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ORIGINAL CONTRIBUTION

Polyunsaturated fatty acid intake and risk of cardiovascular mortality in a low fish-consuming population: a prospective cohort analysis

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Abstract
Purpose The aim of this study was to examine the relationship between polyunsaturated fatty acids (PUFA) intake (n-6 and n-3) and mortality in a population-based sample with a low fish intake.
Methods Cox regression was used to examine the relationships between dietary PUFA intake and all-cause or CVD mortality in the Australian Diabetes, Obesity and Lifestyle Study (AusDiab) cohort, a population of 11,247 Australians aged ≥25 years recruited in 1999/2000 and followed until 2012. Demographic, lifestyle and behavioural information were collected by questionnaire and fasting blood tests undertaken. Dietary intake was collected by a 121-item food frequency questionnaire. Vital status and causes of death were collected by death registry linkage.
Results Those in the highest quintile of n-6 PUFA intake had lower risk of CVD mortality (HR 0.57, 95 % CI 0.38–0.86) after age and sex adjustment, but this failed to retain significance after further risk factor adjustment. Consumption of ≥1 serves/week of non-fried fish was associated with reduced risk of CVD mortality (HR 0.64, 95 % CI 0.45–0.91, $p = 0.013$) compared to those eating less than 1 serve/month, after sex and age adjustment, but did not retain significance after further adjustment. However, long-chain n-3 intake was not associated with CVD mortality, and those in the highest quintile of n-3 intake had a higher risk of all-cause mortality.
Conclusions These findings do not support previous suggestions that n-6 PUFA have adverse effects on CVD risk. Greater intake of non-fried fish was associated with lower risk of CVD mortality, but those with the highest total n-3 intake were at slightly increased risk of all-cause mortality.

Keywords Diet · Fatty acids · Mortality · Cardiovascular disease

Introduction

Since the relationship between dietary saturated fat and plasma cholesterol was discovered mid-last century, cardiovascular disease (CVD) prevention strategies have included recommendations to replace animal-derived saturated fat with vegetable oils high in unsaturated fat [1]. Replacement of dietary saturated fat with polyunsaturated fat has been estimated to have the ability to reduce risk of coronary events and coronary death by 13 and 26 %, respectively [2].

Polyunsaturated fatty acids (PUFA) are classified as either n-3 (omega-3) or n-6 (omega-6). The major in vivo long-chain n-6 PUFA, arachidonic acid, is the precursor of a number of biologically active metabolites including pro-inflammatory eicosanoids. N-3 PUFA of equivalent carbon length compete with n-6 PUFA-forming eicosanoids and metabolites that have lower levels of pro-inflammatory activity and are involved in resolution of inflammation [3]. As inflammation is thought to be involved in the aetiology of CVD, there has been debate as to what effect n-6 PUFA themselves may have on cardiovascular risk and whether

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any risk reduction seen with consumption of n-6 PUFA is driven largely by replacement of saturated fat.

A number of large-scale prospective studies have suggested that modest consumption of fish or very long-chain n-3 PUFA (hereafter described as 'n-3 PUFA') is associated with reduced risk of CVD mortality and in particular sudden cardiac death [4–6]. These findings are supported by the GISSI-Prevenzione randomised trial of fish oil in a post-myocardial infarction population which noted a 45 % reduction in risk of sudden cardiac death [7]. Experimental evidence suggests that incorporation of the n-3 PUFA docosahexaenoic acid [DHA] and eicosapentaenoic acid [EPA] into myocardial membranes drives the anti-arrhythmic effect [8], and this is independent of the background level of dietary n-6 PUFA [9]. However, other studies have found no significant effect on CVD mortality, and these differences are reflected in disparate findings across a series of recent meta-analyses and systematic reviews [10–12].

