P<0.01$ ), but not new CHD events. The GISSI-Prevenzione trial<sup>(103)</sup> (11 324 patients with MI within the previous 3 months before inclusion, followed for 3.5 years) showed that 850 mg/d of EPA+DHA ethyl esters reduced the composite end point (CV death, non-fatal MI, and non-fatal stroke) by 15 % (RR 0.85; 95 % CI 0.68, 0.95;  $P=0.008$ ), and CHD deaths by 35 % (RR 0.65; 95 % CI 0.51, 0.84). The Japan EPA Lipid Intervention Study (JELIS)<sup>(104)</sup> was both a primary ( $n$  14 981) and secondary CHD prevention Japanese study ( $n$  3664 patients, plasma cholesterol ≥ 2.52 g/l, follow-up: mean 4.6 years). EPA (1.8 g/d) plus statin, as compared with statin alone, reduced CHD events by 19 % in the whole group (RR 0.81; 95 % CI 0.69, 0.95;  $P=0.011$ ). The decrease was significant for the 'secondary prevention' group (RR 0.81; 95 % CI 0.66, 1.00;  $P=0.048$ ), but not for the 'primary prevention group' (RR 0.82; 95 % CI 0.63, 1.06;  $P=0.132$ ). In a subsequent analysis, it was shown that EPA decreased risk of CHD by 38 % in subjects who did not achieve the objective of non-HDL and/or of LDL-cholesterol plasma concentrations with statin<sup>(105)</sup>. OMEGA<sup>(106)</sup> (4837 patients; aged 60–80 years with a history of MI; follow-up 40 months) found no effect of a small dose of EPA+DHA (400 mg/d). The Supplementation with FOLate, vitamins B6 and B12 and/or OMEGA-3 fatty acids (SU.FOL.OM3) trial<sup>(107)</sup> assessed the effects of vitamins B and EPA+DHA (600 mg/d) (2501 patients with a recent history of ischaemic stroke; follow-up 4.7 years). No effect of EPA+DHA was observed. The Outcome Reduction with an Initial Glargine Intervention (ORIGIN) trial<sup>(108)</sup> studied the effects of EPA+DHA supplementation (840 mg/d) on CV deaths and CHD events in 12 536 pre-diabetic or diabetic patients, followed for 6.2 years. No effect was observed. The Risk and Prevention Study<sup>(109)</sup> (12 513 patients, 5 years of follow-up) assessed the efficacy of a 1 g/d intake of EPA+DHA (ethyl esters). No benefit was observed for whole patients but the main composite end point (mortality, MI and non-fatal stroke) was decreased in women (RR 0.82; 95 % CI 0.67, 0.99;  $P=0.04$ ). The Cardiovascular Outcome Study, an ancillary study of the Age-Related Eye Disease Study 2 study<sup>(110)</sup> (4203 patients; aged 50–85 years; 4.8 years of follow-up), found no benefit of 1 g/d EPA+DHA. The following studies used pharmaceutical forms of EPA, beyond the scope of our paper, but we decided to include them because of their novelty. The Reduction of Cardiovascular Events with Icosapent Ethyl–Intervention Trial (REDUCE-IT)<sup>(111)</sup> included 8179 patients with a prior CHD history (70 % of patients) or not (secondary + primary prevention subgroups), all with plasma TAG ≥ 2 g/l and receiving a statin; 58 % had diabetes. Patients received either 4 g/d of EPA icosapentethyl or placebo. After a median follow-up of 4.9 years, the primary composite end point (non-fatal CHD, MI and stroke mortality, coronary revascularisation or unstable angina) was reduced by 25 % with EPA icosapentethyl ( $P<0.001$ ). The effect was significant only in the secondary prevention cohort. The key secondary composite end point was reduced by 26 % ( $P<0.001$ ). All pre-specified end points were also reduced with EPA: CV

death or non-fatal MI by 25 % ( $P < 0.001$ ), fatal or non-fatal MI by 31 % ( $P < 0.001$ ), urgent or emergency revascularisation by 35 % ( $P < 0.001$ ), CV death by 20 % ( $P = 0.03$ ), hospitalisation for unstable angina by 32 % ( $P = 0.002$ ), fatal or non-fatal stroke by 38 % ( $P = 0.01$ ). REDUCE-IT USA<sup>(112)</sup> analysed the results of the REDUCE-IT trial in the group of the 3146 US patients only (38.5 % of the trial). The primary composite end point was reduced by 31 % ( $P = 0.000001$ ) as well as the key secondary composite end point ( $P = 0.00008$ ). CHD death was reduced by 34 % ( $P = 0.007$ ), and MI by 28 % ( $P = 0.01$ ). The STRENGTH (STatin Residual Risk Reduction With EpaNova in HiGh CV Risk PatienTs With Hypertriglyceridemia) trial<sup>(113)</sup> aimed to evaluate the effects of 4 g daily of carboxylic acids of EPA+DHA compared with maize oil as a placebo, on major adverse cardiovascular events (MACE) in patients on optimal statin therapy with mixed dyslipidaemia and at high risk for CVD. It has been prematurely halted based on an interim analysis indicating a low probability of clinical benefits of EPA+DHA carboxylic acids. A total of 13 078 patients at high CV risk (aged  $62.5 \pm 9$  years, 70 % with diabetes and at least 50 % with criteria of secondary CV prevention), with mixed dyslipidaemia (hypertriglycerolaemia: 180 mg/dl (2.03 mmol/l) to 500 mg/dl (5.65 mmol/l); HDL-cholesterol  $< 42$  mg/dl ( $< 1.08$  mmol/l) in men and  $< 47$  mg/dl ( $< 1.22$  mmol/l) in women), and all treated with statins (LDL-cholesterol  $< 100$  mg/dl ( $< 2.59$  mmol/l)) were enrolled at 675 sites in twenty-two countries. After 54 months, RR for MACE was 0.99 (95 % CI 0.90, 1.09). Several explanations have been proposed to explain the discrepant results with those of the REDUCE-IT trial: lower duration of STRENGTH, maize oil *v.* mineral oil as placebo, carboxylic acids of EPA + DHA *v.* EPA icosapentethyl, 70 % of patients with diabetes *v.* 58 %. Even if the design of the STRENGTH and REDUCE-IT trials look very similar, the absence of an effect observed in the STRENGTH trial, for whatever reason, does not invalidate the protective effect observed in the REDUCE-IT study. The United States Food & Drug Administration<sup>(114)</sup> approved, in December 2019, the use of icosapentethyl of EPA as an adjunctive therapy to reduce the risk of CV events in patients meeting the inclusion criteria of REDUCE-IT.

### Meta-analyses

We will only pick up the recent largest ones. For others, see Innes & Calder<sup>(115)</sup>. The very large meta-analysis by Alexander *et al.*<sup>(116)</sup>, in 2017, included eighteen RCT and sixteen prospective cohort studies. The overall meta-analysis of RCT found no association of LC *n*-3 PUFA with CHD events (RR 0.94; 95 % CI 0.85, 1.05). In contrast, LC *n*-3 PUFA reduced significantly the risk of CHD events in subgroups of patients at high CV risk with plasma TAG  $\geq 1.5$  g/l (RR 0.84; 95 % CI 0.72, 0.98) or LDL-cholesterol  $\geq 1.30$  g/l (RR 0.86; 95 % CI 0.76, 0.98). An EPA + DHA dose  $> 1$  g/d lowered the risk in those with plasma TAG  $\geq 1.50$  g/l (RR 0.75; 95 % CI 0.64, 0.89). CHD deaths were reduced by 19 % (RR 0.81; 95 % CI 0.65, 1.00) only in secondary prevention (RR 0.80; 95 % CI 0.64, 0.99). As to cohorts, CHD events were reduced by 18 % (RR 0.82; 95 % CI 0.74, 0.92) for the highest intakes, by 23 % for CHD mortality (RR 0.77; 95 % CI 0.66, 0.90), and by 47 % for sudden cardiac death (RR 0.53; 95 % CI 0.41, 0.67). The meta-analysis by Maki *et al.*<sup>(117)</sup>, in 2017, assessed CV

mortality alone and included fourteen RCT (71 899 subjects; follow-up  $\geq 6$  months). The overall analysis concluded a decrease in 8 % of CHD mortality (RR 0.920;  $P = 0.015$ ). The risk reduction was more pronounced ( $-13$  to  $-29$  %; RR 0.709–0.871) for a dose  $> 1$  g/d (28 % of all subjects included), in patient groups with plasma TAG  $\geq 1.50$  g/l, LDL-cholesterol  $\geq 1.30$  g/l, and in RCT where the proportion of patients treated with statins at inclusion was  $< 40$  % (all significant). These results are consistent with those of Alexander *et al.*<sup>(116)</sup>. The meta-analysis by Hu *et al.*<sup>(118)</sup>, in 2019, included thirteen RCT (127 477 participants; mean follow-up 5 years). When excluding REDUCE-IT, LC *n*-3 PUFA were inversely associated with MI (RR 0.92; 95 % CI 0.86, 0.99;  $P = 0.020$ ), CHD deaths (RR 0.92; 95 % CI 0.86, 0.98;  $P = 0.014$ ) and total CHD (RR 0.95; 95 % CI 0.91, 0.99;  $P = 0.008$ ). Including REDUCE-IT reinforced the inverse associations. CHD and CHD death were linearly related to LC *n*-3 PUFA dose. The meta-analysis by Popoff *et al.*<sup>(119)</sup>, in 2019, included eleven RCT, carried out from 1966 to 2016, in patients who had suffered a MI. They had been randomised to receive within 6 weeks after MI to receive *n*-3 FA from very different sources (fish oils, soyabean oils, seeds, refined EPA, DHA, ALA) or, oil or capsule form or as foodstuffs (MedDiet). The authors conclude that there were no benefits to patient important outcome. However, CV mortality was significantly reduced (RR 0.77; 95 % CI 0.65, 0.91), as well as risk of new MI (RR 0.77; 95 % CI 0.6, 0.90). Aung *et al.*<sup>(120)</sup>, in 2018, performed a meta-analysis including ten trials involving 77 917 individuals at high CV risk, followed for a mean of 4.4 years. The range of EPA dose supplementation was 226–1800 mg/d. They found no significant associations with CHD death rate (RR 0.93; 99 % CI 0.83, 1.03;  $P = 0.05$ ), non-fatal MI (RR 0.97; 99 % CI 0.87, 1.08;  $P = 0.43$ ) or any CHD events (RR 0.96; 95 % CI 0.90, 1.01;  $P = 0.12$ ).