Differences in background diet and both the amount and type of fish consumed may be important contributors to variability in the findings of observational studies examining the relationship between CVD mortality and n-3 PUFA. Despite the majority of the population living along the coastal fringe, Australians have previously been reported to have a relatively low intake of fish and very long-chain n-3 PUFA (~180 mg EPA + DHA/day) as compared to Japanese intakes which may be almost an order of magnitude higher (1600 mg EPA + DHA/day) [13, 14]. In contrast, n-6 PUFA intake in the Australian population has been reported to be relatively high (in the order of 5 % of total energy) [15]. There is much debate regarding the ideal n-6 PUFA intake, with some suggesting that the imbalance between n-6 and n-3 PUFA is a risk factor for chronic disease [16]. The evidence from prospective studies for this stance remains inconclusive [17–20]. The aim of the present study was to examine the relationship between PUFA intake and cardiovascular mortality in a population with a relatively low intake of fish.

Methods

The Australian Diabetes, Obesity and Lifestyle Study (AusDiab) is a national Australian prospective population-based cohort study of the prevalence and natural history of diabetes. Baseline measurements were collected during 1999–2000 on 11,247 non-institutionalised men and women aged ≥25 years. Detailed methods and response rates have been previously described [21]. Briefly, individuals were recruited from 42 randomly selected urban and non-urban census districts, six in each state and in the Northern Territory. Of the 17,129 eligible households, 20,347 individuals completed a household interview, and 11,247 (55.3 %) had a biomedical examination, resulting in an overall response rate of 37 %. Vital status and cause of death have been determined via linkage to the Australian National Death Index (NDI). The study was approved by the Human Research Ethics Committees of the International Diabetes Institute and Australian Institute of Health and Welfare, and all participants gave written informed consent.

Usual dietary intake was measured at baseline using a self-administered 121-item semi-quantitative food frequency questionnaire validated against weighed food records for macro- and micronutrients [22] and against plasma fatty acid biomarkers for fish intake [23]. Dietary data were analysed using the Australian NUTTAB 95 database, with fatty acid intakes estimated using the RMIT University fatty acid database, which contains fatty acid composition data for more than 1000 Australian foods [24].

For categorisation of fish intake, participants were classified into the following fish consumption groups: (1) <1 serving per month, (2) 1–3 times per month, (3) 1 time per week, (4) 2 times per week, (5) 3–4 times per week, (6) ≥5 times per week for non-fried fish or total fish intake. For analyses examining 'adequacy' of intake, adequate intake was considered to be ≥500 mg/day of very long-chain n-3 fatty acids or 500 g of fish/week as per National Heart Foundation of Australia recommendations [25]. Total n-6 PUFA intake was calculated as the sum of the major dietary n-6 fatty acid, linoleic acid (18:2n-6), plus the minor dietary n-6 components 20:2n-6 + 20:3n-6 + 20:4n-6 + 20:5n-6 + 22:4n-6. Total long-chain n-3 was calculated as the sum of EPA (20:5n-3) + docosapentaenoic acid (22:5n-3) + DHA (22:6n-3).

Risk factors

Data on age, sex, history of CVD (angina, coronary heart disease or stroke) and smoking (never, ex- or current smoker) were collected by interview. Measurements included blood pressure, anthropometrics, fasting bloods and a 75-g oral glucose tolerance test (OGTT). Plasma glucose, serum total cholesterol, triglycerides and high-density lipoprotein (HDL-C) cholesterol were measured using an Olympus AU600 analyser (Olympus Optical, Tokyo, Japan). All specimens were analysed at a central laboratory. Hypertension was defined a systolic or diastolic blood pressure of 140 mmHg or 90 mmHg, respectively, or prescribed anti-hypertensive medication. Diabetes was defined according to WHO definition [26]. Total physical activity time (exercise) was calculated as the sum of the time spent performing moderate activity (including walking) plus double the time spent in vigorous activity [27].

Statistical methodology

To test differences in means and proportions for baseline characteristics, Student's *t* tests or Chi-square analyses

were used, respectively. The follow-up period for all-cause mortality was up to the date of death or 30 November 2012, whichever occurred first. As cause of death information was not available from the NDI for the same follow-up period as vital status data, the period of follow-up for CVD mortality was up to the date of death or 31 December 2009. Thirteen participants who died could not be matched to the NDI and were considered lost to follow-up and excluded, and 31 deceased individuals had no cause of death available at the time of analysis.