The meta-analysis of RCT by Abdelhamid *et al.*<sup>(76)</sup>, in 2020, focused on the effect of LC *n*-3 PUFA in primary and secondary CHD prevention. LC *n*-3 PUFA reduced CHD events by 9 % (134 116 participants; 8791 events; RR 0.91; 95 % CI 0.85, 0.97; thirty-two RCT), and CHD mortality by 10 % (127 378 participants; 3598 deaths; RR 0.90; 95 % CI 0.81, 1.00; twenty-four RCT). The meta-analysis by Doshi *et al.*<sup>(121)</sup>, in 2020, aimed to compare the effects of combined use of EPA + statin with statin alone. Five RCT were included (27 415 patients; dose of EPA  $\geq 1800$  mg/d) associated or not with a usual dose of statin. EPA + statin resulted in an 18 % reduction in the incidence of MACE (RR 0.78; 95 % CI 0.65, 0.93;  $P < 0.01$ ) and a 30 % reduction in MI (RR 0.71; 95 % CI 0.61, 0.82;  $P < 0.01$ ) as compared with statin alone. Hoang & Kim<sup>(122)</sup>, in 2020, performed a meta-analysis of eighteen RCT using LC *n*-3 supplements for at least 1 year. They decreased CHD by 10 % (RR 0.90; 95 % CI 0.84, 0.96) and MI by 12 % (RR 0.88; 95 % CI 0.83, 0.94). Lombardi *et al.*<sup>(123)</sup> included fourteen RCT (125 763 patients). They compared a high dose ( $> 1$  g/d) of LC *n*-3 PUFA supplementation *v.* control or *v.* a low dose  $\leq 1$  g/d). The high dose was associated with a 21 % decrease in cardiac mortality (RR 0.79; 95 % CI 0.65, 0.96;  $P = 0.03$  *v.* control), a 29 % decrease in MI (RR 0.71 (95 % CI 0.62, 0.82);  $P < 0.0001$  *v.* control; and 0.79 (95 % CI 0.67, 0.92);  $P = 0.003$  *v.* low dose), a 26 % decrease in coronary revascularisation (RR 0.74 (95 % CI 0.66, 0.83);  $P < 0.0001$  *v.* control; and 0.74 (95 % CI 0.66, 0.84);  $P < 0.0001$  *v.* low dose) and a 27 % decrease in unstable angina (RR 0.73 (95 % CI 0.62, 0.86);  $P = 0.0001$  *v.* control; and 0.74 (95 % CI

0.62, 0.89);  $P=0.002$  *v.* low dose). Casula *et al.*<sup>(124)</sup>, in 2020, included sixteen RCT (81 073 patients with previous CV events, dose of LC *n*-3 PUFA  $\geq 1$  g/d for  $\geq 1$  year). LC *n*-3 PUFA supplementation was associated with a 9 % decrease in cardiac mortality (RR 0.91; 95 % CI 0.85, 0.98), a 9 % decrease in MACE (RR 0.90; 95 % CI 0.82, 0.99) and a 17 % decrease in MI (RR 0.83; 95 % CI 0.71, 0.98). Cabiddu *et al.*<sup>(125)</sup>, in 2020, performed a trial-sequential analysis of eleven RCT in primary/secondary prevention (100 609 patients; EPA + DHA dose: 376–2000 mg/d). Cardiac mortality was reduced by 6.3 % (RR 0.937; 95 % CI 0.88, 0.98;  $P=0.018$ ). They also concluded from their analysis that no further trials were needed to better evaluate the efficacy of LC *n*-3 PUFA in preventing cardiac mortality. Bernasconi *et al.*<sup>(126)</sup>, in 2020, performed a meta-analysis and meta-regression of all forty RCT (135 267 participants) published before August 2019 evaluating the effects of an intervention consisting exclusively in dietary supplementation with EPA/DHA (dosage: 400 mg/d to 5500 mg/d, weighted average dosage: 1221 mg/d) on CV outcomes. The authors extracted the data from a meta-analysis by Abdelhamid *et al.*<sup>(76)</sup>, except for four studies with EPA/DHA dietary advice only. Supplementation was associated with a 13 % decrease in MI (RR 0.87; 95 % CI 0.80, 0.96), a 10 % decrease in CHD events (RR 0.90; 95 % CI 0.84, 0.97), a 35 % decrease in fatal MI (RR 0.65; 95 % CI 0.46, 0.91) and a 9 % decrease in CHD mortality (RR 0.91; 95 % CI 0.85, 0.98). Meta-regression showed that the effect was dose dependent for MI (each additional 1 g/d was associated with a significant risk reduction of 9.0 %) and for CHD mortality and fatal MI (protective effect achieved with less than 800–1200 mg/d, then quickly plateauing). The authors were unable to conclude that EPA alone had a superior effect to EPA+DHA. They found no evidence that the effect magnitude for any of the CV outcomes studied depended on the year of publication of the RCT. Regarding the effectiveness of EPA/DHA in primary or secondary prevention, most trials have been performed in patients at high CV risk, so that it can be concluded that EPA/DHA supplementation was protective in secondary prevention. However, the fact that the effect of supplementation did not increase with baseline risk in that meta-analysis provides, according to the authors, some confidence that findings about effectiveness can be generalised to prevention in lower-risk populations.

In summary, prospective cohort studies in primary prevention are all consistent in concluding that LC *n*-3 PUFA are protective in primary prevention for the highest or intermediate intakes, confirmed by meta-analysis by Alexander *et al.*<sup>(116)</sup>. Most large RCT *v.* placebo also support a CHD protective effect in secondary prevention with about a 20 % decrease in CHD death and 30 % decrease in CHD events with doses of EPA + DHA  $\geq 900$  mg/d in secondary CHD prevention. This is confirmed by several recent meta-analyses<sup>(118,120–126)</sup>. Negative studies for some of them can be criticised for their lack of power and/or by the use of OO as a placebo. In addition, it should be noted that CV drugs (statins, aspirin and post-infarction medications) have probably masked partially the effects of LC *n*-3 PUFA. The populations likely to benefit the most in secondary prevention are those at high CV risk with in particular hypertriglycerolaemia ( $\geq 1.50$  g/l) and/or high LDL ( $\geq 1.30$  g/l)<sup>(116)</sup>. The EPA + DHA dose that appears to be effective is the one at least for cardiac mortality that makes it possible to achieve an omega-3 index of  $\geq 8$  %<sup>(127)</sup>,

corresponding to an intake of approximately  $\geq 900$  mg/d of EPA + DHA, which can be achieved by a multiple-week intake of marine products at high concentrations of LC *n*-3 PUFA. The recommendations of the American Heart Association expert panel that revised the previous recommendations in 2017 are in agreement with this<sup>(128)</sup>. The reasons for the controversy on the CV protective effect of EPA/DHA have been cited above, but it is now, in 2020, in view of the consistent results of the seven most recent meta-analyses (the last one including forty RCT), very difficult to assert that LC *n*-3 PUFA do not have a protective effect against CHD, especially in patients at high CV risk. It is very unlikely that the negative result of the recent STRENGTH trial will significantly change this conclusion when included in a further meta-analysis, although it is useful to better understand why no effect was observed. In all cases, LC *n*-3 PUFA should be associated with lifestyle modifications and specific management/treatment of other risk factors.

### Are the recommendations concerning fatty acid intakes for cardiovascular prevention useful today?

The answer is both yes and no. Yes, because it is always useful to assess on the basis of basic, epidemiological and nutritional sciences what specific nutrients can be beneficial or deleterious. Typical examples are the proved deleterious effect of TFA. No, because assessing and/or proposing recommendations for specific nutrient intakes have some pitfalls, especially the discrepant results of CHD effect of SFA, MUFA, *n*-6 PUFA, ALA and even LC *n*-3 PUFA *per se* although for these last controversy is less marked in 2020 than in the past years. From this observation, the whole food pattern may be more important to consider<sup>(129)</sup>. Indeed, as underlined by Martínez-González *et al.*<sup>(130)</sup>, this approach limits confounding by individual dietary factors and captures the synergistic effects of individual foods and nutrients. It may also provide a more powerful tool to assess the effect of dietary habits on CV health because the effect of a single dietary element is likely to be too small as to be detected in epidemiological studies or RCT. Furthermore, specific nutrient recommendations such as an intake of FA as percentage of energy is not very pertinent for counselling a healthy population and individuals at high CHD risk or with a prior history of CHD, because almost nobody is able to calculate a percentage of energy from specific FA in her/his diet. So, if the response for that reason to our question is no, what dietary pattern(s) should we propose to them? Certainly not the Western diet, which increases CHD risk as compared with other types of dietary pattern<sup>(131–134)</sup>.