The accuracy of the NDI for ascertainment of vital status and CVD deaths has been previously established [28]. People who were not matched to the NDI were assumed to be alive. Deaths were attributed to CVD if the underlying cause of death was coded I10–I25, I46.1, I48, I50–I99 or R96 according to the 2006 International Classification of Diseases 10th revision (ICD-10). Cox proportional hazards regression was used to estimate unadjusted and adjusted all-cause and CVD mortality hazard ratios (HR) and 95 % confidence intervals (CI) of n-3 and n-6 intake. N-3 and n-6 intake were modelled in both quintiles, and trends over quintiles were tested by inclusion of quintile (categorical) variable as a continuous variable in the Cox model. Interactions for fish intake and n-3 and n-6 and sex for all-cause and CVD mortality were examined. Given the low statistical power inherent in interaction tests, interaction with a p value <0.2 was considered positive. Other variables known to be confounding factors were included in the model. Multicollinearity between covariates was examined by calculating the mean and individual covariate variance inflation factors (VIF). Neither the individual nor mean covariate VIFs were greater than 2 VIF for results presented herein.

For all-cause and CVD mortality, we adjusted for age, sex, previous CVD (angina, coronary heart disease or stroke), smoking (current or ex-smokers), exercise, education, diabetes and total dietary energy (kJ). Neither waist circumference nor body mass index (BMI) contributed to the models and were thus not included. Similarly, cholesterol or total cholesterol/HDL ratio was not significant predictor of mortality and was not included in the final models. When we modelled the relationship between fish, PUFA and CVD mortality excluding those with CVD, similar patterns were observed compared to the full cohort, but many estimates had lost statistical significance due to a loss of statistical power. We therefore retained original models using the entire population and adjusted for prior CVD. Nonlinear effects were tested by fitting a quadratic terms of n-3 and or n-6 intake to fully adjusted models. The assumptions required for proportional hazards were met, and these were assessed with graphs of log–log plots of the relative hazards by time and scaled Schoenfeld residuals. Analyses were conducted with SPSS version 18.0 (SPSS, Chicago, Illinois, USA) and Stata Statistical Software version 11.2 (StataCorp, College Station, Texas, USA). Values are given as mean ± SD if not otherwise specified.

Results

Study population

The demographic and CVD risk characteristics of the AusDiab population are presented in Table 1. In brief, 55 % were women and at baseline 15.8 % were smokers and 7.4 % had diabetes. Those who died in the follow-up period had significantly lower rates of schooling completion and higher rates of diabetes and hypertension (Table 1).

Dietary n-3 PUFA intake

The AusDiab population had a dietary intake of n-3 fatty acids approximately half of that recommended for primary prevention of CVD, as shown in Table 2. Median intake of long-chain n-3 PUFA (EPA + DPA + DHA, hereafter referred to as 'n-3') in this cohort of Australian adults was 0.26 g/day for women and 0.33 g/day for men. Those in late middle age (55–64 years) were more likely to be meeting recommended intake targets than younger age groups ($p < 0.001$). Across all age ranges, men had higher n-3 intakes than women ($p < 0.001$) (Table 2), and men also had higher mean intakes of n-6 polyunsaturated fat (8.97 and 12.03 g/day for women and men, respectively, $p < 0.001$) and higher total energy and fat intake (data not shown).

All-cause and CVD mortality

Over a median of 12.6 years, there were 1265 deaths (54 % in men) representing a mortality rate of 9.3 per 1000 person years. For CVD mortality, there were 277 deaths (53 % in men) over a median follow-up of 9.7 years. The CVD mortality rate was 2.6 per 1000 person years.