If we come back to the Seven Countries Study, cohorts with the lowest CHD incidence during follow-up were those from Tanushimaru and Ushibuka (Japan) and those from Mediterranean areas (Dalmatia, Montegiorgio, Crevalcore, Crete and Corfu). It is of special interest to note that both the MedDiet and Japanese Dietary Culture were inscribed the same year (2013) in the List of the Intangible Cultural Heritage of Humanity<sup>(135,136)</sup>. Thus, can the MedDiet or the JapDiet be considered as the best one(s) if not panacea(s)?



## Mediterranean diet and CHD

### Randomised clinical trials

Three RCT compared the effects of a MedDiet with another diet on CVD, but two of them for different reasons have been criticised. The Lyon Diet Heart study was a secondary prevention French single-blind trial, first reported in 1994 after a mean of 27 months of follow-up<sup>(137)</sup>. Patients with previous MI were separated into two groups. The experimental group ( $n$  302) received a Mediterranean-type diet: more bread, more root vegetables and green vegetables, more fish, less meat (beef, lamb and pork to be replaced with poultry, no day without fruit. Butter and cream had to be replaced with a rapeseed (canola) oil-based margarine whose composition was comparable with OO with 15 % SFA, 48 % oleic acid but 5–4 % 18 : 1 TFA, 16.4 % LA and 4.8 % ALA). Seasoning oils were rapeseed oil and OO exclusively. Moderate wine consumption was allowed at meals. The control group ( $n$  303) received a low-fat step-1 diet of the National Cholesterol Education Program for secondary prevention. The National Cholesterol Education Program diet recommends less than 30 % of energy from total fat, less than 10 % from saturated fat, and less than 300 mg/d of cholesterol. As compared with the control group, the RR of the 'MedDiet' group for CV deaths and CV deaths + non-fatal MI were 0.24 (95 % CI 0.07, 0.85) and 0.27 (95 % CI 0.12, 0.59), respectively. In a further paper, de Lorgeril *et al.*<sup>(138)</sup> excluded bias possibly due to the single-blind randomisation. The extension to 4 years of follow-up of the trial was published in 1999<sup>(139)</sup> and its results confirm the previous ones. RR for major primary end points (cardiac deaths and cardiac deaths + non-fatal MI) were 0.35 (95 % CI 0.15, 0.83;  $P=0.01$ ) and 0.28 (95 % CI 0.15, 0.53;  $P=0.0001$ ), respectively. It is to note that at the end of the study plasma total cholesterol, LDL-cholesterol, HDL-cholesterol and TAG plasma concentrations were not different between the two groups. However, criticisms have been made. The diet was not strictly a MedDiet because of the addition of the margarine rich in ALA on one hand, and because no sufficient consideration was given to OO with an amount of MUFA of 12.9 %, below the usual 15–20 % of the traditional MedDiet on the other hand. Other criticisms formulated were: wide CI and relatively small number of events. In a statement of three American Heart Association committees<sup>(140)</sup>, these others criticisms have been made: the baseline diet was only assessed in the experimental group at the beginning of the study, and the diet of the control group at baseline was presumed to be comparable. Nutrient intake in the control group was only assessed at the conclusion of the study so that it is not clear whether the control group made any dietary changes. In addition, dietary data were reported for only 27 % of the control group and 48 % of the experimental group. It cannot be excluded that the results of the Lyon Diet Heart Study appeared too good to be true (70 % decreased risk!), but, in spite of criticisms made, it would be excessive to consider this trial as discredited.

The Indo-Mediterranean Diet Heart Study<sup>(141)</sup> was both a primary (patients with surrogate markers of CV risk) and secondary prevention trial (patients with a history of CHD) with a 2-year follow-up. All patients were randomly allocated to two groups: one receiving an Indo-Mediterranean Diet (IMedDiet) ( $n$  499)

and the other as control a low-fat step 1 National Cholesterol Education Program diet ( $n$  501). The IMedDiet was rich in whole grains, fruits, vegetables, walnuts and almonds (250–300 g/d of fruit, 125–150 g/d of vegetables and 25–50 g/d of walnuts or almonds). The experimental group was also encouraged to eat 400–500 g/d of whole grains, legumes, rice, maize and wheat, as well as mustard seed or soyabean oil, in 3–4 servings per d). The RR for non-fatal MI, fatal MI, sudden cardiac death, and total cardiac end points were 0.47 (95 % CI 0.28, 0.79), 0.67 (95 % CI 0.31, 1.42), 0.33 (95 % CI 0.13, 0.86) and 0.48 (95 % CI 0.33, 0.71), respectively. However, *The Lancet* published in 2015 an expression of concern<sup>(142)</sup> because of lack of clarity about original data; other concerns have been addressed by Martínez-González *et al.*<sup>(130)</sup> considering it was reasonable to consider the IMedDiet study as discredited, even though it has been included in many reviews and meta-analyses. Such an inclusion questions the validity of the conclusions of these meta-analyses.

The PREvencion con Dieta MEDiterranea (PREDIMED) Study<sup>(143)</sup> is the more recent RCT assessing the effects of a MedDiet for primary CV prevention in 7447 participants with a high CV risk (median follow-up: 4.8 years). They were assigned to a MedDiet supplemented with extra-virgin OO (increase in extra-virgin OO intake to 50 g/d), or a MedDiet supplemented with mixed nuts (increase in extra-virgin OO intake to 32 g/d plus in nuts from 0.9 to > 6 servings/week), or a control diet (advice to reduce dietary fat) (Table 4). The primary end point was a major CV event (MI, stroke, or CV death). RR for the primary end point was 0.70 (95 % CI 0.55, 0.89). RR for the secondary end points were for stroke: 0.58 (95 % CI 0.42, 0.82), for MI: 0.80 (95 % CI 0.53, 1.21), and for CV deaths: 0.80 (95 % CI 0.51, 1.24). Thus, the RR for the composite end point was reduced by 30 %, but when separating the secondary end points, it was significant for stroke only, with a reduction of 42 % of risk.

### Meta-analyses

Sofi *et al.*<sup>(144)</sup>, in 2013, performed a meta-analysis of prospective cohorts studies (4 172 412 subjects) assessing the association of adherence to a MedDiet with CVD incidence and/or mortality risk. A two-point increase in adherence score to the MedDiet was associated with a 10 % reduction of CV risk (RR 0.90; 95 % CI 0.87, 0.92). In 2017, Grosso *et al.*<sup>(145)</sup> performed a meta-analysis of prospective studies and cohorts comparing adherence to a MedDiet with CHD events and CHD deaths. It is noteworthy that the criticised Indo-Mediterranean Diet Heart Study was included<sup>(134)</sup>, CHD incidence was reported in four prospective studies (RR 0.72; 95 % CI 0.60, 0.86) and MI incidence in three (RR 0.67; 95 % CI 0.54, 0.83). After pooling four RCT, MI incidence, stroke incidence, CVD mortality and composite end point were all significantly decreased by 40, 36, 41 and 45 %, respectively. Liyanage *et al.*<sup>(146)</sup>, in 2016, included six RCT comparing the MedDiet with a control diet (10 950 participants). Three RCT, including 9052 participants and 477 events, reported major CV events (RR 0.63; 95 % CI 0.53, 0.75;  $P<0.001$ ). Exclusion of the Indo-Mediterranean Diet Heart Study did not alter the direction of the effect (RR 0.69; 95 % CI 0.55, 0.86;  $P<0.001$ ). No effect on CV mortality was observed (four studies; 10 623 participants and 315 deaths; RR

**Table 4.** Experimental (Mediterranean) diet used during the PREvencion con Dieta MEDiterranea (PREDIMED) study (adapted from Estruch *et al.*<sup>(143)</sup>)

Recommended	Discouraged
Extra-virgin olive oil rich in polyphenols $\geq 4$ tablespoons/d* Tree nuts and groundnuts $\geq 3$ servings/week†	Soda drinks < 1 drink/d Commercial bakery goods, sweets and pastries (not homemade) < 2 servings/week Spread fats < 1 serving/d Red and processed meats < 1 serving/d
Fresh fruits $\geq 3$ servings/d Vegetables $\geq 2$ servings/d Fish (especially fatty fish), seafood $\geq 3$ servings/week Legumes $\geq 3$ servings/week Sofrito $\geq 2$ servings/week White meat instead of red meat Wine with meals (optionally, only for habitual drinkers) $\geq 7$ glasses/week	

\* In the group assigned to the Mediterranean diet with extra-virgin olive oil, the goal was to consume 50 g (approximately 4 tablespoons) or more per d of the polyphenol-rich olive oil supplied.

† For participants assigned to the Mediterranean diet with nuts, the recommended consumption was one daily serving (30 g, composed of 15 g of walnuts, 7.5 g of almonds and 7.5 g of hazelnuts).