Polyunsaturated fat intake and mortality

Those in the top quintile of n-6 intake were significantly protected from total and CVD mortality in age- and sex-adjusted models, with a significant dose response observed, p for trend = 0.024, which did not retain significance following further adjustment for previous CVD, education, exercise, diabetes, total dietary energy and smoking (Table 3). N-3 intake was not an independent predictor of all-cause or CVD mortality in this population with relatively modest fish intake, and there was no evidence of a relationship between n-3 intake and all-cause mortality and CVD mortality which deviated from linearity ($p > 0.05$). The top quintile of n-3 intake was associated

Table 1 Baseline characteristics of the AusDiab population according to vital status

	Alive	All-cause death	Alive	CVD death
N	9982	1265	10,930	277
Sex (Men)	43.7	54.23	44.5	57.0
Age (years) (SD)	49.2 (13.0)	70.1 (11.5)**	50.9 (14.1)	75.0(9.0)**
Education				
Bachelor degree/post grad	18.1	7.5	17.2	4.8
Associate diploma	12.6	8.7**	12.3	7.8**
Trade/technician	30.2	29.4	30.2	27.0
Secondary school	39.2	54.5**	40.4	60.4**
Smoking				
No smoking	56.0	47.1	55.3	45.9
Ex smoker	28.1	37.7	28.9	42.5
Current smoker	15.9	15.2*	15.8	11.6**
Diabetes	6.7	22.1	7.8	27.7**
History of CVD	5.6	29.6**	7.5	41.2**
BMI (kg/m^2)	27.0 (5.0)	27.2(4.9)	27.0 (5.0)	27.0 (4.5)
Waist (cm)	90.4(13.9)	95.2 (13.7)**	90.8 (14.0)	95.7 (13.5)**
Hypertension	28.1	67.7**	31.2	78.5**
Total cholesterol (mmol/l)	5.6 (1.1)	5.7 (1.1)*	5.7 (1.1)	5.7 (1.1)
HDL cholesterol (mmol/l)	1.4 (0.4)	1.4 (0.4)	1.4 (0.4)	1.4 (0.4)
Triglyceride (mmol/l)	1.3 (0.9,1.9)	1.4 (1.2, 2.0)**	1.3 (0.9,1.9)	1.4 (1.0, 2.0)
VLC n-3 PUFA (g/day)	0.3 (0.2, 0.5)	0.3 (0.1, 0.5)*	0.3 (0.2, 0.5)	0.2 (0.1, 0.4)*
n-6 PUFA (g/day)	9.4 (6.3,13.5)	9.2 (6.1,12.6)	9.4 (6.3,13.4)	8.9 (5.6,11.9)
Saturated fat (g/day)	28.6 (20.1, 39.7)	25.5 (17.5, 35.5)**	28.3 (19.9, 39.4)	23.7 (17.3, 33.0)**

Data given as either mean (SD), median (25th, 75th interquartile range) or percentage of reporting population

Differences between alive and deceased (all-cause or CVD-specific death) groups denoted by * $p < 0.05$ or ** $p < 0.001$

Table 2 Median long-chain omega-3 fatty acid intake in a cohort of Australian adults aged ≥25 years

	N	DHA (g/day)	EPA (g/day)	DHA + EPA + DPA (g/day)
Men (years)				
25–34	560	0.20 (0.10, 0.35)	0.09 (0.04, 0.16)	0.34 (0.16, 0.57)
35–44	1093	0.21 (0.13, 0.33)	0.09 (0.05, 0.16)	0.34 (0.21, 0.55)
45–54	1345	0.21 (0.11, 0.32)	0.09 (0.05, 0.14)	0.34 (0.18, 0.54)
55–64	928	0.20 (0.14, 0.36)	0.09 (0.05, 0.17)	0.32 (0.19, 0.60)
65–74	731	0.18 (0.10, 0.28)	0.07 (0.04, 0.13)	0.28 (0.16, 0.47)
≥75	362	0.15 (0.06, 0.27)	0.06 (0.03, 0.12)	0.25 (0.10, 0.45)
All men	5019	0.20 (0.11, 0.33)	0.09 (0.04, 0.15)	0.32 (0.18, 0.55)
Women (years)				
25–34	803	0.16 (0.09, 0.28)	0.07 (0.04, 0.13)	0.26 (0.14, 0.46)
35–44	1465	0.16 (0.09, 0.26)	0.07 (0.04, 0.12)	0.26 (0.14, 0.43)
45–54	1546	0.15 (0.09, 0.29)	0.07 (0.04, 0.13)	0.25 (0.15, 0.47)
55–64	1096	0.18 (0.10, 0.30)	0.08 (0.04, 0.14)	0.29 (0.16, 0.49)
65–74	837	0.14 (0.08, 0.25)	0.07 (0.03, 0.12)	0.24 (0.13, 0.43)
≥75	451	0.13 (0.06, 0.23)	0.06 (0.03, 0.11)	0.22 (0.11, 0.35)
All women	6198	0.16 (0.09, 0.27)	0.07 (0.04, 0.13)	0.25 (0.14, 0.45)