0.90; 95 % CI 0.72, 1.11;  $P=0.32$ ). Three RCT reported 221 CHD events. The MedDiet was associated with a 35 % decrease in CHD events (RR 0.65; 95 % CI 0.50, 0.85) but the RR was no more significant when the Indo-Mediterranean Diet Heart Study was excluded from analysis. Rosato *et al.*<sup>(147)</sup>, in 2017 (published in 2019), included twenty-nine observational studies. Comparing the highest with the lowest MedDiet score, they found for total CVD (eleven studies) a RR of 0.81 (95 % CI 0.74, 0.88), and for CHD/MI (eleven studies) a RR of 0.70 (95 % CI 0.62, 0.80). Galbete *et al.*<sup>(148)</sup>, in 2018, performed an umbrella review of meta-analyses of cohort studies evaluating the association of the MedDiet (by using adherence to a Mediterranean Diet Score) with type 2 diabetes, CVD, cancer and cognitive-related diseases. They included twenty-seven meta-analyses based on seventy primary studies. Regarding CVD end points, they found twelve meta-analyses including a total of thirty-one primary studies. A two-point increase in adherence to the MedDiet score was associated with a 28 % lower risk of CHD (RR 0.72; 95 % CI 0.60, 0.86), MI (RR 0.67; 95 % CI 0.54, 0.83) and a 26 % lower risk of acute MI (RR 0.74; 95 % CI 0.66, 0.83). The authors indicate that the MedDiet adherence score utilised was heterogeneous; the two more used scores in primary studies were that proposed by Trichopoulou *et al.*<sup>(149)</sup> and that proposed by Fung *et al.*<sup>(150)</sup>, but this did not alter significantly the results. Rees *et al.*<sup>(151)</sup>, in 2019, performed a Cochrane meta-analysis of RCT aiming to study the effects of a Mediterranean-style diet in primary prevention in subjects at high CV risk and in secondary prevention. The intervention could be dietary advice, provision of relevant foods, or both. The comparison group received no intervention, minimal intervention, usual care or another dietary intervention. Outcomes included clinical events and CVD risk factors. The follow-up periods were of 3 months or more defined as the intervention period plus post-intervention follow-up. The meta-analysis included for primary prevention nine trials (1337 participants) comparing a Mediterranean dietary intervention *v.* no intervention or minimal intervention for primary prevention and thirteen trials comparing the MedDiet with another diet (8687 participants), and for secondary prevention two trials (706 participants) comparing the MedDiet with usual care and six trials (1731 participants). The authors observed a great heterogeneity and high risk of bias.

The authors' conclusion is that the quality of evidence for the modest benefits on CVD risk factors in primary prevention is low or moderate, with a small number of studies reporting minimal harms and that there is a paucity of evidence for secondary prevention. Recently, O'Keefe *et al.*<sup>(152)</sup> proposed that a pescos-Mediterranean diet could be more cardioprotective because of associating the benefits of seafood rich in LC *n*-3 PUFA with that of the traditional MedDiet.

In summary, the MedDiet has clearly a protective effect towards CHD in primary prevention (decreased risk by 10 to 30 %). In secondary prevention, the paucity of trials necessitates further ones.

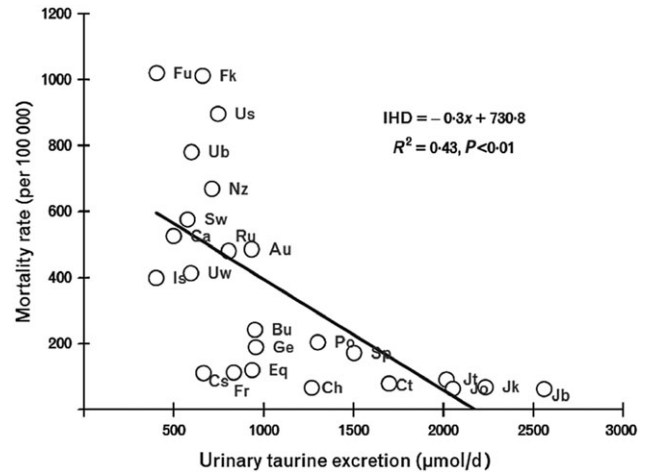
### Japanese diet and CHD

In 1958, at the initiation of the Seven Countries Study, the incidence of CHD and plasma cholesterol levels of the two Japanese cohorts were the lowest among the eleven included<sup>(153)</sup>. Adachi *et al.*<sup>(154)</sup> have recently studied over 60 years the trends in nutritional intake and CHD risk factors among Japanese men in Tanushimaru, one of the two Japanese cities included in the Seven Countries Study. Tanushimaru is a farming city located on Kyushu island, in the south-western island of Japan. For that purpose, they compared the data of seven independent successive cohorts from 1958 to 2018 (1958, 1977, 1982, 1989, 1999, 2009 and 2018). In parallel, total plasma cholesterol increased from 1.68 g/l to 2.09 g/l, and BMI from 21.7 to 24.4 kg/m<sup>2</sup>. By comparing, in three cohorts (1958, 1977 and 1999), 15-year mortality of stroke and MI, the authors found a decrease in stroke mortality from 4.6/1000 participants in cohort 1958 to 0.8/1000 participants and 0.7/1000 participants in cohorts 1977 and 1999, respectively. MI mortality and sudden deaths remained relatively stable. Thus, stroke and MI deaths drastically decreased or remained stable, respectively, in regard of an increase in fat intake, plasma cholesterol and BMI. Other Japanese studies showed no significant increase in CHD incidence over 20 years since the mid-1960s<sup>(155,156)</sup> except for the metropolitan cities of Tokyo and Osaka where westernisation is more marked than in the rest of Japan<sup>(157)</sup>. Furthermore, while the incidence of CHD decreased over the same period in the USA<sup>(158,159)</sup>, CHD in Japan remains approximately one-fourth of that of USA<sup>(160)</sup>, in regard of a higher prevalence of hypertension, smoking, stress

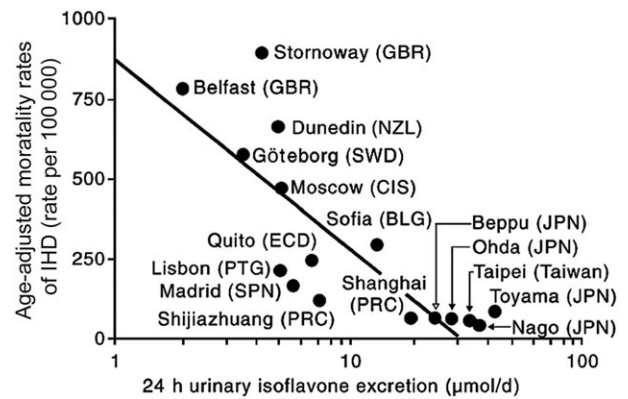
and lower physical activity in Japanese than in their American counterparts<sup>(154)</sup>, Japanese have also almost the same level of plasma cholesterol as Americans<sup>(154)</sup>. Altogether, these observations suggest that the whole dietary pattern of Japanese could be protective. The WHO-Coordinated Cardiovascular Diseases and Alimentary Comparison (CARDIAC) Study<sup>(161)</sup> has been carried out in sixty-one communities from twenty-five countries around the world (1983–2005). It aimed to confirm the association of diets rich in seafood and soyabean with the healthy longevity of Japanese. It showed a positive association between plasma cholesterol and CHD on one hand, and an inverse association with 24 h urinary excretion of taurine (an amino acid contained in fish proteins) (Fig. 2), plasma LC *n*-3 PUFA, and 24 h urinary excretion of isoflavones (an index of soya product intake) (Fig. 3) on the other hand. It is noteworthy that a high seafood/fish consumption ( $\geq 86$  g/d) abolished the positive relationship between heavy smoking and CHD risk in a population of Japanese without history of CHD followed for 5 years (878 163 person-years)<sup>(161–163)</sup>.

Characteristic components of the traditional JapDiet (Washoku) include rice, miso soup, soyabean products, vegetables, fruits, fish, Japanese pickles, seaweed, mushrooms and green tea<sup>(154)</sup> customarily eaten using chopsticks and wooden bowls. Gabriel *et al.*<sup>(164)</sup> detailed the arguments in favour of the beneficial health benefits of the JapDiet. 'The style of eating in Washoku – a large variety of foods, small portions, the inclusion of soups, abundant vegetables, the cooking method, the large content of water, and the effective usage of umami taste – promotes not only the pleasant experience of eating, combined with the large incorporation of bioactive compounds from vegetables, but also ensures an adequate signal for satiety that prevents over-eating.' The other components of diet are various types of seafood/fish that are a rich source of high-quality proteins (rich in taurine) as well as LC *n*-3 PUFA and soyabean-based foods (fermented miso and tofu). Miso soup and salted vegetables account for a persistent high intake of Na of Japanese even if it has decreased markedly since the 1950s. Conversely, the high intake of K is protective. 'In summary, the main elements of Washoku that promote positive health outcomes are: (1) the great variety of seasonal foodstuffs, including vegetables and fishes; (2) the way of cooking dishes based on large amounts of high quality water; (3) the well-balanced nutrition; and finally (4) the value of its connection with health and family ties.'<sup>(164)</sup>

When compared with the MedDiet, we lack interventional studies or RCT demonstrating the protective effect of the traditional JapDiet towards CHD. Beyond that, the two diets have common characteristics but also different ones. The JapDiet has a lower amount of fat and a higher amount of seafood/fish, soya and salt than the MedDiet<sup>(163)</sup>. The MedDiet has been proved to be sustainable<sup>(165)</sup> while the JapDiet has not yet demonstrated it is, even if Gabriel *et al.*<sup>(164)</sup> suggested that because of its composition it could be. The MedDiet is more easily transposable to Westerners because it is more easily acceptable owing to their usual habits and foodstuffs available than the traditional JapDiet. On the other hand, the JapDiet itself should be revitalised for Asians because several epidemiological data plead for its contribution to the low incidence of CHD disease especially in Japanese provided that salt intake reach the current recommendations (about 6 g/d).



**Fig. 2.** Relationship between age-adjusted IHD mortality rate and 24 h urinary taurine excretion (from Yamori *et al.*<sup>(163)</sup>). Au, Perth, Australia; Bu, Sofia, Bulgaria; Ca, St Johns, Canada; Cs, Shijiazhuang, China; Ch, Shanghai, China; Ct, Taiwan, China; Eq, Quito, Ecuador; Fk, Kuopio, Finland, rural; Fu, Kuopio, Finland, urban; Fr, Orleans, France; Ge, Tbilisi, Georgia; Is, Tel Aviv, Israel; Ja, Amino, Japan; Jb, Beppu, Japan; Jo, Ohda, Japan; Jk, Okinawa, Japan; Jt, Toyama, Japan; Nz, Dunedin, New Zealand; Po, Lisbon, Portugal; Ru, Moscow, Russia; Sp, Madrid, Spain; Sw, Göteborg, Sweden; Us, Stornoway, UK; Ub, Belfast, UK; Uw, Western Isles, UK.