Intake data given as weighted medians (25th, 75th interquartile range)

Table 3 Crude and adjusted hazard ratios for n-3 and n-6 PUFA consumption and all-cause and CVD mortality

	All-cause mortality				CVD mortality				Median PUFA intake (g)
	Cases/total (*n*/*N*)	Person (years)	Model 1	Model 2	Cases/total (*n*/*N*)	Person (years)	Model 1	Model 2	
Intake in quintiles									
N-3	350/2382	28,479	1.00	1.00	90/2374	22,334	1.00	1.00	0.09
2	214/2117	25,862	0.82 (0.69–0.97)	0.98 (0.81–1.19)	47/2111	20,167	0.75 (0.53–1.08)	0.92 (0.61–1.37)	0.19
3	237/2250	27,358	0.90 (0.78–1.06)	1.07 (0.89–1.30)	58/2248	21,382	0.93 (0.64–1.30)	1.04 (0.70–1.53)	0.29
4	213/2249	27,440	0.82 (0.69–0.98)*	0.98 (0.80–1.20)	41/2240	21,353	0.69 (0.48–1.00)	0.88 (0.57–1.36)	0.44
5	251/2249	27,338	1.05 (0.89–1.24)	1.39 (1.13–1.70)*	41/2234	21,262	0.79 (0.54–1.14)	1.00 (0.62–1.60)	0.81
N-6	286/2250	27,148	1.00	1.00	70/2245	21,259	1.00	1.00	4.12
2	247/2249	27,279	0.92 (0.77–1.09)	1.04 (0.85–1.28)	54/2244	21,291	0.84 (0.58–1.19)	1.12 (0.73–1.75)	6.91
3	268/2250	27,171	0.94 (0.80–1.11)	1.13 (0.92–1.39)	60/2244	21,222	0.85 (0.60–1.20)	1.22 (0.79–1.88)	9.39
4	249/2249	27,261	0.89 (0.75–1.05)	1.04 (0.83–1.29)	58/2239	21,254	0.85 (0.60–1.20)	1.14 (0.72–1.81)	12.45
5	215/2249	27,618	0.82 (0.69–0.99)*	1.02 (0.79–1.32)	35/2235	21,472	0.57 (0.38–0.86)**	0.88 (0.38–1.55)	17.68

Model 1 adjusted for age (timescale) and sex. Model 2 adjusted for age (timescale), sex, previous CVD, education, exercise, diabetes, total dietary energy and smoking

* Significant at $p < 0.05$, ** significant dose response, $p = 0.024$

with a significantly increased risk of all-cause mortality, p for trend = 0.009 (Table 3, Model 2). Compared to those in the lowest quintile, those in the highest quintile of n-3 intake were younger (51.0 ± 13.7 vs 52.6 ± 15.9 years, $p = 0.0002$), but had significantly higher total energy intake (10,229 ± 4410 vs 6322 ± 2247 kJ/day, $p < 0.0001$), saturated fat intake (46.3 ± 23.8 vs 25.0 ± 11.7 g/day, $p < 0.0001$) and higher BMI (27.4 ± 5.0 vs 26.9 ± 5.3 kg/m^2, $p = 0.0001$). We detected significant interactions for the relationship of n-3 intake ($p = 0.032$) and n-6 intake ($p = 0.083$) and sex and CVD mortality which met our criteria. Figure 1 shows adjusted sex-specific HRs (95 % CI) for the relationship between n-3 and n-6 intake in quintiles and all-cause and CVD mortality, and while not significant, the effects of n-6 PUFA on CVD mortality showed divergent trends in men and women.