**Fig. 3.** Relationship between 24 h urinary isoflavone excretion and age-adjusted mortality rates of CHD (from Yamori *et al.*<sup>(161)</sup>). GBR, Great Britain; NZ, New Zealand; SW, Sweden; CIS, Commonwealth of Independent States (Russia); BLG, Belgium; ECD, Ecuador; PO, Portugal; SPN, Spain; PRC, People's Republic of China; JPN, Japan.

## Conclusion

With the exception of TFA, whose harmfulness has been unanimously recognised, discrepancies remain as to the effect of other types of FA on CHD. These discrepancies explain some of the criticisms that have been made for many years against specific recommendations for the use of FA in primary/secondary CHD prevention. They also prevent us from proposing consensual recommendations adapted to all populations or to populations as a whole; personalised recommendations could be an alternative but are currently not so easy to propose in a pragmatic way. We propose that, instead of continuing to issue specific recommendations on FA intake, new updated recommendations should better promote/revitalise dietary

habits that are proved or reasonably suspected to be protective, taking into account regional specificities to facilitate their acceptance by individuals. The traditional MedDiet and traditional JapDiet meet the criteria for a protective effect against CHD. These diets are low in SFA (both diets), high in MUFA (MedDiet), high in plant PUFA (both diets) and LC *n*-3 PUFA (both diets) and also contain polyphenols (both diets) and isoflavones (JapDiet) consistent with epidemiological data on the protective effects of these nutrients against CHD. Of course, this should not prevent further research on the effects of specific FA on CHD and, where possible, promote intervention studies on these FA or on dietary patterns.

### Acknowledgements

This research received no specific grant from any funding agency, commercial or not-for-profit sectors.

J. D. is the sole author who planned and wrote this article.

There are no conflicts of interest.

### References

- Nestle M (2018) Perspective: Challenges and controversial issues in the dietary guidelines for Americans, 1980–2015. *Adv Nutr* **9**, 148–150.
- Harcombe Z, Baker JS, DiNicolantonio JJ, *et al.* (2016) Evidence from randomised controlled trials does not support current dietary fat guidelines: a systematic review and meta-analysis. *Open Heart* **3**, e000409.
- Keys A, Menotti A, Karvonen MJ, *et al.* (1986) The diet and 15-year death rate in the Seven Countries Study. *Am J Epidemiol* **124**, 903–915.
- Kromhout D, Menotti A, Bloemberg B, *et al.* (1995) Dietary saturated and *trans* fatty acids and cholesterol and 25-year mortality from coronary heart disease: the Seven Countries Study. *Prev Med* **24**, 308–315.
- Menotti A, Kromhout D, Blackburn H, *et al.* (1999) Food intake patterns and 25-year mortality from coronary heart disease: cross-cultural correlations in the Seven Countries Study. The Seven Countries Study Research Group. *Eur J Epidemiol* **15**, 507–515.
- Kromhout D, Menotti A, Alberti-Fidanza A, *et al.* (2018) Comparative ecologic associations 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* **72**, 1103–1110.
- Bang HO, Dyerberg J & Nielsen AB (1971) Plasma lipid and lipoprotein pattern in Greenlandic West-coast Eskimos. *Lancet* **i**, 1143–1145.
- Dyerberg J, Bang HO & Hjørne N (1975) Fatty acid composition of the plasma lipids in Greenland Eskimos. *Am J Clin Nutr* **28**, 958–966.
- Bang HO, Dyerberg J & Hjøorne N (1976) The composition of food consumed by Greenland Eskimos. *Acta Med Scand* **200**, 69–73.
- Bang HO, Dyerberg J & Sinclair HM (1980) The composition of the Eskimo food in north western Greenland. *Am J Clin Nutr* **33**, 2657–2661.
- Dyerberg J & Bang HO (1979) Haemostatic function and platelet polyunsaturated fatty acids in Eskimos. *Lancet* **ii**, 433–435.
- Bang HO (1990) Lipid research in Greenland: preventive and therapeutic consequences. *Scand J Soc Med* **18**, 3–7.
- Dyerberg J & Bang HO (1982) A hypothesis on the development of acute myocardial infarction in Greenlanders. *Scand J Clin Lab Invest Suppl* **161**, 7–13.
- Nelson AM (1972) Diet therapy in coronary disease. Effect on mortality of high-protein, high seafood, fat controlled diet. *Geriatrics* **27**, 27–103.
- Bang HO & Dyerberg J (1981) Personal reflections on the incidence of ischaemic heart disease in Oslo during the Second World War. *Acta Med Scand* **210**, 235–248.
- Hirai A, Hamazaki T, Terano T, *et al.* (1980) Eicosapentaenoic acid and platelet function in Japanese. *Lancet* **ii**, 1132–1133.
- Sho H (2001) History and characteristics of Okinawan longevity food. *Asia Pac J Clin Nutr* **10**, 159–164.
- Toyokawa H (1976) *Public Nutrition*. Tokyo: Koseikan Publisher.
- Nagata N (1963) *Elderly Population of Okinawa*. Tokyo: Chukoron Jigyō.
- United States Department of the Office of the Civil Administrator of the Ryukyu Islands: Records of Health, Education and Welfare (1949) U.S. Occupation Headquarters, World War II. Record Group 260-12.5. National Archives at College Park, 8601 Adelphi Road, College Park, MD 20740-6001.
- Division of Health, Labor and Welfare, Okinawa Prefectural Government (1976) *Health and Welfare Dataset 1880–1976*. Okinawa: Okinawa Prefectural Government.
- Japanese Ministry of Health, Labour and Welfare (2017) Table 5. Life expectancies at birth in some countries. <https://www.mhlw.go.jp/english/database/db-hw/lifetb18/dl/lifetb18-03.pdf> (accessed August 2020).
- Wong CX, Brown A, Lau DH, *et al.* (2019) Epidemiology of sudden cardiac death: global and regional perspectives. *Heart Lung Circ* **28**, 6–14.
- Marmot MG, Syme SL, Kagan A, *et al.* (1975) Epidemiologic studies of coronary heart disease and stroke in Japanese men living in Japan, Hawaii and California: prevalence of coronary and hypertensive heart disease and associated risk factors. *Am J Epidemiol* **102**, 514–525.
- Benfante R (1992) Studies of cardiovascular disease and cause-specific mortality trends in Japanese-American men living in Hawaii and risk factor comparisons with other Japanese populations in the Pacific region: a review. *Hum Biol* **64**, 791–805.
- Tada N, Maruyama C, Koba S, *et al.* (2011) Japanese dietary lifestyle and cardiovascular disease. *J Atheroscler Thromb* **18**, 723–734.
- Mozaffarian D, Katan MB, Ascherio A, *et al.* (2006) *Trans* fatty acids and cardiovascular disease. *N Engl J Med* **354**, 1601–1613.
- Wang DD, Li Y, Chiuve SE, *et al.* (2016) Association of specific dietary fats with total and cause-specific mortality. *JAMA Intern Med* **176**, 1134–1145.
- Zhu Y, Bo Y & Liu Y (2019) Dietary total fat, fatty acids intake, and risk of cardiovascular disease: a dose–response meta-analysis of cohort studies. *Lipids Health Dis* **18**, 91.
- World Health Organization (2018) REPLACE *trans* fat: an action package to eliminate industrially-produced *trans*-fatty acids. <https://www.who.int/docs/default-source/documents/replace-transfats/replace-action-package.pdf> (accessed August 2020).
- United States Food & Drug Administration (2018) Final determination regarding partially hydrogenated oils (removing *trans* fat). <https://www.fda.gov/food/food-additives-petitions/final-determination-regarding-partially-hydrogenated-oils-removing-trans-fat> (accessed August 2020).
- Zong G, Li Y, Wanders AJ, *et al.* (2016) Intake of individual saturated fatty acids and risk of coronary heart disease in US men and women: two prospective longitudinal cohort studies. *BMJ* **355**, i5796.



33. Neelakantan N, Seah JYH & van Dam RM (2020) The effect of coconut oil consumption on cardiovascular risk factors: a systematic review and meta-analysis of clinical trials. *Circulation* **141**, 803–814.
34. Wu JHY, Micha R & Mozaffarian D (2019) Dietary fats and cardiometabolic disease: mechanisms and effects on risk factors and outcomes. *Nat Rev Cardiol* **6**, 581–601.
35. Dehghan M, Mente A, Zhang X, *et al.* (2017) Prospective Urban Rural Epidemiology (PURE) study investigators. Associations of fats and carbohydrate intake with cardiovascular disease and mortality in 18 countries from five continents (PURE): a prospective cohort study. *Lancet* **390**, 2050–2062.
36. Zhuang P, Zhang Y, He W, *et al.* (2019) Dietary fats in relation to total and cause-specific mortality in a prospective cohort of 521 120 individuals with 16 years of follow-up. *Circ Res* **124**, 757–768.
37. Skeaff CM & Miller J (2009) Dietary fat and coronary heart disease: summary of evidence from prospective cohort and randomised controlled trials. *Ann Nutr Metab* **55**, 173–201.
38. Siri-Tarino PW, Sun Q, Hu FB, *et al.* (2010) Meta-analysis of prospective cohort studies evaluating the association of saturated fat with cardiovascular disease. *Am J Clin Nutr* **91**, 535–546.
39. Chowdhury R, Warnakula S, Kunutsor S, *et al.* (2014) Association of dietary, circulating, and supplement fatty acids with coronary risk: a systematic review and meta-analysis. *Ann Intern Med* **160**, 398–406. Erratum in: *Ann Intern Med* (2014), 160, 658.
40. Bittman M (2014) Butter Is Back, *The New York Times*. <https://www.nytimes.com/2014/03/26/opinion/bittman-butter-is-back.html> (accessed August 2020).
41. Walsh B (2014). Eat Butter. Scientists labelled fat the enemy. Why they were wrong. *Time*, vol. 183, no. 24, 23 June 2014. <https://time.com/magazine/us/2863200/june-23rd-2014-vol-183-no-24-u-s/> (accessed August 2020).
42. Li Y, Hruby A, Bernstein AM, *et al.* (2015) Saturated fats compared with unsaturated fats and sources of carbohydrates in relation to risk of coronary heart disease: a prospective cohort study. *J Am Coll Cardiol* **66**, 1538–1548.
43. Chen M, Li Y, Sun Q, *et al.* (2016) Dairy fat and risk of cardiovascular disease in 3 cohorts of US adults. *Am J Clin Nutr* **104**, 1209–1217.
44. Prentice RL, Aragaki AK, Van Horn L, *et al.* (2017) Low-fat dietary pattern and cardiovascular disease: results from the Women's Health Initiative randomized controlled trial. *Am J Clin Nutr* **106**, 35–43.
45. Mozaffarian D, Micha R & Wallace S (2010) 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* **23**, e1000252.
46. Hooper L, Martin N, Abdelhamid A, *et al.* (2015) Reduction in saturated fat intake for cardiovascular disease. *Cochrane Database Syst Rev*, issue 6, CD011737.
47. Hegsted DM, McGandy RB, Myers ML, *et al.* (1965) Quantitative effects of dietary fat on serum cholesterol in man. *Am J Clin Nutr* **17**, 281–295.
48. Mensink RP, Zock PL, Kester AD, *et al.* (2003) Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. *Am J Clin Nutr* **77**, 1146–1155.
49. Hu FB, Stampfer MJ, Manson JE, *et al.* (1997) Dietary fat intake and the risk of coronary heart disease in women. *N Engl J Med* **337**, 1491–1499.
50. Oh K, Hu FB, Manson JE, *et al.* (2005) Dietary fat intake and risk of coronary heart disease in women: 20 years of follow-up of the Nurses' Health Study. *Am J Epidemiol* **161**, 672–679.
51. Guasch-Ferré M, Babio N, Martínez-González MA, *et al.* (2015) PREDIMED Study Investigators. Dietary fat intake and risk of cardiovascular disease and all-cause mortality in a population at high risk of cardiovascular disease. *Am J Clin Nutr* **102**, 1563–1573.
52. Jakobsen MU, O'Reilly EJ, Heitmann BL, *et al.* (2009) Major types of dietary fat and risk of coronary heart disease: a pooled analysis of 11 cohort studies. *Am J Clin Nutr* **89**, 1425–1432.
53. Schwingshackl L & Hoffmann G (2014) Dietary fatty acids in the secondary prevention of coronary heart disease: a systematic review, meta-analysis and meta-regression. *BMJ Open* **4**, e004487.
54. Schwingshackl L & Hoffmann G (2014) Monounsaturated fatty acids, olive oil and health status: a systematic review and meta-analysis of cohort studies. *Lipids Health Dis* **13**, 154.
55. Martínez-González MA, Dominguez LJ & Delgado-Rodríguez M (2014) Olive oil consumption and risk of CHD and/or stroke: a meta-analysis of case-control, cohort and intervention studies. *Br J Nutr* **112**, 248–259.
56. Dolecek T (1992) Epidemiological evidence of relationships between dietary polyunsaturated fatty acids and mortality in the multiple risk factor intervention trial. *Proc Soc Exp Biol Med* **200**, 177–182.
57. Ascherio A, Rimm EB, Giovannucci EL, *et al.* (1996) Dietary fat and risk of coronary heart disease in men: cohort follow up study in the United States. *Br Med J* **313**, 84–90.
58. Mozaffarian D, Ascherio A, Hu FB, *et al.* (2005) Interplay between different polyunsaturated fatty acids and risk of coronary heart disease in men. *Circulation* **111**, 157–164.
59. Pietinen P, Ascherio A, Korhonen P, *et al.* (1997) Intake of fatty acids and risk of coronary heart disease in a cohort of Finnish men. The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study. *Am J Epidemiol* **145**, 876–887.
60. Koh AS, Pan A, Wang R, *et al.* (2015) The association between dietary omega-3 fatty acids and cardiovascular death: the Singapore Chinese Health Study. *Eur J Prev Cardiol* **22**, 364–372.
61. Hu FB, Stampfer MJ, Manson JE, *et al.* (1999) Dietary intake of  $\alpha$ -linolenic acid and risk of fatal ischemic heart disease among women. *Am J Clin Nutr* **69**, 890–897.
62. Albert CM, Oh K, Whang W, *et al.* (2005) Dietary  $\alpha$ -linolenic acid intake and risk of sudden cardiac death and coronary heart disease. *Circulation* **112**, 3232–3238.
63. de Goede J, Verschuren WM, Boer JM, *et al.* (2011) Alpha-linolenic acid intake and 10-year incidence of coronary heart disease and stroke in 20,000 middle-aged men and women in the Netherlands. *PLoS ONE* **6**, e17967.
64. Vedtofte MS, Jakobsen MU, Lauritzen L, *et al.* (2014) Association between the intake of  $\alpha$ -linolenic acid and the risk of CHD. *Br J Nutr* **112**, 735–743.
65. Oomen CM, Ocké MC, Feskens EJ, *et al.* (2001)  $\alpha$ -Linolenic acid intake is not beneficially associated with 10-y risk of coronary artery disease incidence: the Zutphen Elderly Study. *Am J Clin Nutr* **74**, 457–463.
66. Fretts AM, Mozaffarian D, Siscovick DS, *et al.* (2014) Plasma phospholipid and dietary  $\alpha$ -linolenic acid, mortality, CHD and stroke: the Cardiovascular Health Study. *Br J Nutr* **112**, 1206–1213.
67. de Oliveira Otto MC, Wu JH, Baylin A, *et al.* (2013) Circulating and dietary omega-3 and omega-6 polyunsaturated fatty acids and incidence of CVD in the Multi-Ethnic Study of Atherosclerosis. *J Am Heart Assoc* **2**, e000506.