Frequency of fish consumption and mortality

In sex- and age-adjusted models, frequency of intake of non-fried fish was associated with lower risk of cardiovascular mortality (1–3 serves/month HR 0.64 (95 % CI 0.43–0.94), 1 serve per week: 0.64 (0.45–0.91) and 2 or more serves per week: 0.63 (0.44–0.90), all $p < 0.05$. But as can been seen in Fig. 2, after adjustment for sex, previous CVD, education, exercise, diabetes, total energy intake and smoking, the effect of non-fried fish consumption just failed to reach significance [HR 0.70 (95 % CI 0.47–1.02)]. The addition of BMI or waist did not materially alter the results. We detected significant interactions for the relationships of total fish consumption and non-fried fish consumption and sex for all-cause mortality ($p = 0.021$) and CVD

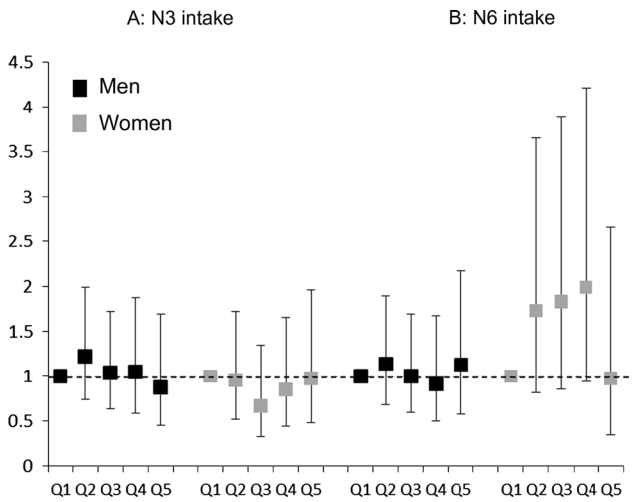

Fig. 1 CVD mortality by n-3 and n-6 intake in men and women. Data presented as adjusted HR ± 95 % CI. Model adjusted for age (time scale), previous CVD, diabetes, smoking, total dietary energy, education and alternately adjusted for n-3 and n-6 intake

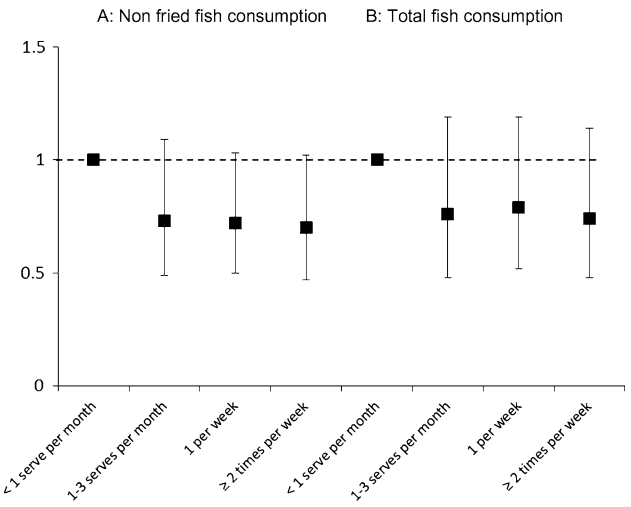

Fig. 2 CVD mortality by frequency of fish intake. Data presented as adjusted HR ± 95 % CI. HRs are adjusted for age, sex, previous CVD, smoking, total dietary energy, exercise and education

mortality ($p = 0.001$), and Table 3 gives the adjusted HR (95 % CI) for non-fried and total fish consumption by sex. As total fish consumption increased, the risk of all-cause mortality increased, but these trends were not significant (Fig. 2; Table 4).