68. Sala-Vila A, Guasch-Ferré M, Hu FB, *et al.* (2016) Dietary  $\alpha$ -linolenic acid, marine  $\omega$ -3 fatty acids, and mortality in a population with high fish consumption: findings from the PREvención con Dieta MEDiterránea (PREDIMED) study. *J Am Heart Assoc* **5**, e002543.
69. Bork CS, Jakobsen MU, Lundbye-Christensen S, *et al.* (2016) Dietary intake and adipose tissue content of  $\alpha$ -linolenic acid and risk of myocardial infarction: a Danish cohort study. *Am J Clin Nutr* **104**, 41–48.
70. Campos H, Baylin A & Willett WC (2008)  $\alpha$ -Linolenic acid and risk of nonfatal acute myocardial infarction. *Circulation* **118**, 339–345.
71. Guallar E, Aro A, Jiménez FJ, *et al.* (1999) Omega-3 fatty acids in adipose tissue and risk of myocardial infarction: the EURAMIC study. *Arterioscler Thromb Vasc Biol* **19**, 1111–1118.
72. Pan A, Chen M, Chowdhury R, *et al.* (2012)  $\alpha$ -Linolenic acid and risk of cardiovascular disease: a systematic review and meta-analysis. *Am J Clin Nutr* **96**, 1262–1273.
73. Del Gobbo LC, Imamura F, Aslibekyan S, *et al.* (2016) Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Fatty Acids and Outcomes Research Consortium (FORCe).  $\omega$ -3 Polyunsaturated fatty acid biomarkers and coronary heart disease: pooling project of 19 cohort studies. *JAMA Intern Med* **176**, 1155–1166.
74. Balk EM & Lichtenstein AH (2017) Omega-3 fatty acids and cardiovascular disease: summary of the 2016 Agency of Healthcare Research and Quality evidence review. *Nutrients* **9**, 865.
75. Wei J, Hou R, Xi Y, *et al.* (2018) The association and dose-response relationship between dietary intake of  $\alpha$ -linolenic acid and risk of CHD: a systematic review and meta-analysis of cohort studies. *Br J Nutr* **119**, 83–89.
76. Abdelhamid AS, Brown TJ, Brainard JS, *et al.* (2020) Omega-3 fatty acids for the primary and secondary prevention of cardiovascular disease. *Cochrane Databas Syst Rev*, issue 3, CD003177.
77. Rose GA, Thomson WB & Williams RT (1965) Corn oil in treatment of ischaemic heart disease. *Br Med J* **1**, 1531–1533.
78. Leren P (1970) The Oslo Diet-Heart Study. Eleven-year report. *Circulation* **42**, 935–942.
79. Turpeinen O (1979) Effect of cholesterol-lowering diet on mortality from coronary heart disease and other causes. *Circulation* **59**, 1–7.
80. Frantz ID Jr, Dawson EA, Ashman PL, *et al.* (1989) Test of effect of lipid lowering by diet on cardiovascular risk. *The Minnesota Coronary Survey. Arteriosclerosis* **9**, 129–135.
81. Ramsden CE, Zamora D, Majchrzak-Hong S, *et al.* (2016) Re-evaluation of the traditional diet-heart hypothesis: analysis of recovered data from Minnesota Coronary Experiment (1968–73). *BMJ* **353**, i1246.
82. Ramsden CE, Zamora D, Leelarthaepin B, *et al.* (2013) Use of dietary linoleic acid for secondary prevention of coronary heart disease and death: evaluation of recovered data from the Sydney Diet Heart Study and updated meta-analysis. *BMJ* **346**, e8707.
83. Ramsden CE, Hibbeln JR, Majchrzak SF, *et al.* (2010)  $n$ -6 Fatty acid-specific and mixed polyunsaturate dietary interventions have different effects on CHD risk: a meta-analysis of randomised controlled trials. *Br J Nutr* **104**, 1586–1600.
84. Farvid MS, Ding M, Pan A, *et al.* (2014) Dietary linoleic acid and risk of coronary heart disease: a systematic review and meta-analysis of prospective cohort studies. *Circulation* **130**, 1568–1578.
85. Hamley S (2017) The effect of replacing saturated fat with mostly  $n$ -6 polyunsaturated fat on coronary heart disease: a meta-analysis of randomized controlled trials. *Nutr J* **16**, 30.
86. Hooper L, Al-Khudairy L, Abdelhamid AS, *et al.* (2018) Omega-6 fats for the primary and secondary prevention of cardiovascular disease. *Cochrane Database Syst Rev*, issue 11, CD011094.
87. Marklund M, Wu JHY, Imamura F, *et al.* (2019) Biomarkers of dietary omega-6 fatty acids and incident cardiovascular disease and mortality. *Circulation* **139**, 2422–2436.
88. Li J, Guasch-Ferré M, Li Y, *et al.* (2020) Dietary intake and biomarkers of linoleic acid and mortality: systematic review and meta-analysis of prospective cohort studies. *Am J Clin Nutr* **112**, 150–167.
89. Hu FB, Bronner L, Willett WC, *et al.* (2002) Fish and omega-3 fatty acid intake and risk of coronary heart disease in women. *JAMA* **287**, 1815–1821.
90. Sun Q, Ma J, Campos H, Rexrode, *et al.* (2008) Blood concentrations of individual long-chain  $n$ -3 fatty acids and risk of nonfatal myocardial infarction. *Am J Clin Nutr* **88**, 216–223.
91. Chiuve SE, Rimm EB, Sandhu RK, *et al.* (2012) Dietary fat quality and risk of sudden cardiac death in women. *Am J Clin Nutr* **96**, 498–507.
92. Albert CM, Campos H, Stampfer MJ, *et al.* (2002) Blood levels of long-chain  $n$ -3 fatty acids and the risk of sudden death. *N Engl J Med* **346**, 1113–1118.
93. Lemaitre RN, King IB, Mozaffarian D, *et al.* (2003)  $n$ -3 Polyunsaturated fatty acids, fatal ischemic heart disease, and nonfatal myocardial infarction in older adults: the Cardiovascular Health Study. *Am J Clin Nutr* **77**, 319–325.
94. Kleber ME, Delgado GE, Lorkowski S, *et al.* (2016) Omega-3 fatty acids and mortality in patients referred for coronary angiography. The Ludwigshafen Risk and Cardiovascular Health Study. *Atherosclerosis* **252**, 175–181.
95. von Schacky C (2014) Omega-3 index and cardiovascular health. *Nutrients* **6**, 799–814.
96. Harris WS, Tintle NL, Etherton MR, *et al.* (2018) Erythrocyte long-chain omega-3 fatty acid levels are inversely associated with mortality and with incident cardiovascular disease: The Framingham Heart Study. *J Clin Lipidol* **12**, 718–727.
97. Hamazaki K, Iso H, Eshak ES, *et al.* (2018) Plasma levels of  $n$ -3 fatty acids and risk of coronary heart disease among Japanese: The Japan Public Health Center-based (JPHC) study. *Atherosclerosis* **272**, 226–232.
98. ASCEND Study Collaborative Group (2018) Effects of  $n$ -3 fatty acid supplements in diabetes mellitus. *N Engl J Med* **379**, 1540–1550.
99. Manson JE, Cook NR, Lee IM, *et al.* (2019) Marine  $n$ -3 fatty acids and prevention of cardiovascular disease and cancer. *N Engl J Med* **80**, 23–32.
100. Manson JE, Bassuk SS, Cook NR, *et al.* (2020) Vitamin D, marine  $n$ -3 fatty acids, and primary prevention of cardiovascular disease current evidence. *Circ Res* **126**, 112–128.
101. Li ZH, Zhong WF, Liu S, *et al.* (2020) Associations of habitual fish oil supplementation with cardiovascular outcomes and all cause mortality: evidence from a large population based cohort study. *BMJ* **368**, m456.
102. Burr ML, Gilbert JF, Holliday RA, *et al.* (1989) Effects of changes in fat, fish, and fibre intakes on death and myocardial reinfarction: Diet and Reinfarction Trial (DART). *Lancet* **334**, 757–761.
103. Anonymous (1999) Dietary supplementation with  $n$ -3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico (Italian Group for the Study of Survival in Myocardial Infarction). *Lancet* **354**, 447–455.
104. Yokoyama M, Origasa H, Matsuzaki M, *et al.* (2007) Effects of eicosapentaenoic acid on major coronary events in



- hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis. *Lancet* **369**, 1090–1098.
105. Sasaki J, Yokoyama M, Matsuzaki M, *et al.* (2012) Relationship between coronary artery disease and non-HDL-C, and effect of highly purified EPA on the risk of coronary artery disease in hypercholesterolemic patients treated with statins: sub-analysis of the Japan EPA Lipid Intervention Study (JELIS). *J Atheroscler Thromb* **19**, 194–204.
  106. Rauch B, Schiele R, Schneider S, *et al.* (2010) OMEGA, a randomized, placebo-controlled trial to test the effect of highly purified omega-3 fatty acids on top of modern guideline-adjusted therapy after myocardial infarction. *Circulation* **122**, 2152–2159.
  107. Galan P, Kesse-Guyot E, Czernichow S, *et al.* (2010) Effects of B vitamins and omega 3 fatty acids on cardiovascular diseases: a randomised placebo controlled trial. *BMJ* **341**, c6273.
  108. ORIGIN Trial Investigators (2012) *n*-3 Fatty acids and cardiovascular outcomes in patients with dysglycemia. *N Engl J Med* **367**, 309–318.
  109. Risk and Prevention Study Collaborative Group (2013) *n*-3 Fatty acids in patients with multiple cardiovascular risk factors. *N Engl J Med* **368**, 1800–1808.
  110. Bonds DE, Harrington M, Worrall BB, *et al.* (2014) Effect of long-chain  $\omega$ -3 fatty acids and lutein + zeaxanthin supplements on cardiovascular outcomes: results of the Age-Related Eye Disease Study 2 (AREDS) randomized clinical trial. *JAMA Intern Med* **174**, 763–771.
  111. Bhatt DL, Steg PG, Miller M, *et al.* (2019) Cardiovascular risk reduction with icosapent ethyl for hypertriglyceridemia. *N Engl J Med* **380**, 11–22.
  112. Bhatt DL, Miller M, Brinton EA, *et al.* (2020) REDUCE-IT USA: results from the 3146 patients randomized in the United States. *Circulation* **141**, 367–375.
  113. Nicholls SJ, Lincoff AM, Garcia M, *et al.* (2020) Effect of high-dose omega-3 fatty acids vs corn oil on major adverse cardiovascular events in patients at high cardiovascular risk: the STRENGTH Randomized Clinical Trial. *JAMA* **324**, 2268–2280.
  114. United States Food & Drug Administration (2019) FDA approves use of drug to reduce risk of cardiovascular events in certain adult patient groups. <https://www.fda.gov/news-events/press-announcements/fda-approves-use-drug-reduce-risk-cardiovascular-events-certain-adult-patient-groups> (accessed December 2020).
  115. Innes JK & Calder PC (2020) Marine omega-3 (*n*-3) fatty acids for cardiovascular health: an update for 2020. *Int J Mol Sci* **21**, 1362.
  116. Alexander DD, Miller PE, Van Elswyk ME, *et al.* (2017) A meta-analysis of randomized controlled trials and prospective cohort studies of eicosapentaenoic and docosahexaenoic long-chain omega-3 fatty acids and coronary heart disease risk. *Mayo Clin Proc* **92**, 15–29.
  117. Maki KC, Palacios OM, Bell M, *et al.* (2017) Use of supplemental long-chain omega-3 fatty acids and risk for cardiac death: an updated meta-analysis and review of research gaps. *J Clin Lipidol* **11**, 1152–1160.
  118. Hu Y, Hu FB & Manson JE (2019) Marine omega-3 supplementation and cardiovascular disease: an updated meta-analysis of 13 randomized controlled trials involving 127 477 participants. *J Am Heart Assoc* **8**, e013543.
  119. Popoff F, Balaciano G, Bardach A, *et al.* (2019) Omega 3 fatty acid supplementation after myocardial infarction: a systematic review and meta-analysis. *BMC Cardiovasc Disord* **19**, 136.
  120. Aung T, Halsey J, Kromhout D, *et al.* (2018) Associations of omega-3 fatty acid supplement use with cardiovascular disease risks: meta-analysis of 10 trials involving 77 917 individuals. *JAMA Cardiol* **3**, 225–234.
  121. Doshi R, Kumar A, Thakkar S, *et al.* (2020) Meta-analysis comparing combined use of eicosapentaenoic acid and statin to statin alone. *Am J Cardiol* **125**, 198–204.
  122. Hoang T & Kim J (2020) Comparative effect of statins and omega-3 supplementation on cardiovascular events: meta-analysis and network meta-analysis of 63 randomized controlled trials including 264,516 participants. *Nutrients* **12**, E2218.
  123. Lombardi M, Chiabrando JG, Vescovo GM, *et al.* (2020) Impact of different doses of omega-3 fatty acids on cardiovascular outcomes: a pairwise and network meta-analysis. *Curr Atheroscler Rep* **22**, 45.
  124. Casula M, Olmastroni E, Gazzotti M, *et al.* (2020) Omega-3 polyunsaturated fatty acids supplementation and cardiovascular outcomes: do formulation, dosage, and baseline cardiovascular risk matter? An updated meta-analysis of randomized controlled trials. *Pharmacol Res* **160**, 105060.
  125. Cabiddu MF, Russi A, Appolloni L, *et al.* (2020) Omega-3 for the prevention of cardiovascular diseases: meta-analysis and trial-sequential analysis. *Eur J Hosp Pharm* 2020, ejhpharm-2020-002207.
  126. Bernasconi AA, Wiest MM, Lavie CJ, *et al.* (2020) Effect of omega-3 dosage on cardiovascular outcomes: an updated meta-analysis and meta-regression of interventional trials. *Mayo Clin Proc* 2020, S0025-6196(20)30985-X.
  127. von Schacky C (2020) Omega-3 index in 2018/19. *Proc Nutr Soc* **79**, 381–387.
  128. Siscovick DS, Barringer TA, Fretts AM, *et al.* (2017) Omega-3 polyunsaturated fatty acid (fish oil) supplementation and the prevention of clinical cardiovascular disease: a Science Advisory from the American Heart Association. *Circulation* **135**, e867–e884.
  129. Hu FB (2002) Dietary pattern analysis: a new direction in nutritional epidemiology. *Curr Opin Lipidol* **13**, 3–9.
  130. Martínez-González MA, Gea A & Ruiz-Canela M (2019) The Mediterranean diet and cardiovascular health. *Circ Res* **124**, 779–798.
  131. Iqbal R, Anand S, Ounpuu S, *et al.* (2008) Dietary patterns and the risk of acute myocardial infarction in 52 countries: results of the INTERHEART study. *Circulation* **118**, 1929–1937.
  132. Hu FB, Rimm EB, Stampfer MJ, *et al.* (2000) Prospective study of major dietary patterns and risk of coronary heart disease in men. *Am J Clin Nutr* **72**, 912–921.
  133. Fung TT, Willett WC, Stampfer MJ, *et al.* (2001) Dietary patterns and the risk of coronary heart disease in women. *Arch Intern Med* **161**, 1857–1862.
  134. Brunner EJ, Mosdøl A, Witte DR, *et al.* (2008) Dietary patterns and 15-y risks of major coronary events, diabetes, and mortality. *Am J Clin Nutr* **87**, 1414–1421.
  135. United Nations Educational, Scientific and Cultural Organization (UNESCO) (2013) Intangible Cultural Heritage: Washoku, traditional dietary cultures of the Japanese, notably for the celebration of New Year. <https://ich.unesco.org/en/RL/washoku-traditional-dietary-cultures-of-the-japanese-notably-for-the-celebration-of-new-year-00869> (accessed August 2020).
  136. United Nations Educational, Scientific and Cultural Organization (UNESCO) (2013) Intangible Cultural Heritage: Mediterranean diet. <https://ich.unesco.org/en/RL/mediterranean-diet-00884> (accessed August 2020).
  137. de Lorgeril M, Renaud S, Mamelle N, *et al.* (1994) Mediterranean alpha-linolenic acid-rich diet in secondary prevention of coronary heart disease. *Lancet* **343**, 1454–1459.
  138. de Lorgeril M, Salen P, Caillat-Vallet E, *et al.* (1997) Control of bias in dietary trial to prevent coronary recurrences: The Lyon Diet Heart Study. *Eur J Clin Nutr* **51**, 116–122.



139. de Lorgeril M, Salen P, Martin JL, *et al.* (1999) Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: final report of the Lyon Diet Heart Study. *Circulation* **99**, 779–785.
140. Kris-Etherton P, Eckel RH, Howard BV, *et al.* (2001) Nutrition Committee Population Science Committee and Clinical Science Committee of the American Heart Association. AHA Science Advisory: Lyon Diet Heart Study. Benefits of a Mediterranean-style, National Cholesterol Education Program/American Heart Association step I dietary pattern on cardiovascular disease. *Circulation* **103**, 1823–1825.
141. Singh RB, Dubnov G, Niaz MA, *et al.* (2002) Effect of an Indo-Mediterranean diet on progression of coronary artery disease in high risk patients (Indo-Mediterranean Diet Heart Study): a randomized single-blind trial. *Lancet* **360**, 1455–1461.
142. Horton R (2005) Expression of concern: Indo-Mediterranean Diet Heart Study. *Lancet* **366**, 354–356.
143. Estruch R, Ros E, Salas-Salvadó J, *et al.* (2018) Primary prevention of cardiovascular disease with a Mediterranean diet supplemented with extra-virgin olive oil or nuts. *N Engl J Med* **378**, e34.
144. Sofi F, Macchi C, Abbate R, *et al.* (2014) Mediterranean diet and health status: an updated meta-analysis and a proposal for a literature-based adherence score. *Public Health Nutr* **17**, 2769–2782.
145. Grosso G, Marventano S, Yang J, *et al.* (2017) A comprehensive meta-analysis on evidence of Mediterranean diet and cardiovascular disease: are individual components equal? *Crit Rev Food Sci Nutr* **57**, 3218–3232.
146. Liyanage T, Ninomiya T, Wang A, *et al.* (2016) Effects of the Mediterranean diet on cardiovascular outcomes – a systematic review and meta-analysis. *PLOS ONE* **11**, e0159252.
147. Rosato V, Temple NJ, La Vecchia C, *et al.* (2019) Mediterranean diet and cardiovascular disease: a systematic review and meta-analysis of observational studies. *Eur J Nutr* **58**, 173–191.
148. Galbete C, Schwingshackl L, Schwedhelm C, *et al.* (2018) Evaluating Mediterranean diet and risk of chronic disease in cohort studies: an umbrella review of meta-analyses. *Eur J Epidemiol* **33**, 909–931.
149. Trichopoulou A, Costacou T, Bamia C, *et al.* (2003) Adherence to a Mediterranean diet and survival in a Greek population. *N Engl J Med* **348**, 2599–2608.
150. Fung TT, Rexrode KM, Mantzoros CS, *et al.* (2009) Mediterranean diet and incidence of and mortality from coronary heart disease and stroke in women. *Circulation* **119**, 1093–1100.
151. Rees K, Takeda A, Martin N, *et al.* (2019) Mediterranean-style diet for the primary and secondary prevention of cardiovascular disease. *Cochrane Database Syst Rev*, issue 3, CD009825.
152. O’Keefe JH, Torres-Acosta N, O’Keefe EL, *et al.* (2020) A pesco-Mediterranean diet with intermittent fasting: JACC review topic of the week. *J Am Coll Cardiol* **76**, 1484–1493.
153. Keys A, Aravanis C, Blackburn H, *et al.* (1980) *Seven Countries Study. A Multivariate Analysis of Death and Coronary Artery Disease*. Cambridge, MA: Harvard University Press.
154. Adachi H, Enomoto M, Fukami A, *et al.* (2020) Trends in nutritional intake and coronary risk factors over 60 years among Japanese men in Tanushimaru. *Heart Vessels* **35**, 901–908.
155. Toshima H (1994) Coronary artery disease trends in Japan. *Jpn Circ J* **58**, 166–172.
156. Tanizaki Y, Kiyohara Y, Kato I, *et al.* (2000) Incidence and risk factors for subtypes of cerebral infarction in a general population: the Hisayama study. *Stroke* **31**, 2616–2622.
157. Iso H (2011) Lifestyle and cardiovascular disease in Japan. *J Atheroscler Thromb* **18**, 83–88.
158. Weir HK, Anderson RN, Coleman King SM, *et al.* (2016) Heart Disease and Cancer Deaths – Trends and Projections in the United States, 1969–2020. [https://www.cdc.gov/Pcd/issues/2016/16\\_0211.htm](https://www.cdc.gov/Pcd/issues/2016/16_0211.htm) (accessed August 2020).
159. Benjamin EJ, Muntner P, Alonso A, *et al.* (2019) Heart disease and stroke statistics – 2019 update: a report from the American Heart Association. *Circulation* **139**, e56–e528.
160. Finegold JA, Asaria P & Francis DP (2013) Mortality from ischaemic heart disease by country region, and age: statistics from World Health Organisation and United Nations. *Int J Cardiol* **168**, 934–945.
161. Yamori Y, Miura A & Taira K (2001) Implications from and for food cultures for cardiovascular diseases: Japanese food, particularly Okinawan diets. *Asia Pac J Clin Nutr* **10**, 144–145.
162. Yamori Y (2006) Food factors for atherosclerosis prevention: Asian perspective derived from analyses of worldwide dietary biomarkers. *Exp Clin Cardiol* **11**, 94–98.
163. Yamori Y, Liu L, Mizushima S, *et al.* (2006) Male cardiovascular mortality and dietary markers in 25 population samples of 16 countries. *J Hypertens* **24**, 1499–1505.
164. Gabriel AS, Ninomiya K & Uneyama H (2018) The role of the Japanese traditional diet in healthy and sustainable dietary patterns around the world. *Nutrients* **10**, 173.
165. Dernini S, Berry EM, Serra-Majem L, *et al.* (2017) Med Diet 4.0: the Mediterranean diet with four sustainable benefits. *Public Health Nutr* **20**, 1322–1330.