Discussion

The present study found that n-6 PUFA intake was inversely associated with risk of all-cause and cardiovascular mortality, although the significance of these associations was not maintained after multivariate adjustment. The findings of this study make an important contribution to the ongoing debate surrounding PUFA intake and CVD risk, contrasting a recent finding from long-term follow-up of another Australian cohort (the Sydney Diet Heart Study, a randomised dietary intervention trial) which found significantly higher rates of cardiovascular mortality in participants randomised to replacement of saturated fat with n-6 PUFA compared to those who maintained their usual diet [29]. Other prospective studies have found no significant association between n-6 PUFA and mortality [17, 18], while the Nurses' Health Study is among the largest of the prospective studies which have found protective effects of n-6 PUFA on cardiovascular mortality [30]. The complexity of measuring and manipulating diet is a major limitation in long-term outcome studies, and this is reflected in contrasting conclusions from recent meta-analyses and reviews. Two recent meta-analyses highlight this equivocacy, and one meta-analysis of randomised controlled trials concluded that diets high in n-6 PUFA might in fact increase the risk of CHD and mortality [20], whereas another recent meta-analysis including the Nurses' Health Study data has concluded that dietary linoleic acid (the major n-6 PUFA) was dose dependently inversely associated with cardiovascular mortality [31]. The findings of the present study strengthen support for the American Heart Association review which suggested that high intakes of n-6 PUFA were safe or possibly even cardioprotective [32].

The mechanisms governing cardioprotective effects of n-6 PUFA remain to be fully established. Early studies highlighted the cholesterol-lowering effects of n-6 PUFA-rich diets [33], while more recent studies have noted that increased linoleic acid (18:2 n-6) intake is associated with reduced levels of C-reactive protein [34] and lower blood pressure [35], although the findings of the current study do not suggest that blood pressure was a driver of lower risk by quintile of n-6 intake. Conversely, carriers of risk alleles for hyperhomocysteinemia show increased levels of

Table 4 Adjusted HR (95 % CI) for total fish consumption and non-fried fish consumption and all-cause and CVD mortality by sex

	All-cause mortality		CVD mortality	
	Men	Women	Men	Women
Total fish consumption				
<1 serve per month	1.00	1.00	1.00	1.00
1–3 serves per month	0.73 (0.55–0.98)*	0.76 (0.54–1.08)	0.56 (0.31–1.02)	1.12 (0.53–2.33)
1 serve per week	0.81 (0.62–1.05)	0.84 (0.62–1.15)	0.77 (0.47–1.29)	0.88 (0.44–1.77)
2 or more times per week	0.96 (0.73–1.26)	0.97 (0.72–1.32)	0.69 (0.40–1.20)	0.85 (0.42–1.73)
Non-fried fish consumption				
<1 serve per month	1.00	1.00	1.00	1.00
1–3 serves per month	0.93 (0.76–1.27)	0.73 (0.54–0.98)	0.85 (0.51–1.41)	0.59 (0.31–1.31)
1 serve per week	0.98 (0.77–1.24)	0.78 (0.60–1.01)	0.77 (0.48–1.24)	0.60 (0.35–1.03)
2 or more times per week	1.11 (0.87–1.42)	0.91 (0.70–1.17)	0.72 (0.44–1.19)	0.60 (0.34–1.03)

Adjusted for age (timescale), previous CVD, education, exercise, diabetes, total dietary energy and smoking

There were no significant dose responses

plasma homocysteine with increased n-6 PUFA intake [36], and linoleic acid intake was found to be associated with increased ex vivo lipoprotein oxidisability [37]. Despite concerns surrounding pro-inflammatory and pro-atherogenic effects of n-6 PUFA which have been shown experimentally, to date there is a little evidence of an increase in inflammatory-related CVD with high n-6 PUFA intake [31, 38]. While not significant, the findings of the present study suggested the possibility of sex differences in cardioprotection associated with n-6 PUFA. Sex was also identified as a significant source of heterogeneity in the meta-analysis of linoleic acid intake and coronary heart disease risk by Farvid and colleagues [30], but the nature and magnitude of any sex differences in cardioprotection by n-6 PUFA remain to be clarified. There is some evidence to suggest that there may be sex differences in incorporation of n-6 PUFA into platelet cell membrane phospholipids [39].

In the AusDiab cohort, non-fried fish consumption at a frequency of 2 or more times per week was associated with a significant reduction in CVD mortality risk. This effect was consistent, though more modest, than large cohort studies such as the US Physician's Health Study (RR 0.47, CI 0.23–0.98) [40] and the Cardiovascular Health Study (HR 0.53, CI 0.30–0.96) [4]. However, those in the highest quintile of n-3 intake had a significantly increased risk of all-cause mortality, which raises a number of considerations. Fish and seafood are a major source of long-chain n-3 PUFA in the diet, although meat sources also make a significant contribution in the Australian diet [24], raising the possibility of confounding arising from high red meat intake [41]. The dietary questionnaire used in the AusDiab Study was a 121-item food frequency questionnaire which had previously been validated for measurement of fish intake against plasma fatty acid biomarkers [22], but dietary intake is notoriously difficult to measure and this may contribute to an increase in error when examining relationships between health outcomes and nutrients found in small amounts (such as n-3 PUFA). Although not available in the present study, red blood cell fatty acid composition would be a more robust biomarker of usual long-chain n-3 intake [42]. Another possibility is that there may be other nutrients or contaminants in fish influencing risk of cardiovascular mortality. Fish is a good source of protein and selenium, but may also contain amounts of compounds with adverse effects, such as methylmercury, polychlorinated biphenols or dioxins, sometimes in large concentrations [43].

There remains considerable experimental evidence to support independent anti-arrhythmic and anti-inflammatory effects of n-3 [8, 44], and the results of the GISSI-P randomised controlled trial of fish oil also strongly support the theory that EPA + DHA reduce risk of cardiovascular mortality [7]. Experimental evidence suggests that there is a threshold of incorporation into cellular membranes [9], and the body of clinical and human trial evidence suggests that the threshold for prevention of CVD mortality may be around 500 mg EPA + DHA per day (equivalent to around 2–3 servings of fatty fish per week) [45]. The intake of long-chain n-3 PUFA in the AusDiab cohort was slightly higher than that reported in the last published national survey of Australian dietary intake conducted in 1995 (which was in the order of ~0.25 g/day n-3 PUFA) [46], although intakes remained around half of that recommended by Australian and international bodies for primary prevention of CVD and less than a third of that recommended for secondary CVD prevention.

The present study found a trend towards sex differences in n-3 PUFA-mediated protection from CVD mortality, with a non-significant n-3-mediated cardioprotection noted in men, but not women. Whether sex differences exist in the effects of n-3 PUFA on CVD has yet to be clarified. Although some studies examining n-3 PUFA and CVD mortality have found no differences between men and women [47], others have noted sex effects [48, 49]. Mechanistically, it has been shown that sex differences may exist in the anti-inflammatory effects of VLC n-3 PUFA, with EPA more effectively reducing platelet aggregation in males, whereas an anti-inflammatory effect of DHA is noted in females, but not males [50]. In addition, males and females may differ in metabolism of VLC n-3 PUFA post-ingestion [51].

The prospective study design is a strength of the present analyses; however, there are some limitations to be considered. The assumption was made that the dietary patterns measured represent usual and relatively stable dietary intake for the population under study, and in addition, use of dietary supplements was not assessed. While the assessment of diet using FFQs has limitations, in nutritional epidemiology FFQs remain widely used and accepted tools. One cannot discount the possibility of residual confounding from factors which were not measured in the present study, although attempts were made to include known plausible confounding factors.

In conclusion, this study suggests that increased dietary n-6 PUFA intake lowers risk of cardiovascular mortality. While the present study found intake of non-fried fish to be associated with a lower risk of cardiovascular mortality, cardiovascular benefits of n-3 PUFA were not evident.

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Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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